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Article

Effect of *Sophora japonica* Extract on Lipid Content in High Fat Diet Fed Rats

Byung-Yong Ahn[1] and Sung-Jin Park[2]*

[1]*Department of Oriental Medicine Resources, Chonbuk National University, Iksan 570-749, Republic of Korea*
[2]*Department of Tourism Food Service Cuisine, Hallym College, Chuncheon 200-711, Republic of Korea*

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The effect of hot water extract of *Sophora japonica* Linne (Koehwa) flower on lipid content were studied in terms of hematological variables in rats fed with high fat diet. Experimental rats were divided into basal diet only (BDG), high fat diet control (FDC), high fat diet and 6% *Sophora japonica* extract powder (FD6S), and high fat diet and 12% *Sophora japonica* extract powder (FD12S) groups. The levels of hematological variables were not significantly different among the four groups, whereas transferrin concentration and glutamic oxaloacetate transaminase (GOT) activity in serum metabolic variables were significantly different. Transferrin concentration was higher in the FD6S and FD12S groups than in BDG and FDC groups. FD6S and FD12S groups showed significantly lower level of GOT activity. Total cholesterol levels of FD6S and FD12S groups were 220.38 and 205.02 mg/dL in the serum, respectively. Total cholesterol levels of FD6S and FD12S groups were lower than that of FDC group (341.38 mg/dL) and the same level as that of FDG group (216.18 mg/dL). HDL- and LDL-cholesterol levels of FDC group were 26.84 and 62.91 mg/dL, whereas those of FD12S group were 38.02 and 44.16 mg/dL. Supplementation of 12% Koehwa extract powder remarkably increased HDL-cholesterol level and greatly decreased LDL-cholesterol level. Atherogenic indices in FD6S and FD12S groups were significantly lower than those of yje FDG group. The FD12S group supplemented with Koehwa extract showed lower triglyceride concentration than that of the FDC group. These results suggested that dietary supplementation of Koehwa extracts did not have any adversary effect on the hematological variables, but improved the lipid content and reduced hepatic damage of the high fat fed rats.

Key words: *HDL-cholesterol, high fat diet,* Sophora japonica*, total cholesterol, triglyceride*

With rapid changes in the epidemiology of diseases in Korea caused by the improvement in living standards and westernization of diet, dietary lifestyle diseases have become the biggest health problems today. In particular, hyperlipidemia, the main risk factor of atherosclerosis, is known to be one of the three highest risk factors of coronary heart diseases together with high blood pressure and smoking. Related dietary factors include cholesterol and total lipid intake [Anderson, 2006], ratio of unsaturated to saturated fatty acids in dietary fat [Shepherd, 1980], and excessive sugar intake [Mettson and Grundy, 1985]. The type of lipid taken is known to be more closely related to the health than the total amount of lipid intake. Accordingly, recent prevention and treatment efforts for hyperlipidemia emphasize more on the development of health-functional food products with the underlying scientific basis than the use of medicines [Kim *et al*., 2002; Park and Han, 2003; Park *et al*,, 2003; Park *et al*., 2005a, 2005b. With the increase in the interest on the development of health food products based on herbal drugs, scientific studies are being actively conducted on the active principles and the functionalities of herbs [Wu *et al*., 1998, 2001; Cha *et al*., 2002; Choi *et al*., 2002]. In particular, studies reported reduction in the serum lipid by intake of various herbals; the green tea and oolong tea in

*Corresponding author
Phone: +82-33-240-9234; Fax: +82-33-240-9119
E-mail: sjpark@hsc.ac.kr

Abbreviations: BDG, basal diet group; FD6S, high fat diet plus 6% extract powder group; FD12S, high fat diet plus 12% extract powder group; FDC, high fat diet control group; GOT, glutamic oxaloacetate transaminase; Hct, hematocrit; HDL, high density lipoprotein; LDL, low density lipoprotein; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RBC, red cell count; WBC, white cell corpuscle

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hyperlipidemia caused by high sugar diet [Yang *et al*., 2001], blueberry (*Vaccinium myrtillus* L.) [Andrea, 1996], *Coriandrum sativum* [Hwang *et al*., 2001], the water extract of oyster mushroom (*Pleurotus ostreatus*) [Kim *et al*., 1999], and sedum [Kim *et al*., 2002]. Park and Park [2001] reported that *Dioscorea batatas* and *Gastrodia rhizoma* reduced total lipid concentration in serum and liver of white rats. Another study reported that the carrot extract lowered the concentration of total cholesterol and low density lipoprotein (LDL)-cholesterol, while increasing the concentration of high density lipoprotein (HDL)-cholesterol of white rats with resected ovary [Kim *et al*., 2000]. A recent study showed the beneficial effect of the polyphenol fraction of Korean pears on lipid metablism [Choi *et al*., 2004].

