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Effects of *Schizandra chinensis* BAILL on Lipid Lowering and Antioxidant in Hyperlipidemic Rat

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ABSTRACT

The present study examined the effects of *Schizandra chinensis* extract on the serum lipid composition and the antioxidant of rats in which obesity was induced through high fat diet. Fifty male Sprague-Dawely rats weighing 163.91±4.17g on the average were adjusted to basic diet and laboratory environment and were fed with high fat diet freely for 6 weeks to induce obesity. Forty rats, the final weight of which was 400g, were selected and were divided into a control group(C), treated groups(T I ; body weight of 100mg/kg, T II ; 150mg/kg and T III ; 200mg/kg), 10 heads of similar weight for each, and test breeding was performed for 4 weeks. During the test breeding, all treated groups were fed with basic diet and difference in intake among the treated groups were maintained to be less than 5%. According to the result, the quantity of Triglyceride in serum was lower in all of the groups treated with *Schizandra chinensis* than the control group, but the difference was not significant except the treated group of 200mg ($P>0.05$). The quantity of Total cholesterol in serum was significantly lower in all the groups treated with *Schizandra chinensis* than in the control group ($P<0.05$) but differences according to the quantity of *Schizandra chinensis* applied were not observed. The quantity of HDL-cholesterol was not significantly different among all the groups including the control group ($P>0.05$) and no regular tendency of change in the quantity was observed according to the quantity of *Schizandra chinensis* applied. The quantity of LDL-cholesterol was lower in all the groups treated with *Schizandra chinensis*, but the treated group of 100mg was not significantly different from the control group. The quantity of TBARS in serum was lower in all the groups treated with *Schizandra chinensis* than in the control group ($P<0.05$), but no regular tendency of change in the quantity was observed according to the quantity of *Schizandra chinensis* applied. The quantity of liver TBARS was not significantly different among all the treated groups ($P>0.05$). The levels of glutathione peroxidase activity (GSH-Px), superoxide dismutase activity (SOD) and catalase activity were higher in all the groups treated with *Schizandra chinensis* treated group than in the control group ($P<0.05$), and the treated group of 200mg showed the highest activity among the treated groups.

Key words **:** Schizandra chinensis, lipid lowering, antioxidant, plant extract. obesity.

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INTRODUCTION

Recently with the increase of high energy food, obesity is causing many problems in contemporary people's health. In particular, the excessive accumulation of body fat by obesity causes abnormalities such as the reduction of calorie consumption in the body, the inhibition of fat oxidation, excessive free fat acid in blood, the promotion of lipid peroxidation in blood, the increase of neutral fat and abnormal sugar metabolism, inducing diabetes, Hyperlipidemic, respiratory diseases, cancers, liver diseases, heart diseases and various metabolic disorders (Takayama, 1988; Roden, 1996). In addition, the excessive accumulation of body fat increases lipid peroxide in the body, which induces the retrogression of cells and tissues and has negative effects on body functions (Bidlack and Tappel, 1973; Saito, 1988; Vergroeson, 1997).

There can be many causes of obesity but the most common one comes from imbalance between energy supply and energy consumption. That is, excessive energy from high-energy food and insufficient exercise is obesity resulting from the accumulation of unconsumed energy in the form of fat in the body (Younes and Siegers, 1980). This type of obesity can be prevented and treated through diet therapy and exercise therapy but if it is overlooked out of contemporary people' complicated life environment, it may cause diseases even engendering life. Considering this, contemporary people who lack exercise are in pressing need of functional food that disintegrates and removes energy remaining in the body or partially cuts off the absorption of fat, which is a main source of high energy.

Many researches have made researches on preventing obesity in many ways (Schwartz, 1980; Langanier and Yu, 1987; Powell and Connoly 1988; Yamaoka, *et al.*, 1996). However, the results have been unsatisfactory and further researches are necessary.

This is a basic study on the development of functional food that improves or prevent obesity using *schizandra chinensis*, which is widely used as an ingredient of Chinese medicine or folk medicine. This study fed rats, in which obesity was induced, with different quantities of *schizandra chinensis* extract by group, and compared lipid composition, the quantity of TBARS and the activity of anti-oxidant enzymes among the groups.

