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Anti-Obesity Effects of Extracts from Sulfur-Grain Maggot In Obesity Model Rats

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ABSTRACT

This research tried to explain only a part of mechanisms possibly involved in anti-obesity effects of the Extracts of Sulfur-grain Maggot (ESM). Animals were classified into a normal diet group (NC, normal control), HFD (high-fat diet without ESM), HFD 15 (high-fat diet+oral administration of 15 mg of ESM extract/100 g body weight) and HFD 30 (high-fat diet+oral administration of 30 mg of ESM extract/100 g body weight). The body weight gain declined in HFD 15 and HFD 30 groups compared with the HFD group, even though the diet intake increased significantly. The weight of liver and adipose tissue decreased significantly in HFD 15 and 30 groups compared with the control HFD. Triglyceride, total cholesterol, low density lipoprotein-cholesterol (LDL-C) and Atherogenic Index (AI) decreased in HFD 15 and HFD 30 groups compared with in the HFD group, but the contents of high density lipoprotein-cholesterol (HDL-C) increased significantly. Expression of sterol regulatory element binding proteins (SREBP-1α, SREBP-2)-mRNA in the liver was decreased in HFD 15 and 30 groups compared with the control HFD but the expression of lipoprotein lipase (LPL) and peroxisome proliferator-activated receptors(PPARα)-mRNAin adipose tissue increased significantly. Fat accumulation in the liver tissues and liver damage were greatly reduced in HFD 15 and HFD 30 groups compared with the HFD group. In conclusion, this research discovered for the first time that grain maggot has anti-obesity effects, by reducing the abdominal fat of obese model animals and lowering blood lipid level through the down-regulation of SREBP-1α, SREBP-2 and the up-regulation of LPL-mRNA, not only PPARα-mRNA.

Key words: Sulfur-grain maggot, lipid profile, genes, obesity

INTRODUCTION

Today, we are witnessing a growing incidence rate of hyperlipidemia, CVD (coronary vascular disease), obesity and type 2 diabetes, all of which are metabolic diseases caused by dietary habits. Obesity is caused by an imbalance of energy metabolism, with excessive energy stored in the adipocytes and accumulated within the body. Obesity can cause hyperlipidemia, hypertension, fatty liver, diabetes, CVD and carcinogenesis by inducing insulin resistance and increasing inflammatory response (Bray *et al*., 2004; Adaramoye *et al*., 2008). The rise of blood LDL-C caused by the intake of a high-fat diet has been identified as a main cause of death from CVD (Yamaguchi *et al*., 2012). Gene expression of SREBP-1α, SREBP-2, PPARα and LPL regulates lipid metabolism, so it has a close relationship with obesity (Wang and Eckel, 2009; Rodriguez-Cantu *et al*., 2011; Rotllan and Carlos, 2012). Sulfur has been known to have antioxidation (Atmaca, 2004), anti-obesity (Ban *et al*., 2012) and anti-cancer effects (Wu *et al*., 2005; Lee *et al*., 2008), as well as preventive effects for rheumatoid arthritis (Hasegawa *et al*., 2004; Kim *et al*., 2006). It also has other diverse bioactive effects related to hemostasis, neural paralysis and cold limbs. To increase the efficacy of sulfur, sulfur toxicity clearance technology has been developed along with poultry production technology using sulfur

(Komarnisky *et al*., 2003; Park *et al*., 2010a). Biotherapies began to be applied to medicine such as the treatment of patients with chronic wound infection and the treatment of burns or pressure sores by utilizing *Musca domestica* L. (Sherman *et al*., 2000; Scavee *et al*., 2003). In particular, *Musca domestica* L. treatment is being recommended for patients infected with gram positive bacteria including *Staphylococcus aureus*. It has been reported that *Musca domestica* L. extract contains strong antibacterial peptide. In Republic of Korea, *Musca domestica* L. has been known as grain maggot since a long time ago and has been used as a folk medicine (Jaklic *et al*., 2008; Park *et al*., 2010b). The grain maggot is non-toxic and its diverse pharmacological effects are well known. The effects of the grain maggot are described in old books as follows: Grain maggot purifies the blood and lowers fever (Chinese Dictionary) and effective for lowering fever (Ben Cao Gang Mu or Compendium of MateriaMedica), prescribed for the treatment of malignant boil in lips in Prime Pyeondok (Park *et al*., 2010a). Grain maggot extract is known to have the following effects: antibacterial activation of antibiotics resistant bacteria (MRSA, VRE), reduction of blood lipid and sugar and protection of alcoholic liver damage (Park *et al*., 2010b). Recently, in an attempt to create a synergy between the bioactive effects of sulfur and grain maggot, bioconversion technology was developed to produce high-functional sulfur-grain maggot (Park and Park, 2012). However, the bioactive effects of sulfur-grain maggot in terms of anti-obesity are not well known. This study orally administered the extract of sulfur-grain maggot to obesity model rats to identify its effect on the anti-obesity and the level of blood lipid, as well as the mechanism by which it functions.

MATERIALS AND METHODS

Preparation of extracts: Insect bio (Chuncheon, Gangwon Province, Republic of Korea) provided sulfur-maggot (grain maggot produced with 2.5% of sulfur powder in medium) that was grown in an artificial environment using corn, sugar and milk powdered formula as medium which was dried in an oven at 70°C. A defatted sample was obtained by compressing for 30 min with 1,000 PSI at a high temperature of 150°C and removing the lipid completely with hexane. After mixing a defatted sample with ethanol (w/v 1:10), reflux condensing system was used for repeated extraction three times for 3 h each at 60°C to obtain an ethanol extract sample. The extract underwent vacuum evaporation using a rotary vacuum evaporator (Eyela N-1000, Tokyo Rikakikai Co., Japan) and was stored in a refrigerator to be used as a sample. Through this method, we secured 3.50 g extract of ESM containing 0.37% of sulfur from 100 g of maggots dried.

