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# Lowering Lipid Mechanism of the Ethanol Extracts from Maggot of *Musca domestica* in Rats Fed a High-Cholesterol Diet

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ABSTRACT

The purpose of this study was to determine the effects of oral administration of ethanol extracts from maggots (EM) on lowering blood lipids in rats that are placed on a high-cholesterol diet. Sprague Dawley male rats were randomly divided into the following 4 groups based on the amount of EM administration in mg 100 g of per body weight over the period of 6 weeks: Normal control without EM (NC), EM 5.0, 7.0 and 9.0. EM groups had significantly lower levels of serum triglyceride, total cholesterol and low-density lipoprotein-cholesterol, compared to the NC. HMG-CoA reductase activity in EM groups was significantly lower than those of the control group but total sterol, neutral sterol and acid sterol excretion were significantly increased in EM groups, when compared to the control group. To identify the biological mechanism of EM towards the hypocholesterolemic effect, Sterol Response Element Binding Proteins (SREBPs) and the Peroxisome Proliferator-Activated Receptors (PPARα) transcription system were determined in rats on a high-cholesterol diet. It was discovered that EM suppresses the expression of SREBP-1α and SREBP-2 mRNA in the liver tissues of these rats while simultaneously increasing the expression of PPARα mRNA. Therefore, result of this study provide the first evidence that EM may have hypocholesterolemic effects in rats on high-cholesterol diet, by regulating cholesterol metabolism-related biochemical parameters and SREBP-1α, SREPB-2 and PPARα gene expressions.

**Key words:** Blood lipid, HMG-CoA reductase, sterol, SREPBs, PPARα

## INTRODUCTION

It was reported in 2013 that the highest death rates in Koreans are associated with cancer, followed by cerebrovascular and cardiovascular diseases attributed to abnormal lipid metabolism. Therefore, nutritional and medical attention is centered on the reduction of blood triglyceride and harmful cholesterol levels along with the development of biomaterials from insects. Elevated blood levels of Low Density Lipoprotein Cholesterol (LDL-C) and triglyceride can increase the risk of cardiovascular disease (CVD) as well as metabolic syndrome including type 2 diabetes and obesity (Quehenberger and Dennis, 2011). Blood cholesterol and triglyceride levels are regulated by genetic factors and diet (Belguith-Hadriche *et al.*, 2010; Liu *et al.*, 2012).

Among the various target genes included in lipid metabolism, sterol regulatory element binding proteins (SREPBs: SREPB-1α, SREPB-1c, SREPB-2) are important transcription activator genes which directly activate the expression of more than 30 genes included in the synthesis and uptake of cholesterol, fatty acids, triglycerides and phospholipids (Horton *et al.*, 1998; Rodriguez-Cantu *et al.*, 2011). SREBP-1α up-regulates the expression of cholesterol, fatty acid biosynthesis enzyme and LDL receptor transcription while SREPB-1c selectively activates the genes included in the synthesis of triglyceride and fatty acid. SREPB-2 regulates the genes important for cholesterol homeostasis by up-regulating the expression of HMG-CoA synthase, HMG-CoA reductase and LDL receptor transcription (Rotllan and Fernandez-Hernando, 2012). Peroxisome

proliferator-activated receptors (PPARs: Alpha, gamma, delta) are nuclear receptor proteins that function as gene expression-regulating transcription factors. PPARα, a major regulator of lipid metabolism in the liver, promotes the uptake, utilization and catabolism of fatty acids by up-regulating the genes included in fatty acid transport and peroxisomal and mitochondrial fatty acid β-oxidation (Berger and Moller, 2002; Frazier-Wood *et al.*, 2013).

Housefly (*Musca domestica* L.) maggots which are called grain larvae in Korea, are a potential biomedical material that contains non-specific antibacterial peptide, one of the components of innate immunity (Wang *et al.*, 2007; Ratcliffe *et al.*, 2011). Biotherapeutic technology, in which patients with burns and bedsores are treated using fly maggots, has been introduced in the medical field because of the appearance of super bacteria (Sherman *et al.*, 2000; Jaklic *et al.*, 2008). Strong antibacterial peptides against MRSA, *Staphylococcus aureus* and *Bacillus subtilis* strains were found in fly maggot excretions and extracts (An *et al.*, 2004; Bexfield *et al.*, 2004). In the previous studies, it was confirmed that ethanol extracts from fly maggot which were artificially bred using soybean meal, corn, sugar and skim milk powder as a medium in the standard environment, contained 5 kDa of antibacterial peptides (Jang *et al.*, 2007). This showed *in vitro* antibacterial activity against super bacteria such as MRSA and VRE (Park *et al.*, 2010), by lowering blood lipid and glucose levels and increasing IgG, cecal *Bifidobacteria, Lactobacillus* and total organic acids in normal rats fed with chow diet (Park and Park, 2014). Although many studies have been conducted regarding *in vitro* antitumor and antibacterial activity as well as *in vivo* blood lipid reduction from fly maggot extract in normal rats, almost no reports of hypocholesterolemic effects have yet been made for humans and animals with high-cholesterol diets (Yong *et al.*, 2012; Park *et al.*, 2010; Park and Park, 2014). Therefore, this study investigated the lowering lipid mechanism by oral administration of ethanol extracts from maggots (EM) to rats on high-cholesterol diet. The focus of this study was to examine the biochemical parameters and relevant gene expressions of SREPB-1α, SREPB-2 and PARRα which reduce blood cholesterol levels.

