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Effects of the *Cynanchum wilfordii* Ethanol Extract on the Serum Lipid Profile in Hypercholesterolemic Rats

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ABSTRACT: The purpose of this study was to investigate the effects of the ethanol extract of *Cynanchum wilfordii* (ECW) on the blood lipid profile of hypercholesterolemic rats. Thirty 7-week-old male Sprague-Dawley rats were allowed free access to either a normal diet (AIN-93 diet), or 1% high-cholesterol diet with or without 0.5% or 1% ECW for 5 weeks. After sacrifice, the rat serum lipid profile was analyzed. The diets containing ECW decreased body weight gains compared to the normal diet. Serum HDL-cholesterol levels of ECW-fed groups were significantly increased in the hypercholesterolemic groups and normal groups ($P<0.05$). When 1% ECW was fed to the normal group, total cholesterol level was increased. Moreover, treatment of ECW in hypercholesterolemic groups yielded a dose-dependent and highly significant decrease in the atherogenic index as compared to the control. These results suggest that intake of *Cynanchum wilfordii* may help reduce the risks of hypercholesterolemia by increasing blood HDL-cholesterol and lowering the atherogenic index.

Keywords: atherogenic index, *Cynanchum wilfordii*, HDL-cholesterol, hypercholesterolemia, LDL-cholesterol

INTRODUCTION

Populations of obesity and associated diseases are rising and becoming a global burden. The rapid increase in wealth of Asian countries has been associated with dramatic increases in the prevalence of obesity related diseases such as diabetes, and coronary heart disease (1,2) During the last few decades, obesity, hyperlipidemia, and hypercholesterolemia are rapidly rising in Korea. The 2010 report of the national data from the Korea National Health and Nutrition Examination Survey (KNHANES) revealed that the rate of hypercholesterolemia among the male population increased slightly, from 10% in 1998 to 13.5% by 2010, while the rate of obesity in the population dramatically increased from 25.1% to 36.5% during the same time period. Also, the rate of hypo-HDL-cholesterolemia in the population was 35.8% in men and 17.4% in women (3).

Hypercholesterolemia is associated with diseases such as atherosclerosis (deposition of cholesterol in the artery walls), cardiovascular disease, and diabetes. Epidemiological studies revealed that the risk factors of cardiovascular disease and diabetes are hypercholesterolemia and hyperlipidemia (4). Diet modification, involving reduction in cholesterol consumption, is usually the initial step in the management of hypercholesterolemia. However, diet modification alone may not reduce blood cholesterol to target levels.

Several pharmacological agents used to lower cholesterol levels such as statins, niacin, resins, and fibrates, are used along with diet modification (5). Statins have been used to reduce LDL-cholesterol levels and have a positive effect on the risk of cardiovascular events (6-8). Furthermore, statin therapy, which reduces LDL-cholesterol levels, is associated with a reduction in the regression of atherosclerosis (9). Although statins are the most effective lipid-lowering drugs, they have adverse effects such as myopathy, rhabdomyolysis, and polyneuropathy (10-16). Even though statin-associated side-effects are rare, there still remains a safety issue, and therefore, other agents with atherogenic index-reducing effects are being researched.

Cynanchum wilfordii Hemsley belongs to the Asclepiadaceae family and is widely distributed in Korea, north-

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ern China, and Japan. This medicinal plant has long been used as a folk remedy in Korea and exhibits beneficial effects on diabetes mellitus, gastric disorders, anti-inflammatory activity, and vascular relaxation (17-19). *C. wilfordii* contains an important class of biologically active compounds, including gagaminine and its glycosides, various wilfosides and cynauricuosides, as well as sarcotine, penupogenin, and cynandione A (20).

The purpose of this study was to investigate the dose response of the blood lipid profile by using different concentrations of the ethanol extract of *Cynanchum wilfordii* in hypercholesterolemic Sprague-Dawley rats. Furthermore, the effect of hypercholesterolemic rats were compared to rats fed a cholesterol-free diet.

MATERIALS AND METHODS

Ethanol extraction of *Cynanchum wilfordii*

Cynanchum wilfordii specimens were obtained from Byeongnyeongdo Clean Food and Agricultural Cooperative (Incheon, Korea). Dried roots of *Cynanchum wilfordii* were extracted by adding 10 volumes of 80% ethanol; extraction took place for 6 h at 80°C. The resulting ethanol extract was concentrated under reduced pressure by vacuum evaporator (COSMOS-660; Kyung Seo Machine Co., Incheon, Korea). The resulting extract was frozen and lyophilized using freeze-drier (FDCF type, OPERON Co. Ltd., Gyeonggido, Korea). The 25.31% of solid content was used in further experiments.

