Effect of methanol extract of Schisandraceae Fructus on high fat diet induced hyperlipidemic mice

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Accepted: February 16, 2019

Abstract

OBJECTIVE: To investigate the effects and molecular targets of Schisandraceae Fructus (SF) methanol extract (SFme) in mice with hyperlipidemia induced by high fat diet.

METHODS: We observed changes in body weight, blood serum content of total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglyceride. The extent of accumulation of lipid peroxide due to lipid metabolism disorder also evaluated by measuring malondialdehyde (MDA) level. In addition, after getting gene expression in hepatic tissues, target protein of SFme was identified using a protein interaction database.

RESULTS: SFme significantly decreased total cholesterol and triglyceride levels without alteration of body weight in mice, and the liver content of MDA was statistically decreased by SFme. And expression changes of cyclin-dependent kinase 1 (Cdk1) and leucine-rich repeat kinase 2 (Lrrk2) were restored by SFme.

CONCLUSION: The effect of SFme on the high-fat-diet induced hyperlipidemia via decreasing total cholesterol and triglyceride levels may involve the expression of Cdk1 and Lrrk2 proteins.

Keywords: Schisandra; hyperlipidemias; Obesity; Cardiovascular diseases; CDC2 protein kinase

INTRODUCTION

In recent years, drastic changes in living environment and excessive nutritional intake have been increasing due to various metabolic diseases such as diabetes, hypertension, hyperlipidemia, and cardiovascular diseases. Among the main causes of death in Korea, cerebrovascular disease, heart disease occupies second and third place after cancer. Frequent intake of fat and carbohydrates might cause elevation of blood cholesterol, accumulation of fat, increase of insulin resistance, weight gain, and metabolic abnormalities in the body such as hyperlipidemia can cause diabetes, hypertension, and other various diseases such as cardiovascular disease, stroke, and cancer. Thus, hyperlipidemia became a threatening to human health and a social problem. Because hyperlipidemia is recognized as a direct cause of cerebrovascular disease and heart disease, therapeutic methods for hyperlipidemia have been studied diversely. It is clear that there is a direct correlation between diabetes and hyperlipidemia among these risk factors. Indeed, cardiovascular
disease is the leading cause of death in diabetic patients, and it is known that 31% to 34% of diabetic patients are associated with coronary artery disease.\(^1\)\(^,\)\(^2\)\(^,\)\(^9\)\(^,\)\(^12\)\(^,\)\(^13\) Recently traditional medicine industry has been increasingly interested in raising the added value of the pharmaceutical industry through the development of new drugs. Schisandrae Fructus (SF), the dried berries of Schisandra chinensis Baillon, is one of the most popularly used medicinal herbs. Previous reports on SF are very diverse such as effects on anti-oxidative,\(^3\)\(^,\)\(^4\)\(^,\)\(^14\) hepatoprotective,\(^5\) and memory impairment.\(^4\)\(^,\)\(^15\)\(^,\)\(^16\) Recent reports related to this study were effects of SF ethanol extract in hypercholesterolemic mice,\(^7\) effects of schisandrin B in hypertriglyceridemia,\(^8\) and effects of SF in serum and hepatic lipid in high cholesterol diet.\(^7\)\(^,\)\(^19\)

Schisandra chinensis Baillon is a plant that can be used for both food and medicine, and currently it is recognized as one of highly valuable drugs to use various forms of processed foods and traditional medicine clinically. Well known major bioactive constituents of SF are gomisin and schisandrin.\(^11\)\(^,\)\(^12\)\(^,\)\(^13\) But like many other herbal medicines, SF is composed of various kinds of phytochemicals; it would be difficult to identify major components having pharmaceutical effect. Therefore high throughput screening systems such as microarray analysis could be a proper tool to elucidate the molecular effects of herbal extract on disease animal model. As depicted above, SF had various pharmacological activities, thus it has great potential as pharmacological agent. In this study, we aimed to confirm the anti-hyperlipidemic effect in mice with high-fat induced hyperlipidemia, and to study molecular target which SF methanol extract might work on.