Experimental design and feeding management: Animal testing was conducted in compliance with EEC Directive of 1986; 86/609/EEC and approval was obtained from IACUC (Institutional Animal Care and Use Committees) of Kangwon National University (No. 20100017). We purchased 40 eight-week old (average weight: 200±2.50 g) Sprague-Dawley strain male rats from Daehan Bio Link (Eumseong, Chungbuk). In order to verify the varying clinical effectiveness per gender of subject animals, we used male animals primarily and the 2nd phase with female ones is under way. Reasons for selecting males first were: Hormonal changes from estrous cycles of females may influence the results and researchers prefer males as they reduce deviations in results and improve reliability. After a week of acclimatization, they were raised for 40 days with oral administration of the extract. Treatment groups were divided into NC (normal control group with purified pellet diet), HFD (high-fat diet control group without ESM), HFD 15 (high-fat diet group with oral administration of ESM 15 mg/100 g body weight) and HFD 30 (high-fat diet group with oral administration of ESM 30 mg/100 g body weight) and rats were raised separately in individual cages with 10 repetitions. Purified pellet diet (g/100 g) was made by mixing casein 20.0, corn starch 51.15, maltodextrin10.0, sugar 7.0, soybean oil 5.0, powdered cellulose 5.0, AIN 93G mineral mixture 1.00, AIN 93G vitamin mixture 0.30, L-cystine 0.30, Choline bitartrate 0.25 and t-Butylhydroquinone 0.0014. For obese model animals, a high-fat diet containing 40 g/100 g lipid was used (Park *et al*., 2013). High-fat diet (w/w, g/100 g diet) was made by mixing soybean oil 5and lard 35 gand the contents of added corn were adjusted. Temperature of the experimental animal breeding room was maintained at 20±2 with relative humidity of 60±5%. Lighting was adjusted every 12 h. Experimental diet and water were provided without limit. Average daily body weight gain and diet intake were investigated during the experimental period.

Oral administration: Purified pellet diet group (NC group) and high-fat diet groups (HFD, HFD 15 and HFD 30 groups) were fed separately and ESM was orally administered through a stomach tube 1 mm in diameter at a particular time every day. One milliliter of saline and the contents of ESM corresponding to each treatment group were orally administered to the NC group and high-fat diet groups, respectively, by dissolving in 1 mL of saline.

Collection of blood and organs and biochemical analysis: Concerning animal sacrifice, diet was withdrawn from 12 h before termination and only water was provided for the convenience of anesthesia and anatomy. The animals were anesthetized with zoletil/rompun (10 and 5 mg kg^{-1}, respectively). The blood was collected from abdominal aorta using heparinized vacuum tube (Becton Dickinson Vacutainer System, Franklin lakes, NJ 07417, USA). Centrifugation was performed for 15 min at 3,000 g to separate plasma which was quickly frozen at -196°C by liquid nitrogen and stored at -20°C until the next biochemical analysis. Liver, kidney, heart, spleen and abdominal fat were taken off immediately after blood collecting and blood was washed away with saline. Then they were absorbed onto Whatman filter paper to remove water and measure the weight of these organs. Among blood lipid fractions, triglyceride, total cholesterol, HDL-C and LDL-C were analyzed using Diagnostic Kit (Sigma chemical Co, St, Louis, MO, USA). AI that is used to determine the risk level of CVD was calculated using the following formula: [(total cholesterol)-(HDL-C)]/HDL-C (Mildner-Szkudlarz and Bajerska, 2013). Liver function enzymes such as AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) were measured using a biochemical blood autoanalyzer (Fuji Dri-Chem 3500, Japan).

Gene expression of SREBPs and PPARα: Real-Time Polymerase Chain Reaction (RT-PCR) was used to measure the relative level of LPL-mRNA transcripts for -Actin in adipose tissue and SREBP-1α, SREBP-2 and PPARα-mRNA for Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in liver (Park and Park, 2014). In summary, liver and adipose tissues were collected and quickly frozen using liquid nitrogen to be stored at -80°C. Lysis buffer of Xprep Tissue RNA Mini Kit (Philekorea Technology, PKT) was used to 30 mg of liver and adipose tissue to extract total RNA. The RNA concentration was measured by absorbance at 260 nm using a NanoDrop ND-1000 Spectrophotometer (USA) to obtain extracted RNA 300-500 ng μL^{-1}. cDNA synthesis kit (PKT) was used for the extracted 1 µg RNA to synthesize the 1st strand cDNA. The cDNA was amplified by being grown at 70°C for 5 min, at 42°C for 30 min and at 85°C for 5 min. Finally, the amount of 500 ng cDNA was used as template by RT-PCR and relative concentration was identified as to the mRNA transcription of each target gene for reference gene GAPDH. QuantiMix SYBR Kit (PKT) was used for RT-PCR and cDNA was diluted at a ratio of 1:5. Then, each primer (Forward and Reverse) was mixed. Gene expression was performed in accordance with the gene expression protocol manual suggested by Eco Real-Timc PCR (Illumina Inc.). The GAPDH and β-actin were used to regulate the concentration of mRNA as the housekeeping gene. Specific oligonucleotide primers are as follows:

GAPDH forward5'-TGCACCACCAACTGCTTAG3',reverse5' GGATGCAGGGATGATGTTC-3';SREBP-1α forward 5'-ATGGACGAGCTGCCCTTCGGTGAGGCGGCT-3', reverse 5'-CCTGGCGATGGCTGTGTGCTG-3'; SREBP-2forward 5'-TTTGTCAGCAATCAAGTGGGAGAGTTC-3', reverse 5'-GCTGCGTTCTGGTATATCAAAGGCTGC-3'; PPARα forward 5'-CCCTCTCTCCAGCTTCCAGCCC-3', reverse 5'-CCACAAGCGTCTTCTCAGCCATG-3'; β-actin forward 5'-CTCTTCCAGCCTTCCTTCCT-3', reverse 5'-AGCAC TGTGTTGGCGTACAG-3'; LPL forward 5'-CAGCTG GGCCTAACTTTGAG-3', reverse 5'-AATGGCTTC TCCAATGTTGC-3'.

