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Effect of Rhodiola Extract Supplementation on Blood Lipid Concentrations and Anti-Oxidant Status in Rats Fed Highly Oxidized Linoleic Acid Diets*

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ABSTRACT

The influence of Rhodiola extract on tissue antioxidant status, plasma lipid levels, cholesterol contents of liver and feces were investigated in rats fed oxidized linoleic acid. Groups of five-week old male Sprague-Dawley rats fed *ad libitum* with a diet containing 20% oxidized linoleic acid with or without 300 mg/kg body weight freeze-dried Rhodiola water extract. The antioxidant effect of dietary Rhodiola extract supplementation on the peroxidation potential of rats was investigated. The microsomal thiobarbituric acid reactive substance (TBARS) contents were changed significantly by Rhodiola extract supplementation. Hepatic Catalase activities were increased in Rhodiola supplemented rats, whereas hepatic Manganese Superoxide Dismutase (MnSOD) or Copper Zinc Superoxide Dismutase (CuZnSOD) were not elevated. In addition, plasma cholesterol lowering effect was observed along with the stimulated excretion of cholesterol through the feces were observed with Rhodiola feeding. Supplementation with Rhodiola extract did not alter high density lipoprotein (HDL) cholesterol. These results support that Rhodiola extract may be effective in protection against oxidative stress, and prevention and treatment of blood dyslipidemia. It demonstrates that Rhodiola extract has a potential to exert anti-atherogenic properties antioxidative capacities.

KEY WORDS: oxidized linoleic acid, lipid peroxy radical, rhodiola extract supplementation, anti-oxidant status, lipid concentrations in blood, liver and feces.

INTRODUCTION

A significant attention has been placed on the role of the antioxidative defense system in oxidative stress.[1,2] Free radicals are produced in the body as by products of normal metabolism and can be accumulated as a result of exposure to environmental pollutants, smoking or certain kinds of dietary compounds. Because they are highly reactive, they can damage cellular components. Free radicals may play a role in a wide variety of pathologic conditions and may also be important in the aging process. If their target is DNA, the likelihood of cancer increases; if their target is low-density lipoprotein (LDL) in blood, atherosclerosis may result. One of the most frequently consumed oxidized components is thermally oxidized dietary fat.[3] Thermally oxidized fat is generally considered to contain potentially toxic lipid peroxidation products, and lipid peroxidates occurring during frying has been demonstrated as products of free radical-mediated reactions.[4,5] Also, it has been shown in experimental animals that oxidized lipids may have some implication in the pathogenesis of atherosclerosis since oxidized lipids in the diet can be incorporated into very low density lipoprotein.[6] Furthermore, a strong possibility of lipid peroxyl radicals from oxidized oils to be involved in colon carcinogenesis has been discussed in the inducible colon cancer experiments with rodents.[7] A significant increase in tumor initiating and promoting activities of oxidized fat has been observed.[8] On the other hand, high consumption of peroxidized lipid has been shown to cause rapid depletion of anti-oxidant vitamins such as vitamin E and vitamin C in serum.[9,10]

Plant origin antioxidants can neutralize many types of free radicals and thus may protect from many types of diseases.[11-13] One of the antioxidant phytochemicals, Rhodiola (*Rhodiola rosea*), a medicinal herb widely used in Asia and Eastern Europe, has been used traditionally for release of fatigue, metabolic toxicity and anti-aging.[14-17] Recently its effectiveness as an antioxidant has been reported in experiment of which Rhodiola extract decreased oxidative stress caused by high fat diet.[18] Its antioxidant properties have been also demonstrated in rats treated with CCl_4.[19] Strong *in vitro* active-oxygen scavenging activity of Rhodiola extract has been shown in the study by Ohsugi *et al.* evaluated by electron spin resonance technique.[20] Other effects of Rhodiola include the enhance-

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ment of the swimming duration in rats and the doubling of platelet aggregation time.[21]

In the present study the free radical scavenging effect of Rhodiola extract was examined in rats fed highly oxidized linoleic acid. Also, the cholesterol-lowering effect in plasma, liver and feces of rats possibly through antioxidant activities were investigated to evaluate the antioxidant potential of Rhodiola extract against consuming oxidized fat.

MATERIALS AND METHODS

1. Chemicals

Linolcic acid, 2-thiobarbituric acid, butylated hydroxy toluene, 1,1,3,3,-tetraethoxypropane, sodium xanthine, hydroxylamine hydrochloride, sodium xanthine, xanthine oxidase, N-(1-naphthyl)-ethylenediamine, sulfanilamide, 30% hydrogen peroxide, potassium cyanide, bovine serum albumin, L-cysteine, α-cellulose, choline bitartrate and tert-butylhydroquinone were purchased from Sigma Chemical, St. Louis, USA. Bio-rad protein assay reagent was purchased from Bio-rad Chemicals, Richimond, USA. Other reagents are all chemical grades and purchased from the commercial reagent suppliers. Corn starch was supplied by Miwon Co, Seoul, Korea, casein was a product of New Zealand Dairy Board (Wellington, New Zealand), and soybean oil and lard were commercial brands.