Sophora japonica contains triterpenoid saponin, betulin, and flavonoids such as rutin and tannin [Lee and Shin, 1997]. The effects of flavonoids on the elasticity of blood vessel, improved coronary flow, and lipid content reduction have been known for a long time; however, no systematic studies on *S. japonica* are available. Existing studies on *S. japonica* were mostly focused on the antioxidant activities. Thus, the objectives of the present study were to examine the effect(s) of the *S. japonica* water extract on the lipid content and the hematologic properties of white rats on high fat diet for the development of a wide-ranged functional food products using this herbal drug

Materials and Methods

Materials. The dried flowers of *S. japonica* (Korean name, Koehwa) were purchased from Geumodang, Jeonju, Korea. *S. japonica* powder was suspended in distilled water (9:1, v/v) and refluxed for 4 h. The extracted solution was filtered, concentrated under reduced presssure (CCA-1100, Eyela, Tokyo, Japan), and finally freeze-dried (PVTFA 10AT, Ilsin, Korea).

Animal study and experimental diet. White Sprague-Dawley male rats weighing 150±5 g each were obtained

Table 1. Composition of experimental diet

Ingredient (g)	Group			
	BDG[1)]	FDC[2)]	FD6S[3)]	FD12S[4)]
Starch[5)]	22.68	21.34	21.34	21.34
Wheat-powder[6)]	22.68	21.34	21.34	21.34
Sucrose[7)]	20.18	18.26	18.26	18.26
Corn oil[8)]	2.14	3.64	3.64	3.64
Beef tallow[9)]	4.28	10.94	10.94	10.94
Casein[10)]	20.18	16.62	16.62	16.62
Cellulose[11)]	4.60	4.60	4.60	4.60
Mineral mixture[12)]	1.41	1.41	1.41	1.41
Vitamin mixture[13)]	1.85	1.85	1.85	1.85
SPE[14)]	-	-	6%	12%
Carbohydrate (g)[15)]	16.25(65%)	13.75(55%)	13.75(55%)	13.75(55%)
Lipid (g)[15)]	1.60(15%)	3.30(30%)	3.30(30%)	3.30(30%)
Protein (g)[15)]	5.00(20%)	3.75(15%)	3.75(15%)	3.75(15%)

1)BDG: Basal diet only group
2)FDC: High fat diet control group
3)FD6S: High fat diet+*Sophora japonica* powder 6% of total intake(g)
4)FD12S: High fat diet+*Sophora japonica* powder 12% of total intake(g)
5)Starch: Woo-li Food, Korea
6)Wheat-powder: CJ Food, Korea
7)Sucrose: Sigma Co. LTD., USA
8)Corn oil: CJ Food, Korea
9)Beef tallow: Lotte Samkang, Korea
10)Casein: Naarden Agro products BV, Holland
11)Cellulose : Sigma Co. LTD., USA
12)AIN-Mineral mixture 76: ICN Biomedicals, Germany
13)AIN-Vitamin mixture 76: ICN Biomedicals, Germany
14)SPE: *Sophora japonica* extract
15)( ): Energy construction ratio

from Samtako Co., Ltd. (Daejeon, Korea) and raised individually in a stainless steal cage (thermo-hygrostat, temperature 22±2°C, humidity 50±5%). After 1 week of adaptation period, the rats were given the experimental diet for 7 weeks. The acclimated white rats were divided into four groups using a randomized block design, each composed of ten rats (Table 1); BDG, FDC, FD6S, and FD12S. Contribution of the macronutrients from *S. japonica* in the diet had insignificant effect on the dietary composition.

