

Original Article

Synergic Effect of *GamiSamgieum* (SGMX) and Lipitor on Hyperlipidemia in Animal Model

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Objectives: To investigate the possibility of *GamiSamgieum* (SGMX) as a combination therapy with statins on hyperlipidemia using an animal model.

Methods: Forty eight ICR mice (male) were divided into six groups of eight mice each: naive, induced, Lipitor 5 mg/kg, Lipitor 5 mg/kg plus SGMX 100 mg/kg, Lipitor 10 mg/kg, and Lipitor 10 mg/kg plus SGMX mg/kg treatment group. Hyperlipidemia was induced by feeding a purified high fat diet for all groups (except naive) along with treatment of drugs for 6 weeks, and then biological parameters were examined on the last experiential day.

Results: Lipitor treatment lowered total cholesterol and increased HDL-cholesterol compared to the induced group with no statistical significance. However, co-treatment of SGMX with Lipitor revealed synergic effects on total cholesterol and HDL-cholesterol significantly ($P < 0.05$) in both. SGMX co-treatment also significantly protected liver tissues from the oxidative stress in liver tissues ($P < 0.05$) and augmented inhibitory effect of Lipitor against fat accumulation in the body.

Conclusion: These results indicate the possibility of that SGMX can be used for patients having hyperlipidemia as a combination therapy with statin drugs

Key Words : hyperlipidemia, SGMX, Lipitor, cholesterol, traditional Korean medicine

Introduction

Hyperlipidemia is a pathologic status characterized as an excess of fatty substances in the blood stream^{1,2}. These lipid disorders are an important risk factor in developing heart disease and stroke, which in turn are leading causes of death in most developed countries, including Korea^{3,4}. Hyperlipemia is very prevalent because of modern lifestyle elements such as lack of physical activity and high intake of fat, and due to a rapidly aging population^{5,6}.

So far, there are currently four major classes of lipid-lowering drugs, being statins, bile acid sequestrants, nicotinic acid, and fibric acids^{7,8}. These agents

have therapeutic effects against cholesterol, HDL/LDL cholesterol and triglyceride. However, these drugs all have a limitations owing to adverse effects such as myopathy or gastrointestinal distress^{9,10}. Therefore, lipid-lowering agents are practically prescribed with others, reducing adverse effects but enhancing clinical efficacies.

On the other hand, various herbal plants were reported to show anti-cholesterolemic activity¹¹⁻¹³. SGMX is a modified formula composed of fourteen herbal plants. SGMX has been prescribed for patients with cerebrovascular accident, hypertension or hyperlipidemia-associated symptoms, and showed anti-hyperlipidemic effect in a previous study using an

• Received : 10 October 2009

• Revised : 27 October 2009

• Accepted : 10 November 2009

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animal model¹⁴⁾.

The present study aimed to investigate the synergic effects of SGMX and statins on hyperlipidemia, because this combination therapy could be a very practical treatment regarding lowering lipid levels.

Materials and Methods

1. Preparation of SGMX

SGMX is composed of fourteen medicinal herbs (Table 1). SGMX was manufactured in KyungBang Pharmacy (Incheon, Korea) according to over-the-counter Korean monographs. Briefly, a total of [180 kg of the thirteen herbs were boiled in 1,800 L] of distilled water for 2 h at 100 °C, and then filtered using a 300-mesh filter (50 µm). The extract was concentrated into syrup containing around 30% water at 60 °C under 760 mmHg. For this study, some of the syrup was filtered through filter paper (Advantec, Toyo Roshi Kaisha, Tokyo, Japan) and lyophilized. The final extraction gave a yield of 14.7 % (w/w).

2. Fingerprinting of SGMX

Two-dimensional high-performance liquid chromatography (HPLC) was used to produce a fingerprint for SGMX using eight major compositional herbs (*Rehmannia gietinosa* Eucommia ulmoides Cortex, *Achyranthes japonica* Radix, *Paeonia lactiflora* Radix, *Glycyrrhiza uralensis* Radix, and *Coptis japonica* Radix) and their main compounds (5-hydroxymethyl furaldehyde and geniposidic acid, chlorogenic acid, 20-hydroxyecdysone, paeoniflorin, glycyrrhizin and berberine, and coptisine) respectively (Fig. 1). The HPLC system consisted of a SCL-10A system controller, SPD-10AVP diode array detector and CTO-10AS column temperature controller (Shimadzu, Kyoto, Japan). An Eclipse-DB-C18 (2.1 × 150 mm) column was eluted with solvents A (5 % acetonitrile in water containing 0.05 % formic acid) and B (90 % acetonitrile in water) at a flow rate of 0.4 ml/min. Solutions 100 % A and 0 % B changing over 30 min to 30 % B, 40 min to 50 % B were used. All chromatograms were obtained using wavelength of 250 nm, X-and Y-axis mean time (min).