## MATERIALS AND METHODS

**Preparation of EM:** Fly maggots (2-3 days old) were obtained from Dr. InsectBio Co., Ltd. (Chuncheon, Republic of Korea). Approximately 98% of the lipid was removed at the primary stage after drying at 28°C convection dry oven and by pressing and extracting for 30 min at 1,000 PSI and 150°C. The remaining lipid was removed completely with hexane and defatted maggots were obtained. After mixing the defatted maggots and pure ethanol at a ratio of 1:10, an ethanol extract was obtained using a reflux condensing system. Then, after concentrating the reflux under reduced pressure with a rotary evaporator in a water bath at 40°C, the 4.30% ethanol extracts from maggots (EM) was obtained which contained a 5 kDa antibacterial peptide (Jang *et al.*, 2007; Park *et al.*, 2010).

**Animal experiments:** Animal testing was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and approval was obtained from the Institutional Animal Care and Use Committee at Kangwon National University (Approval No.; 017/07/2009). Forty Sprague-Dawley strain male rats weighing $200\pm7.85$ g (Daehanbio, Co., Chungcheong, Korea) were divided into 4 groups: Normal control without EM (NC), EM 5.0, 7.0, 9.0 mg 100 g per body weight) with 10 rats in each group, by employing a randomized complete block design which assigns one rat per cage. For a basal experimental diet, a purified diet was prepared based on AIN-93 and was made into pellets containing regulated equal levels of crude protein and gross energy. The composition of basal experimental diet (w/w, g 100 g$^{-1}$) includes casein-vitamin free 20.0, corn starch 39.75, maltodextrin 13.20, sugar 10.0, soybean oil 7.0, powered cellulose 5.0, AIN 93G mineral mix 3.50, AIN 93G vitamin mix 1.00, L-cystine 0.30, choline bitartrate 0.25 and t-butylhydroquinone 0.0014. A high-cholesterol diet (w/w, g 100 g$^{-1}$) was prepared by adding sodium cholate 0.25 and cholesterol (Sigma Chemical Co., St, Louis, MO, USA) 0.25, after reducing the amount of corn in the basal chow diet in order to induce hyper-VLDL-cholesterolaemia. At the same time each day, 1 mL of saline was orally administered through gavage to the EM negative control group and different doses of EM dissolved in 1 mL of saline was administered to EM groups. The rats were treated for 6 week in a standard environment, where water and food were freely accessible. The room was maintained at 21-23°C with a relative humidity of 55-57% and a 12 h light/dark cycle (lights on from 07:00 to 19:00).

**Serum lipid profile:** Food was taken away from the rats 10 h prior to sacrifice on the experiment day. From the rats lightly anesthetized with ether, 2 mL of whole blood was collected with a cardiac puncher into a serum separating tube (SST tube, BD, Falcon, SanJose, CA, USA) which was centrifuged at 3,000 g to obtain serum. Triacylglyceride, total cholesterol, High-Density Lipoprotein Cholesterol (HDL-C) and Low-density Lipoprotein Cholesterol (LDL-C) concentration were measured with a commercial enzyme kit (Sigma Co., Louis, MO, USA) in an automatic analyzer (Hitachi 917, Japan).

**HMG-CoA reductase activity:** HMG-CoA reductase activity was measured by slightly modifying the method of Tamasawa *et al.* (1997). Microsome 20 mg extracted by triple centrifugation at 105,000 g with a homogenizing buffer was suspended in a potassium phosphate buffer (100 mM, pH 7.4), EDTA (1 mM) and didithiothreitol 1 mM and then homogenized to obtain 20 μL (microsomal protein 50-300 μg) of homogenate. It was incubated using Triton X-100 (0.5%, v/v) at 0°C for 60 min and then centrifuged at 8,000 g for 10 min to obtain a supernatant which was stored at -80°C before biochemical analysis. After adding each mixture prepared with NADPH and HMG-CoA reductase, the

supernatant was incubated at 37°C for 30 min in an incubator. Optical density before and after the reaction was measured at 340 nm with the Jasco-UVIDEC-610 double-beam spectrophotometer (Tokyo, Japan). Endogenous oxidation of NADPH was measured and its value was corrected. HMG-CoA reductase activity was expressed in a reduced amount due to the oxidation of NADPH.

$$\text{Specific activity} = \text{pmoles NADPH oxidized mg microsomal protein}^{-1} \text{min}^{-1}$$

**Steroid excretions:** Neutral sterol and bile acids excreted through feces were investigated according to the method of Park and Jang (2008). Feces were collected for 5 days after the completion of the experiment and were freeze-dried in a laboratory freeze drier (SFDSF12, Samwon Co. Ltd., Busan, Korea). Neutral steroids and bile acids in dried feces were extracted twice with 2×10 volumes of alkaline ethanol (KOH 0.5 mol $L^{-1}$) at 70°C for 2 h. Bile acids were quantified by catalyzing with 3-α-hydroxysteroid dehydrogenase (Sigma, USA). Neutral steroids in alkaline ethanol solution (100 µL) which is used as internal standards, were added by 5-α-cholestane (Sigma, USA) and were then extracted three times with hexane (500 µL). Hexane extracts were concentrated to 200 µL which was induced with trimethylsilyl (TMS) ethers and injected to the Gas-Liquid Chromatographer (GLC, Packard model 439, USA), to which 2 µL of DBM capillary column (0.25 mm ×30 m, J and W Scientific, Folsom, CA) and a flame ionization detector were attached. Helium was used as a carrier gas and the isocratic temperature (260°C) was maintained for steroid separation. The split ratio was 10:1 and the sterol concentration was calculated from the peak area relative to the area of the internal standard.

