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Effects of Scutellariae Radix Extract on Lipid Metabolism, Oxidation and Production of Pro-Inflammatory Cytokines in Rats Fed Highly Oxidized Fat

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Objectives: This study investigated the effects of Scutellariae Radix extract (SRE) on lipids metabolism, oxidation and the production of pro-inflammatory cytokines in rats fed highly oxidized fat.

Methods: To induce obesity, male Sprague-Dawley rats were fed a highly oxidized fat diet for 10 weeks. SRE at 100 mg/kg were administered orally to obesity-induced rats for 6 weeks, and their lipid metabolism, oxidation and production of pro-inflammatory cytokines were examined.

Results: The concentrations of free fatty acid, triglyceride, total cholesterol, and low density lipoprotein-cholesterol in plasma decreased in SRE-treated groups, although the difference was not significant between control and SRE-treated groups, while that of high density lipoprotein-cholesterol significantly increased in SRE group. The concentrations of total cholesterol and triglyceride in the liver were tended to decrease in SRE-treated group than in control group. The levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in plasma were decreased in SRE group. Activities of glutathione peroxidase, superoxide dismutase, and catalase in liver were tended to increase in the SRE group. The plasma concentrations of interleukin (IL)-1 β , tumor necrosis factor (TNF)- α and IL-6 were lower in SRE group than in control group, while that of IL-10 was higher. The liver concentrations of IL-1 β , TNF- α , and IL-6 were tended to decrease in SRE group.

Conclusions: Finally SRE could be used in the production of nutraceuticals for lowering lipids and exerting anti-oxidation and anti-inflammatory effects in obesity rats fed highly oxidized rat.

Key Words: Scutellaria baicalensis, Pro-inflammatory cytokines, Oxidized fat, Anti-oxidation

Introduction

Highly oxidized fat in foodstuffs causes abnormal lipid metabolism and produces a high concentration of lipid peroxide¹⁻³⁾, which in turn can cause biofunctional abnormalities such as degenerative problems, cancer, senescence, and membranous change and injury⁴⁻⁶⁾.

A high concentration of lipid peroxide also increases inflammation-mediating substances, abnormal cytokine production, and the activation of the inflammatory signaling mechanism, and these can exert negative effects on the immune system⁷⁻⁹.

The recent increases in the consumption of high-fat and high-calorie foods are increasing the problem of abnormal lipid metabolism, which has prompted many studies investigating the use of natural products in the development of nutraceuticals¹⁰⁻¹³⁾. Such nutraceuticals could be useful in lowering lipids and exerting antioxidant and anti-inflammatory effects.

Scutellariae Radix is the root of the perennial herb

Scutellaria baicalensis Georgi. Its major components are flavonoid compounds, of which around 30 elements have been identified¹⁴⁾. The main biological functions of Scutellariae Radix, which are mainly attributable to flavonoids, include antihistamine effects¹⁵⁾, antiliver toxin¹⁶⁾, antibiosis¹⁷⁾, anticancer¹⁸⁾, antibacterial activation¹⁹⁾, and anti-inflammatory effects²⁰⁾. For example, baicalein (5, 6, 7-trihydroxyflavone) from Scutellariae Radix showed the same strong anti-inflammatory effect as sulfasalazine against dextran sodium sulfate exposure in animals²¹⁾. With the aim of developing nutraceuticals for lowering lipids and with antioxidant and anti-inflammatory effects, we administered Scutellariae Radix extract (SRE) to rats being fed highly oxidized fat. The lipid composition, anti-oxidation, and production of pro-inflammatory cytokines were examined.

Materials and Methods

1. Animals and treatment

Sixty male Sprague–Dawley rats weighing $173.59\pm$ 6.21 g (mean±standard deviation [SD]) were fed a highly oxidized fat diet (Table 1) for 10 weeks. Forty rats that weighed at least 400 g were randomly assigned to four groups containing 10 rats each: a control group in which physiological saline solution (100 mg/kg) was administered, and three treatment groups in which SRE was administered at 100, 200, and 300 mg/kg by Zonde.

2. Feeding

A baseline diet was supplied equally to all groups (Table 1) for 6 weeks for experiment, with a maximum intragroup difference in the amount consumed of 5%. The rats were allowed free access to water.