Table 1. Prescription of SGMX

General Name	Medical Use	Relative Amount (g)
<i>Rehmannia glutinosa</i>	Radix	12
<i>Eucommia ulmoides</i>	Cortex	4
<i>Achyranthes japonica</i>	Radix	4
<i>Lycium chinese</i>	Fructus	4
<i>Poria cocos</i>	Flos	4
<i>Paeonia lactiflora</i>	Radix	4
<i>Aconitum carmichaell</i>	Radix	4
<i>Aralia continentalis</i>	Radix	4
<i>Glycyrrhiza uralensis</i>	Radix	4
<i>Cinnamomum cassia</i>	Cortex	4
<i>Angelica gigas</i>	Radix	4
<i>Coptis japonica</i>	Radix	2
<i>Ohylostachys nigra</i>	Concretio	2
<i>Zingiber officinale</i>	Rhizoma	2
Total		60

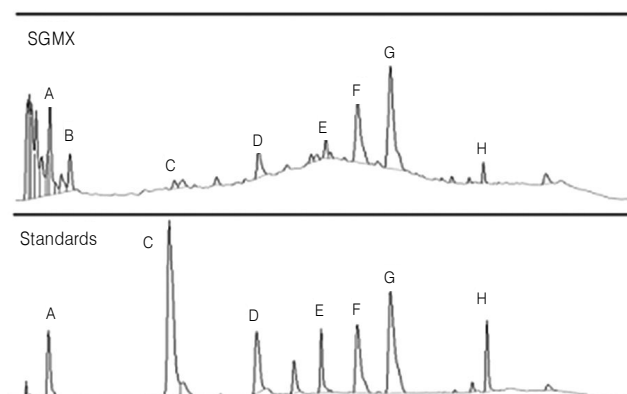


Fig. 1. HPLC-based fingerprinting for SGMX.

A: 5-hydroxymethyl furaldehyde, B: geniposidic acid, C: chlorogenic acid, D: paeoniflorin, E: 20-hydroxy ecdysone, F: coptisine, G: berberine, H: glycyrrhizic acid

3. Animal and experimental design

Forty-eight ICR male mice (six weeks old) were purchased from Orientbio (Gyeonggido, Korea). After seven days of acclimation under environment of 22 ± 2 °C, 12 h light/dark cycle, free access to water and food, hyperlipidemia was induced by feeding a purified high fat diet for all groups (except naive) for 6 weeks. The animals were divided into six groups of eight mice each: naive, induced, Lipitor 5, Lipitor 10, Lipitor 5 plus SGMX 100, and Lipitor 10 plus SGMX 100. The mice were administrated orally with Lipitor (5 or 10 mg/10 ml/kg) with or without SGMX (100 mg/10 ml/kg). On the last day of experiment were mice fasted for 4 h and sacrificed under ether anesthesia.

4. Preparation of high fat diet and reagents

The purified high fat diet was made by FeedLab Korea (Gyeonggido, Korea) for adjusting to fat 45 % calorie in total diet (Table 2). Lipitor was purchased from Pfizer Inc (Seoul, Korea). Other reagents, including potassium chloride (KCl), phosphoric acid, and 1.1.1.3.3-tetraethoxypropane (TEP) were purchased from sigma (Milwaukee, WI, USA). N-butanol was purchased from Daejung Chemical (Gyeonggido,

Korea), and thiobarbituric acid (TBA) was purchased from Lancaster (Lancashire, England).

5. Weight analysis of body, fat, and liver

Mice body weights were measured once a week for 6 weeks. At the end of experiment epididymal, visceral and perirenal fats as well as livers were removed and weighted separately. Then an individual fat or liver weight index was calculated according to the formula, as follows:

$$(\text{Fat or liver weight} / \text{body weight}) \times 100 \%$$

6. Analysis of serum cholesterol and lipid peroxidation products in liver

On the last day of experiment whole blood was collected from the abdominal aorta, and then centrifuged at 3000 rpm for 15 min to separate serum. Sera were used to determine total cholesterol and high density lipoprotein (HDL)-cholesterol using an Auto Chemistry Analyzer (Chiron, Emeryville, CA, USA).