**SREPBs and PPARα gene expression:** Relative levels of sterol regulatory element binding proteins (SREPB-1α, SREPB-2) and peroxisome proliferator-activated receptors (PPARα), mRNA transcripts to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in liver tissue were measured by Real-Time Polymerase Chain Reaction (RT-PCR). In summary, liver tissue were collected, freeze-dried in liquid nitrogen and then stored at -80°C. Total RNA was extracted from 30 mg of liver tissue using a lysis buffer in the Xprep Tissue RNA Mini Kit (Philekorea Technology, PKT). RNA concentrations were measured by absorbance at 260 nm using the NanoDrop ND-1000 Spectrophotometer (USA) and 300-500 ng µL$^{-1}$ of RNA were extracted. First strand cDNA was synthesized from 1 µg of extracted RNA using the cDNA synthesis kit (PKT). cDNA was amplified by incubating for 5 min at 70°C, 30 min at 42°C and 5 min at 85°C. Finally, cDNA was used by RT-PCR as a template to confirm the relative levels of mRNA transcripts of each target gene to GAPDH, a reference gene. RT-PCR was conducted through the QuantiMix SYBR Kit (PKT), using cDNA that had been diluted in distilled water (1:5, w/v) and mixed with each primer (forward and reverse). A gene expression assay was conducted using the gene expression protocol manual proposed by Eco Real-Time PCR (Illumina Inc.). GAPDH were used as housekeeping genes to control mRNA levels, respectively. Specific oligonuclotide primers were as follows:

| GAPDH | forward | 5'-TGCACCACCAACTGCTTAG-3', |
| | reverse | 5'-GGATGCAGGGATGATGTTC-3' |
| SREPB-1α | forward | 5'-ATGGACGAGCTGCCCTTCGGTGAGGCGGCT-3', |
| | reverse | 5'-CCTGGCGATGGCTGTGTGCTG-3' |
| SREPB-1α | forward | 5'-TTTGTCAGCAATCAAGTGGGAGAGTTC-3', |
| | reverse | 5'-GCTGCGTTCTGGTATATCAAAGGCTGC-3' |
| PPAR | forward | 5'-CCCTCTCTCCAGCTTCCAGCCC-3', |
| | reverse | 5'-CCACAAGCGTCTTCTCAGCCATG-3' |

**Statistical analysis:** The results of the study were analyzed with one-way analysis of variance (ANOVA) using the SAS program. All the data was presented as means±standard deviation (n = 10 rats group$^{-1}$) and the differences between the groups were tested for significance with Duncan's multiple range test at p<0.05 (SAS., 2005).

## RESULTS AND DISCUSSION

Diet consumption (24.12±0.20 g day$^{-1}$) and weight gain (63.74±0.28 g week$^{-1}$) of rats during the experimental period were not different among treatment groups. The serum lipid profiles, except for that of HDL-C, were lower in EM groups than in the control group. Also, they were shown to be lower in EM 7 and 9 mg groups than in EM 5 mg group, revealing a statistically significant difference among the treatment groups (p<0.05) (Table 1). Triglyceride, total cholesterol and LDL-C concentrations in EM groups decreased by 20.15, 18.77 and 27.60%, respectively (Fig. 1) while HDL-C increased by 141.43% in EM groups compared to the control group (p<0.05) (Fig. 2).

The level of activity of HMG-CoA reductase, an enzyme important for cholesterol synthesis in the liver, significantly decreased by 19.85% in EM groups compared to the control group (p<0.05). The EM 9 mg group had a lower enzyme activity than EM 5 and 7 mg groups, showing a statistically significant difference among treatment groups (p<0.05) (Table 2, Fig. 3).

Elimination of total sterol increased by 126.78% depending on the amount of EM administration while total neutral sterol, total cholesterol and coprostanol concentrations significantly increased by 123.8, 136.2 and 105.6% (only EM 5 mg group) in EM groups, respectively (p<0.05) (Table 2, Fig. 4). Total acid sterol and bile acid excretion increased

Table 1: Effects of the oral dosing of fly maggot extract on concentration of serum lipid in rats fed high-cholesterol diet (mg dL$^{-1}$)

| Lipid profiles | Oral dose (EM mg 100 g$^{-1}$ body weight) | | | |
| | 0 | 5 | 7 | 9 |
| --- | --- | --- | --- | --- |
| Triglyceride | 123.18±1.25[a] | 104.15±0.76[b] | 103.31±0.81[b] | 98.36±1.17[c] |
| Total cholesterol | 90.41±1.43[a] | 78.51±1.93[b] | 74.04±2.12[c] | 73.44±2.17[c] |
| HDL-C | 15.51±0.44[c] | 18.85±0.24[b] | 19.18±0.33[b] | 21.42±0.47[a] |
| LDL-C | 38.17±1.44[a] | 30.17±1.38[b] | 27.77±1.09[c] | 27.69±1.27[c] |

Values are Mean±SD (n = 10), [a,b,c]p<0.05, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol
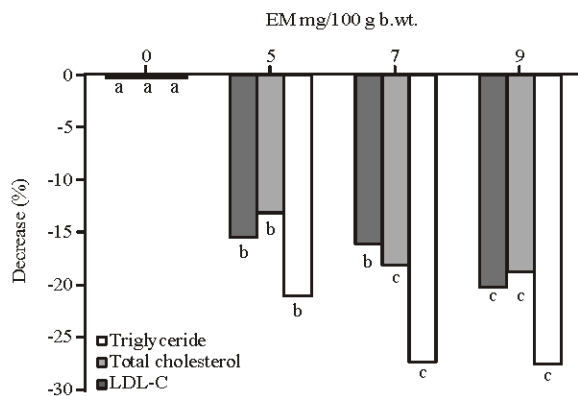
EM mg/100 g b.wt.



