대한한의학 방제학회지 제27권 제1호
Herbal Formula Science
2019;27(1):1~6
pISSN 1229-1218, eISSN 2288-5641 https://doi.org/10.14374/HFS.2019.27.1.1
Official Journal of The Korean Medicine Society For The Herbal Formula Study
Available at http://www.formulastudy.com HFS

Original Article / 원저

山楂淸血湯이 Triton WR1339로 유발된 흰쥐의 高脂血症에 미치는 영향

장문석, 주승훈, 김은이, 박성규*

경희대학교 대학원 한의과대학 처방제형학교실

Effects of Sansachunghyul-tang (SCT) on blood cholesterol levels in Triton WR1339-induced hyperlipidemic rats

Mun Seog Chang, Seung-Hun Joo, Eun Yi Kim, Seong Kyu Park*

Department of Prescriptionology, College of Korean Medicine, Graduate School, Kyung Hee University

ABSTRACT

Objectives : Sansachunghyul-tang (山楂淸血湯) is a new formula consist of some herbs to treatment of hyperlipidemia. The lipid-lowering effects of the water extract of Sansachunghyul-tang (SCT) were examined using Triton WR1339-induced hyperlipidemic rats.

Methods : Hyperlipidemia was induced by intravenous injection of Triton WR1339 (tyloxapol) at 200 mg/kg body weight. Four groups of experimental animals were used: normal, control, and two sample groups. Sample groups were injected with SCT (100 and 1000 mg/kg) for 7 days.

Results : SCT produced statistically significant lowering effects on levels of total cholesterol (81.8 and 83.1%, $p < 0.01$, respectively), triglyceride (88.9 and 93.4%, $p < 0.01$, respectively), and phospholipid (86.7 and 94.3%, $p < 0.01$, respectively) than the control group. Taken together, SCT improved parameters of the HDL-cholesterol and LDL-cholesterol levels.

Conclusions : In conclusion, These results suggest that SCT could act as a potent antihyperlipidemic in therapeutics for hyperlipidemia.

Key words : Hyperlipidemia, Sansachunghyul-tang (SCT), Triton WR1339.

Ⅰ. Introduction

Atherosclerosis is a multi factorial process of plaque formation that results in coronary heart disease[1]. Hypercholesterolemia and high levels of low-density lipoproteins (LDL) are the major risk factors for atherosclerosis and coronary heart disease[2,3]. When lipid metabolism is imbalanced, hypertriglyceridemia and hypercholesterolemia may lead to a variety of serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, and functional depression of some organs[4].

Triton WR1339 is a detergent that blocks the hepatic and peripheral clearances of triglyceride-rich lipoproteins[5]. To investigate the efficacy of hypolipidemic agents from natural herbal resources, this study attempted to evaluate and compare the hypolipidemic activities of Sansachunghyul-tang (SCT) using Triton WR1339-induced hyperlipidemic rats.

Ⅱ. Materials and Methods

1. SCT composition and preparation

The following herbs were collected for SCT from the Wonkwang Herbal Drug Co. Ltd. (Seoul, Korea): Crataegi Fructus (*Crataegus pinnatifida* Bung *var. typica* Schneider, Rosaceae), Polygoni Multiflori Radix (*Polygonum multiflorum* Thunb., Polygonaceae), Acanthopanacis Senticosi Radix et Caulis (*Acanthopanax senticosus* Rupe et Maxim, Araliaceae), Bambusae Caulis in Taeniam (*Phyllostachys nigra* (Lodd.) Munro *var. henonis* (Bean) Stapf, Bambusaceae), Chrysanthemi Flos (*Chrysanthemum indicum* Linne, Compositae), Schisandrae Fructus (*Schisandra chinensis* (Turcz.) Baill., Magnoliaceae). Researchers have identified many chemical constituents of the herbs composing this formula: triterpenes and flavonoids from Crataegi Fructus[6,7]; anthraquinones, gallic acid, catechin, and emodin from Polygoni Multiflori Radix[8,9]; acanthoside, eleutheroside, chiisanoside, senticoside, triterpenic saponin, syringin, flavone, vitamins, minerals, ß-sitosterol, sesamine, and savinine from Acanthopanacis Senticosi Radix et Caulis[10]; orientin, isoorientin, isovitexin, and vitexin from Bambusae Caulis in Taeniam[11]; flavonoids, terpenoids, and phenolic compounds from Chrysanthemi Flos[12]; dibenzocyclooctadiene lignans (deoxyschisandrin, gomisin N, gomisin A, schisandrin, and wuweizisu C); and flavonols (quercetin and kaempferol) and cinnamic acid from Schisandrae Fructus[13].