Electron microscopic examination of liver and adipose cells: To observe liver and adipose cells, liver and abdominal fat tissues were obtained after blood collecting and were cut into 1 mm^3 samples within 3 min. Then, the samples were buffered with 0.1 M cacodylate (pH 7.4) and fixed and washed with 4% glutaraldehyde. After fixing with 2% osmium tetroxide (OsO_4, 2%), they were washed with buffer solution and dried with ethanol. The samples were substituted with propylene oxide and dehydration solution within tissue was infiltrated and embedded with EPON resin. They underwent embedding and polymerization at 60°C and a 300 nm thick slice was made with ultramicrotome, which was dyed with toluidine blue. Liver and adipose cells were observed using an Energy Filtering-Transmission Electron Microscope (EF-TEM, Leo 912AB, Carl Zeiss Inc., Germany).

Statistics analysis: For statistical interpretation of the analyzed data, the SAS software package was used. After calculating average and standard error of each treatment group and performing analysis of variance, significance ($p<0.05$) was examined at a level of 95% through Duncan's multiple range test (SAS., 2004).

RESULTS

Growth performance: When ESM was administered orally to obesity animals that ingested a high-fat diet, diet intake, the body weight gain and dieting efficiency are as summarized in Table 1. Diet intake was significantly higher in HFD 30, HFD 15 and NC groups compared with HFD group. The HFD 15 and HFD 30 groups recorded a lower value than NC group but there was not a difference between HFD 15 and HFD 30 groups. Body weight gain was significantly lower in HFD 15, HFD 30 and NC groups compared with the HFD group but there was no difference among HFD 15, HFD 30 and NC groups. The HFD 15 and HFD 30 groups showed body weight decrease by 25.55% compared with the HFD group which was similar to the NC group. Diet efficiency was lower among the HFD 15, HFD 30 and NC groups compared with the HFD group and there was a significant difference among the treatment groups.

Weight of organs: Weight change of liver, kidney, heart, spleen and adipose tissue after ESM administration is described in Table 2. Except for kidney, heart and spleen, which showed no difference among the treatment groups, the weights of liver and adipose tissues were significantly lower among HFD 15, HFD 30 and NC groups compared with HFD group but there was no significant difference between HFD 30 and NC groups. Compared with HFD group, liver weight

Table 1: Effect of oral administration of ESM on diet intake, body weight gain and diet efficiency ratio in obesity rats

Items	NC	HFD	HFD 15	HFD 30	p-value
Diet intake (g/day/head)	29.74 ± 0.58^{a}	21.33 ± 0.42^{c}	25.46 ± 0.63^{b}	26.05 ± 0.47^{b}	0.0231
Body weight gain (g/day/head)	5.87 ± 0.51^{b}	8.18 ± 0.33^{a}	6.37 ± 0.42^{b}	6.09 ± 0.37^{b}	0.0122
DER	0.20 ± 0.01^{d}	0.39 ± 0.01^{a}	0.27 ± 0.01^{b}	0.25 ± 0.01^{c}	0.0028

NC: Normal control, HFD: High-fat diet induced obesity rats, HFD15: High-fat diet induced obesity rats with ESM 15.0 mg/100 g body weight HFD30: high-fat diet, induced obesity rats with ESM 30.0 mg/100 g body weight. Values are Mean±Standard errors (n = 10), DER: Diet efficiency ratio, body weight gain/diet intake, ^{a,b,c,d}values with different superscripts within the same row are significantly different at $p<0.05$