**MATERIALS AND METHODS**

**Animals**

For the induction of hyperlipidemia, male ICR mice (SAMTAKO, Osan, Korea) with a weight of 20 to 25 g at 6 weeks of age were used. All the mice used in this experiment were obtained from a specific pathogen-free barrier facility (supplementary document 1), and were adapted to laboratory environment [room temperature (24 ± 2 °C), humidity 55% ± 5%, 12 h dark/light cycle] for 1 week or more while supplying sufficient amount of solid feed and water. The experimental protocol involving animals was approved by the ethics committee of PNU (Pusan National University; approval number PNU- 2013-0311).

**Preparation of SF methanol extract**

SF used in this study was purchased from an authorized pharmaceutical company (Naemomedah Co., Korea) and authenticated by Dr. Cho (Pusan National University School of Korean Medicine, Yangsan, Korea). A voucher specimen (No. SF14-0217) was deposited in the low temperature room (4 °C) of the laboratory, and fingerprinting of the specimen was shown in Figure 1. 500 g of SF was immersed in methanol at room temperature for 5 d, the process of obtaining the filtrate was repeated twice, and the dry extract obtained was 96.8 g, thus the yield was 19.4%.

**Induction of hyperlipidemia and classification of experimental groups**

For induction of hyperlipidemia, we fed high-fat diet to mice of control group (CON) and SFme treated group (SFG) for 4 weeks, and mice of normal group (NOR) were supplied general feed. At the fifth week of the experiment, high-fat diet fed mice were distributed to the CON and SFG randomly basically based on the body weight. From the fifth week of the experiment, SFG mice were fed high fat diet with SFme while CON mice were fed high fat died for additional 2 weeks. And the food for rodents was made from Daol Biotech (Daejeon, Korea) (Table 1, Figure 2).

**Harvesting liver tissues, preparation for gene expression analysis, and MDA measurement**

After sacrificing the experimental animal, the liver tissue was excised, and blood was removed using cold (4 °C) perfusion solution containing 130 mM NaCl, 5 mM KCl, 10 mM Tris-HCl (pH 7.4). In order to observe gene expression, total RNA was isolated using a Qiagen RNeasy Kit (Qiagen Korea Ltd., Seoul, Korea) according to the manufacturer’s instructions. Then, an Agilent microarray containing approximately 45 000 oligo-spots (Agilent Technologies Co., Santa Clara, CA, USA) was used for hybridization. RNA from NOR was used as a reference, and we considered 3-fold of expression change as baseline of up- or down-regulation. Hierarchical clustering of gene were analyzed using a multiple experiment viewer (MeV ver. 4.9, ebiogen, Seoul, Korea), and a functional protein association networks database (STRING, https://string-db.org) was applied for interaction network analysis.

For MDA measurement, Stadie-Riggs microtome (Thomas Scientific, NJ, USA) was used to prepare a tissue slice having a width of about 1 mm and a thickness of about 0.4-0.5 mm with a horizontal length and a vertical length of 1 cm each to measure MDA content. Then add 3 mL phosphoric acid and 0.6% thiobarbituric acid solution, and boil for 60 min. Then add 4 mL of 1-butanol, mix well, and centrifuge at 800 xg for 25 min. Absorbance of supernatant of the mixed solution was evaluated as measuring at 534 and 510 nm.

**Blood collection, measurement of blood cholesterol and triglyceride**

After 2 weeks of SFme administration finished, blood was taken from the mouse’s abdominal vein. The
Figure 1 High performance chromatography (HPLC) images of Schisandra Fructus (SF) and its standard compounds.

A: chromatogram of standard compounds, schizandrin (1), gomisin A (2) and gomisin N (3); B: chromatogram of SF. HPLC, Shimadzu, pump, LC-20AD; auto-sampler, SIL-20A; detector, SPD-M20A; column oven, CTO-20A. Mobile phase, mixture of acetonitrile, water, and formic acid (70:30:0.1). Column, YMC-Triart C18, 250 mm x 4.6 mm, 5 μm; column temperature, 33 °C; flow rate, 0.6 mL/min; injection volume, 10 μL.