Blood sampling. Upon completion of the extraction of blood from the heart under anesthesia, the rats were fasted for 16 h. Three milliliters each of the extracted blood samples were individually placed in a complete blood cell tube, and the remaining blood samples were centrifuged (US-5500CF, Vision, Seoul, Korea) to separate the serum for cryopreservation at −80°C.

Hematologic analyses. Measurement and analysis of red cell count (RBC), white cell corpuscle (WBC), hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and lymphocytes were performed by Green Cross (Jeonju, Korea).

Clinical chemistry. Bayer kit (BL, R-208, Bayer, New York, NY) was used for the analysis of total protein, albumin, total bilirubin, and alkaline phosphatase. Boehringer Mannheim kit (AL-712, Boehringer Mannheim, Marberg, Germany) was used for analysis of uric acid, blood urea nitrogen, glutamic oxaloacetate transaminase (GOT), glutamic pyruvate transaminase (GTP), lactate dehydrogenase (LDH), HDL-cholesterol, and triglyceride. Young dong diagnostics kit (Chole 21, Youngin, Seoul, Korea) was used for total cholesterol, and Daichi kit (Da-401, Daichi, Tokyo, Japan) was used for LDL-cholesterol [Lee and Lee, 1993].

Statistics. All collected data were analyzed using SPSS (version 10.0). Means±standard deviation were computed for all measurements, and significance of differences among the four groups were tested using ANOVA and Duncan's multiple range test (p<0.05).

Results and Discussion

Changes in concentration of blood metabolism index. Hematologic properties and clinical chemistry on the serum of the experimental rats that went through 7 weeks of *S. japonica* extract diet with high fat are shown in Tables 2 and 3. No significant differences in hematologic properties among the four groups were indicated. Though it is difficult to address the health improvement effects only in terms of hematologic properties, 7 weeks of *S. japonica* intake did not result in the reduction of RBC, WBC, Hct, hemoglobin, MCV, MCH, and MVHC. The results suggest that intake of *S. japonica* up to 12% at least does not have a harmful influence on hematologic factors.

The clinical serum chemistry values, that sensitively reflect the nutritional status of the body, showed significant differences in transferrin and GOT vlaues among the four groups. Transferrin that moves iron from tisuue to tissue showed concentrations of 49.35 and 52.06

Table 2. Hematological profile levels in rats fed experimental diets

Variable	Group			
	BDG[1)]	FDC[2)]	FD6S[3)]	FD12S[4)]
RBC (×10^6/mm^3)	3.87±0.32	3.67±0.41	3.07±0.42	3.13±0.38
WBC (×10^3/mm^3)	3.57±0.28	3.02±0.19	2.91±0.09	3.12±0.07
Hematocrit (%)	57.38±4.92	57.00±3.19	58.00±5.10	57.63±3.72
Hemoglobin (g/dL)	16.35±1.01	15.99±0.99	16.39±1.06	16.70±0.96
MCV[5)] (fl)	64.63±0.91	63.10±0.54	64.25±0.12	63.13±0.85
MCH[6)] (pg)	18.50±0.31	18.39±0.45	18.00±0.29	18.50±1.48
MCHC[7)] (g/dL)	28.63±1.09	28.59±0.89	28.00±3.02	28.88±1.52
Lymphocyte (%)	72.88±5.02	73.00±4.02	69.63±4.75	72.88±5.55

Values are mean±SD.
Alphabet: Significantly different at the p<0.05 level by Duncan’s multiple range test.
[1)]BDG: Basal diet only group
[2)]FDC: High fat diet control group
[3)]FD6S: High fat diet+*Sophora japonica* powder 6% of total intake(g)
[4)]FD12S: High fat diet+*Sophora japonica* powder 12% of total intake(g)
[5)]Mean corpuscular volume
[6)]Mean corpuscular hemoglobin
[7)]Mean corpuscular hemoglobin concentration