2. Animals and feeding regiments

Male Sprague-Dawley rats, five-weeks old, were fed a standard laboratory diet (manufactured by Cheil Feed Co., Seoul, Korea) for one week. Using a randomized complete block design, rats were divided by initial body weight into two groups of nine rats each. Rats were housed individually in an environmentally controlled animal laboratory with a 12-h light:dark cycle. For 4 weeks, rats were fed one of the two diet regiments (Table 1) *ad libitum*. Freeze dried Rhodiola extract was supplemented at the level of 300 mg per kg body weight of rats. The level of Rhodiola extract of 300 mg per kg body weight were selected on the basis of the experiment of Ryu *et al.*,[16] in which 200 mg/kg Rhodiola methanol extract significantly reduced malondialdehyde formations in liver microsomes from rats treated with CCl_4, and the concentration was increased to 300 mg considering water extraction procedure used in this study. The diet also contained 20.5 g of oxidized linoleic acid per kg basis. The diets were stored at −40℃ before use.

Table 1. Composition of the experimental diets[1] (g/kg)

Ingredients	
Corn starch	558.5
Sucrose	100.0
Soybean oil	41.0

1) Other ingredients are casein, 200: L-cysteine, 3.0: α-cellulose, 50: choline bitartrate, 2.5: tert-butylhydroquinone, 0.014: AIN Modified 93G salt mix[2], 35.0: Modified AIN 93G vitamin mix[3] 10.0
2) AIN 93G salt mix (g/kg): calcium carbonate, 357.0: potassium phosphate monobasic, 196.0: potassium citrate, 70.78: sodium chloride, 74.0: potassium sulfate, 46.6: magnesium oxide, 24.4: ferric citrate, 6.08: zinc carbonate, 1.65: manganous carbonate, 0.63: cupric carbonate, 0.3: potassium iodate, 0.01: sodium selenate, 0.01025: ammonium paramolybdate, 0.00795: chromium potassium sulfate, 0.275: sodium meta-silicate, 1.45: powdered sucrose, 221.2268
3) AIN 93G vitamin mix (g/kg): nicotinic acid, 3.0: calcium pantothenate, 1.6: pyridoxine hydrochloride, 0.7: thiamin hydrochloride, 0.6: riboflavin, 0.6: D-biotin, 0.02: folic acid, 0.2: vitamin B_{12}, 0.025: α-tocopherol acetate, 15.0: retinyl acetate, 0.8: vitamin D_3, 0.25: vitamin K, 0.075: powdered sucrose, 974.655

3. Preparation of Rhodiola extract

Rhodiola extract was prepared by the following method. Rhodiola roots were obtained from Yunbyun, China. One hundred grams of washed and sliced Rhodiola roots were extracted for three hours with 1,000 ml water at 80℃ total of two times and freeze-dried, and the extraction yield was 10%.

4. Preparation of oxidized linoleic acid

One hundred gram of linoleic acid were maintained at 25℃ for 3hr to have a peroxide value of 30 meq/kg. The resultant oxdized linoleic acid was stored at −20℃ until the diets were prepared.

5. Sample preparation for lipid analysis, TBARS contents, and enzyme activity measurements

After fasting 14 hr, rats were anesthetized and blood from the portal vein was collected into heparinized tubes. Plasma was prepared by centrifuging the blood at 600×g for 15 min 4℃, and stored at −80℃ for analysis. Feces were collected for three days of later part of the experiemental period and dried at 60℃ until the constant weights were obtained. The fecal lipid for cholesterol determination was extracted with 1 : 2 choloform and methanol. Liver was removed promptly, blotted and weighed, and a one g/L liver homogenate was prepared in ice-cold 10 mmol sucrose EDTA Hepes buffer (0.25 mol sucrose, 1 mmol EDTA, 1 mmol $MgCl_2$, pH 7.4). The homogenates were centrifuged at 12,000 × g for 10min, and then the supernatant portion was centrifuged at 105,000 × g for 1 hr 4℃. The resulted pellet contained the microsomes, whereas the supernatant represented the cytosol. The microsomal pellet was suspended in 1 ml of ice-cold phos-

phate buffered saline. The cytosolic and microsomal fractions were then stored at −80℃ until analysis.