Table 1 Experimental groups and compositions of normal and high fat diet

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<th>Diet (g/kg)</th>
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<td>Cholic acid</td>
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<td>SFme</td>
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Notes: SFme: Schisandrae Fructus methanol extract. *Typical analysis of cholesterol in lard = 0.95 mg/g.
obtained blood was centrifuged at 5000 \( \times g \) for 20 min, and the supernatant was taken to measure blood cholesterol and triglyceride contents. Content of serum total cholesterol, HDL-cholesterol, and triglyceride were measured using measurement kit (FUJIFILM, Tokyo, Japan).

**Statistical analysis**

For the statistical analysis of the experimental material, a statistical package SigmaPlot ver. 12 (SigmaStat, San Jose, CA, USA) was used. Experimental results are expressed as mean ± standard deviation (\( \bar{x} \pm s \)), and statistical significance among the groups was determined using One-way analysis of variance test followed by Tukey’s post-hoc analysis with SigmaPlot. Statistical significance was determined when the \( P \) value was less than 0.05.

**RESULTS**

**Influence on body weight change**

A slight weight gain was observed in the CON when compared to NOR as a result of observing changes in body weight through experiment periods, but there was no statistical significance among groups (Figure 3). And there was no difference among groups in food intake during experimental periods (data not shown).

![Figure 2 Design of the hyperlipidemia induction](image)

The mice were pretreated with normal diet or high fat diet up to their group for 6 weeks. Schisandrae Fructus methanol extract (SFme) mixed with high fat diet was fed to SFme administration group (SFG) for the last 2 weeks. NOR: normal diet-fed naive mice (\( n = 8 \)); CON: high fat diet (HFD)-fed hyperlipidemic mice (\( n = 8 \)); SFG: HFD-fed and SFme administered mice (\( n = 8 \)).

**Effect of change of serum lipid content**

Observation of change in total cholesterol content from mouse blood showed a significant increase when NOR and CON were compared which appeared (118 ± 13) and (163 ± 7) mg/kg respectively. And in the SFG, it appeared (138 ± 11) mg/kg and showed significant change compared with the CON (Figure 4A). As a result of observing changes in HDL-cholesterol content from mouse blood, no special differences could be found in all groups (Figure 4B). In serum level of triglyceride, statistically significant increase was observed when NOR and CON were compared which appeared (89 ± 12) mg/kg and (168 ± 22) mg/kg respectively. And in the SFG, it appeared (120 ± 25) mg/kg and showed significant change compared with the CON (Figure 4C).

![Figure 3 Effects of SFme on changes in body weights in hyperlipidemic mice](image)

Body weights were measured every 2 weeks NOR: normal diet-fed naive mice (\( n = 8 \)); CON: HFD-fed hyperlipidemic mice (\( n = 8 \)); SFG: HFD-fed and SFme administered mice (\( n = 8 \)). SFme: Schisandrae Fructus methanol extract; SFG: SFme administration group; HFD: high fat diet. Values are presented as mean ± standard deviation.

![Figure 4 Effects of SFme on levels of total cholesterol, HDL-cholesterol, and triglyceride in hyperlipidemic mice](image)

A: total cholesterol; B: HDL-cholesterol; C: triglyceride. NOR: normal diet-fed naive mice (\( n = 8 \)); CON: HFD-fed hyperlipidemic mice (\( n = 8 \)); SFG: HFD-fed and SFme administered mice (\( n = 8 \)). SFme: Schisandrae Fructus methanol extract; HDL: high-density lipoprotein; SFG: SFme administration group; HFD: high fat diet. Values are presented as mean ± standard deviation. *\( P < 0.001 \) vs NOR; "\( P < 0.001 \) as compared to CON.
Changes in lipid peroxide content in liver tissue

As a result of measuring the content of MDA which is a lipid peroxide from mouse liver tissue, in hyperlipidemic condition, CON was found to be significant as compared with the NOR showing (180 ± 32) and (118 ± 13) pmole MDA/mg protein respectively. And SFG showed significant change compared to CON which appears (152 ± 14) pmole MDA/mg protein (Figure 5).