Fig. 1: Decrease percentage serum lipid profiles after EM oral administration in rats fed high-cholesterol diets. Bars±SD (n=10). [a,b,c]p<0.05 among groups



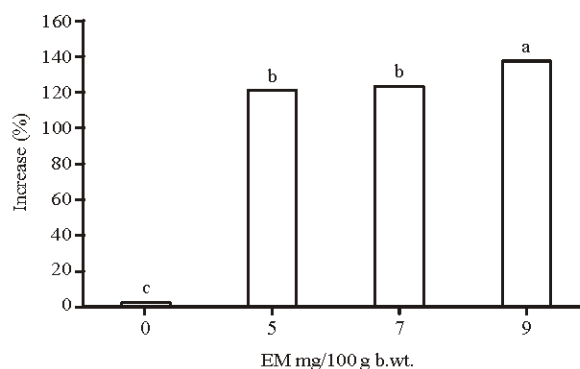Fig. 2: Increase percentage in serum HDL-C levels after EM oral administration in rats fed high-cholesterol diets. Bars±SD (n=10). [a,b,c]p<0.05 among groups
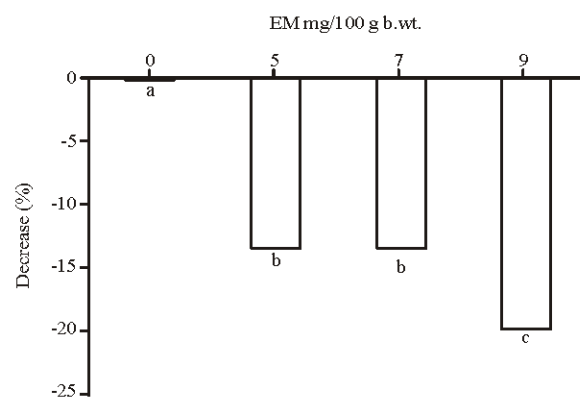
EM mg/100 g b.wt.



Fig. 3: Decrease percentage in HMG-CoA reductase activity after EM oral administration in rats fed high-cholesterol diets. Bars±SD (n=10). HMG-CoA reductase activity: pmole NADPH oxidized mg microsomal protein$^{-1}$ min$^{-1}$. [a,b,c]p<0.05 among groups

by 131.2 and 133.4%, respectively, when EM was orally administered and EM 7 and 9 mg groups excreted larger amounts of total acid sterol and bile acid than the 5 mg group (p<0.05). Concentrations of lithocholic acid, deoxycholic acid,

Table 2: HMG-CoA reductase activity (pmole NADPH oxidized mg microsomal protein$^{-1}$ min$^{-1}$) and sterol excretion (mg day$^{-1}$ rat$^{-1}$) in rats fed high-cholesterol diets

| Item | Oral dose (EM mg 100 g$^{-1}$ body weight) | | | |
| | 0 | 5 | 7 | 9 |
| --- | --- | --- | --- | --- |
| HMG-CoA reductase activity | 209.44±0.22[a] | 180.97±0.27[b] | 181.04±0.20[b] | 167.87±0.26[c] |
| Total neutral sterol | 33.17±0.28[b] | 40.68±0.32[a] | 41.01±1.00[a] | 41.05±0.91[a] |
| Total cholesterol | 23.21±0.58[c] | 30.17±1.09[b] | 31.37±0.49[ab] | 31.62±0.57[a] |
| Coprostanol | 9.96±0.18[b] | 10.51±0.07[a] | 9.64±0.06[c] | 9.43±0.05[d] |
| Total acid sterol | 22.84±0.37[c] | 26.75±0.53[b] | 28.87±0.63[a] | 29.96±0.91[a] |
| Total bile acids | 13.64±0.50[c] | 15.14±0.78[b] | 17.06±0.92[a] | 18.19±0.85[a] |
| Lithocholic acid | 4.89±0.09[c] | 5.48±0.03[b] | 5.55±0.07[b] | 5.75±0.07[a] |
| Deoxycholic acid | 0.33±0.03[c] | 0.40±0.02[b] | 0.47±0.04[a] | 0.48±0.04[a] |
| Cholic acid | 3.09±0.07[c] | 3.88±0.09[b] | 4.05±0.08[a] | 4.02±0.09[ab] |
| Chenodeoxycholic acid | 0.89±0.05[d] | 1.85±0.06[a] | 1.69±0.06[b] | 1.52±0.05[c] |
| Total sterol | 56.01±1.01[c] | 67.43±0.58[b] | 69.88±1.15[a] | 71.01±1.88[a] |

cholic acid and chenodeoxycholic acid were 117.6, 145.5, 131.1 and 207.9%, respectively and they were higher in the EM group than in the control groups (p<0.05) (Table 2, Fig. 5).