Lipid peroxidation in liver tissue was examined using the method of thiobarbituric acid reactive substances (TBARS), as previously described¹⁵⁾. The concentration of TBARS was determined as μmol of

Table 2. Experimental diet compositions

Formulation	Normal		High fat diet (45% cal)	
	gm %	kcal %	gm %	kcal %
Protein	20	20	24	20
Carbohydrate	64	64	41	35
Fat	7	16	24	45
kcal/kg	4,000		4,776	
Ingredient	g	kcal	g	kcal
Casein (from milk)	200	800	200	800
Corn starch	397.486	1590	155.036	620
Sucrose	100	400	50	200
Dextrose	132	528	132	528
Cellulose	50	0	50	0
Soybean oil	70	630	25	225
Lard	0	0	175	1575
Mineral Mixture	35	0	35	0
Vitamin Mixture	10	40	10	40
TBHQ	0.014	0	0.014	0
L-Cystine	3	12	3	12
Choline bitartrate	2.5	0	2.5	0
Total	1,000	4,000	837.6	4,000

MDA per mg of tissue using TEP as a standard. Briefly, 0.15 g of Liver tissue was homogenized in 1.5 ml of ice-cold 0.15 % KCl, then using 0.13 ml of homogenate mixed with 0.08 ml of 1 % phosphoric acid and 0.26 ml of 0.67 % TBA. The mixture was incubated for 45 min at 100 °C, after that, 1.03 ml of n-butanol was added, followed by vigorous mixing and centrifugation at 3000 rpm for 15 min. The absorbance of the supernatant was measured at 535 nm by spectrophotometer.

7. Statistical analysis

All data were expressed as the mean \pm S.D. (n = 8). Significant differences between the groups were statistically analyzed using one-way analysis of variance (ANOVA), followed by a two pairs Student's t-test. $P < 0.05$, $P < 0.01$, and $P < 0.001$ were regarded as statistically significant.

Results

1. Change of body weight

Mice of all six groups showed increased body weight throughout the experimental period. There was no significant difference in changes of body weight among the groups (Fig. 2).

2. Changes of body fat weight

In induced group, epididymal, visceral and perirenal fat weight and total body fat weight were markedly increased compared to the naive group ($P < 0.01$, $P < 0.05$). Treatment with Lipitor inhibited the increase of total fat weight significantly in the Lipitor 10 group ($P < 0.01$). The combined treatment with Lipitor plus SGMX synergically reduced the fat weight compared especially to the Lipitor 5 group ($P < 0.01$, $P < 0.05$) (Fig. 3).

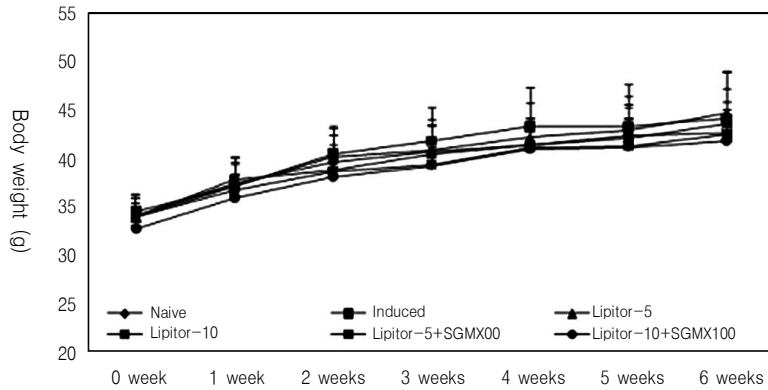


Fig. 2. Changes of body weight.

All the groups except naive were fed with high fat diet for 6 weeks, and treated with Lipitor (5 or 10 mg/kg) with or without SGMX (100 mg/kg).

3. Change of liver weight

The relative liver weight of the induced groups significantly increased compared to the naive group ($P < 0.01$). In all of the treatment groups, the increase of relative weight of liver was smaller than in the induced group. In particular, the Lipitor 5 plus SGMX 100 group decreased significantly compared to the induced group ($P < 0.01$) or Lipitor 5 group (Fig. 4).