3. Preparation of SRE

Scutellariae Radix (500 g, dried weight, purchase from Korean medicinal store) was divided and extracted three times for 5 hours each in a cooling water reflux cistern using decompression concentrated and then extracted using methanol yield (120 g, 24%). SRE was administered orally via a Jones tube at 5 p.m. daily for 6 weeks. The control group was given normal saline in the same form.

4. Lipid peroxide assay

Lipid peroxide was prepared by aeration and stirred with soybean oil heated to 60°C for 72 hours. The peroxide level was more than 200 meq/kg²²⁾.

5. Sampling

Blood was collected after 14 hours of fasting using the heart puncture method on the last day of the experiment. Samples of approximately 7 ml were collected and divided for individual analysis. Liver samples collected after blood sampling were processed according to specific biochemical analyses and stored at -80° C.

Table 1. Composition	of Experimental Diets
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Ingredients (%)	Diets		
Ingredients (%)	Basal diet	Oxidized lipid diet	
Sugar	50.00	44.74	
Corn starch	12.00	10.74	
Casein	20.00	17.89	
Corn oil	8.00	7.16	
Cellulose	5.00	4.47	
AIN-76 Mineral mix.	3.50	3.50	
AIN-76 Vitamin mix.	1.00	1.00	
DL-methionine	0.30	0.30	
Choline chloride	0.20	0.20	
Oxidized soybean oil		10.00	
Total	100.00	100.00	

AlN-76 Mineral mix (g/kg): CaHPO₄ (500), NaCl (74), K citrate monohydrate: 220, K₂SO₄: 52, MgO: 24, Mn carbohydrate: 3,5, Fe citrate: 6,0, Zn cabonate: 1,6, Cu Carbonate: 0,3, KIO₃: 0,01, Na₂SeO₃,5H₂O: 0,01, CrK(SO₄)₂,12H₂O: 0,55, Sucrose: 118, AlN-76 Vitamin mix (g/kg): thiamin, HCI: 0,6, riboflavin: 0,6, pyridoxine, HCI: 0,7, nicotinic acid: 3, D-calcium pantothenate: 1,6, folic acid: 0,2, D-biotin: 0,02, cyanocobalamin: 0,001, retinyl palmitate: 0,8 (500,000 IU/g), DL- α -tocopheryl acetate: 20 (250 IU/g), cholecalferol: 0,00025, menaquinone: 0,005.

6. Serological analysis

Total plasma concentrations of cholesterol, total hepatic cholesterol, high density lipoprotein (HDL)-cholesterol, and triglyceride were measured using commercial kits (Wako, Japan). The plasma thiobarbituric acid (TBARS) concentration was quantified using the method of Buege and Aust²³⁾. The hepatic TBARS concentration was measured by the method of Ohkawa et al.24, and glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in plasma were measured using an automatic blood-analyzing device (Boehringer Mannheim, Mannheim, Germany). The activities of glutathione peroxidase (GSH-Px), liver superoxide dismutase (SOD), and liver catalase (CAT) were measured using the methods of Levander et al.²⁵, Flohe et al.²⁶⁾, and Johnson and Hakan Borg²⁷⁾, respectively. The concentration of plasma free fatty acid was measured by the enzyme technique using the V-NEFA kit (Mitsui, Tokyo, Japan). The concentrations of cytokines (interleukin [IL]-1 β , tumor necrosis factor [TNF]- α , IL-6, and IL-10) were determined by enzyme-linked immunosorbent assays using commercial kits (Biosource International, Camarillo, CA, USA). The minimum detectable concentration of TNF- α was 0.7 pg/ml, and those of the other cytokines were 3~8 pg/ml. Hepatic amounts of cytokines were calculated per 1 g of wet tissue in 5 ml of phosphate buffer saline. The concentrations of cytokines in plasma were expressed in pictograms per milliliter (pg/mg), and hepatic cytokine amounts were measured

 Table 2. Effects of SRE on Plasma FFA and Triglyceride Concentration in Rat Fed Oxidized Fat

Treatment	FFA (uEql)	Triglyceride (mg/dl)
Control SRE (100 mg/kg) SRE (200 mg/kg) SRE (300 mg/kg)	$\begin{array}{c} 872.74 \pm 43.25^{\circ} \\ 811.72 \pm 45.41^{b\circ} \\ 783.44 \pm 37.95^{b} \\ 652.38 \pm 39.72^{a} \end{array}$	$\begin{array}{c} 298.31 \pm 37.92^{b} \\ 257.11 \pm 41.65^{ab} \\ 232.48 \pm 35.51^{ab} \\ 203.52 \pm 31.11^{a} \end{array}$

Values are presented as mean \pm standard deviation.

