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Effects of *Coptidis Rhizoma* on Lowering Lipid and Oxidative Stress

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Abstract - The effects of hwangryun (*Coptidis Rhizoma*) on lowering lipid and oxidative stress in the induced obesity rat was observed. The concentration of plasma triglyceride in hwangryun treatment groups showed the low values compared to the control group, and as the increased hwangryun, the concentration of triglyceride decreased. The concentration of plasma total cholesterol decreased in all hwangryun treatment groups. However the concentration of plasma HDL-cholesterol and LDL-cholesterol showed no significantly difference in all the treatment groups. Thiobarbituric acid reactive substance (TBARS) concentration in plasma and liver showed low values in all hwangryun treatment groups compared to the control group. Glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities showed no significantly difference in all the treatment groups. However catalase (CAT) activity showed a tendence to increase in hwangryun groups, and in 200mg/kg hwangryun treatment group showed significantly a high value than the control group. Summarizing the results above, hwangryun has the functional materials that lowering lipid and works with oxidative stress.

Key words - *Coptidis Rhizoma*, Glutathione peroxidase, Superoxide dismutase, Catalase, Thiobarbituric acid reactive substance

Introduction

Obesity can be the cause of diabetes, hypertension, arteriosclerosis, myocardial infarction, and other adult diseases. Also in vivo peroxide over accumulation from obesity may cause degenerative process, and it can be the cause for cancer, aging and other sickness. (Kasuga, 1997; Masugi, 1997; Saito, 1988; Vergroeson, 1997; Bidlack and Tappel, 1973). Therefore, recently, many researchers have been researching to control peroxide accumulation and lowering lipid. (Younes and Siegers, 1980; Ishikawa and Suzukawa, 1997; Ueda and Tanoue, 2000; Langanier and Yu, 1987). But until now, there is no satisfying result, and it had made us realize the need for more various researches. Hwangryun (*Coptidis Rhizoma*) is used as an oriental medicine, and the major elements are isoquinoline group's alkaloids berberin, jateorrhizine, palmatine, coptisine, magnoflorine, epiberberin, berbestine, ferulic acid and many more had been known (Chung and Paik, 1997), anti-microorganism reaction, acetylcholine enhancement effect, febricide, antidiuresis effect had been known (Tang and Eisenbrand, 1992). Also, there was a report of experiment result in which ververine sulfate decreases serum cholesterol concentration in rat (Vad *et al.*, 1971), and increased bile secretion in dog (Oshiba *et al.*, 1974). With these research results, Hwangryun has the possibility of lowering lipid and antioxidant in vivo. Therefore, this research is the basic resarch of developing a functional foods with lowering lipid and oxidative stress by administration of hwangryun extract to the rat with induced obesity, and compared and analysed the lipid composition, TBARS concentration and enzyme activities.

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Materials and Methods

Experimental animals

Twenty-one of male Sprague-Dawley rats that were fed high fat diet (Table 1) for 8 weeks had been divided to control group (normal saline 100mg/kg), treatment group I (Hwangryun extract 100mg/kg) and treatment group II (Hwangryun extract 200mg/kg), and each group was placed 7 rats with similar weight.

Diet

Diet was given the basic diet (Table 1.) for 4 weeks to all groups equally, and the feeding amount was made equally so that the difference is within 5% of each. Water was ad libitum.

Hwangryun extract and administration

The natural dried hwangryun 300g was extracted 3 times at each

Table 1. Composition of experimental diets

Ingredients (%)	Basal diet	High fat diet
Casein	20.0	20.0
a-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
Cellurose 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-B_{12}, 0.005; Ascorbic acid, 300; D-biotin, 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, 5000 (IU/kg diet); Cholecalciferol, 250 (IU/kg diet).

5 hours, filtered, and decompression pressed and made MeOH ext. 80g. Diet was given at 5pm everyday orally with Jone's tube.

Sampling and analysis

Blood sampling was made after 12 hours fast before the ending experiment and with cardiac puncture method. Plasma TBARS content was determined from EDTA treated blood, after cultivating it for 120 minutes at 38℃, with Buege and Aust (1978) method and liver TBARS content was determined with Ohkawa *et al.* (1979) method. Liver glutathione peroxidase (GSH-Px) activity was measured in Levander *et al.* (1992) method, liver superoxide dismutase (SOD) activity measurement was done in Flohe *et al.* (1992) method and liver catalase (CAT) activity measurement was based on Johnson and Hkan Borg (1988) method. Total cholesterol, HDL-cholesterol, triglyceride content in the plasma were quantified by kit (Wako Co. Japan).

Statistics

Results are analyzed by one-way ANOVA using SPSS package, and each group's significance was done by Duncan' s multiple range test at $P<0.05$.