MATERIALS AND METHODS

experimental rats

Fifty male sprague-Dawely rats weighing 163.91 ± 4.17g on the average were adjusted to basic diet(Tab. 1) and laboratory environment during 10 days before experiment.

Inducing obesity and experiment group organization

Fifty rats were fed with high fat diet freely for 6 weeks to induce obesity. Forty rats, the final weight of which was 400g, were selected and were divided into a control group, treated group I (body weight of 100mg/kg), treated group II (150mg/kg) and treated group III (200mg/kg), 10 heads of similar weight for each.

Feeding of experimental diet

During the test breeding, 4 weeks, all treated groups were fed with basic diet and difference in intake among the treated groups were maintained to be less than 5%.

Extraction of blood

After fasting 12hours at that time of end, extract 5ml of blood per 1 head by the use of injector from the heart.

Biochemical assay

1) Serum cholesterol

Serum total cholesterol(TC), LDL-cholesterol, HDL-cholesterol and triglyceride were analyzed by automatic blood analyzer(Boehringer Manheim, German)

2) Serum Thiobarbituric acid reactive substance (TBARS) in liver

To measure the quantity of TBARS in the liver, we extracted a certain size of liver slice, and washed it with 0.9% physiological salt solution to remove blood. The liver slice was grinded thoroughly together with 1.15% KCl water solution using a Teflon-Elvelijem homogenizer and 10% homogenate was prepared. Out of homogenate, 0.1 ㎖ was taken and put into a screw cap tube and to the tube were added 0.2 ㎖ of 8.0% sodium dodecyl sulfate, 1.5 ㎖ of 20% acetic acid solution (pH 3.5) and 1.5 ㎖ of 0.8% TBA solution. Distilled water was added so that the resulting volume became 4 ㎖. The resulting solution was shaken and heated in 95℃ water bath for an hour. The heated test tube was cooled in running tap water. Then 1 ㎖ of distilled water and 5 ㎖ of mixture of n-butanol : pyridine (15 : 1, v/v) were added and voltex was carried out. After centrifuged at 1,500xg for 10 minutes, the supernatant (n-butanol : pyridine layer) was taken and its absorbance was measured at 532 nm. The reference material used was TMP (1,1,3,3-tetraamitoxypropane) and the level of lipid peroxide was indicated in nmol MDA (malondialdehyde) (Ohkawa, *et al.*, 1979).

3) Liver glutathione peroxidase activity (GSH-Px)

The activity of liver glutathione peroxidase (GSH - Px) was analyzed by the method suggested by Levander *et al.* (1983). We dissected rats under anesthesia, extracted a certain size of liver slice, washed it with physiological salt solution to remove blood, ground it together with 0.15 M KCl using a Teflon-Elvelijem homogenizer to prepare 20% homogenate, and centrifuged the homogenate at 9,000xg for 15 minutes. The supernatant was again centrifuged at 15,000xg for an hour and supernatant to be used in

Table 1. Composition of experimental diets.

Ingredients(%)	Basal diet	High fat diet
Casein	20.0	20.0
α- Corn starch	35.0	30.0
Sucrose	11.0	10.0
Lard	4.0	25.0
Corn oil	1.0	5.0
Mineral mix[1)]	3.5	3.5
Vitamin mix[2)]	1.0	1.0
Cellulose powder	23.5	5.2
DL-methione	0.3	0.3

[1)] Mineral mix.(g/kg diet) : $CaCO_3$, 29.29 ; $CaHPO_4 \cdot 2H_2O$, 0.43 ; KH_2PO_4, 34.30 ; NaCl, 25.06 ; $MgSO_4 \cdot 7H_2O$, 9.98 ; Feric citrate hexahydrate, 0.623 ; $CuSO_4 \cdot 5H_2O$, 0.516 ; $MnSO_4 \cdot H_2O$, 0.121 ; $ZnCl_2$, 0.02 ; KI, 0.005 ; $(NH_4)_6 MO_7O_{24} \cdot 4H_2O$, 0.0025.