Table 3. Serum metabolic profile levels in rats fed experimental diets

Variable	Group			
	BDG[1)]	FDC[2)]	FD6S[3)]	FD12S[4)]
Total protein (g/dL)	4.86±0.32	4.95±0.29	4.91±0.57	5.21±0.33
Albumin (g/dL)	3.34±0.10	3.60±0.17	3.40±0.12	4.11±0.24
Total bilirubin(mg/dL)	0.18±0.04	0.25±0.06	0.20±0.04	0.21±0.03
Creatinine (mg/dL)	0.58±0.04	0.60±0.06	0.59±0.02	0.47±0.05
Transferrin (mg/dL)	38.41±5.11[a]	40.59±3.99[a]	49.35±2.62[b]	52.06±4.00[b]
Uric acid (mg/dL)	1.75±0.44	1.94±0.32	2.51±0.59	2.81±0.61
BUN[5)] (mg/dL)	12.05±3.00	11.94±2.62	12.18±2.75	11.71±2.11
ALP[6)] (U)	109.63±5.19	115.12±3.76	125.13±4.92	126.36±7.01
GOT[7)] (U)	31.00±14.00[a]	45.06±10.17[b]	21.75±16.61[c]	20.25±13.27[c]
GPT[8)] (U)	27.13±5.15	29.00±4.10	25.13±9.16	28.75±4.92
LDH[9)] (U)	139.65±30.09	167.09±19.21	118.58±41.72	109.61±37.72

Values are mean±SD.
Alphabet: Significantly different at p<0.05 level by Duncan's multiple range test.
[1)]BDG: Basal diet only group
[2)]FDC: High fat diet control group
[3)]F6E: High fat diet+extract powder 6% of total intake(g)
[4)]F12E: High fat diet+extract powder 12% of total intake(g)
[5)]BUN: Blood uea nitrogen
[6)]ALP: Alkaline phosphatase
[7)]GOT: Glutamic oxaloacetate transaminase
[8)]GPT: Glutamic pyruvate transaminase
[9)]LDH: Lactate dehydrogenase

mg/dL respectively for 6 and 12% *S. japonica* extract, significantly higher than the concentration (38.41 mg/dL) of BDG. However, the increase of transferrin concentration was not accompanied by the changes in Hct, hemoglobin, MCV, MCH and MCHC. Therefore, this result is not sufficient to be interpreted as an increase in absorption of iron content.

GOT activity as index of liver damage showed a significant increase in FDC to 45.06 U as compared to 31.00 U of BDG (Table 3). However, the GOT values of the FD6S and FD12S respectively were 21.75 and 20.25 U, significantly lower than both FDC and BDG. Normally GTP activity does not increase in the animals on high fat diet, and in the present study no change was found among the four groups. The reduction in GOT activity was thus considered as indication of the liver protection from hyperlipidemia by *S. Japonica.*

Changes in lipid serum composition. Table 4 shows total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations as well as the atherogenic indices of the four groups. Significant differences among the four groups were shown by the composition of every lipid species. In particular, total cholesterol concentration was 341.38 mg/dL in FDC but decreased in FD6S and FD12S respectively to 220.38 and 205.022 mg/dL, which are similar to the level of BDG (216.18 mg/dL). Therefore, one can conclude that intake of 6% *S. japonica* extract strongly reduced total cholesterol concentration of the serum.

HDL-cholesterol concentration of high fat control group at 26.84 mg/dL was significantly lower than that of BDG at 30.76 mg/dL. Furthermore, the HDL concentration of the 12% group was significantly high at 38.02 mg/dL compared to the FDC at 26.84 mg/dL. Similarily, FD6S also showed significantly higher HDL-cholesterol value than FDC. The intake of the 6% extract restored the HDL-cholesterol level of the animals on high fat diet to that of BDG.

When it comes to the comparison of LDL-cholesterol concentrations of BDG and FDC, the LDL-cholesterol level of the latter group at 62.91 mg/dL was significantly higher than that of the former group at 46.24 mg/dL. LDL-cholesterol concentration of the FD12S was 44.16 mg/dL, which was significantly lower than FDC (62.91 mg/dL) and similar to the level of BDG.