SRE: Scutellariae Radix extract, FFA: free fatty acid.

 $^{\rm a,b,c}$ Means in the same column with different superscripts are significantly different (P < 0.05).

in picograms per milligram (pg/mg) of tissue.

7. Statistical analysis

Statistical analyses (mean \pm SD) were performed using one-way ANOVA with the PASW Statistics ver. 18.0[®] for Windows (IBM Co., Armonk, NY, USA), and the cutoff for significance was P<0.05 by Duncan's multiple range test.

Results and Discussion

Highly oxidized fat causes abnormal lipid metabolism and produces a high concentration of lipid peroxide¹⁻³, which in turn can cause biofunctional abnormalities⁴⁻⁶.

A high concentration of lipid peroxide also increases inflammation-mediating substances, abnormal cytokine production, and the activation of the inflammatory signaling mechanism, and these can exert negative effects on the immune system⁷⁻⁹⁾. With the aim of developing nutraceuticals for lowering lipids and with antioxidant and anti-inflammatory effects, this study investigated the effects of SRE on lipids, anti-oxidation, and the concentration of pro-inflammatory cytokines in rats fed highly oxidized fat.

The concentrations of plasma free fatty acid and triglyceride (Table 2) were lower in the SRE groups than the control group, although the difference was not significant between control and 100 mg/kg SRE. The con-

 Table 3. Effects of SRE on Plasma Total Cholesterol, HDL-Cholesterol

 and LDL-Cholesterol Concentration in Rat Fed Oxidized Fat

Treatment	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Control	217.52±17.65°	25.18±5.79 ^b	58.71±5.23 ^b
SRE (100 mg/kg)	188.49±21.52 ^{bc}	34.25 ± 3.47^{a}	54.19±5.54 ^{ab}
SRE (200 mg/kg)	167.33±18.22 ^{ab}	35.11±3.95 ^a	52.38±5.47 ^a
SRE (300 mg/kg)	$145.34\!\pm\!19.53^a$	35.86±3.11 ^a	43.52±5.11 ^a

Values are presented as mean±standard deviation.

SRE: Scutellariae Radix extract, HDL: high density lipoprotein, LDL: low density lipoprotein.

 a,b,c Means in the same column with different superscripts are significantly different (P<0.05).

centrations of total plasma cholesterol and low density lipoprotein-cholesterol (Table 3) also decreased as the concentration of SRE increased, although the difference was not significant between control and SRE (100 mg/kg)-treated groups. The concentrations of blood free fatty acid, total cholesterol, and triglyceride are closely related to the degree of intracorporeal lipid accumulation, showing that SRE can decrease the lipid level to prevent adult diseases, and optimally inhibit intracorporeal lipid accumulation. The HDL-cholesterol concentration was significantly higher in all of the SRE groups than in the control group. These results showed that SRE has positive effects on intracorporeal lipid metabolism and is effective in preventing adult diseases. The liver total cholesterol and triglyceride concentrations (Table 4) were lower in the SRE groups than in the control group, although the difference was not significant between control and 100 mg/kg SRE. Since lipid is

 Table 4. Effects of SRE on Liver Total Cholesterol and Triglyceride

 Concentration in Rat Fed Oxidized Fat

Treatment	Total cholesterol (mg/g)	Triglyceride (mg/dl)
Control SRE (100 mg/kg) SRE (200 mg/kg) SRE (300 mg/kg)	19.11±1.85° 17.34±1.53 ^{bc} 14.55±1.62 ^{ab} 13.98±1.74 ^a	$\begin{array}{c} 28.34 {\pm} 2.12^{b} \\ 25.19 {\pm} 1.95^{ab} \\ 22.48 {\pm} 2.51^{a} \\ 21.52 {\pm} 1.91^{a} \end{array}$

Values are presented as mean±standard deviation.

SRE: Scutellariae Radix extract.

^{a,b,c}Means in the same column with different superscripts are significantly different (P<0,05).