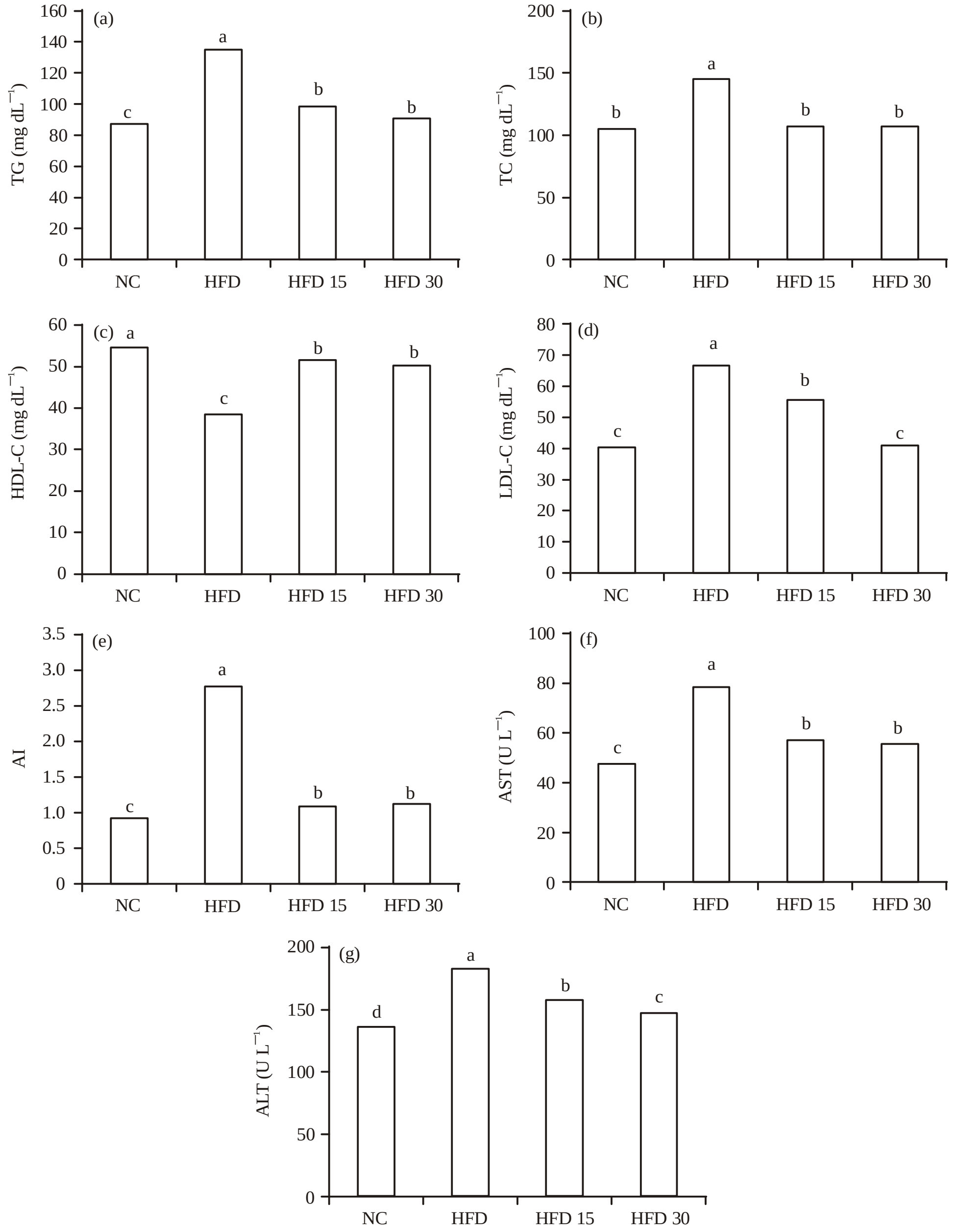

Fig. 1(a-g): Effect of oral administration of ESM on blood lipid profiles, (a) $T6_1$, (b) Tc, (c) HDL, (d) LDL, (e) AI, (f) AST and (g) ALT in obesity rats. Bars are Mean±standard errors ($n = 10$), ^{a,b,c,d}Values are significantly different at $p<0.05$

Table 2: Effect of oral administration of ESM on weight of organ and adipose tissues in obesity rats (g/100 g body weight)

Items	NC	HFD	HFD 15	HFD 30	p-value
Liver	3.24 ± 0.17^{c}	4.53 ± 0.22^{a}	3.84 ± 0.23^{b}	3.30 ± 0.27^{c}	0.0207
Kidney	0.60±0.21	0.63±0.23	0.61±0.32	0.59±0.27	0.0105
Heart	0.30±0.08	0.39±0.10	0.37±0.10	0.35±0.14	0.0028
Spleen	0.31±0.04	0.32±0.11	0.30±0.08	0.33±0.07	0.0017
Adipose tissue	3.84 ± 0.27^{c}	7.05 ± 0.28^{a}	6.18 ± 0.20^{b}	5.30 ± 0.22^{c}	0.0337

Values are Mean±Standard errors ($n = 10$), ^{a,b,c,d}values with different superscripts within the same row are significantly different at $p<0.05$

declined by 15.23, 27.15 and 28.48% in the HFD 15, HFD 30 and NC groups, respectively. The weight of adipose tissue went down by 12.43, 24.82 and 45.53%, respectively, in the above groups.

Blood lipid profiles, AST and ALT: The change of blood lipid profiles, AST and ALT in the event of ESM administration to high-fat diet induced obesity animals is described in Fig. 1. Triglyceride was significantly lower in the

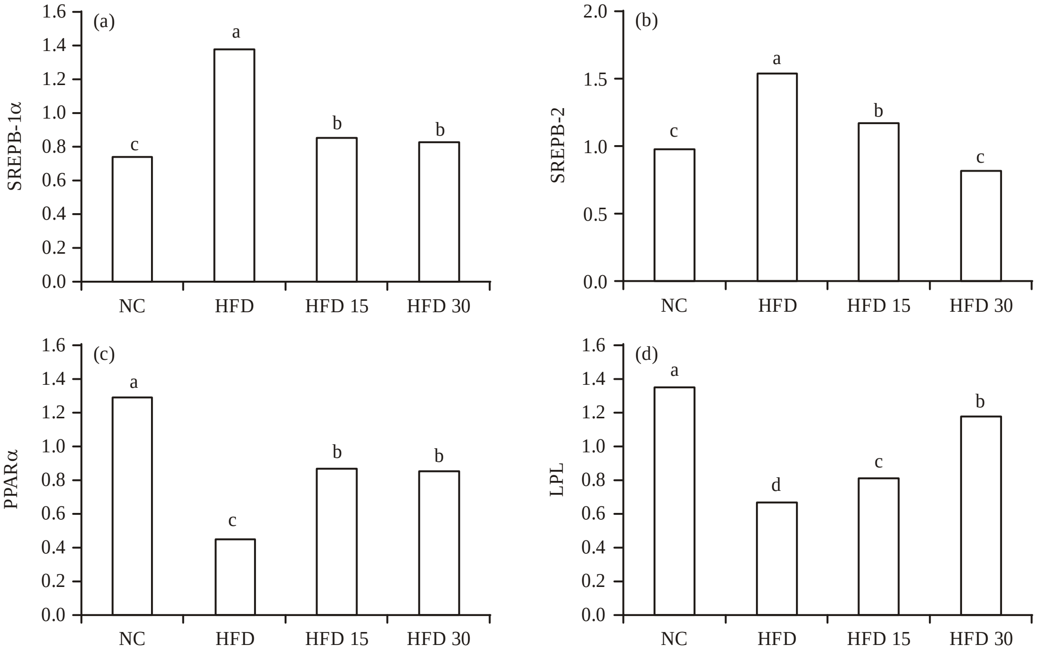

Fig. 2(a-d): Effect of oral administration of ESM on gene expressions of (a) SREBP-1α and (b) SREBP-2 mRNA in liver or (c) PPAR-α and (d) LPL mRNA in adipose tissue in obesity rats. Bars are Mean±Standard errors (n = 10). ^{a,b,c,d}Values are significantly different at $p<0.05$