Relative levels of SREPB-1α and SREPB-2 were lower by 0.25-0.54 and 0.22-0.59, respectively but those of PPARα were significantly higher by 0.55 in EM groups compared to the control group (p<0.05). Among EM oral administration groups, relative levels of SREPB-1α and SREPB-2 were lower by 0.25-0.29 and 0.32-0.37 in 7 and 9 mg groups, respectively, compared to the 5 mg group. On the other hand, relative levels of PPARα mRNA transcripts were higher by 0.16-0.20 in 7 and 9 mg groups, compared to the 5 mg group (p<0.05). Relative levels of SREPB-1α, SREPB-2 and PPARα among EM 7 and 9 mg oral administration groups showed no statistically significant difference (Fig. 6).

One important fact discovered through this experiment is that the oral administration of EM might decrease serum harmful LDL-C in rats fed with high-cholesterol diet. It is reported that EM could significantly decrease serum LDL-C levels in normal rats which supports the result of this study (Park, 2007). The results showed that when different levels of EM were orally administered to normal rats for 6 weeks, hypolipidemic effects were accompanied by decreased serum triglyceride and cholesterol. The mechanism of EM lowering serum cholesterol is not fully understood. Since EM contains antibacterial peptides, it is possible that hypocholesterolemic effects are bifidogenic effects caused by an increase of *Bifidobacteria* and *Lactobacillus* in the cecum of animals (Park and Park, 2014). The antibacterial peptides are known to increase the number of *Bifidobacteria* and *Lactobacillus* by destruction of the transmembrane potential of harmful bacteria and the enhancement of immunity (Sanchez *et al.*, 2010). *Bifidobacteria* decreases triglyceride and cholesterol concentrations by regulating blood lipid metabolism (Roberfroid, 2000). In this study, we suggested a hypothesis that the hypocholesterolemic effect causes the metabolic adaption of liver tissues which can be induced by Short Chain Fatty Acid (SCFA) and this might cause the changes in lipid parameters. We reported recently that SCFAs, such as acetic acid and propionic acid, can be increased by selectively increasing cecal *Bifidobacteria* and *Lactobacillus* in normal rats with orally administered EM (Park and Park, 2014). In isolated rat hepatocytes, acetic acid and propionic acid can

suppress the synthesis of *de novo* cholesterol and SCFA can lower blood cholesterol (Cho *et al*., 1999). It was reported that triglyceride synthesis from free fatty acid and acetic acid as well as that from fatty acyl synthase activity, is actually decreased in the hepatocytes which are separated from oligofructose. It is assumed that decreased LDL-C may dominantly contribute to changes in the VLDL fraction but it

is not determined yet (Roberfroid, 2000). The mechanism of EM for hypocholesterolemic effects may include EM's possible influence on the increases in LDL decomposition, peripheral LDL receptor and LDL clearance (Cho *et al*., 1999).

Since oral administration of EM showed a decrease in LDL-C in rats fed with high-cholesterol diet, it can be considered to have bioactivity effects of improving a lipid
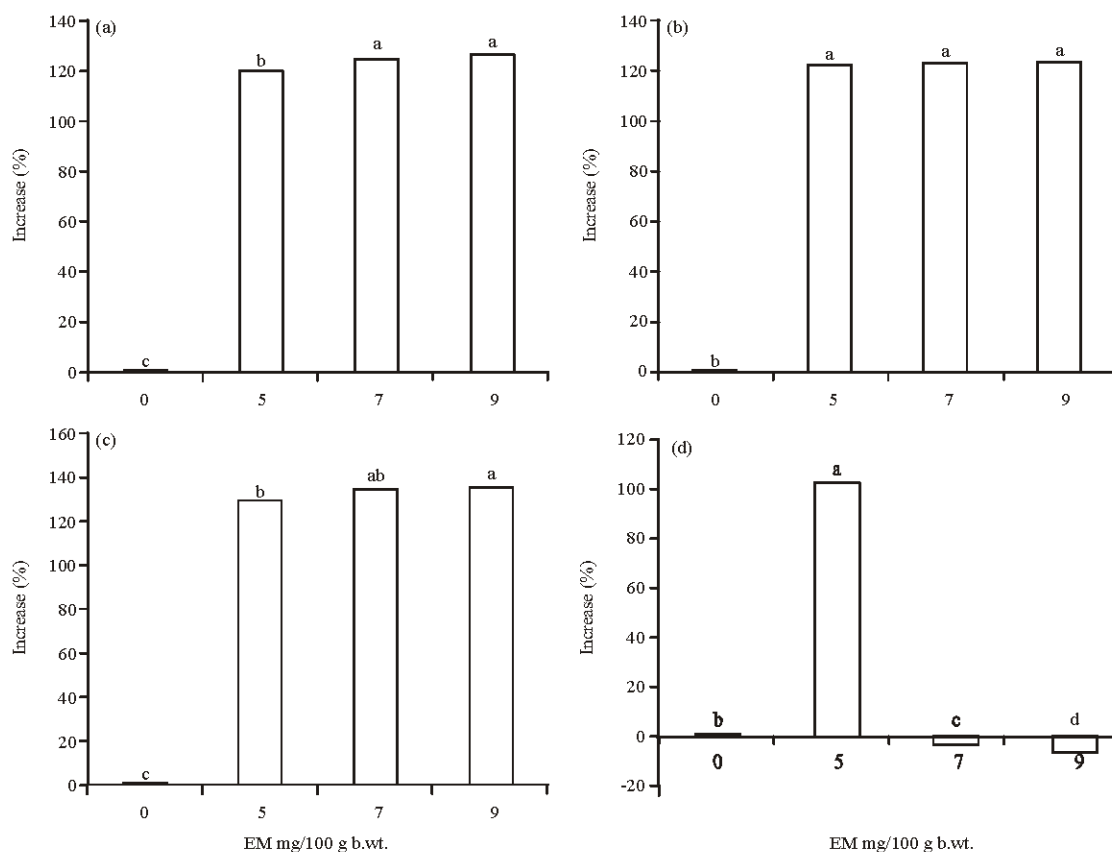