Animals and maintenance

All aspects of this study were conducted according to the standards of the Committee on Care and Use of Laboratory Animal of Korea Food Research Institute.

Seven-week-old male Sprague-Dawley rats (n=30) were purchased at 230~250 g body weight from Nara Biotech Co. (Seoul, Korea). The rats were housed in pairs in plastic cages with paper floors, in a controlled environment at 24±2°C, 55% relative humidity, and a 12-h light-dark cycle (lights on at 7 am). The rats were acclimated to the facility for a week and given free access to a commercial diet (G-Bio, Gyeonggi, Korea) and deionized water *ad libitum*. After a week of acclimation, rats were grouped into 6 groups of 5 rats each, by randomized complete block design, based on body weight. The animals were allowed free access to either a normal diet, or a 1% high-cholesterol diet containing 0.5% or 1% ECW, and to deionized water *ad libitum*. Food intake and body weight were measured weekly.

Experimental diets

Table 1 presents the diet composition of experimental diets. The normal diet did not contain cholesterol; however, 1% cholesterol was added to the cholesterol-rich diet to induce hypercholesterolemia. The animals were divided into the following 6 groups of 5 rats each and were fed these diets for 5 weeks: Normal diet (N), 0.5% *Cynanchum wilfordii* diet (containing 0.5% ECW; CW-0.5), 1% *Cynanchum wilfordii* diet (containing 1% ECW; CW-1), cholesterol control diet (containing 1% cholesterol; CC), cholesterol diet containing 0.5% *Cynanchum wilfordii* (containing 1% cholesterol and 0.5% ECW; CCW-0.5), and cholesterol diet containing 1% *Cynanchum wilfordii* (containing 1% cholesterol and 1% ECW; CCW-1).

Blood and tissue collection

The day before sacrifice, the animals were deprived of food for 16~19 h. The animals were sacrificed in random order by cervical dislocation. The anterior chamber

Table 1. Composition of experimental diets

Ingredient (g/kg)	Normal diets (cholesterol-free)			Cholesterol-rich diets		
	N	CW-0.5	CW-1	CC	CCW-0.5	CCW-1
Casein	140	140	140	140	140	140
Corn starch	465.692	465.692	465.692	465.692	465.692	465.692
Dyetrose[1)]	155	155	155	155	155	155
Sucrose	100	100	100	100	100	100
Cellulose	50	45	40	40	35	30
Soybean oil	40	40	40	40	40	40
Cholesterol	-	-	-	10	10	10
Cynanchum wilfordii	-	5	10	-	5	10
TBHQ[2)]	0.008	0.008	0.008	0.008	0.008	0.008
Mineral Mix[3)]	35	35	35	35	35	35
Vitamin Mix[4)]	10	10	10	10	10	10
L-cystein	1.8	1.8	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Total	1,000	1,000	1,000	1,000	1,000	1,000

[1)]Dyetrose (Dextrinized cornstarch, 90~94% tetrasaccharides).
[2)]TBHQ (*tert*-butylhydroquinone).
[3)]Mineral Mix [AIN-93M-MX (21)].
[4)]Vitamin Mix [AIN-93-VX (21)].

Table 2. Initial body weight, final body weight, total body weight gain, food intake, and food efficiency ratio in rats

Group[1)]	Initial body weight (g)	Final body weight (g)	Total body weight gain (g)	Food intake (g/day)	Food efficiency ratio[2)]
N	331.94±9.49^{A}	507.53±16.41$^{B3)}$	175.59±9.53^{B}	22.50±1.05^{B}	0.21±0.01^{B}
CW-0.5	335.54±4.75^{A}	503.28±16.60^{B}	167.74±14.80^{B}	21.57±0.70^{AB}	0.22±0.02^{B}
CW-1	328.76±6.32^{A}	449.16±10.37^{A}	120.41±4.69^{A}	20.04±0.58^{A}	0.17±0.01^{A}
CC	332.05±5.23^{b}	504.19±16.66^{b}	172.14±12.23^{b}	22.49±1.22^{b}	0.22±0.02^{b}
CCW-0.5	328.57±2.57^{ab}	514.19±11.12^{b}	185.62±11.85^{b}	23.07±0.83^{b}	0.23±0.01^{b}
CCW-1	320.65±3.00^{a}	394.41±6.99^{a}	73.75±6.34^{a}	16.32±0.28^{a}	0.13±0.01^{a}