HDL-cholesterol is known to reduce the blood concentration of cholesterol by delivering cholesterol from peripheral tissues and blood vessel walls to the liver, thereby removing the cholesterol the body in the form of bile acids. It is also known as a factor that improves

Table 4. Serum lipid profile in rats fed experimental diets

Variable	Group			
	BDG[1)]	FDC[2)]	FD6S[3)]	FD12S[4)]
Total cholesterol (mg/dL)	216.18±19.24[a]	341.38±1.02[b]	220.38±20.10[a]	205.02±17.62[a]
HDL-cholesterol (mg/dL)	30.57±4.09[a]	26.84±2.92[b]	30.76±4.11[a]	38.02±2.11[c]
LDL-cholesterol (mg/dL)	46.24±8.75[a]	62.91±5.12[b]	52.16±8.75[c]	44.16±4.44[a]
Triglyceride (mg/dL)	47.31±11.41[a]	146.07±18.20[b]	99.75±9.82[b]	43.25±12.91[a]
Atherogenic index[5)]	6.07±1.02[a]	11.72±0.98[b]	6.16±0.62[a]	4.39±0.39[c]

Values are mean±SD.
Alphabet: Significantly different at $p<0.05$ level by Duncan's multiple range test.
[1)]BDG: Basal diet only group
[2)]FDC: High fat diet control group
[3)]FD6S: High fat diet+*Sophora japonica* powder 6% of total intake(g)
[4)]FD12S: High fat diet+*Sophora japonica* powder 12% of total intake(g)
[5)]Atherogenic index: [Totalcholesterol-(HDL-cholesterol)/HDL-cholesterol]

circulatory diseases such as atherosclerosis and high blood pressure by disturbing the formation of foam cells [Castelli *et al.,* 1986]. Sharrett *et al.* [2001] conducted an epidemiological investigation for 10 years on 12,339 males and females aged between 45-64 years and reported that the risk of cardiovascular diseases is reduced with the increase in the HDL-cholesterol level in blood. Therefore, intake of 12% *S. japonica* extract by white rats on high fat diet reduced total cholesterol and LDL-cholesterol concentrations while increasing the content of HDL-cholesterol. These changes in the serum cholesterol distribution can reduce the risk of cardiovascular diseases. Moreover, the extent of the cholesterol level change could serve as a major indication in titer determination of intake in clinical tests.

S. japonica contains triterpenoid saponin, betulin, sophoradiol, glucose, glucuronic acid, and flavonoids, such as rutin and tannin. Rutin, quercetin, and quercitrin operate on capillaries to recover the elasticity of blood vessels, expand heart blood vessels, and increase coronary flow [Lee and Shin, 1997]. This suggests the possibility that the active ingredients showing an improvement of of the blood chemistry in the present study are flavonoids.

Triglyceride concentration of FDC was significantly increased to 146.07 mg/dL from that of BDG at 47.31 mg/dL due to the high fat intake. However, triglyceride concentration was reduced to 43.25 mg/dL in FD12S, which was even lower than the concentration shown by BDG at 47.31 mg/dL. On the other hand, no significant difference was observed between FD6S and FDC. Among the Koreans with high sugar intake, many incidents of hypertriglyceridemia accompanied by hyperglyceridemia have been reported [Whang *et al.,* 1996; Kim and Whang, 1997; Kim, 2000]. *S. japonica* can be thus used as an important functional food material for the prevention of hypertriglyceridemia.

In the case of atherogenic index, significantly differerent values were found among the groups; 6.07% in BDG, 11.72% in FDC, 6.16% in FD6S and 4.39% in FD12S. This different index values resulted from the reduction in total cholesterol concentration caused by *S. japonica* extract intake that led ti the increase in the HDL-cholesterol concentration.

No reports have yet been published on the relationship between lipid metabolism and intake of *S. japonica* flower. The present study demonstrated that the reduction in total cholesterol, LDL-cholesterol and triglyceride concentrations and the increase in HDL-cholesterol concentration in the serum were brought on by the intake of *S. japonica* flower extract. The extrapolation of the results obtained with rats to the humans signifies improvements in the lipid metabolism of hyperlipidemia patients. However, in-depth follow up study must be accompanied to examine whether such phenomena resulted from the suppression of cholesterol absorption, acceleration of excretion or suppression of biosynthesis in the liver. Studies on the isolation of active principle(s) are also deemed necessary.

In summary, intake of *S. japonica* flower extract up to 12% in diet by white rats on high fat diet was effective in reducing the liver damage from high fat diet and improving the serum lipid profile in a beneficial way without negative effects on the hematological factors.

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