Fig. 4(a-d): Percentage increase in fecal (a) Total sterol, (b) Neutral sterol, (c) Total cholesterol and (d) Coprostanol profiles after EM oral administration in rats fed high-cholesterol diets. Fecal excretions: mg day$^{-1}$ rat$^{-1}$. [a,b,c]p<0.05 among treatment groups (n = 10)
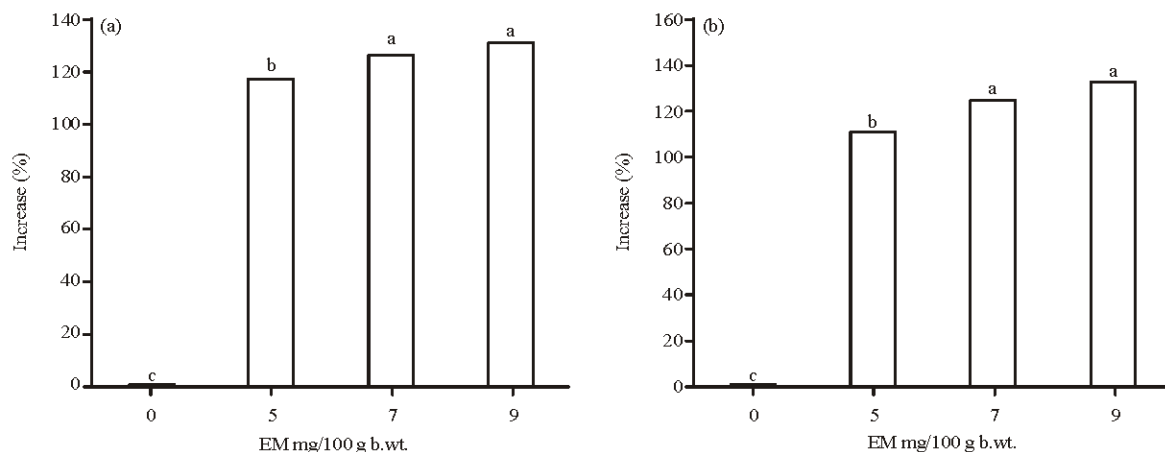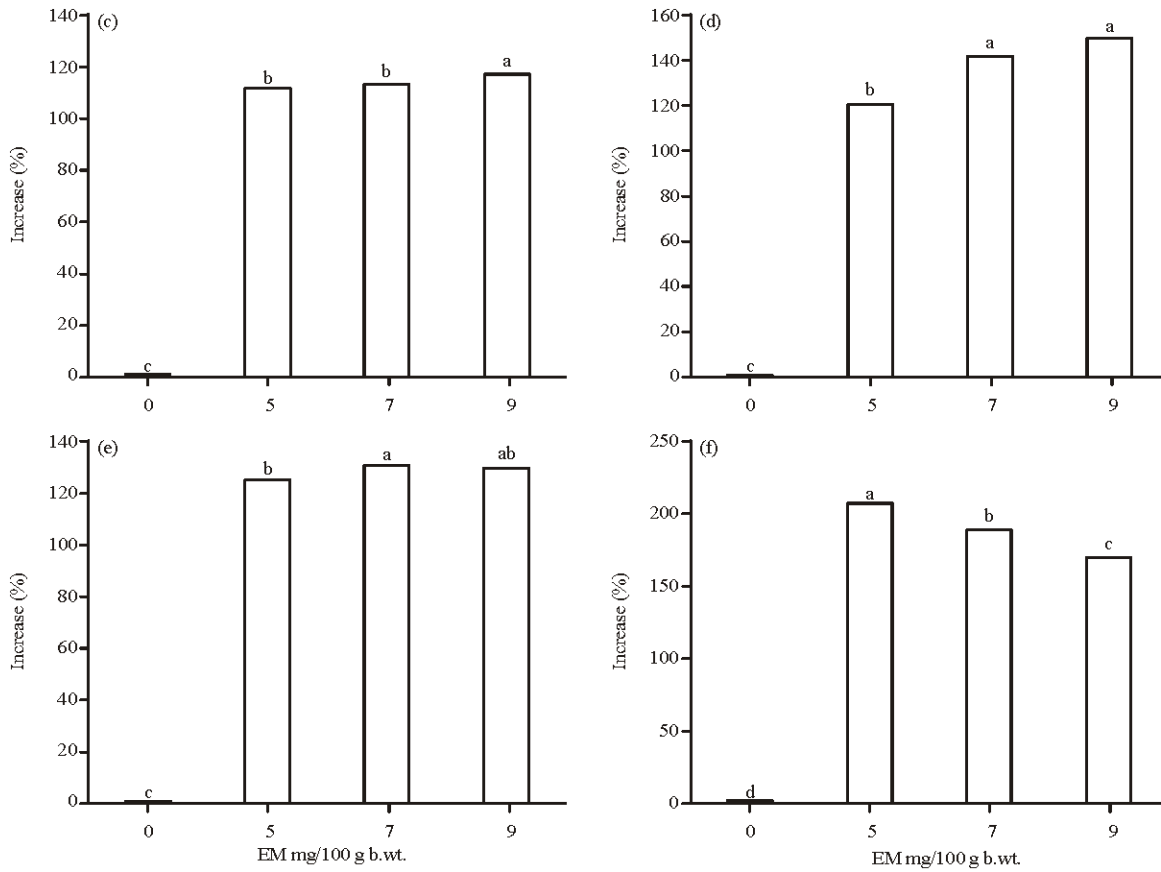


