

Experimental Study on the Effects of *GamiSamgieum* (SGMX) on Hyperlipidemia

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Objectives : This study was aimed to investigate the effects of *GamiSamgieum* (SGMX) on hyperlipidemia using an animal model. Additionally, the underlying mechanisms were investigated by determination of gene expressions related with lipid metabolism.

Methods : Forty mice were selected for use in this experiment, and then divided into five groups; Naive, Induced, SGMX 100, SGMX 200, and Lovastatin group as positive control with 8 mice each, and their blood was collected for analysis of blood and serum parameters.

Results : Administration of SGMX significantly inhibited the increase of liver weight and the macrovacuolar cytoplasmic alterations in histopathologic finding. SGMX administration significantly protected the liver from pathologic elevation of AST and ALT and from lipid peroxidation. SGMX administration significantly lowered total cholesterol levels and TG, and partially upregulated the gene expression of LCAT, CYP7A1 and LDL-R receptor.

Conclusion : SGMX is suggested to possess hypolipidemic effect, and thus could be a potential candidate for herbal hypolipidemic via further studies.

Key Words : *GamiSamgieum* (SGMX), hyperlipidemia

Introduction

Hyperlipidemia is a pathologic status characterized as an excess of fatty substances including cholesterol, triglycerides or lipoproteins in the blood¹⁾. Cholesterol is an essential element of human cell membranes and a structural component of steroid hormones, but it also plays a major role in the development of plaque and resulting atherosclerosis²⁾. Triglycerides, a main constituent of animal fats, play an important role in metabolism as energy sources and transporters of dietary fat, however, a high level of triglycerides in the bloodstream has been linked to atherosclerosis, too³⁾. Accordingly, lipid disorders are an important

risk factor in developing heart disease and stroke, which are leading causes of death in the most developed countries⁴⁾. It is well known that the quantitative problems as well as qualitative troubles of blood lipids are equivalently important in hyperlipidemia-derived diseases^{5,6)}.

Cholesterol control is thus becoming more stringently required. So far, the current major drugs for hyperlipidemia belong in one of four classes: 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors commonly referred to statins, bile acid sequestrants, nicotinic acid, and fibric acids⁷⁾. The statins are the most potent lipid-lowering agents currently available, which can lower LDL and triglyceride levels, but may induce myopathy

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and an elevation of liver enzyme levels⁸). The bile acid sequestrants lower LDL levels and raise HDL levels but show side effects of gastrointestinal distress, constipation or interruption of other drugs⁹. In addition, nicotinic acid and fibric acids lower LDL and triglyceride levels while showing adverse effects such as flushing, hyperglycemia, hyperuricemia, GI distress, or hepatotoxicity¹⁰. Many researchers have shifted attention to oriental medicine-derived therapeutics or drug developments for lipid management, especially focusing on drugs of less toxicity along with improvement of lipid disorders. Several herbal plants have shown anti-cholesterolemic activity¹¹. In traditional medicine, hyperlipidemia is regarded as retention of phlegm and fluid disease. Etiology and pathogenesis of hyperlipidemia are explained as status of spleen-deficiency and phlegm-stagnation, accumulation and stasis of damp-heat, and qi & blood stagnation¹².

GamiSamgieum (SGMX), a modified traditional herbal prescription, has been used for patients with cerebrovascular accident, hypertension or hyperlipidemia-associated symptoms since 2002 at Daejeon Oriental Hospital. This drug has been planned for

development as an herbal hypolipidemic. Although SGMX has been used to treat hyperlipidemia in clinic, the effect and mechanisms of this drug should be further examined from a laboratory basis.

Therefore, the present study aimed to elucidate the effects of SGMX on hyperlipidemia using an animal model. Additionally, the underlying mechanisms were investigated by determination of gene expressions related with lipid metabolism.