 $\ensuremath{\text{Table 5.}}$ Effects of SRE on Plasma and Liver TBARS in Rat Fed Oxidized Fat

Treatment	Plasma TBARS (nmoles MDA/ml)	Liver TBARS (nmoles MDA/g)
Control SRE (100 mg/kg) SRE (200 mg/kg) SRE (300 mg/kg)	$\begin{array}{c} 29.75 \pm 4.14^{b} \\ 25.22 \pm 4.39^{ab} \\ 21.14 \pm 3.75^{a} \\ 20.18 \pm 3.49^{a} \end{array}$	$\begin{array}{r} 34.11 \pm 5.93^{b} \\ 25.44 \pm 4.57^{a} \\ 24.38 \pm 4.21^{a} \\ 21.71 \pm 3.58^{a} \end{array}$

Values are presented as mean±standard deviation.

SRE: Scutellariae Radix extract, TBARS: thiobarbituric acid, MDA: malone dialdehyde.

 $^{a,b}\mbox{Means in the same column with different superscripts are significantly different (P<0.05).$

synthesized as well as analyzed in the liver, the hepatic lipid concentration is usually kept within quantum satis. But in some cases it may differ according to the diet or enzyme-activity-related factors. In this study, since all of the rats had the same intake of the same diet, the results suggest that SRE can influence hepatic lipid synthesis or lipolysis.

Table 5 indicates that the plasma and hepatic TBARS concentrations decreased in the SRE groups, although the difference of the plasma TBARS was not significant between control and 100 mg/kg SRE. This demonstrates that SRE contains functional substances that decrease the concentration of intracorporeal peroxide, and are similar to previous findings of natural plant resources containing antioxidant ingredients^{10,11)}.

Table 6 indicates that the activities of GOT and GPT decreased in the SRE groups. The present results show that SRE exerts effects to improve liver function.

 Table 6. Effects of SRE on levels of GOT and GPT in the Plasma of

 Rat Fed Oxidized Fat

Treatment	GOT (karmen unit)	GPT (karmen unit)
Control	45.17±3.59 ^b	48.29±3.37 ^b
SRE (100 mg/kg)	37.55±3.27 ^a	42.11±3.24 ^{ab}
SRE (200 mg/kg)	37.31±3.62 ^a	41.57±3.65 ^a
SRE (300 mg/kg)	32.38 ± 4.12^{a}	37.83±3.15 ^a

Values are presented as mean ± standard deviation,

SRE: Scutellariae Radix extract, GOT: glutamic oxaloacetic transaminase, GPT: and glutamic pyruvic transaminase.

 $^{a,b}\mbox{Means in the same column with different superscripts are significantly different (P<0.05).$

 Table 7. Effects of SRE on Glutathione Peroxidase, Superoxide Dismutase and Catalase Activity in Lives of Rat Fed Oxidized Fat

Treatment	GSH-px (nM/n/mg/protein)	SOD (unit/mg protein)	CAT (µM H ₂ O ₂ /min/mg protein)
Control SRE (100 mg/kg) SRE (200 mg/kg)	121.83±21.22 ^a 173.11±24.38 ^b 189.45±25.61 ^b		$\begin{array}{c} 61.54 {\pm} 10.72^{a} \\ 87.14 {\pm} 11.38^{b} \\ 95.17 {\pm} 15.38^{b} \end{array}$
SRE (300 mg/kg)	207.61 ± 25.27^{b}	6.24 ± 1.75^{b}	98.65 ± 13.21^{b}

Values are presented as mean ± standard deviation.

SRE: Scutellariae Radix extract, GSH-px: glutathione peroxidase, SOD: liver superoxide dismutase, CAT: liver catalase.

^{a,b}Means in the same column with different superscripts are significantly different (P < 0.05).

Treatment	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	IL-10 (pg/ml)
Control	21.88±1.14 ^b	31,92±3,15 ^b	32.11±2.73 ^b	18.17±2.05ª
SRE (100 mg/kg)	20.75±1.59 ^b	29,58±3,17 ^{ab}	29.43±3.27 ^b	21,55±1,98ª
SRE (200 mg/kg)	19,57±1,38 ^{ab}	28,23±3,21ª	27.95±3.11 ^{ab}	22,37±2,13ª
SRE (300 mg/kg)	17.18±1.23 ^a	23.76±2.43 ^a	21.58±2.92 ^a	27.48±1.95 ^b

Table 8. Effects of SRE on Cytokines Levels in the Plasma of Rat Fed Oxidized Fat

Values are presented as mean ± standard deviation.