[1)]N, basal diet; CW-0.5, containing 0.5% *Cynanchum wilfordii* (g/kg); CW-1, containing 1% *Cynanchum wilfordii* (g/kg); CC, 1% cholesterol; CCW-0.5, 1% cholesterol+0.5% *Cynanchum wilfordii* (g/kg) and CCW-1, 1% cholesterol+1% *Cynanchum wilfordii* (g/kg).
[2)]Food efficiency ratio=weight gain (g/day)/food intake (g/day).
[3)]Different superscript letters within a column indicate significantly different values as assessed by Duncan's multiple range test (P<0.05); uppercase, normal diet group; lowercase, cholesterol diet group.

of the eye was punctured with a needle, and a blood sample was collected into an SGS green Vac-Tube (SPM Co. Ltd., Seoul, Korea) to separate the serum. Blood samples were centrifuged (Hanil Sci. Ind., Seoul, Korea) for 15 min at 65.5 g, and the supernatants retained and stored at −80°C until analysis. Liver was rapidly dissected from the body, washed gently with 0.9% saline 3 times and then weighed.

Biochemical analysis

Serum cholesterol, triglyceride, and glucose levels were determined in individual rats using a glucose hexokinase kit (Bayer, Leverkusen, Germany), a cholesterol reagent kit (Bayer), a triglyceride reagents kit (Bayer), an LDL-cholesterol kit (Bayer), and a Direct HDL-cholesterol kit (Bayer), using an automatic biochemical analyzer (ADVIA 1650, Bayer). Total serum lipid was determined using a total lipid reagent kit (D-Tek Llc, Bensalem, PA, USA) and a Agilent 8453 UV-visible spectrophotometer (Agilent Technology, Santa Clara, CA, USA). The serum levels of AST and ALT were determined using a Fuji dri-chem slide kit (Fuji Film Co., Tokyo, Japan).

Statistical analysis

Data are expressed as means±SEM in all figures and tables. One-way analysis of variance was used to determine whether significant differences existed among the groups. Duncan's multiple range test was further utilized to determine which means were significantly different. All significance levels were set as P<0.05. All statistical analyses were performed using SPSS 12.0 package (Chicago, IL, USA).

RESULTS AND DISCUSSION

Food intakes, body weight gains, and food efficiency ratio

Food intakes and body weight gains are illustrated in Table 2. Initial body weights were not significantly different in all diet groups. Supplementing of the diets with ECW affected food intake during the experimental period. The mean food intake of the CW-1 group (20.04±0.58 g/day) was significantly lower than that of the N group (22.50±1.05 g/day), and the CCW-1 group (16.23±0.28 g/day) was significantly lower than the CC group (22.49±1.22 g/day). Reduction in food intake, total body weight gain, and final body weight were significantly lower (P<0.05) in rats fed the diet containing 1% ECW (groups CW-1 and CCW-1), resulting in a lower food efficiency ratio compared to that of the N and CC groups (Table 2). But these data may not provide adequate explanation whether the reduction of food intake and body weight gain due to palatability of ECW or the effect of ECW on the brain system. Further research is needed to elucidate relationship between CW and brain signal system.

Relative liver weight

The increase of liver weight and size following high-cholesterol feeding relates to the large expansion of the pool of hepatic cholesterol ester stores. Therefore, cholesterol overload initiates the development of nonalcoholic fatty

Table 3. Liver weight, serum AST and ALT of SD male rats

Group[1)]	Liver (g/100 g bw)	AST (u/L)	ALT (u/L)
N	2.24±0.05$^{A2)}$	97.20±2.90^{A}	36.67±2.67^{B}
CW-0.5	2.37±0.05^{AB}	94.25±4.48^{A}	29.75±1.75^{B}
CW-1	2.53±0.11^{B}	94.00±3.11^{A}	33.75±1.11^{AB}
CC	3.09±0.04^{a}	105.20±3.15^{a}	37.50±3.77^{a}
CCW-0.5	3.04±0.17^{a}	99.00±5.97^{a}	35.33±2.03^{a}
CCW-1	3.10±0.08^{a}	92.20±5.08^{a}	33.25±0.48^{a}