Materials and Methods

1. Preparation of *GamiSamgieum* (SGMX)

Fifteen medicinal herbs for SGMX were purchased from Daejeon oriental hospital. SGMX comprises: 12 g of *Rehmannia glutinosa*; 4 g each of *Paeonia lactiflora*, *Cinnamomum cassia*, *Eucommia ulmoides*, *Achyranthes bidentata*, *Poria cocos*, *Angelica gigas*, *Lycium chinese*, *Angelica dahurica*, *Aconitum camichaeli*, *Coptis japonica*, *Bambusa silicea*, *Glycyrrhiza uralensis*, and *Zingiber officinale* (Table 1). In this study we used a double amount (128g) and 100 mg/kg of SGMX (SGMX 100) and 200 mg/kg of

Table 1. Prescription of *GamiSamgieum* (SGMX)

General name	Part used	Voucher specimen number	Relative Amount(g)
<i>Rehmannia glutinosa</i> (熟地黄)	Radix	01104	12
<i>Paeonia lactiflora</i> (芍药)	Radix	01125	4
<i>Cinnamomum cassia</i> (桂枝)	Ramulus	01939	4
<i>Eucommia ulmoides</i> (杜仲)	Cortex	01785	4
<i>Achyranthes bidentata</i> (牛膝)	Radix	01127	4
<i>Poria cocos</i> (白茯苓)	Flos	01747	4
<i>Angelica gigas</i> (当归)	Radix	01129	4
<i>Lycium chinese</i> (枸杞子)	Fructus	01126	4
<i>Angelica dahurica</i> (白芷)	Radix		4
<i>Aconitum camichaeli</i> (附子)	Radix		4
<i>Coptis japonica</i> (日黄连)	Radix	01057	4
<i>Bambusa silicea</i> (竹茹)	Concretio		4
<i>Glycyrrhiza uralensis</i> (甘草)	Radix	01449	4
<i>Zingiber officinale</i> (生薑)	Rhizoma		4
			64

SGMX (SGMX 200) were used. Dried SGMX was mixed with 2 ℓ of distilled water and then the whole mixture was boiled for 2 h. The SGMX extract was filtered and freeze dried. The yield of SGMX was 29.42% (w/v) in terms of the dried medicinal herbs.

2. Animals and experimental design

Six-week old male ICR mice (within 30 to 35 g of body weight) were purchased from a commercial animal breeder (Daehan BioLink, Korea). Forty mice of healthy appearance were selected for use in this experiment, and then divided into five groups of 8 mice each: Naive, Induced, SGMX 100, SGMX 200, and Lovastatin group as a positive control. Hyperlipidemia was induced by feeding a home-made high cholesterol diet for all groups except Naive throughout the experimental period, four weeks.

3. Preparation of high cholesterol diet and reagents

The high cholesterol diet was made by adding three fatty materials into ground commercial feed (Samyang Feed Co., Korea) for adjusting to 1% cholesterol, 0.25% cholic acid and 2.55% olive oil (Kanto Chemical Co., Inc. Tokyo, Japan).

Lovastatin (purchased from Samyang Feed Co., Korea) was used as a positive control. DNA Taq polymerase was obtained from Bioneer (Cheongwon, Korea), M-MLV reverse transcriptase was obtained from Promega (Madison, USA). TRIzol[®] reagent was obtained from Gibco (Maryland, USA). The other reagents were purchased from Sigma Inc. (St. Louis, USA).

4. Measure of organ weight and histopathological observations

All mice were measured for their body weight every week. On the last day of experiments, liver and spleen were removed after collection of whole blood from the abdominal aorta. Then, the weights of the two organs were carefully measured, and the

liver was separately stocked for RNA extraction and determination of lipid peroxidation products.

In order to evaluate the effects of SGMX through histomorphological findings, a portion of liver tissue was removed and fixed in 10% phosphate buffered formalin. The Paraplast-embedded liver sections (4 μm in thickness) were stained with hematoxylin & eosin (HE). The histopathological features were examined under microscope (Nikon, Japan).

5. Analysis of serum biochemistry including cholesterol and TG

On the last day of experiments, mice were fasted for 4 h and whole blood was collected from the abdominal aorta, then used for measurement of serum chemistries. The blood was centrifuged at 3000 rpm for 15 min to separate serum. Sera were prepared from the blood, and serum levels of alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total cholesterol (T-chol), high density lipoprotein (HDL)-cholesterol, and triglyceride (TG) were determined using an Auto Chemistry Analyzer (Chiron, Emeryville, CA, USA).