Result and Discussion

Each group's triglyceride concentration of plasma is listed in Table 2. Amount of triglyceride compared to control group, the hwangryun (*Coptidis Rhizoma*) treatment groups showed significantly low values, and as the hwangryun was increased, it decreased. This result is thought to be caused by fiber and other functional elements within hwangryun had caused it, and the lowering lipid level showed similar result with the other researcher who had researched on functional elements of plants (Sin and Han, 1997). In Table 3., total Cholesterol, HDL-cholesterol and LDL-cholesterol results are listed. The concentration of total cholesterol showed decrease in hwangryun extract treatment groups compared to the control group. However HDL cholesterol and LDL-cholesterol concentration decreased in all hwangryun treatment groups, but it did not show significantly difference. When total cholesterol and LDL-cholesterol increases abnormally within the blood, atherosclerosis, hypertension and other circulatory disease can occur (Vergroeson, 1997; Hang and Hostmark, 1987; Harris *et al.*, 1983; Sanders and Hochland, 1983). In the hwangryun extract treatment groups, the decrease of plasma total cholesterol concentration stated that hwangryun can be used to develop effective functional elements to prevent obesity and other various circulatory disease and cure. HDL-cholesterol and LDL-cholesterol showed little decrease in hwangryun extract treatment

Table 2. Effects of *Coptidis Rhizoma* ext. on plasma triglyceride concentration in rat fed high fat diet

Treatment	Triglyceride (mg/dℓ)
I	201.33 ± 18.45^{b}
II	145.00 ± 23.27^{a}
III	123.75 ± 15.95^{a}

[a,b]Means in the same column with different superscripts are significantly different ($P<0.05$). I: Control, II: 100mg/kg *Coptidis Rhizoma* ext., III: 200mg/kg *cotischonensin* ext.

Table 3. Effect of *Coptidis Rhizoma* ext. on plasma total cholesterol, HDL-cholesterol and LDL -cholesterol in rat fed high fat diet

Treatment	Total Cholesterol (mg/dℓ)	HDL-cholesterol (mg/dℓ)	LDL-cholesterol (mg/dℓ)
I	184.00 ± 8.89^{c}	34.33 ± 4.53^{NS}	15.25 ± 3.86^{NS}
II	131.00 ± 11.92^{b}	33.50 ± 3.32^{NS}	12.75 ± 2.22^{NS}
III	109.00 ± 9.49^{a}	29.00 ± 3.56^{NS}	10.67 ± 2.52^{NS}

[a,b]Means in the same column with different superscripts are significantly different ($P<0.05$). I : Control, II : 100mg/kg *Coptidis Rhizoma* ext., III : 200mg/kg *Coptidis Rhizoma* ext. [NS]: Not significantly different ($P>0.05$).

Table 4. Effect of *Coptidis Rhizoma* ext. on plasma and liver TBARS concentration in rat fed high fat diet

Treatment	Plasma TBARS (nmoles MDA/ml)	Liver TBARS (nmoles MDA/g)
I	27.35±2.46[b]	16.64±3.89[b]
II	11.99±4.69[a]	9.30±1.92[a]
III	8.22±1.70[a]	6.91±1.23[a]

[a,b]Means in the same column with different superscripts are significantly different (P<0.05). I: Control, II: 100mg/kg *Coptidis Rhizoma* ext., III: 200mg/kg *Coptidis Rhizoma* ext.

groups and showed no significantly difference in all groups. This result is thought to be caused by HDL-cholesterol and LDL-cholesterol concentration related other factors (Satio, 1988) and in the similar experiment, total cholesterol decreased but, HDL-cholesterol and LDL-cholesterol concentration did not showed noticeable difference and it was same with the other experiment results (Lee and Choi, 2000). In table 4., plasma and liver TBARS concentration is listed. TBARS concentration of plasma and liver decreased in all hwangryun extract treatment groups compared to control group. TBARS concentration increases when lipid over accumulation or lipid metabolism is abnormal, and it shows lipid peroxide in vivo accumulation amount. With these results, an element within hwangryun have reacted positive to lipid metabolism and antioxidant substances. Table 5. shows the activities of GSH-Px, SOD and CAT. GSH-Px activity showed increase or decrease not related to hwangryun treatment, however these values showed no significantly difference with control group. SOD activity showed increase in hwangryun treatment groups, but it did not show significantly difference with control group. CAT activity showed slightly increase in hwangryun treatment groups, and only in 200mg/kg group, CAT activity showed significantly difference with control group. Activities of these enzyme indirectly places antioxidation level, and the result may effect TBARS concentration. In this experiment, each group's TBARS concentration change and enzyme activity change is similar but statistical significance were not acceptable. These results can be thought to have been made by reaction of other relational enzyme activity increase and peroxide storage and other elements.

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Table 5. Effect of *Coptidis Rhizoma* ext. on antioxidase (GSH-Px,SOD,CAT) activities in rat fed high fat diet

Treatment	GSH-Px (nmoles/min/mg protein)	SOD (unit/mg protein)	CAT (μmoles(H_2O_2)/min/mg protein)
I	150.17± 1.51[NS]	12.49±1.81[NS]	65.12±4.35[a]
II	150.63±11.67[NS]	12.50±2.71[NS]	75.37±5.15[ab]
III	145.64± 6.27[NS]	12.87±2.25[NS]	79.28±4.59[b]

[a,b]Means in the same column with different superscripts are significantly different (P<0.05). I: Control, II: 100mg/kg *Coptidis Rhizoma* ext., III: 200mg/kg *Coptidis Rhizoma* ext. [NS]Not significantly different (P>0.05).

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