Fig. 5(a-f): Continue

Fig. 5(a-f): Percentage increase in (a) Total sterol, (b) Total bile acid, (c) Lithocholic acid, (d) Deoxycholic acid, (e) Cholic acid and (f) Chenodeoxy cholic acid profiles after EM oral administration in rats fed high-cholesterol diets. [a,b,c]p<0.05 among treatment groups (n = 10)
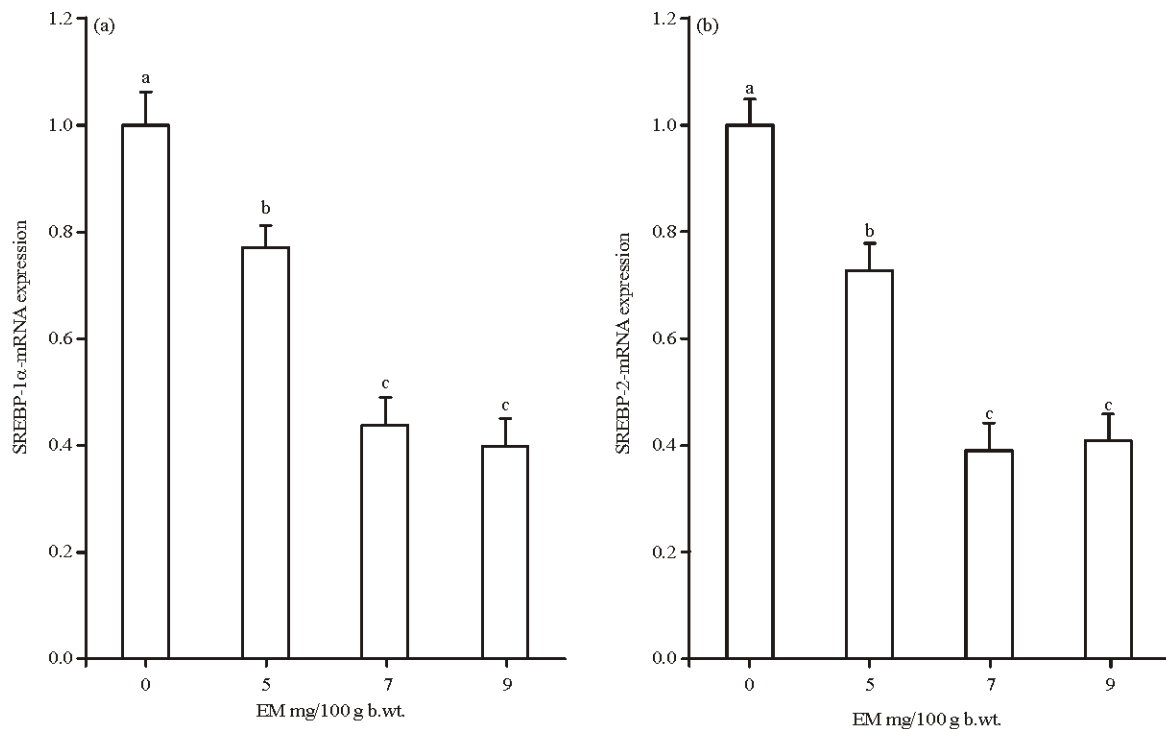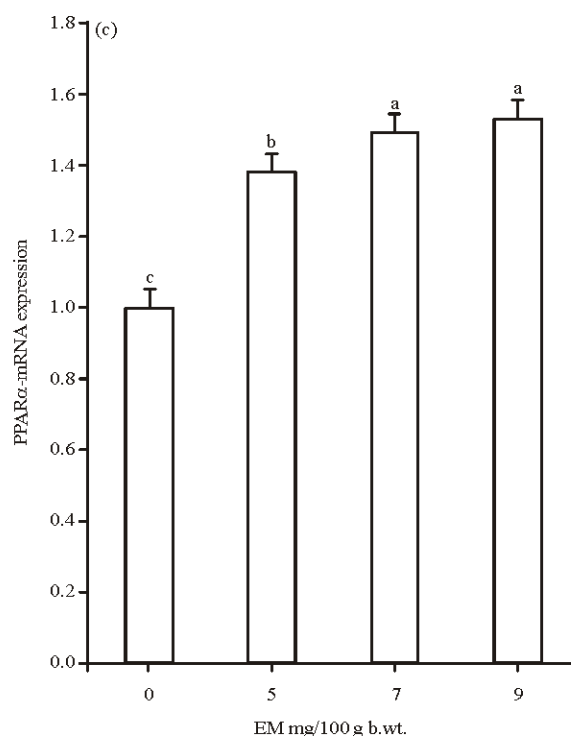