6. Measurement of cholesterol and TG in liver tissues

Determination of total cholesterol and TG in liver tissues was performed using an Auto Chemistry Analyzer (Chiron, Emeryville, CA, USA). Briefly, the hepatic lipids were extracted using slightly modified Folch's method¹³⁾ from one portion of liver stored in deep freeze. A liver specimen (200 mg) was homogenized with a 3:1 ethanol:ether mixture. The samples were centrifuged and supernatants were then evaporated at 90°C to dryness. Residues were resolved with 1.5 ml of 2-propanol.

7. Measurement of liver lipid peroxidation products

0.2 g of liver tissue was homogenized in ice-cold KCl (2 ml, 11.5 g/l); then, 0.13 ml of the homog-

enate was mixed with phosphoric acid (0.08 ml, 10 g/l) and thiobarbituric acid (0.26 ml, 0.67%). After heating the mixture for 45 min at 100 °C, 1.03 ml of n-butanol was added, and then was vigorously mixed. The mixture was then centrifuged at 3000 rpm for 15 min, and the absorbance value of the super organic layer was measured with a spectrophotometer at 535 and 520 nm with comparison to the standard solution (freshly prepared TEP).

8. RT-PCR for analysis gene expression

Total cellular RNA was isolated by the TRIzol[®] reagent (Gibco, Maryland, USA) according to the manufacturer's instructions. The mRNA levels were fixed quantity at 260 nm by spectrophotometer (Cary50, Varian, USA).

Total RNA was extracted from homogenized liver sample of SD female rats. The RNA (1 µg) was reverse-transcribed (RT) into first strand cDNA in a RT mixture containing 2 µl 10 mM dNTPs mix, 1 µl oligo-dT primer (20 pmol/µl), 2 µl 100 mM DTT, 4 µl 5×RT buffer (250 mM Tris-Cl, pH 8.3, 375 mM KCl, 15 mM MgCl₂, RNase inhibitor 20 U), 1 µl M-MLV RT (200 U/µl; Promega, U.S.A) and 2 µlDDW. The RT mixture was incubated at 42°C for 60 min, heated to 72°C for 10 min to inactivate the reverse transcriptase activity, and chilled to 4°C for 5 min. A portion of the RT product (1 µl) was then subjected to the polymerase chain reaction (PCR) in a DNA thermal cycler (TaKaRa, Tokyo, Japan).

PCR amplification was carried out in the thermal cycler using a protocol of initial denaturing step at 95°C for 10 min; then 35 cycles at 95°C for 1 min (denaturing), at 60°C for 40s (annealing), and at 72°C for 10 min. The PCR products were run on a 1% agarose gel in 0.5× TBE buffer.

9. Statistical analysis

Results were expressed as the mean ± S.E.. Statistical analysis of the data was carried out by Student's t-test. Differences from the respective control data at the levels of *p*<0.05 and *p*<0.01 were regarded as statistically significant.

Results

1. Changes in body weight

During the entire four weeks of the experimental period, there were no specific observed changes in general appearance nor animal deaths. Every mouse had gained body weight. The induced group showed the most increased weight while the naive group gained the least. There were no statistically significant changes among those five groups.

2. Absolute and relative weights of liver and spleen

On the last day of experiment, body weight as well as liver and spleen weight were measured (Table 2). Hypertrophy of the liver was observed in

Table 2. Absolute and Relative Organ Weights

Groups	Liver		Spleen	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)
Naive	2.1 ± 0.23	5.0 ± 0.2	0.14 ± 0.02	0.34 ± 0.06
Induced	2.9 ± 0.33	7.0 ± 0.8	0.17 ± 0.05	0.40 ± 0.12
SGMX 100	2.6 ± 0.18*	6.3 ± 0.5*	0.14 ± 0.02	0.34 ± 0.05
SGMX 200	2.9 ± 0.25	6.9 ± 0.4	0.18 ± 0.07	0.44 ± 0.16
Lovastatin 20	2.8 ± 0.29	6.6 ± 0.7	0.15 ± 0.03	0.35 ± 0.07

After treatment with SGMX (100 or 200mg/10ml/kg) or Lovastatin (20 mg/10ml/kg) for four weeks, body weight as well as liver and spleen weight were measured. Data expressed as mean ± SD. (n = 8). *: *p* < 0.05; significant differences compared with the induced group administrated distilled water only.