As shown in Table 1, the SCT used in this study was prepared from six different medicinal herbs. This mixture was boiled in water (50 g/1000 ml) for 2 h at 100°C and then filtered, after which the extract was evaporated and lyophilized. The resulting SCT extract had a yield of 26%.

Table 1. Composition of Sansachunghyul-tang (SCT)

Botanical name	Family	Part used	Ratio (g)
Crataegi Fuctus	Rosaceae	Fruit	8.0
Polygoni Multiflori Radix	Polygonaceae	Root	6.0
Acanthopanacis Senticosi Radix et Caulis	Araliaceae	Root and Caulis	6.0
Bambusae Caulus in Taeniam	Bambusaceae	Caulis	4.0
Chrysanthemi Flos	Compositae	Flower	4.0
Schizandrae Fructus	Magnoliaceae	Fruit	1.5

*Corresponding author : Seong Kyu Park. Department of Prescriptionology, College of Korean Medicine, Graduate School, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Republic of Korea.
Tel : +82-2-961-0330, Fax : +82-2-961-0536, E-mail : comskp@khu.ac.kr
• Received : November 27, 2018 / Revised : December 18, 2018 / Accepted : December 21, 2018

2. Animals and experimental protocol

Male eight-week-old Sprague-Dawley rats (200 ± 20 g) were obtained from Semtako (Osan, Korea). The rats were housed in a specific-pathogen-free environment with a 12-h light/12-h dark cycle at the Center for Laboratory Animal Care and Use, Kyung Hee University, Korea. Animal care and experimental procedures conformed to the Guide for the Care and Use of Laboratory Animals (Department of Health, Education, and Welfare, publication no. NIH 78-23, 1996). The animals were provided water and standard rodent pellet food (Purina Korea Inc., Seoul, Korea) ad libitum. They were randomly divided into four groups of eight animals each (normal, control, and two sample groups). The two sample groups were given SCT solution via gastric gavages for 7 days in the quantities of 100 and 1000 mg/kg body weight, respectively. Control rats received the vehicle only. On day 7, Triton WR1339 (Tyloxapol; Sigma, St. Louis, MO, USA) was injected after the regular 16-h fasting period as a 10% solution in saline (200 mg/kg body weight) into rat tail veins. To regulate body condition, all food was removed from the cages 6 h before administration of SCT, and was returned immediately afterwards. Rats were anesthetized with ether 18 h after injection with Triton WR1339, and blood was withdrawn by cardiac puncture to determine concentrations of total cholesterol, triglyceride, phospholipid, HDL-cholesterol, and LDL-cholesterol. Serum was obtained by centrifugation (1500 × g, 10 min). Serum lipid concentrations were measured using commercial kits (Roche, Basel, Switzerland; Randox, Crumlin, UK).

3. Statistical analysis

All data were expressed as the mean ± SD, and statistical significance was determined using Student's t-test. Improved activity was calculated using the following formula: Improved activity (%) = [(Cv − Sv)/ (Nv − Cv)] × 100, where Nv is the value of the normal group, Cv is the value of the control group, and Sv is the value of the sample group.

Ⅲ. Results

As shown in Table 2, 18 h after injection with Triton WR1339, total cholesterol levels in the Triton-treated control group were significantly higher than the normal group ($p < 0.001$). Levels in the groups treated with SCT dosages of 100 and 1000 mg/kg doses were significantly lower (81.8 and 83.1%, $p < 0.01$, respectively) than the Triton-treated control group. Triglyceride levels in the Triton-treated control group were significantly higher than the normal group ($p < 0.01$), whereas levels in groups treated with SCT at dosages of 100 and 1000 mg/kg doses were significantly lower (88.9 and 93.4%, $p < 0.01$, respectively) than the Triton-treated control group.