HFD 15, HFD 30 and NC groups compared to the HFD group, by 27.36, 32.40 and 35.12%, respectively and HFD 15 and HFD 30 group registered higher values than NC group. Total cholesterol was significantly lower in HFD 30, HFD 15 and NC groups compared to HFD group, by 26.33, 26.70 and 27.53%, respectively but there was no difference among these 3 groups. LDL-C was significantly lower in HFD 15, HFD 30 and NC groups compared to HFD group, by 17.06, 38.70 and 39.27%, respectively. HFD 30 and NC groups showed lower value than HFD 15 group, but there was no difference between the two groups. The HDL-C was significantly higher in HFD 15, HFD 30, compared to HFD group by 142.27, 106.17 and 109.09%, respectively. The value of HFD 15 and HFD 30 groups was significantly lower than that of NC group, by 5.81 and 8.33%, respectively. The AI was significantly lower in HFD 30, HFD 15 and NC groups, by 11.12, 59.72 and 66.91%, respectively, compared to HFD group. AI and AST was significantly lower in HFD 15, HFD 30 and NC groups, by 27.30, 28.97 and 39.22%, respectively, compared to HFD group. The HFD 15 and HFD 30 groups registered a significantly higher value than NC group, by 119.61 and 116.86%, respectively, but there was no difference between the two groups. ALT was significantly lower in HFD 15, HFD 30 and NC groups compared to HFD group, by 13.51, 18.97 and 24.94%, respectively and there was a significant difference between the three groups.

Gene expression of SREBPs, PPAR-α and LPL mRNA: Relative gene expression level of SREBP-1α, SREBP-2, PPAR-α and LPL mRNA in ESM administered rats is shown in Fig. 2. Expression of SREBP-1α and SREBP-2 mRNA in liver was lower in all other groups compared to in the HFD group but on the other hand, expression of PPARα ad LPL mRNA in adipose tissue increased significantly.

SREBP-1α mRNA decreased significantly in the HFD 15, HFD 30 and NC groups compared to HFD group, by 37.68, 39.86 and 46.38%, respectively. The HFD 15 and HFD 30 groups registered significantly higher values compared to NC group, by 116.22 and 112.12%, respectively, but there was no difference the between the two groups. The SREBP-2 mRNA decreased significantly in the HFD 15, NC and HFD 30 groups, by 24.02 and 46.75%, respectively. The HFD 15 group showed a significantly higher value than NC group, by 119.39% but there was no difference between NC and HFD 30 groups. The PPAR-α mRNA increased significantly in NC, HFD 15 and HFD 30 groups compared to HFD group, by 286.67, 193.33 and 188.89%, respectively. The HFD 15 and HFD 30 groups showed a significantly lower value than that of NC group by 32.56 and 34.11%, respectively but there was no difference between the two groups. The LPL mRNA expression of adipose tissue increased in the NC, HFD 30 and HFD 15 groups compared to HFD group, by 120.90, 176.12 and 201.49%, respectively and there was a significant difference among the three groups.

Morphological change of liver and fat cells: The morphological change of liver and fat cells as a result of ESM administration is shown in Fig. 3 and 4. In the liver cells of HFD group, porous-coated fats and very large fats are accumulated compared to normal diet fed NC group. As the

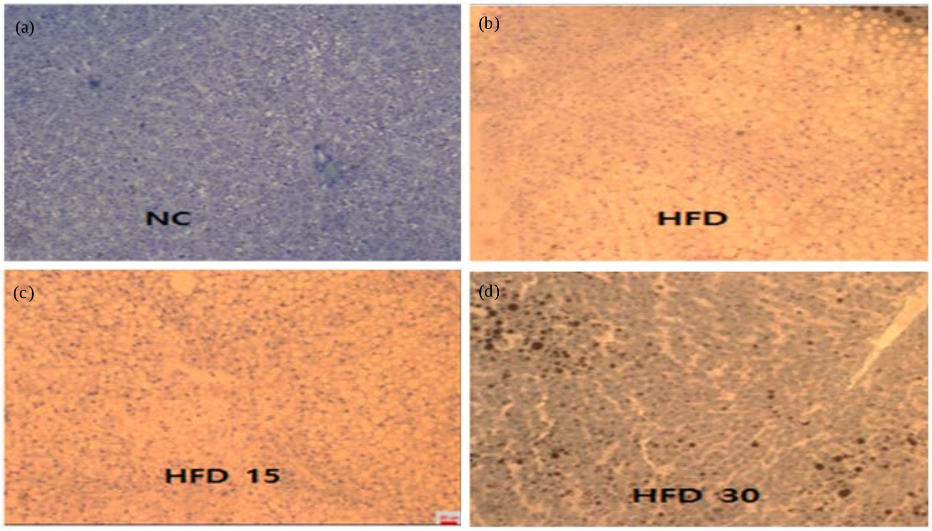

Fig. 3(a-d): Morphological change of liver by oral administration of ESM in obesity rats