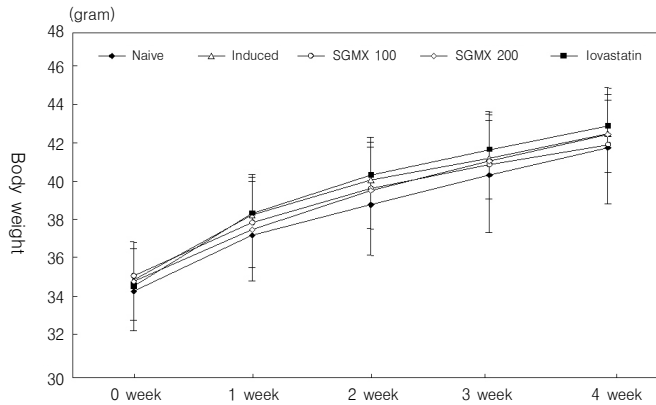


Fig. 1. Changes in body weight of mice given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia. SGMX (100 or 200 mg/kg), lovastatin (20 mg/kg) or distilled water given to appropriate group. The body weight was measured every week (n=8).

all groups given a high cholesterol diet. However, the administration of SGMX or lovastatin showed a pattern of slight protection against the increase of liver weight. The group of SGMX 100 had statistical significance compared to the induced group ($p<0.05$). This pattern was almost the same in relative liver weight to body weight. The absolute and relative spleen weight didn't show any differences among the five groups.

3. Histopathological observations

After finishing induction of hyperlipidemia by high cholesterol diet for 4 weeks, H & E stain for

liver tissues was performed and pathologic findings were examined under microscope. The macrovacuolar cytoplasmic alterations of hepatocytes were detected in the periarterial area in all groups given a high cholesterol diet. However, the severity was remarkably decreased in SGMX (100 or 200 mg/kg) and lovastatin (20 mg/kg) groups compared to the groups given a high cholesterol diet only (Fig. 2).

4. Serum biochemical analysis

On the last day of experiments, sera from all mice were collected for analysis of biochemistry. AST, ALT, T-cholesterol, HDL-cholesterol, and TG

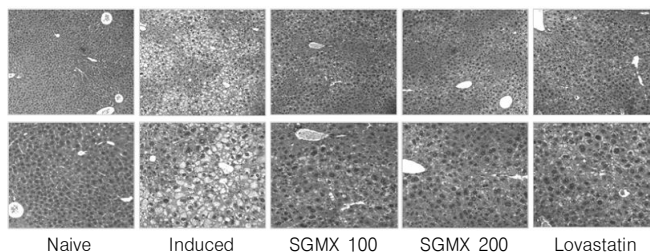


Fig. 2. Histopathological findings in liver tissues given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia. SGMX (100 or 200 mg/kg), lovastatin (20 mg/kg) or distilled water given to appropriate group. On the last day of experiment, liver tissues were removed and performed for H & E stain. The top is observation under $\times 200$ and the bottom is under $\times 400$ of microscope.

Table 3. Analysis of Serum Chemistry

Groups	AST (IU/L)	ALT (IU/L)	T-chol (mg/dl)	HDL (mg/dl)	HDL to LDL (%)	TG (mg/dl)
Naive	41.3 ± 18.2	34.3 ± 6.1	108.9 ± 27.6	61.8 ± 10.4	131.2 ± 20.4	84.2 ± 10.0
Induced	74.8 ± 11.3	63.7 ± 20.5	220.0 ± 39.3	58.0 ± 10.0	35.8 ± 6.0	138.7 ± 29.2
SGMX 100	60.8 ± 11.6*	64.0 ± 14.6	152.0 ± 30.1 [†]	65.1 ± 10.7	74.8 ± 11.7 [†]	105.0 ± 15.5*
SGMX 200	63.8 ± 13.2	64.8 ± 18.1	166.4 ± 52.5*	64.1 ± 18.5	62.6 ± 15.5 [†]	102.5 ± 21.8*
Lovastatin 20	65.8 ± 16.5	60.0 ± 21.5	154.7 ± 37.0 [†]	65.5 ± 11.8	73.4 ± 13.8 [†]	113.4 ± 29.1*

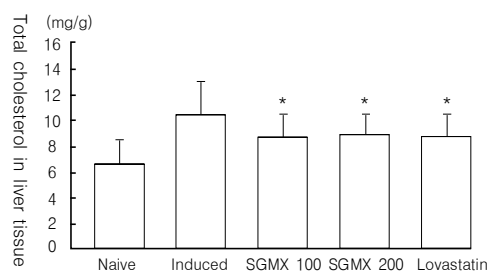
Data expressed as mean ± SD. (n = 8). *: p < 0.05, [†]: p < 0.01

were analyzed (Table 3).