Fig. 6(a-c): Continue

Fig. 6(a-c): Relative (a) SREPB-1α mRNA, (b) SREPB-2 mRNA and (c) PPARα mRNA expression after EM oral administration in rats fed high-cholesterol diets. Values are expressed as the relative ratio to that of GAPDH (internal control). [a,b,c]p<0.05 among treatment groups (n = 10)

metabolism in humans. It is widely known that high levels of blood triglyceride and LDL-C increase death rates by causing cardiovascular diseases including myocardial infarction and arteriosclerosis as well as obesity, whereas an increase in HDL-C greatly helps to prevent them (Jensen *et al.*, 1999). LDL, the most important lipid carrier for cholesterol accumulation in the arteries, transports cholesterol ester from the liver to blood and peripheral tissues throughout the body, whereas HDL is a lipidprotein that carries cholesterol from the artery to the liver. Therefore, increased blood HDL-C will result in increased capacity of cholesterol transport from blood and tissues to the liver. In other words, it will eliminate blood cholesterol by reversely transporting cholesterol. LDL-C is hence called 'bad cholesterol' as a high level of blood LDL-C is harmful to health while HDL-C is called 'good cholesterol' (Quehenberger and Dennis, 2011).

The result of this study reconfirmed that oral administration of EM decreases harmful LDL-C in blood, by suppressing the activity of HMG-CoA reductase in the animal liver and simultaneously promoting sterol excretion through feces. As a result, we can conclude that the suppressed activity of HMG-CoA reductase in the liver and increased sterol excretions through feces are bifidogenic effects of antibacterial peptides in EM which selectively increase *Bifidobacteria* (Cho *et al.*, 1999; Sanchez *et al.*, 2010; Park and Park, 2014). Suppression of the activity of HMG-CoA reductase which is a rate-limiting enzyme for cholesterol biosynthesis in the liver, decreases blood cholesterol levels. It was reported that as HMG-CoA gene expression (not determined) was more

suppressed, blood triglyceride and LDL-cholesterol were more decreased in hypercholesterolemic rats compared to the rats on normal diet (Rogi *et al.*, 2011). EM oral administration prominently increased the percentages of primary bile acids (choile acid and chenodeoxycholic acid) formed through cholesterol in the liver and the percentage of the secondary bile acids (lithocholic acid as insoluble and not resorbed and deoxycholic acid) made from intestinal bacteria, resulting in changes in the excretion patterns of fecal bile acids (Huang *et al.*, 2009). Higaki *et al.* (2006) reported that in normal rats, peptides of soy protein are combined with bile acid which are then excreted through feces. It is reported that the mechanism of hypocholesteremic effects of *Bifidobacteria* and *Lactobacillus* in rats and humans can be explained as follows. The fermentation products of these microflora decrease cholesterol generation and promote the elimination of cholesterol through feces by suppressing cholesterol synthesis enzymes. *Bifidobacteria* and *Lactobacillus* suppresses the absorption of cholesterol into the body by combining with cholesterol, simultaneously interfering with the recycling of bile salt (a metabolic product of cholesterol) and promoting its elimination which increases the demand of bile acid made from cholesterol (An *et al.*, 2011). Increased excretion of bile acid through feces decreases blood cholesterol levels in rats with high-cholesterol diet (Nishimura *et al.*, 2009). Because of the increased excretion of bile acid, a small amount of cholesterol used for lipoprotein synthesis in the liver can be switched to bile acid synthesis (Cho *et al.*, 1999).

In this study, the hypocholesterolemic effects were compared between the rats with oral administration of EM and high-cholesterol diet. It was confirmed that oral administration of EM decreases LDL-C by regulating mRNA levels of SREPB-1α, SREPB-2 and PPARα genes which regulate cholesterol metabolism related to high cholesterol in liver tissues. SREPBs activate the transcription of genes for HMG-CoA and other enzymes included in cholesterol synthesis. If blood cholesterol levels rise, the proteolytic cleavage of SREPBs from the membrane ceases and proteins in the nuclei are degraded (Ji *et al.*, 2011). The result of this study showed that low HMG-CoA reductase activity and low gene expression of SREPB-1α and SREPB-2 in EM administration groups might be related to the findings above. SREPB-1α and SREPB-2 can regulate the expression of majority of genes included in lipoprotein uptake, such as cholesterol, triglyceride and LDL synthesis (Rotllan and Fernandez-Hernando, 2012). PPARα regulates the expression of genes included in mitochondrial and liver fatty acid β-oxidation (Ji *et al.*, 2011). Compared to rats on normal diet, the gene expression of SREPBs and HMG-CoA was more suppressed in hypercholesterolemic rats which in turn, can decrease the levels of blood triglyceride and LDL cholesterol (Rogi *et al.*, 2011). It is assumed that the oral administration of EM down-regulates gene expression of cholesterol biosynthesis by suppressing SREBP-1α and SREBP-2 in the liver of rats while up-regulating lipid catabolic genes through activation of PPARα. It was consistently found that in high-cholesterol diet fed rats, SREPB-1α and SREPB-2 mRNA transcription was suppressed, whereas PPARα mRNA transcription was multiplied. Therefore, it can be concluded that the down-regulated expression of SREPB-1α and SREPB-2 which cause lipogenesis and up-regulated expression of PPARα which causes lipid decomposition, are attributed to the bifidogenic effects of antibacterial peptides in EM.

In summary, it was confirmed that oral administration of EM to rats on high-cholesterol diet has hypocholesterolemic effects. These effects were expressed by the suppression of HMG CoA reductase activity in cholesterol metabolism which is an increased steroids excretion through feces, down-regulation of SREPB-1α and SREPB-2 mRNA and up-regulation of PPARα mRNA.

## ACKNOWLEDGMENTS

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