SRE: Scutellariae Radix extract, IL: interleukin, TNF: tumor necrosis factor.

^{a,b}Means in the same row with different superscripts are significantly different (P < 0.05).

Table 9. Effects of SRE on Cytokines in Rat Fed Oxidized Fat

Treatment	IL-1β (pg/mg)	IL-6 (pg/mg)	TNF-α (pg/mg)	IL-10 (pg/mg)
Control	26.91±2.14 ^b	17.24±1.91 ^b	2.49±0.93 ^{NS}	1.54±0.55 ^{NS}
SRE (100 mg/kg)	21.39±2.21 ^a	13.88±1.67 ^{ab}	1.96±0.84 ^{NS}	1.71±0.63 ^{NS}
SRE (200 mg/kg)	21.15±1.85 ^a	12.95±1.58 ^a	1.85±0.91 ^{NS}	1.78±0.72 ^{NS}
SRE (300 mg/kg)	19.38±1.79 ^a	11.39±1.25 ^a	1.51 ± 0.73^{NS}	1.95 ± 0.71^{NS}

Values are presented as mean ± standard deviation.

SRE: Scutellariae Radix extract, IL: interleukin, TNF: tumor necrosis factor, NS: not significantly different (P>0.05).

^{a,b}Means in the same row with different superscripts are significantly different (P<0.05).

Table 7 indicates that the activities of GSH-Px, SOD, and CAT were significantly increased in the SRE groups compared to the control group. Intracorporeal peroxide accumulation is closely interrelated with the activities of antioxidant enzymes. In this study, the activities of GSH-Px, SOD, and CAT corresponded to the changes in TBARS concentrations. These results suggest that functional substances in SRE act on the activities of antioxidant enzymes and the biologic responses in the production inhibition and lysis promotion of intracorporeal peroxide. Table 8 lists the plasma concentrations of IL-1 β , IL-6, TNF- α , and IL-10. The concentrations of the pro-inflammatory cytokines IL-1B, IL-6, and TNF- α were decreased in the SRE groups, while that of the anti-inflammatory cytokine IL-10 was increased. Although the concentrations of TNF- α and IL-10 did not differ significantly among the treatment groups, the liver cytokines (Table 9) were consistent with those found in plasma cytokines, with IL-1β, IL-6, and TNF- α being decreased in the SRE groups and IL-10 being increased.

Decreased pro-inflammatory cytokines indicates an

inhibitory effect inflammatory response, thus functional substances in SRE contributed to improving the inflammatory response. These results indicate that SRE could be used in the production of nutraceuticals for lowering lipids and exerting anti-oxidation and anti-inflammatory effects.

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황금추출물이 과산화지질을 급여한 흰쥐의 지질대사, 산화반응 및 전염증성 Cytokine의 생산에 미치는 영향

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목적: 본 연구는 황금추출물이 과산화지질을 장기간 급여한 흰쥐의 지질대사, 산화반응 및 전염증성 cytokine의 생산에 미치는 영 향을 검토하기 위해 수행되었다.

방법: 10주 동안 흰쥐에게 과산화지질을 과량으로 급여하여 생체 내에 과잉의 지질을 축적시킨 후, 황금추출물을 6주간 급여하였다. 그 후, 각 처리군별로 지질대사, 산화반응 및 전염성 cytokine의 생산에 미치는 영향을 조사했다.

결과: 혈액 내 유리지방산, 총지질, 총콜레스테롤, low density lipoprotein-cholesterol과 간장 내 총지질, 총콜레스테롤이 황금추출 물 처리군에서 하락했다. 혈액 및 간장의 thiobarbituric acid 농도가 황금추출물 처리군에서 하락했다. 간장 내 항산화효소들 (glutathione peroxidase, liver catalase, liver superoxide dismutase)의 활성치가 황금처리군에서 상승하였다. 전염증성 cytokine (interleukin [IL]-1β, tumor necrosis factor-α, IL-6)의 농도는 황금추출물 처리군에서 하락했다. 반면에 IL-10의 농도는 황금추출물 처리군에서 증가했다.

결론: 이상의 결과들을 종합해 보면, 황금 추출물은 과산화지질을 급여한 비만쥐의 지질대사, 항산화반응 및 항염증반응에 긍정적 인 효과를 나타내었음을 인식시켜 준다.