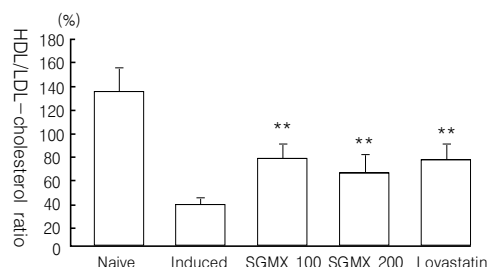
The groups fed with a high cholesterol diet showed a significantly higher level of total cholesterol and TG but had a lower level of HDL-cholesterol. Additionally, this hyperlipidemia model induced the

slight elevation of hepatic enzymes such as AST and ALT.

On the other hand, administration of SGMX slightly protected the liver against mild distortion of hepatic enzymes. The group of SGMX (100) had


Fig. 3. Total cholesterol in liver tissues given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia. SGMX (100 or 200 mg/ka), lovastatin (20 mg/ka) or distilled water given to appropriate group. On the last day of experiment, total cholesterol in liver tissue was determined. The values are expressed as the mean ± SD. (n=8) *: p < 0.05 significant differences compared with the induced group administered distilled water only.


Fig. 4. The ratio of HDL-cholesterol to LDL-cholesterol in serum given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia. SGMX (100 or 200 mg/ka), lovastatin (20 mg/ka) or distilled water given to appropriate group. On the last day of experiment, sera from every mouse were collected, and analysis of biochemistry was performed. LDL-cholesterol was calculated by subtraction of HDL-cholesterol from total cholesterol. The values are expressed as the mean ± SD. (n=8) **p < 0.01 significant differences compared with the induced group administered distilled water only.

statistical significance for AST ($p < 0.05$). Also, administration of SGMX and lovastatin showed a pattern of anti-hyperlipidemia. Total cholesterol was significantly lowered by SGMX (100, 200) and lovastatin ($p < 0.001$ or $p < 0.05$) compared to the induced group. These drugs also increased the HDL-cholesterol ($p > 0.05$) and its ratio ($p < 0.001$) to LDL-cholesterol. TG was significantly lowered by SGMX (100, 200) and lovastatin ($p < 0.05$) (Fig. 3, 4).

5. Total cholesterol and TG levels in liver tissues

Total cholesterol and TG in liver tissues were determined. The content of cholesterol was significantly lowered by SGMX (100 or 200) and lovastatin ($p < 0.05$) compared to the induced group (Fig. 5).

6. Lipid peroxidation products in liver tissues

To examine the effects of SGMX on lipid accumulation-induced distortion of liver tissue via lipid peroxidation process, MDA content was determined. The content of MDA was significantly lowered by SGMX (100 or 200) and lovastatin ($p < 0.05$ or $p < 0.01$) comparing to the induced group (Fig. 6).

7. Lipid metabolism-associated gene expressions

To examine the effects of SGMX on lipid metabolism, six gene expressions being HMG-CoA reductase, ACAT, DGAT, LCAT, CYP-7A1, and LDL-R in liver tissues were measured (Fig. 7-9). These gene expressions were calculated by comparing to expression of corresponding housekeeping gene, GAPDH.

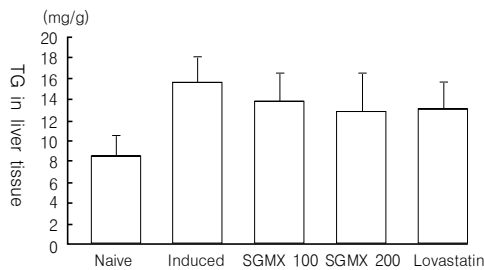


Fig. 5. TG in liver tissues given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia. SGMX (100 or 200 mg/ka), lovastatin (20 mg/ka) or distilled water given to appropriate group. On the last day of experiment, TG in liver tissue was determined. The values are expressed as the mean \pm SD. (n=8)