[1)]N, basal diet; CW-0.5, containing 0.5% *Cynanchum wilfordii* (g/kg); CW-1, containing 1% *Cynanchum wilfordii* (g/kg); CC, 1% cholesterol; CCW-0.5, 1% cholesterol+0.5% *Cynanchum wilfordii* (g/kg) and CCW-1, 1% cholesterol+1% *Cynanchum wilfordii* (g/kg).
[2)]Different letters in a column indicate significantly different values as assessed by Duncan's multiple range test (P<0.05); uppercase, normal diet group; lowercase, cholesterol diet group.
AST, aspartate aminotransferase; ALT, alanine aminotransferase.

liver disease (NAFLD). Therefore, we measure liver weight because ECW may have an effect on improving fatty liver condition due to 1% cholesterol diet.

The dietary effect of ECW on liver weight and serum levels of AST and ALT are represented in Table 3. Final liver weights were significantly higher when 1% cholesterol was added to the basal diet (CC: 3.09±0.04 g/100 g body weight). Feeding the basal diet resulted in liver weights of 2.24±0.05 g/100 g body weight. This result is similar to that of Anderson et al. (22), where feeding rats the 1% cholesterol diet led to a significant increase in final liver weights. No significant differences were observed in liver weights of animals in the groups fed ECW diets in high-cholesterol groups. The serum levels of AST and ALT were determined to evaluate the effects of ECW on liver injury. No significant difference was seen in serum levels of AST and ALT except for the CW-0.5 group, which slightly decreased in the ALT level.

Changes in lipid profile

The serum lipid profile of the CC group showed increased concentrations of total cholesterol (TC) and LDL-cholesterol, and decreased HDL-cholesterol, compared to the N group, which was fed the cholesterol-free diet (Table 4). Total cholesterol levels were slightly increased in hypercholesterolemic groups (CC, CCW-0.5, and CCW-1), resulting in higher atherogenic indexes in the cholesterol-rich diet groups than in the normal diet groups (cholesterol-free). The LDL-cholesterol level of the CC group (10.00±2.00 mg/dL) was significantly higher than that of the N group (5.50±0.29 mg/dL). Therefore, the cholesterol-rich diet groups (CC, CCW-0.5, and CCW-1) had altered serum lipid profiles, resulting in hypercholesterolemia.

No significant difference in total cholesterol levels were found except for the CW-1 group; total cholesterol level of the CW-1 group was slightly increased than that of the N group. The average total cholesterol level of a 10-week-old rat is 82.0±10.05 mg/mL, whereas the total cholesterol level of CW-1 rats (69.67±3.84 mg/dL) and CCW-1 rats (87.00±1.00 mg/dL) increased over that of N (52.75±2.69 mg/dL) and CC rats (69.75±9.51 mg/dL).

Supplementing the diets with *Cynanchum wilfordii* extract generated a dose-dependent increase in HDL-cholesterol levels in both normal and hypercholesterolemic groups as compared to their controls. In normal groups, CW-1 group (18.40±1.44 mg/dL) were significantly higher than in the N group (12.40±0.75 mg/dL), whereas HDL-cholesterol levels of the CW-0.5 group (13.00±0.55 mg/dL) were not significant. Moreover, in hypercholesterolemia groups, CCW-1 group (24.80±2.18 mg/dL) were significantly increased over that of the CC group (10.40±0.68 mg/dL) ($P<0.05$). Our results are similar to a previous study where hypercholesterolemic rats that had been provided water containing *Cynanchum wilfordii* extract showed increased HDL-cholesterol levels (23).

Changes in HDL-cholesterol to total cholesterol ratios are illustrated in Table 4. HDL-cholesterol/total cholesterol ratios were not significantly different among basal diet groups (N, CW-0.5, and CW-1). However, in the hypercholesterolemic diet groups, HDL-cholesterol/total cholesterol ratios of the CCW-0.5 (19.36±1.78%) and CCW-1 groups (26.56±1.02%) were significantly higher than that of the CC group (15.05±2.17%). Moreover, the effect of supplementation of *Cynanchum wilfordii* was dose-dependent; this effect was much stronger in the CCW-1 than in the CCW-0.5 group. In a later Framingham study (2000), the HDL-cholesterol/total cholesterol ratio was demonstrated to be the most efficient lipid profile for predicting coronary heart disease (CHD) (24). Furthermore, the Strong Heart study found the ratio of HDL-cholesterol/total cholesterol to be a good predictor of cardiovascular risk in both men and women with type 2 diabetes, although it was a stronger predictor in men (25). Barter et al. (26) also reported that the cardiovascular disease (CVD) event rate was reduced by 40% in the highest HDL-cholesterol group relative to the lowest and that the frequency of major CVD events increased with decreasing levels of HDL-cholesterol.