4. Change of serum total cholesterol and HDL-cholesterol

The induced groups showed increase of serum total cholesterol and decrease of HDL-cholesterol compared to the naive group. The treatment with Lipitor 5 and 10 mg/kg showed a reduction of cholesterol and elevation of HDL-cholesterol. However, its effect didn't reach statistical significance in either total cholesterol or HDL-cholesterol ($P > 0.05$).

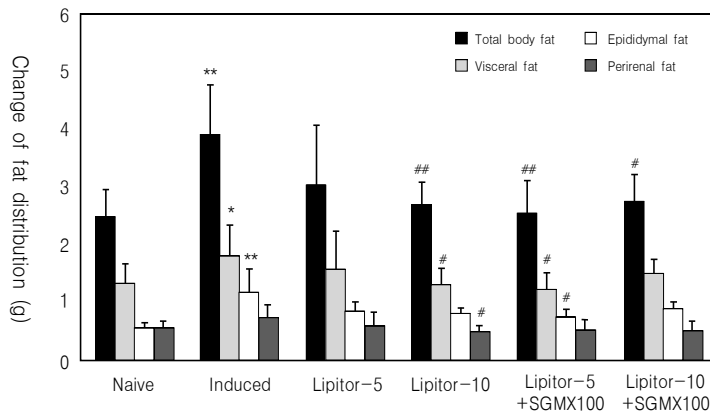


Fig. 3. Change of body fat weight.

At the end of the last experimental day, epididymal, visceral and perirenal adipose fat were removed and weighted.

** $P < 0.01$, * $P < 0.05$ vs. naive group

$P < 0.01$, # $P < 0.05$ vs. induced group

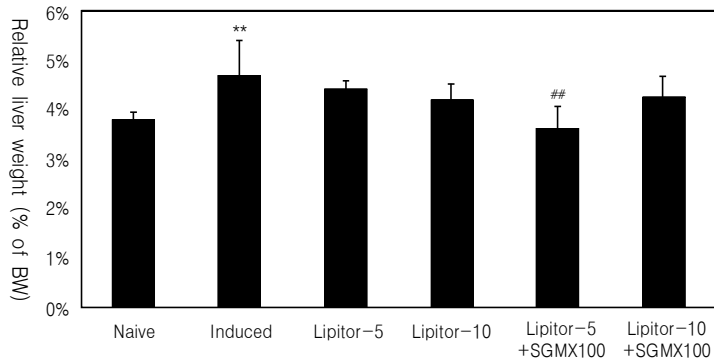


Fig. 4. Relative liver weight.

At the end of the last experiment day, the removed liver weight was measured, and then relative liver weight was calculated.

** $P < 0.01$ vs. naive group

$P < 0.01$ vs. induced group

In contrast, combined treatment with Lipitor plus SGMX significantly ameliorated the increase of total cholesterol and reduction of HDL-cholesterol ($P < 0.05$) (Fig. 5).

5. Change of lipid peroxidation in liver tissues

The lipid peroxidation was examined in liver tissue. The content of MDA significantly increased in induced groups whereas it was inhibited by Lipitor treatment ($P < 0.01$ in Lipitor 10 group). This effect was synergically augmented by treatment with Lipitor plus SGMX ($P < 0.05$, $P < 0.01$) (Fig. 6).

Discussion

Blood lipids come from the diet or endogenous fatty acid synthesis. The morbidity of hyperlipidemia is continually increasing, so the drug market for hyperlipidemia is rapidly growing¹⁶. So far, four classes of lipid-lowering drugs are used, and these drugs play a role in one process such as lipid absorption, synthesis, secretion or metabolism^{17,18}.

Lipitor used in this experiment is one of typical statins, inhibiting HMG-CoA reductase which is a rate-limiting enzyme of cholesterol synthesis in the

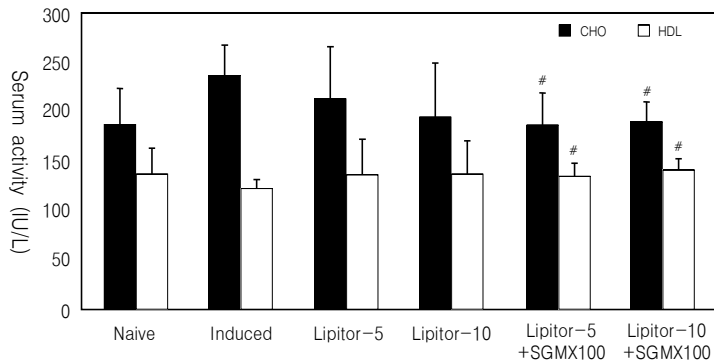


Fig. 5. Serum total cholesterol and HDL-cholesterol

At the end of the last experiment day, sera were analyzed using an Auto Chemistry Analyzer. # $P < 0.05$ vs. induced group.