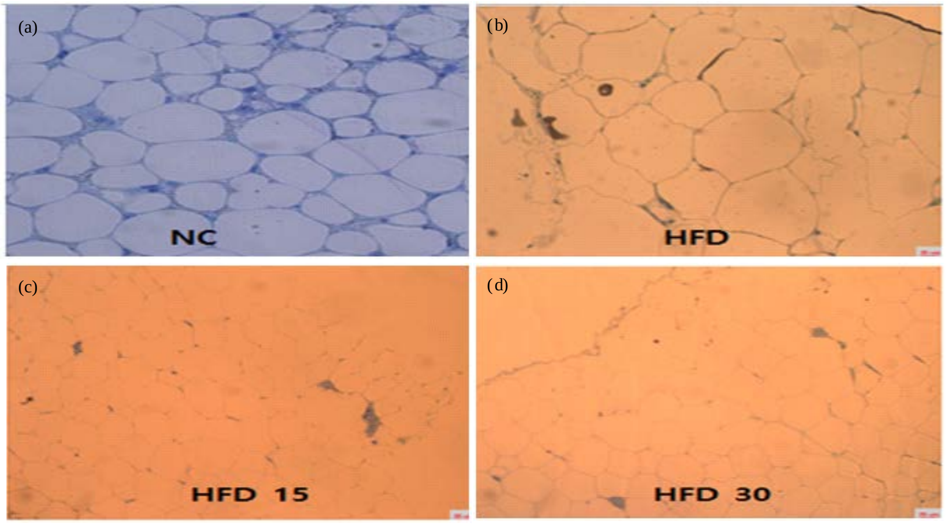

Fig. 4(a-d): Morphological change of fat cells by oral administration of ESM in obesity rats

entire liver cell was covered with fat globule, it was impossible to observe the form of the cell and the cytoplasm looked white due to the accumulation of excessive fat inside. It induced fatty liver, with an unclear membrane and form of cell organelle and the size of adipocyte within adipose tissue increased (The number and size of adipocytes were not measured). In the liver cells of HFD 15 and HFD 30 groups, fat accumulation was reduced compared with the HFD group and Kupffer's cell was activated. Normal liver mesenchymal cell is seen clearly surrounding the central vein and liver damage resulting from a high-fat diet was greatly alleviated. Concerning adipose tissue of HFD 15 and HFD 30 groups, the size of adipocytes within adipose tissue became diminished compared with the HFD group and the size of adipocytes displayed a tendency of decreasing, to close to the NC group.

DISCUSSION

The result of this study has found that ESM administration to obesity model animals can reduce body weight significantly. These findings are supported by the anti-obesity effects of sulfur-containing thiacremonone separated from garlic for obese model animals through body weight loss and the reduction of blood triglyceride and glucose (Ban *et al*., 2012). These are also supported by the paper by Park and Park (2012), which observed body weight decrease in obesity model animals fed with grain maggot extract. The result was affected by high energy intake through the high-fat diet during testing period. Furthermore, body weight increased despite a relatively low diet intake because of the increase of body fat accumulated along with hepatomegaly instead of muscle increase (Tzang *et al*., 2009). Since, dietary intake of animals is determined by energy requirements, it is known that dietary intake decreases when a high-energy diet is ingested, compared with a low-energy diet (Park *et al*., 2008) observed a higher dietary intake among animals consuming a chow diet compared with the high-fat diet group.

This result newly found that ESM administration has the effect of remarkably lowering body fat accumulation in obesity model animals. Compared with the animals that ingested a chow diet, the weight of liver and adipose tissue was increased in high-fat diet obesity model animals and the weight decreased when grain maggot was administered. The findings of scientists support these testing results (Park and Park, 2012). It is generally known that high-fat diet ingestion causes lipid metabolism disturbance in the liver, leading to lipid accumulation in liver tissues which means that the weight of the liver increases compared with normal diet ingestion (Park *et al*., 2008). The highlighted matters were the weights of liver and adipose tissues which became reduced in a dose-dependent manner in the sulfur-grain maggot extracts group. This is because the amount of blood lipid decreased due to the synergy created between the antibacterial peptide contained in ESM and sulfur which ultimately resulted in less lipid being moved to peritoneal tissue (Park *et al*., 2010b). Since antibacterial peptide regulates blood lipid metabolism by increasing *Bifidobacteria* and *Lactobacillus* count, it reduces triglyceride and cholesterol. Animal fatness occurs with excessive energy consumption being switched over to fat increase and fats are accumulated in many body parts in the form of hypodermic fats or abdominal fats. Excessive fat accumulation has adverse effects on the physiological and biochemical functions of the body (Son *et al*., 2012). Meanwhile, it is known that increased intake of saturated fat including pork belly can induce obesity by increasing the number and size of adipocytes and accumulating abdominal fat (Adaramoye *et al*., 2008).

One of the significant findings of this test result is that ESM administration to high-fat diet induced obesity animals lowers blood LDL-C. These results are supported by the following: the reduction of blood lipid by *Musca domestica* L. extract in animals that ingested a chow diet (Park, 2007); the increase of blood lipid among high-fat diet obesity model animals (Park *et al*., 2008) and the report that sulfur-containing thiacremonone brings about an anti-obesity effect through the reduction of blood triglyceride and blood glucose in obesity model animals (Ban *et al*., 2012). It is known that a high level of blood triglyceride and LDL-C raises the mortality of CVD such as myocardial infarction and atherosclerosis, while the increase of HDL-C level is helpful in preventing this CVD (Yamaguchi *et al*., 2012). The LDL is the most important lipid carrier for cholesterol accumulation within the artery. The LDL transports cholesterol esters from liver to the blood and the cells of peripheral tissues in many parts of body. Meanwhile, HDL is a lipid protein transporting cholesterol from the artery to the liver. The increase of blood HDL-C enhances cholesterol transport capability from blood and tissues to liver which removes blood cholesterol through the reverse transport of cholesterol. The AI indicates the amount of glyceride against HDL-C. If the value in a clinical setting is 3.0 or more, there is a risk of atherosclerosis (An *et al*., 2011). Blood LDL-C and ALT showed a tendency of decreased dependence as a result of dosing sulfur-fed grain larvae extract. Blood AST and ALT are indicators of the level of liver damage. Given that they were high in HFD group but decreased in HFD 15 and HFD 30 groups, sulfur-grain maggot extract has a liver protection function to some extent (Alqasoumi, 2014). In conclusion, considering the fact that a decrease of blood lipid, AST and ALT was observed in animals administered with sulfur-grain maggot extract, it is fair to say that it would have bioactive effects related to lipid metabolism and liver function protection among obese people.