Taken together, phospholipid levels in groups treated with SCT were significantly lower (86.7 and 94.3%, $p < 0.01$, respectively) than the Triton-treated group. In contrast, no differences appeared in HDL-cholesterol levels between the Triton-treated control group and the normal group, while the Triton-treated control group exhibited significantly higher LDL-cholesterol levels than the normal group ($p < 0.001$). Groups treated with SCT tended to have higher levels of HDL-cholesterol and lower levels of LDL-cholesterol than the Triton-treated group, although the differences were not significant.

Table 2. Effects of Sansachunghyul-tang (SCT) on Serum Total Cholesterol, Triglyceride, Phospholipid, HDL and LDL-Cholesterol Levels in Triton WR1339-induced Hyperlipidemic Rats

Group	Dose (mg/kg, p.o.)	TC level (mg/dl)	TG level (mg/dl)	Phospholipid level (mg/dl)	HDL level (mg/dl)	LDL level (mg/dl)
Normal	-	85.3 ± 15.9	89.5 ± 32.0	130.0 ± 19.2	62.8 ± 10.2	7.2 ± 1.5
Control	-	270.7 ± 88.7$^{###}$	1164.5 ± 729.8$^{##}$	304.8 ± 116.3$^{##}$	51.2 ± 13.5	42.5 ± 18.7$^{###}$
SCT	100	119.0 ± 38.2**	208.0 ± 95.1**	153.2 ± 14.9**	63.7 ± 12.9	25.2 ± 15.2
SCT	1,000	116.7 ± 34.4**	160.3 ± 77.9**	139.8 ± 18.6**	69.2 ± 15.1	24.0 ± 10.8

$p < 0.01$, ### $p < 0.001$ compared to normal group, and ** $p < 0.01$ compared to control group.

Ⅳ. Discussion

Sansachunghyul-tang (SCT) is a new herbal medicine formula composed mainly of Crataegi Fructus and five other herbs. The constituents of SCT have been found to have various biological functions in disease models. The main herb in SCT, *Crataegus pinnatifida*, reportedly improves heart function and produces an inhibitory effect on LDL oxidation in both cell and cell-free systems[6]. *Polygonum multiflorum* reportedly has antioxidant and free radical scavenging properties, which may explain its protective effect in atherosclerosis and the cardiovascular system[14]. *Acanthopanax senticosus* is used to treat rheumatism; as a prophylaxis for various diseases including chronic bronchitis, hypertension, ischemia; to relieve stress or fatigue; and to treat symptoms associated with diabetes[15]. Both *Polygonum multiflorum* and *Acanthopanax senticosus* may participate in the antihyperlipidemia activity of *C. pinnatifida* as observed in this study. In addition, *Phyllostachys nigra* reportedly protects against myocardial ischemia, restrains blood platelet aggregation, increases coronal flow, and has a vasorelaxant effect on rabbit thoracic aortic rings, thereby reducing arterial blood pressure[11]. *Chrysanthemum indicum* reportedly has antibacterial, antiviral, antioxidant, anti-inflammatory, and immunomodulatory properties and has been used to treat inflammation, hypertension, and respiratory diseases[16]. Finally, the fruit of *Schisandra chinensis* reportedly has hepatoprotective properties[17,18]. Crataegi Fructus could inhibit the atherogenesis formation and development, which might be due to regulating the lipid metabolism, enhancing the antioxidation, and reducing the release of inflammatory factors[19]. Based on previous reports, the prescriptions containing Crataegi Fructus was providing a basis for its clinically scientific application and the research of new antihyperlipidemic medicines.

Ⅴ. Conclusion

In this study, the hypolipidemic effects of SCT were measured in Triton WR1339- induced hyperlipidemic rats. Results using blood-driven parameters such as total cholesterol, triglyceride, and phospholipid levels indicated that SCT has potent antihyperlipidemic properties. Therefore, we conclude that SCT is an effective hypolipidemic agent. Further studies are needed to clarify the mechanisms underlying SCT's suppression of blood cholesterol and the molecule(s) responsible for this effect.

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