![Figure 5 Effects of SFme on lipid peroxidation levels in hyperlipidemic mice. Lipid peroxidation in liver tissues were measured using spectrophotometry.](image)

**NOR**: normal diet-fed naive mice (n = 8); **CON**: HFD-fed hyperlipidemic mice (n = 8); **SFG**: HFD-fed and SFme administered mice (n = 8). SFme: Schisandraceae Fructus methanol extract; SFG: SFme administration group; HFD: high fat diet. Values are presented as mean ± standard deviation. *P < 0.001 vs NOR; †P < 0.05 as compared to CON.

Expression profile of genes

Expression pattern of genes in the livers of mice were observed, and a total of 285 genes showing at least 3-fold ratio variation in CON when compared with that of NOR was hierarchically clustered as shown in Figure 6. It is evident that the expression of 285 genes was significantly changed in the livers of hyperlipidemic mice compared with NOR and CON. Among the altered genes, we selected 45 genes (19-down regulated, 26 up-regulated) which the expression was restored by SFme based on hierarchical clustering using MeV software (Figure 6). Tendency of alteration and restoration of the genes were shown as Figure 7.

By using STRING database, assessing functional genomics, exploring predicted interaction networks can suggest new directions for future experimental research. In this study, by assessing 45 genes restored by SFme administration, the changes in pathway activities in liver tissues were obtained, and p53 pathway was thought to have an important role in hyperlipidemic conditions (Table 2). And main target proteins having key roles in pathways above were identified as Cdk1 and Lrrk2 (Figure 8).

**DISCUSSION**

In this study, SFme significantly decreased total cholesterol and triglyceride levels without alteration of body weight in mice. In our preliminary study, food intake was observed because rodents’ chow mixed with SFme might affect food intake which might influence on change of body weight and total lipid content in blood. But SFme mixed chow did not affect food intake, and it may reflect the restoration of body weight among experimental groups in mice. And the liver content of MDA was statistically decreased by SFme, and this result was thought to be related hepatoprotective activities of SFme.

By applying hundreds differentially expressed genes in hyperlipidemic mice, we identified p53 signaling pathway, which was recently reported to act as a novel regulator of hepatic lipid metabolism and consequently of systemic lipid homeostasis and atherosclerosis development. And by using protein network database such as STRING, we also obtained important target proteins such as Cdk1 which regulates mitochondrial development.

In conclusion, SFme suppressed hyperlipidemia by regulation of serum total lipid and triglyceride, and lipid peroxidation of liver tissues not affecting body weight gain of hyperlipidemia-induced mice. In addition, we can expect the effect of SFme on hyperlipidemia by restoring genes and proteins related to of hepatic lipid metabolism, and Cdk1 protein was identified as molecular target which play key role in ameliorating hyperlipidemia.

**ACKNOWLEDGEMENTS**

The present authors are grateful to Oh SR and Jeong SY who very kindly provided fingerprinting of SF shown as in Figure 1.

**REFERENCES**


**Figure 6** Changes in lipid peroxide content in liver tissue of systemic lipid homeostasis and atherosclerosis development. And SFme down-regulated the altered gene expression of Lrrk2. These results meet the data of molecular pathway identification.
Figure 6 Effects of SFme on expression patterns of genes of liver tissues in hyperlipidemic mice
To identify the genes using the quantitative analysis and clustering of their expression, MeV ver. 4.0 software was used. Genes colored red were up-regulated genes compared to NOR (N); green, down-regulated genes compared to NOR. A and B groups indicates genes showing ≥ 3-fold up-regulated expression compared to that in the NOR group; C and D groups indicates genes showing ≥ 3-fold down-regulated expression compared with that in the NOR group. N: normal diet-fed naive mice; C: HFD-fed hyperlipidemic mice (n = 8); S: HFD-fed and SFme administered mice. SFme: Schisandrae Fructus methanol extract; NOR: normal group; HFD: high fat diet.

Figure 7 Line plot of alteration of gene expression liver tissues in hyperlipidemic mice
The resultant SFme-responsive genes are plotted as log values for each differentially expressed gene. NOR: normal group, naive mice; CON: control group, hyperlipidemic mice; SFG: Schisandrae Fructus methanol extract (SFme) treated group.

10 Sanders TA, Oakley FR, Miller GJ, et al. Influence of n-6
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Notes: false discovery rate (FDR) corrections were calculated using the Benjamini-Hochberg procedure.