The result of gene expression is supported by the following report: Administration of *Musca domestica* L. extract to high-cholesterol diet fed animals inhibits the expression of SREBP-1α and SREBP-2 mRNA in liver while, facilitating the expression of PPARα mRNA in adipose tissue (Park and Park, 2014). Of the target genes relating to lipid metabolism, Sterol regulatory element binding proteins (SREBPs: SREBP-1α, SREBP-1c, SREBP-2) is an important transcription activating gene that activates the expression of more than 30 genes involved with biosynthesis of neutral fat and cholesterol (Ji *et al*., 2011; Rodriguez-Cantu *et al*., 2011). SREBP-1α facilitates and regulates the expression of LDL receptor transcription gene and biosynthesis enzyme of cholesterol and fatty acid, while SREBP-2 controls a gene that is essential for the homeostasis of cholesterol by facilitating and regulating the expression of LDL receptor transcription gene (Frazier-Wood *et al*., 2013; Rotllan and Carlos, 2012). Peroxisome proliferator-activated receptors (PPARs: alpha, gamma, delta) are nuclear receptor proteins that have a close relationship with obesity as a major regulator of lipid metabolism in liver. The activation of PPAR-α enhances the transport of fatty acids and the securing, use and decomposition of fatty acids by facilitating and modulating the gene involved with -oxidation of fatty acid in peroxisome and mitochondria (Kelley and Azhar, 2005; Frazier-Wood *et al*., 2013). Gene expression of SREBPs mRNA is inhibited among hyperlipidemia model animals compared to chow diet rats and this may be a reason behind the reduction of blood lipid (Rogi *et al*., 2011). The LPL lowers blood neutral fat by hydrolyzing neutral fat and providing free fatty acids to adipocyte (Yamaguchi *et al*., 2012). The LPL, as a rate-limiting enzyme in charge of lipolysis, is involved with weight control by increasing lipid partitioning in obese tissues (Wang and Eckel, 2009). In conclusion, ESM administration down-regulates gene expression of cholesterol biosynthesis by inhibiting SREBP-1α and SREBP-2 mRNA in animal liver and up-regulates the lipolysis gene by activating PPARα mRNA and LPL mRNA. This will ultimately helping prevents obesity by regulating gene expression.

The testing newly discovered that ESM administration can be a great help in protecting animal liver from being damaged and reducing the number of adipocytes. It is considered that fatty liver occurred in the HFD group, because excess fat was accumulated in the liver with animals ingesting a high-fat diet. As excess fat continues to be accumulated in adipocytes, the size of adipocytes continues to grow until the fat is consumed as energy. It is known that obesity is caused by an increase in the size of adipocytes rather than in their number (Greenberg and Obin, 2006). Meanwhile, the size of visceral adipocytes becomes huge with the increase in insulin resistance due to the accumulation of visceral fat. Obesity is also known to be a powerful risk factor for metabolic syndrome and CVD (Hanauer, 2005).

CONCLUSION

In conclusion, results of this study suggest that sulfur-grain maggot extracts has anti-obesity effects, by reducing the abdominal fat of obesity model animals and lowering blood lipid level through the down-regulation of SREBP-1α and SREBP-2 mRNA and the up-regulation of PPAR-α mRNA.

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REFERENCES

Adaramoye, O.A., O. Akintayo, J. Achem and M.A. Fafunso, 2008. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. Vasc. Health Risk Manage., 4: 235-241.

Alqasoumi, S.I., 2014. Evaluation of the hepatroprotective and nephroprotective activities of *Scrophularia hypericifolia* growing in Saudi Arabia. Saudi Pharm. J., 22: 258-263.

An, H.M., S.Y. Park, D.K. Lee, J.R. Kim and M.K. Cha *et al*., 2011. Antiobesity and lipid-lowering effects of *Bifidobacterium* spp. in high fat diet-induced obese rats. Lipids Health Dis., Vol. 10. 10.1186/1476-511X-10-116

Atmaca, G., 2004. Antioxidant effects of sulfur-containing amino acids. Yonsei Med. J., 45: 776-788.

Ban, J.O., D.H. Lee, E.J. Kim, J.W. Kang and M.S. Kim *et al*., 2012. Antiobesity effects of a sulfur compound thiacremonone mediated via down-regulation of serum triglyceride and glucose levels and lipid accumulation in the liver of db/db mice. Phytother. Res., 26: 1265-1271.

Bray, G.A., S. Paeratakul and B.M. Popkin, 2004. Dietary fat and obesity: A review of animal, clinical and epidemiological studies. Physiol. Behav., 83: 549-555.

Frazier-Wood, A.C., J.M. Ordovas, R.J. Straka, J.E. Hixson, I.B. Borecki, H.K. Tiwari and D.K. Arnett, 2013. The PPAR alpha gene is associated with triglyceride, low-density cholesterol and inflammation marker response to fenofibrate intervention: The GOLDN study. Pharm. J., 13: 312-317.

Greenberg, A.S. and M.S. Obin, 2006. Obesity and the role of adipose tissue in inflammation and metabolism. Am. J. Clin. Nutr., 83: 461S-465S.

Hanauer, S.B., 2005. Obesity and visceral fat: A growing inflammatory disease. Nat. Clin. Prac. Gastroenterol. Hepatol., 2: 245-249.