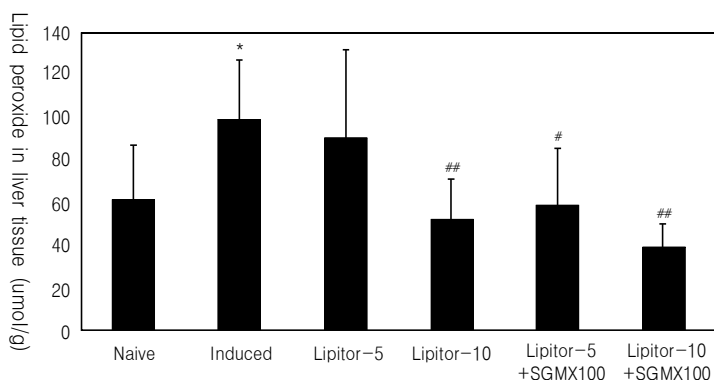


Fig. 6. Measurement of lipid peroxide levels in liver tissues.

At the end of the last experiment day, MDA were determined in the liver tissues.

** P < 0.05 vs. naive group

P < 0.05, ## P < 0.01 vs. induced group.

liver. Statins also stimulate clearance of LDL from the bloodstream, and are most frequently prescribed worldwide¹⁹). However, statins are clinically limited because of notable adverse effects⁹). This drug is known to fail to reduce heart disease and stroke even though it can lower lipid levels by 15 to 30 %. Accordingly, many lipid-lowering agents are usually prescribed together with other drugs supporting its adverse effects and clinical efficacies^{20,21}).

In this context, combination therapy with western and herbal drugs could be a valuable model. SGMX is originated from a traditional formula, Samgieum, which has been used to treat muscle-joint disorders caused by three pathogenic statuses of wind, cold and dampness and stroke sequelae²²). The safety and effect to hyperlipidemia-associated symptoms have been proved in clinical and animal studies^{23,24}).

To investigate the potency of SGMX as a combination therapy, 100 mg/kg of SGMX was co-treated with Lipitor to mice having hyperlipidemia. Treatment with Lipitor reduced the accumulation of body fat and increase of liver weight (Fig. 3, 4). Lipitor also moderately lowered the serum total cholesterol. However, combined treatment with SGMX showed a more significant effect on them than treatment with

Lipitor only. Additionally, co-treatment with SGMX synergically ameliorated the reduction of HDL-cholesterol (Fig. 5). The triglyceride, one of the main lipid components regarding hyperlipidemia, was not changed in this model (data not shown).

Myopathy and hepatotoxicity are known as main side effects of statins⁹). In this study, the level of lipid peroxidation product was very high in the induced groups, whereas SCMx co-treatment significantly reduced its elevation. This result indicates that SGMX has an anti-oxidative property, which is in accordance with previous research¹⁴). The link of oxidative stress to the excess fat diet and hepatotoxicity has been well reported^{25,26}). SGMX also showed an anti-fatigue effect to mice under swimming-induced fatigue after high dose-statin administration (data not shown).

An ideal treatment for hyperlipidemia is improving physical activity of patients, besides lowering blood lipid level. In traditional Korean medicine, hyperlipidemia is regarded as a status of spleen-deficiency, phlegm-stagnation, accumulation and stasis of damp-heat, and Qi and blood stagnation^{27,28}). The herbal medicines treating these pathologic conditions are generally effective on improving whole symptoms as

well as disease-specific condition. Other groups have studied development of anti-lipidemic drugs using traditional herbal formula prescriptions, and reported several positive results²⁹⁾.

Taken together, this study presented a possibility of SGMX application to patients with hyperlipidemia as a combination therapy with western lipid-lowering agents. However, further studies are need for the optimal combination model and its underlying mechanisms responsible for the effects in the future.

Acknowledgement

This study was supported by a grant of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea (B080003).

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