[2)] Vitamin mix(mg/kg diet) : Thiamine-HCl, 12 ; Riboflavin, 40 ; Pyrodoxin-HCl, 8 ; Vitamin-B12, 0.005 ; Ascorbic acid, 300 ; D-bitotin, 0.2 ; Menadione, 52 ; Folic acid, 2 ; D-calcium pantothenate, 50 ; P-aminobenzoic acid, 50 ; Nicotinic acid, 60; Cholin choloride, 2000(IU/kg diet) ; Rethinyl acetae, 5,000(IU/kg diet) ; Cholecalciferol, 250(IU/kg diet).

Table 2. Effects of *schizandra chinensis* ext. on lipid composition in hyperlipidemic rat.

Treatment	Triglyceride (mg/dl)	Totalcholesterol (mg/dl)	HDL-cho. (mg/dl)	LDL-cho. (mg/dl)
Control	270.91 ± 14.57^{b}	219.63 ± 8.21^{b}	42.62 ± 4.01^{NS}	67.44 ± 4.82^{c}
T- I(100)	258.29 ± 17.88^{b}	172.45 ± 6.44^{a}	44.98 ± 3.53^{NS}	60.25 ± 4.04^{bc}
T-II(150)	251.38 ± 15.12^{b}	151.97 ± 7.21^{a}	41.77 ± 4.97^{NS}	55.28 ± 5.11^{ab}
T-III(200)	215.73 ± 15.57^{a}	165.31 ± 7.08^{a}	42.61 ± 5.08^{NS}	51.75 ± 4.74^{a}

T-I : *schizandra chinensis* ext. 100mg/kg body weight, T-2: *schizandra chinensis* ext. 150mg/kg body weight, T-3: *schizandra chinensis* ext. 200mg/kg body weight.

a,b,c: Values with different superscript in the same column are significantly different($P<0.05$).

NS: Not significantly($P>0.05$).

analysis was taken so that the protein content became 100-200㎍. the temperature of the centrifugal machine was maintained at 4℃, and the prepared sample was put in stock solution (K buffer, 40mM glutathione, KH buffer, 1 unit of glutathione reductase for each ㎖ of KH buffer) and incubated at 37℃ for 10 minutes. In addition, 20 mM NADPH was added and left alone for 2 minutes. Again 15 mM t-butyl hydroperoxide was added and the absorbance reduction rate was measured at 340nm for one minute. The activity of GSH-Px was indicated in the number of n mols oxidized from NADPH to NADH for each mg of protein for a minute.

4) Liver superoxide dismutase activity (SOD)

In order to measure liver SOD, superoxide was produced through xanthine oxidase. The superoxide reduces ferricytochrome C (Fe^{3+}) into ferrouscyto-chrome C (Fe^{2+}). At that time, if there is SOD, it competes with superoxide and as a result the reduction rate of cytochrome c decreases. Using the method of Flohe et al. based on the principle, we measured liver SOD> In this experiment, the degree of interference with the reduction of ferriccytochrome c was colorimetrically quantified at 550 nm at intervals of 30 seconds for 3 minutes. The activity was indicated in the quantity of SOD interfering the reduction of ferriccytochrome c by 50%.

5) Liver catalase activity

In order to measure the activity of liver catalase, we homogenized 0.2g of liver into 25 mM KH_2PO_4-NaOH buffer (pH 7.0) and diluted the homogenate with the same buffer in 60 times. Then in a ice bath, the absorbance of the sample was measured twice, for 15 seconds each, with a ultrasonicator (Heat System Ultrasonics. Inc., Ultrasonic Professor W-385) and activity was calculated from a reference curve obtained from reference solution formaldehyde (Johnsson. 1988).