Hasegawa, T., S. Ueno, S. Kumamoto and Y. Yoshikai, 2004. Suppressive effect of methylsulfonylmethane (MSM) on type II collagen-induced arthritis in DBA/1J mice. Jpn. Pharmacol. Ther., 32: 421-427.

Jaklic, D., A. Lapanje, K. Zupancic, D. Smrke and N. Gunde-Cimerman, 2008. Selective antimicrobial activity of maggots against pathogenic bacteria. J. Med. Microbiol., 57: 617-625.

Ji, G., X. Zhao, L. Leng, P. Liu and Z. Jiang, 2011. Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. Lipids Health Dis., Vol. 10. 10.1186/1476-511X-10-23

Kelley, G.L. and S. Azhar, 2005. Reversal of high dietary fructose-induced PPARα suppression by oral administration of lipoxygenase/cyclooxygenase inhibitors. Nutr. Metab., Vol. 2. 10.1186/1743-7075-2-18

Kim, L.S., L.J. Axelrod, P. Howard, N. Buratovich and R.F. Water, 2006. Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: A pilot clinical trial. Osteoarthr. Cartilage, 14: 286-294.

Komarnisky, L.A., R.J. Christopherson and T.K. Basu, 2003. Sulfur: Its clinical and toxicologic aspects. Nutrition, 19: 54-61.

Lee, J., H.J. Lee, J.D. Park, S.K. Lee and S.I. Lee *et al*., 2008. Anti-cancer activity of highly purified sulfur in immortalized and malignant human oral keratinocytes. Toxicol. *In vitro*, 22: 87-95.

Mildner-Szkudlarz, S. and J. Bajerska, 2013. Protective effect of grape by-product-fortified breads against cholesterol/cholic acid diet-induced hypercholesterolaemia in rats. J. Sci. Food Agric., 93: 3271-3278.

Park, B.S., 2007. A study on the bioactive effect of ethanol extracts from fly larvae. J. Environ. Agric. Res., 9: 9-22.

Park, C.H., D.I. Kim, H.K. Jung, G.D. Lee, K.S. Kim and J.H. Hong, 2008. Effect of *Bulnesia sarmienti* single and complex extracts on serum lipid and body fat in rats fed high-fat diet. Korean J. Food Sci. Technol., 40: 449-454.

Park, S.O., J.H. Shin, W.K. Choi and B.S. Park, 2010a. Effect of feeding dietary legislation sulfur as an antibiotic replacement in broiler chickens. Ann. Anim. Res. Sci., 21: 32-39.

Park, S.O., J.H. Shin, W.K. Choi, B.S. Park, J.S. Oh and A. Jang, 2010b. Antibacterial activity of house fly-maggot extracts against MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant *Enterococci*). J. Environ. Biol., 31: 865-871.

Park, B.S. and S.O. Park, 2012. Effects of grain larvae extracts on hepatotoxicity and blood lipid in obese rats. J. Anim. Vet. Adv., 11: 988-994.

Park, B.S., N.K. Singh and A.M.M.T. Reza, 2013. Obesity, obesity-related diseases and application of animal model in obesity research: An overview. Korean J. Oil Chem. Soc., 30: 622-634.

Park, S.O. and B.S. Park, 2014. Extracts of housefly maggot reduces blood cholesterol in hypercholesterolemic rats. Korean J. Oil Chem. Soc., 31: 101-112.

Rodriguez-Cantu, L.N., J.A. Gutierrez-Uribe, J. Arriola-Vucovich, R.I. Diaz-De La Garza, J.W. Fahey and S.O. Serna-Saldivar, 2011. Broccoli (*Brassica oleracea* var. italica) sprouts and extracts rich in glucosinolates and isothiocyanates affect cholesterol metabolism and genes involved in lipid homeostasis in hamsters. J. Agric. Food Chem., 59: 1095-1103.

Rogi, T., N. Tomimori, Y. Ono and Y. Kiso, 2011. The mechanism underlying the synergetic hypocholesterolemic effect of sesamin and α-tocopherol in rats fed a high-cholesterol diet. J. Pharmacol. Sci., 115: 408-416.

Rotllan, N. and C. Fernandez-Hernando, 2012. MicroRNA regulation of cholesterol metabolism. Cholesterol, Vol. 12. 10.1155/2012/847849

SAS., 2004. SAS/STAT User's Guide: Statistics. SAS Institute, Cary, NC., USA.

Scavee, V., F.X. Polis and J.C. Schoevaerdts, 2003. Maggot therapy: Many hands make light work. Acta Chirurgica Belgica, 103: 405-407.

Sherman, R.A., M.J.R. Hall and S. Thomas, 2000. Medicinal maggots: An ancient remedy for some contemporary afflictions. Annu. Rev. Entomol., 45: 55-81.

Son, E.J., U.K. Pal, P.K. Mandal, G.E. Hong, S.K. Kim and C.H. Lee, 2012. Hypolipidaemic effect of processed sulfur, *Allium tuberosum* rottl. and fermented *Allium tuberosum* rottl. In rat. Asian J. Anim. Vet. Adv., 7: 812-821.

Tzang, B.S., S.F. Yang, S.G. Fu, H.C. Yang, H.L. Sun and Y.C. Chen, 2009. Effects of dietary flaxseed oil on cholesterol metabolism of hamsters. Food Chem., 114: 1450-1455.

Wang, H. and R.H. Eckel, 2009. Lipoprotein lipase: From gene to obesity. Am. J. Physiol. Endocrinol. Metab., 297: E271-E288.

Wu, X., F. Kassie and V. Mersch-Sundermann, 2005. Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds. Mutat. Res., 589: 81-102.

Yamaguchi, T., Y. Miyashita, A. Saiki, F. Watanabe, H. Watanabe and K. Shirai, 2012. Formula diet is effective for the reduction and differentiation of visceral adipose tissue in Zucker fatty rats. J. Atheroscl. Thromb., 19: 127-136.