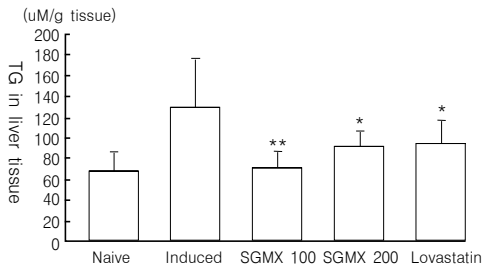


Fig. 6. Lipid peroxidation products in liver tissues given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia. SGMX (100 or 200 mg/ka), lovastatin (20 mg/ka) or distilled water given to appropriate group. On the last day of experiment, liver tissues were removed, and then MDA content was determined. The values are expressed as the mean \pm SE. (n=8) *: $p < 0.05$, **: $p < 0.01$: significant differences compared with the induced group administered distilled water only.

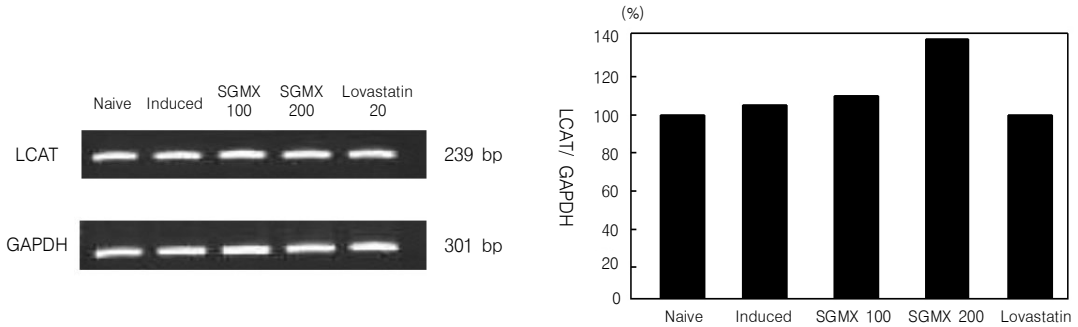


Fig. 7. LCAT gene expression in liver tissue given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia with SGMX (100 or 200 mg/kg), lovastatin (20 mg/kg) or distilled water. On the last day of experiment, liver tissues were removed, RT-PCR was performed.

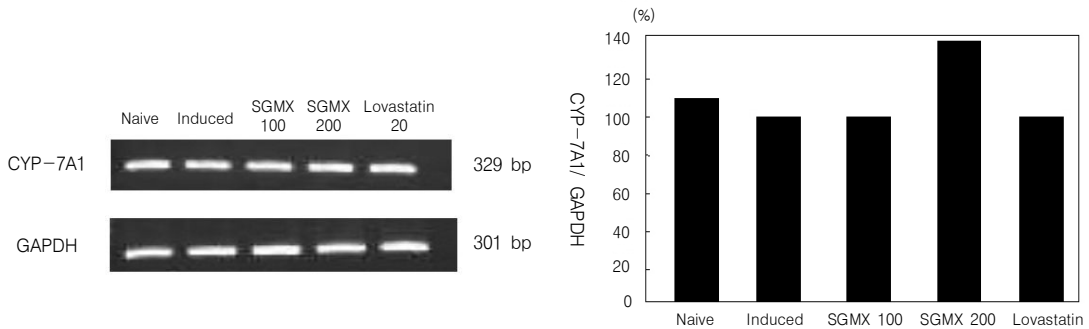


Fig. 8. CYP-7A1 gene expression in liver tissue given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia with SGMX (100 or 200 mg/kg), lovastatin (20 mg/kg) or distilled water. On the last day of experiment, liver tissues were removed, RT-PCR was performed.

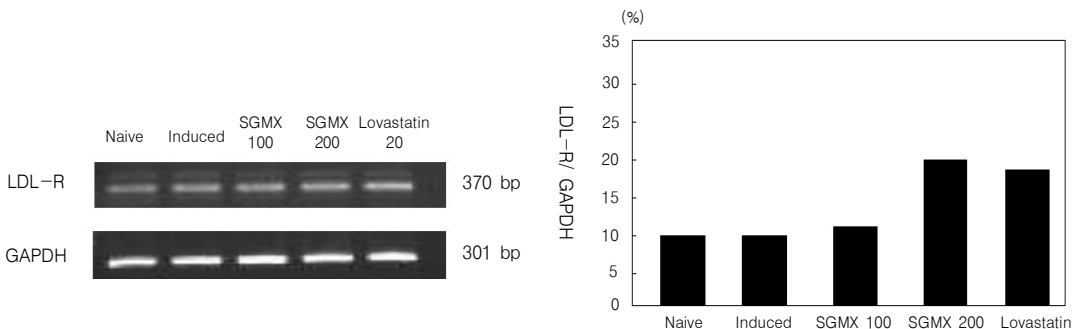


Fig. 9. LDL-R gene expression in liver tissue given high cholesterol diet.