Table 4. Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and atherogenic index in SD male rats

Group[1)]	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	LDL-cholesterol (mg/dL)	Atherogenic index (AI)[2)]	HDL-cholesteol/total cholesterol (%)
N	52.75±2.69[A3)]	12.40±0.75[A]	5.50±0.29[A]	2.99±0.10[A]	24.69±0.56[A]
CW-0.5	53.80±4.43[A]	13.00±0.55[A]	6.00±0.71[A]	2.93±0.20[A]	25.67±1.31[A]
CW-1	69.67±3.84[B]	18.40±1.44[B]	9.75±0.63[B]	3.20±0.07[A]	23.84±0.39[A]
CC	69.75±9.51[a]	10.40±0.68[a]	10.00±2.00[a]	6.08±1.03[b]	15.05±2.17[a]
CCW-0.5	69.33±1.45[a]	14.20±0.86[a]	10.33±1.45[a]	4.28±0.42[ab]	19.36±1.78[a]
CCW-1	87.00±1.00[a]	24.80±2.18[b]	13.75±0.95[a]	3.09±0.33[a]	26.56±1.02[b]

[1)]N, basal diet; CW-0.5, containing 0.5% *Cynanchum wilfordii* (g/kg); CW-1, containing 1% *Cynanchum wilfordii* (g/kg); CC, 1% cholesterol; CCW-0.5, 1% cholesterol+0.5% *Cynanchum wilfordii* (g/kg) and CCW-1, 1% cholesterol+1% *Cynanchum wilfordii* (g/kg).
[2)]AI=(total cholesterol−HDL-cholesterol)/HDL-cholesterol.
[3)]Different letters in a column indicate significantly different values assessed by Duncan's multiple range test ($P<0.05$); uppercase, normal diet group; lowercase, cholesterol diet group.

Rats fed cholesterol-rich diets exhibited markedly increased atherogenic indexes (CC; 6.08±1.03) compared to the basal diet group (N; 2.99±0.10). Supplementing the diet with *Cynanchum wilfordii* in hypercholesterolemic groups yielded a dose-dependent and highly significant decrease in the atherogenic index (CC, 6.08±1.03; CCW-0.5, 4.28±0.42; CCW-1, 3.09±0.33).

Thus, the present study demonstrated that a diet containing 1% cholesterol fed to rats for 5 weeks increased total cholesterol level associated with increase in LDL-cholesterol level and decrease in HDL-cholesterol level. Treatment with *Cynanchum wilfordii* decreased food intake and body weight gain, while increasing HDL-cholesterol level. The ratio of HDL-cholesterol/total cholesterol was significantly increased in a dose-dependent manner when *Cynanchum wilfordii* was added to the hypercholesterolemic diets. Finally, in cholesterol-rich diet groups, supplementing with *Cynanchum wilfordii* decreased the atherogenic index in a dose-dependent manner.

Administration of statins, the most effective lipid-lowering drugs, has been reported to cause an increase in HDL-cholesterol levels from 5% to 8% in humans (27,28); however, the clinical significance of this remains unclear. Population studies have consistently illustrated that CHD and atherosclerosis are strongly related to total blood cholesterol, which is mostly made up of LDL-cholesterol. LDL-cholesterol is the most atherogenic of lipoproteins. HDL-cholesterol also carries cholesterol, but elevated HDL-cholesterol represents cholesterol returning from the rest of the body to the liver, and thus indicates a protective effect against heart disease and atherosclerosis. High LDL-cholesterol and low HDL-cholesterol are associated with heart disease, whereas low LDL-cholesterol and high HDL-cholesterol are inversely associated with increased risk (29); this relationship between HDL-cholesterol and CHD has been shown by a number of previous studies (27,30-34). The Framingham study of the 1980s illustrated that study subjects at the 80th percentile of HDL-cholesterol had half the risk of developing CHD as compared to people at the 20th percentile. Another study also revealed that HDL-cholesterol and total cholesterol levels were significantly related to CHD outcome (35).

In conclusion, the results of our study suggest that intake of ethanolic extract of *Cynanchum wilfordii* has potential to improve the hypercholesterolemic condition by improving blood lipid profile, and further study based on blood lipid modulation mechanism is needed.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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