6. Plasma, liver, total body lipid analyses, and TBARS measurement

Plasma total cholesterol and fecal cholesterol contents were measured enzymatically according to the methods used previously.[22] Plasma HDL cholesterol was measured enzymatically after precipitation of apolipoprotein B containing lipoproteins with dextran sulfate according to the precipitation method of Sjoblom and Eklund.[23] Total liver lipids were determined using the Folch gravimetric method,[24] after extracting the portions of liver homogenates with a 2 : 1 (v/v) chloroform:methanol mixture.

TBARS contents. The thiobarbituric acid test was used to assay the lipid peroxide content of microsomal fraction according to the modified method of Masugi and Nakamura.[25] 1,1,3,3-tetraethoxypropane was used as a standard.

7. Enzyme activity and protein measurements

CuZnSOD activities of cytosol fractions were determined on the basis of its inhibitory action on the rate of superoxide formation of xanthine: xanthine oxidase by the modified method described by Oyanagui.[26] MnSOD activities of microsomal fractions were measured with the same method as CuZnSOD determination in the presence of KCN to inhibit CuZnSOD activities. Catalase activities were determined following H_2O_2 reduction at 240 nm.[27] Protein concentration was determined by the Bio-rad assay using bovine serum albumin as a protein standard.

8. Statistical analyses

Data analysis of estimation of means and standard error of means for each of the groups was carried out with the Statistical Analysis System (SAS).[28] Statistically significant differences between two group means were evaluated primarily by Student's *t*-test.

RESULTS

1. Final weight, food efficiency ration (FER) and organ weights

Final body weights and food efficiency ratio, and organ weights of animals fed experimental diets are shown in Table 2. The final body weight after feeding 4 week period was significantly higher in Rhodiola supplemented group compared to the non-supplemented control group. Food efficiency ratio of the Rhodiola extract supplemented group was higher than that fed the control diet. Among the organ weights of the two groups, liver and kidney showed the heavier trend in weights of rats supplemented with Rhodiola.

2. Liver antioxidative status

The free radical scavenging effect of Rhodiola extract was evaluated in rats (Table 3). The effect on the accumulation of TBARS was observed from liver microsomal fractions of the rats fed highly oxidized linoleic acid, and the administration of this extract affected TBARS formation.

Rhodiola feeding resulted in the decreased activities of MnSOD and increased activities of catalase in liver microsomes. The activities of CuZnSOD in the cytosolic fraction

Table 2. Body weight, food efficiency ratio (FER), organ weights of rats fed an oxidized linoleic acid diet without or with supplementation of Rhodiola extracts for 4 wk

	Control	Rhodiola supplemented
Initial body weight (g)	180.1 ± 4.24[1)]	181.7 ± 4.50
Final body weight (g)	262.6 ± 13.40	278.10 ± 9.80*
FER	0.24 ± 0.14	0.33 ± 0.03*
Liver (g)	8.12 ± 0.76	8.52 ± 0.50*
Heart (g)	1.03 ± 0.06	1.10 ± 0.05
Lung (g)	1.82 ± 0.18	1.67 ± 0.13
Kidney (g)	1.93 ± 0.06	2.16 ± 0.05*
Spleen(g)	0.71 ± 0.07	0.76 ± 0.07
Testis (g)	3.36 ± 0.14	3.38 ± 0.15
Epididymal fat pad (g)	3.50 ± 0.44	3.73 ± 0.34

1) Data are expresses as means and standard errors of the mean, and values in a row with the star marks are significantly different ($p < 0.05$), otherwise statistically not different (NS).

Table 3. Thiobarbituric reactive substance (TABRS) contents in liver microsomal fractions, CopperZinc superoxide dismutase (CuZn SOD) activites in liver cytosol fractions, Manganese superoxide dismutase (Mn-SOD) and catalase activities in liver microsomal fractions of rats fed an oxidized linoleic acid diet without or with supplementation of Rhodiola extracts for 4 wk

	Contol	Rhodiola supplemented
TBARS, nmol/mg protein	2.33 ± 0.13[1)]	2.03 ± 0.16*
CuZnSOD, U/mg protein	151.77 ± 21.71	155.25 ± 18.55
MnSOD, U/mg protein	27.86 ± 6.68	15.71 ± 2.95*
Catalase, U/mg protein	88.05 ± 0.12	96.40 ± 8.90*

1) Data are expresses as means and standard errors of the mean, and values in a row with the star marks are significantly different ($p < 0.05$), otherwise statistically are not different (NS).