All groups were fed with high-cholesterol diet for 4 weeks to induce hyperlipidemia with SGMX (100 or 200 mg/kg), lovastatin (20 mg/kg) or distilled water. On the last day of experiment, liver tissues were removed, RT-PCR was performed.

The overall pattern of the above gene expressions didn't show any specific changes among the groups treated with SGMX (100 or 200) or lovastatin.

HMG-CoA reductase gene expression was not affected by SGMX (100 or 200) or lovastatin. ACAT gene expression was drastically upregulated by high cholesterol feed, and was most significant in the lovastatin group. The expressions of DGAT and LCAT gene weren't changed by high cholesterol diet, and only LCAT was slightly upregulated by administration of SGMX (200) (Fig. 7). CYP-7A1 and LDL-R were upregulated by 20 % in 200 mg/kg of SGMX administration.

Discussion

Hyperlipidemia, also known as hyperlipoproteinemia, is an excess of lipid substances such as cholesterol, cholesterol esters, phospholipids and triglycerides in the bloodstream¹⁴. Along with the change of lifestyle and progress to an aging society, hyperlipidemia has been a major risk, killing people directly or indirectly in the developed countries¹⁵. This is strongly associated with high morbidity and mortality of cardiovascular disease and stroke which are the second and third highest causes of death in Korea¹⁶. Moreover, subjects with hyperlipidemia are consistently increasing among younger generations, so the social interest as well as drug market for hyperlipidemia is rapidly growing.

The supply of lipids comes from the diet in the small intestine or through endogenous fatty acid synthesis, primarily in the liver. Dietary fatty acids are esterified to form triglyceride and cholesterol. In order to travel in the bloodstream, the lipids should be combined with apoprotein resulting in forming one of the lipoproteins: chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), or high-density lipoproteins (HDL)¹⁷. The quantitative overload or qualitative unbalance of lipoproteins promotes pathologic changes in blood vessels¹⁸.

So far, four classes of drugs, HMG-CoA reduc-

tase inhibitors, bile acid sequestrants, nicotinic acid and fibric acids, are used for hyperlipidemia. These drugs play a role in one of the processes such as lipid absorption, synthesis, secretion or metabolism¹⁹.

The current hypolipidemic agents provide general benefits lowering lipid levels and consequently somewhat reducing the risk of cardiovascular disease and stroke. However, these drugs have limitations due to lacking efficacy with unwanted adverse effects like increase in hepatotoxicity, myopathy and concerns regarding noncardiovascular death²⁰. Therefore, there are still needs for new drugs for hyperlipidemia having high efficacy, especially without severe adverse effects^{21,22}.

The safety and therapeutic effects of Samgjeum were examined in animal models, and then SGMX has been used mainly for patients suffering from hyperlipidemia-associated symptoms and sequela of stroke since 2002²³. However, more animal-based studies are still wanted to supporting clinical efficacies and explain its underlying mechanisms.

The current study adapted a hyperlipidemia mouse model. After eating a high cholesterol diet for four weeks, mice had a twofold high cholesterol level in serum. Then, administration of 100 or 200 mg/kg of SGMX significantly inhibited the increase of serum total cholesterol level. The elevated TG level also decreased while HDL-cholesterol was likely to be increased by SGMX treatment. Accordingly, the ratio of HDL/LDL-cholesterol was directed into physiological condition compared to the induced group. Basically, those results were shown as the same pattern in liver tissues for total cholesterol ($p < 0.05$) and TG contents (Fig. 4, 5).