RESULTS AND DISCUSSION

Table 2 shows the tendency of change of lipid composition in each treated group's serum. The quantity of Triglyceride decreased more in the groups treated with *schizandra chinensis* than in the control group, but the differences were not significant except in the treated group of 200mg. The quantity of Triglyceride in serum is mainly affected by diet intake and synthesis and disintegration in the liver. This experiment adjusted diet intake to be equal among the treated groups. Accordingly, the reduction of Triglyceride in the groups treated with *schizandra chinensis* implies the possibility that *schizandra chinensis* may affect the synthesis and disintegration of Triglyceride in the liver. The quantity of total cholesterol was between 151.97mg/dl~219.63mg/dl,

Table 3. Effects of schizandra chinensis ext. on TBARS contents in hyperlipidemic rat.

Treatment	Plasma TBARS (n mols MDA/ml)	Liver TBARS (n mols MDA/g)
Control	26.85 ± 2.77^{b}	27.36 ± 1.77^{NS}
T- I(100)	18.35 ± 2.51^{a}	26.92 ± 1.85^{NS}
T-II(150)	20.32 ± 3.05^{a}	28.44 ± 1.09^{NS}
T-III(200)	18.95 ± 2.71^{a}	26.29 ± 1.95^{NS}

Table 4. Effects of *schizandra chinensis* ext. on glutathione peroxidase(GSH-Px)activity, superoxide dismutase(SOD) activity and catalase(CAT) activity in hyperlipidemic rat.

Treatment	GSH-Px (nmole/min/mg protein)	SOD (unit/g tissue)	CAT (nmole H_2O_2 decompose/min/mg protein)
Control	127.59 ± 17.88^{a}	171.08 ± 24.22^{a}	120.75 ± 18.51^{a}
T- I(100)	183.59 ± 20.17^{b}	249.58 ± 25.71^{b}	161.05 ± 20.55^{b}
T-II(150)	192.44 ± 17.62^{b}	237.96 ± 27.65^{b}	173.69 ± 19.79^{b}
T-III(200)	198.41 ± 18.59^{b}	257.33 ± 25.97^{b}	180.54 ± 21.37^{b}

and its decrease was more significant in all the groups treated with *schizandra chinensis* than in the control group. The quantity of LDL-cholesterol as well decreased more significantly in treated groups of 150mg or heavier. The abnormal increase of the triglyceride concentration, the total cholesterol concentration and the LDL-cholesterol in blood shows the high possibility of obesity, heart diseases and various circulatory disorders (Takayama, 1988; Roden, 1966). This suggests that a specific substance in *schizandra chinensis* may be effective in improving obesity and particularly in preventing heart diseases. The quantity of HDL-cholesterol did not show any significant difference among all the treated groups. The result was observed in other researches (Lee, *et al.*, 2000). Various factors are involved in the synthesis of HDL-cholesterol in the body.

Table 3 shows the quantity of TBARS in serum and liver by group. The quantity of TBARS in serum was between 18.35Unit~26.85Unit for all the treated group, and it decreased more significantly in all the groups treated with *schizandra chinensis* than in the control group. The quantity of TBARS is an important indicator of the accumulation of lipidperoxide in the body (Ohkawa, *et al.*, 1979). In general, the concentration of lipidperoxide increases or decreases in proportion to the accumulation of the body fat (Lee, 2003). Lipidperoxide is known to have negative effects on cancers, aging, change of living membrane, destruction, etc. (Bidlack and Tappel, 1973; Saito, 1988; Vergroeson, 1997). This opinion and the result of the present study suggest that a certain substance in *schizandra chinensis* inhibits the lowering and the oxidation of body lipid. However, the quantity of TBARS in liver was not significantly different among the treated groups. The result indicates that treatment in this experiment was not enough to accumulate peroxide in the liver and the function of the liver is normal, considering that the disintegration and composition of lipid are carried out in the liver and these disintegration and composition materials are discharged with the blood.

Table 4 shows GSH-Px, SOD and CAT activities for each treated group. The activities of all the three anti-oxidant enzymes were significant higher in the control

group. The result suggests that there is a substance fit to the result of TBARS concentration and that there is a substance in *schizandra chinensis* contributes to the increase of the activity of anti-oxide enzymes.

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