The above results are experimental evidence for the anti-hyperlipidemic effect of SGMX. The efficacy was comparable with lovastatin used as positive control drug in this study. Lovastatin is a typical HMG-CoA reductase inhibitor from the group known as statins²⁴. This drug was known to inhibit HMG-CoA reductase which is a rate-limiting enzyme of cholesterol synthesis in the liver. Statins also stimulate LDL receptors in the liver resulting in

clearance of LDL from the bloodstream²⁵). In this study, lovastatin as well as SGMX upregulated the gene expression of LDL receptors in the liver. Other groups explored the same effect of coconut water compared to lovastatin²⁶.

In order to explain some mechanism aspects of hypolipidemic effects, several other gene expressions were measured in the liver. ACAT, an acyltransferase, is the primary enzyme in the intestinal cholesterol absorption and synthesis of cholesterol via ACAT-mediated esterification of cholesterol in liver²⁷. This enzyme is very important because it plays a major role in hepatic production and releasing VLDL, and atherogenesis. Unlike the expectation, SGMX treatment did not affect this enzyme. Treatment with 200 mg/kg of SGMX upregulated the gene expressions of LCAT and CYP7A1 respectively (Fig. 7, 8). LCAT converts free cholesterol into a cholesteryl ester which eventually is sequestered to synthesize HDL-lipoprotein particle, then is known to prevent arteriosclerosis²⁸.

Cholesterol is normally eliminated into bile. CYP 7A1 is the rate-limiting enzyme in the synthesis of bile acid from cholesterol, then induces the secretion of bile acids and reduction of cholesterol level in hepatocytes²⁹. This result corresponds to the result from levels of hepatic total cholesterol. SGMX treatment inhibited the accumulation of cholesterol compared to the induced group (Fig. 3).

The above results partially explain the hypolipidemic effect of SGMX. In addition, SGMX protected the liver from high cholesterol diet-induced alteration of hepatocytes. The induced group showed high level of AST and ALT (Table 3). The macrovacuolar cytoplasmic alterations in micromorphologic finding and severe lipid peroxidation were observed (Fig. 6). However, SGMX treatment significantly ameliorated the pathologic changes. It is strongly assumed that SGMX has an anti-oxidative property which is responsible for hepatoprotective effects in this model. The link of oxidative stress and the excess consumption of high fat is well known³⁰. Moreover, many herbal medicines was reported to exhibit

antioxidant property³¹.

So far, many groups have researched for development of anti-lipidemic drugs using herbal prescriptions, and reported several positive results³¹. The new strategy or ideal purpose of treatment for hyperlipidemia is focused on improving quality of life of patients besides lowering blood lipid levels³². From this point of view, traditional herbal drugs would be a good resource for hypolipidemia. Nevertheless, a new standardized herbal mixture-derived drug has not yet been made.

Taken together, it is suggested that SGMX possess hypolipidemic effect by lowering total cholesterol and TG levels in serum and inside liver. The possible mechanisms may be related with partially upregulation of LDL receptor, LCAT, and CYP7A1 gene expression in this model. This study shows the possibility of SGMX as a candidate for herbal hypolipidemic through further studies in the future.

Conclusion

This study was aimed to investigate the effects of SGMX on hyperlipidemia using high cholesterol diet-fed mice model. The following biomarkers were measured: body weight, liver and spleen weight, histopathologic finding, serum AST and ALT, serum total-cholesterol, HDL-cholesterol and TG level, hepatic total-cholesterol and TG contents, and metabolism -associated gene expressions.

1. SGMX (100) administration significantly inhibited the increase of liver weight ($p < 0.05$).
2. SGMX administration inhibited the macrovacuolar cytoplasmic alterations in histopathologic finding.
3. SGMX (100) administration significantly protected the liver from pathologic elevation of AST ($p < 0.05$).
4. SGMX administration significantly lowered total cholesterol levels ($p < 0.01$) and TG ($p < 0.05$), but insignificantly increased HDL-cholesterol.
5. SGMX administration significantly increased the ratio of HDL to LDL-cholesterol ($p < 0.01$).

6. SGMX administration significantly lowered total cholesterol levels ($p<0.05$).
7. SGMX (100, 200) administration significantly protected liver from lipid peroxidation ($p<0.01$, $p<0.05$ respectively).
8. SGMX administration partially upregulated the gene expression of LCAT, CYP7A1 and LDL-R Receptor.

From this study, SGMX is suggested to possess hypolipidemic effect, and then could be a potential candidate for herbal hypolipidemic via further studies.

Acknowledgment

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