Table 4. Plasma cholesterol concentrations, liver cholesterol and fecal cholesterol contents of rats fed an oxidized linoleic acid diet without or with supplementation of Rhodiola extracts for 4 wk

	Control	Rhodiola supplemented
Plasma cholesterol (mg/dl)		
Total	90.00 ± 6.51[1)]	83.78 ± 3.44*
HDL	42.00 ± 5.46	46.22 ± 5.66
Liver cholesterol (mg/g dry weight)	1.84 ± 0.26	2.35 ± 0.37
Fecal cholesterol (mg/g dry weight)	0.82 ± 0.18	1.26 ± 0.25*

1) Data are expresses as means and standard errors of the mean, and values in a row with star marks are significantly different ($p < 0.05$), otherwise statistically are not different (NS).

were not changed in Rhodiola extract supplemented group.

3. Effect on lipid contents

The effects of dietary treatment on plasma total and HDL cholesterol, plasma triacylglycerol, liver and fecal cholesterol contents are presented in Table 4. Supplementing Rhodiola extract lowered the total plasma cholesterol concentrations. This result suggests that Rhodiola extract exerts a hypocholesterolemic effect in rats. Plasma HDL cholesterol levels were not affected by supplementing Rhodiola extract. Although liver cholesterol concentrations were not changed by Rhodiola extract, the fecal cholesterol excretion was significantly elevated by the addition of Rhodiola.

DISCUSSION

Supplementing Rhodiola significantly reduced TBARS formation in liver of the rats fed highly oxidized linoleic acid. A significant elevation of TBARS in the tissues of the animals were observed by feeding oxidized frying oil and this could be reversed by increasing dietary vitamin C levels up to 1500 mg/kg diet.[18] It has been shown that among the organs studied, the liver and kidney were affected most by oxidized oil, and the higher concentrations of lipid peroxides were observed only in liver and kidney.[29] Since liver and kidney are the organs carrying out important functions such as membrane bound mixed function oxidase functions, the possible alteration of membrane lipid peroxidation status may lead to adverse effect. Also, it has been reported that free radicals from dietary oxidized linoleic acid may result in impairment of membrane function of vital organs, since it alters membrane fluidity.[30]

In this study, body weight gain was significantly increased by supplementation of Rhodiola, and this may indicate that the growth retardation exhibited by the oxidative stress could be reversed by feeding antioxidant such as Rhodiola. In the study of Hochgraf *et al.*[18] the severe growth retardation was observed by feeding oxidized linoleic acid. Increased FER of the Rhodiola group shown in this study appeared to be resulted from the increase in body weight gain. The weights of liver and kidney of Rhodiola supplemented group were significantly higher, and it can be speculated that less accumulation of peroxides in liver and kidney might be involved in the normal growth of these two organs.

Major dietary flavonoids such as quercetin or catchin have shown to be protective against the increased peroxide formation induced by feeding polyunsaturated fatty acids.[31] Present study indicates that Rhodiola can protect liver from peroxide caused damage of liver. In the previous study,[32] persimmon leaf extract increased the activities of catalase in rats fed high-fat or high-sucrose diets. It appears that Rhodiola elevates the antioxidant status of rats fed oxidized linoleic acid as determined by the increase in catalase activities. However, the decrease in MnSOD may not necessarily be related to the decrease in the anti-oxidant capacity, since it has been shown that in the hearts of manganese-deficient rats, the decrease in MnSOD could be compensated by catalase or CuZnSOD.[33]

Elevated levels of plasma cholesterol in rats subjected to adriamycin induced oxidative stress has been reported by Huerta *et al.*[34] It was proposed that the high plasma cholesterol was resulted from enhanced hepatic cholesterol biosynthesis. However, it has not been shown that antioxidant could reduce the elevated cholesterol levels. In this study, Rhodiola lowered plasma cholesterol and increased fecal cholesterol contents. This indicates that Rhodiola extract may play an important role in lowering blood cholesterol concentrations by increased excretion of cholesterol through the feces. Generally, there are several mechanisms proposed for the flavonoids to lower serum cholesterol. Suppression of hepatic cholesterol synthesis has been suggested as one of them.[35] However, for Rhodiola, this mechanism may not apply, since there were no differences in the total liver cholesterol concentrations. The increased fecal loss of sterol and bile acids might be the possible explanation of cholesterol-lowering mechanism in this study. The increase in fecal cholesterol excretion in rats fed Rhodiola extract might be caused by a reduction in cholesterol absorption. The reduced cholesterol absorption could have been resulted in higher cholesterol catabolism in the liver, which leads to lower plasma cholesterol concentration. Kurowska *et al.* reported that citrus juices induce the increase in fecal cholesterol excretion in rabbits.[36]

In other studies[37,38] on lipid-lowering effect of green tea, black tea, jasmine tea, oolung tea mostly in hyerlipidemic conditions, it has been suggested that flavonoids including gallate compounds account for their hypocholesterolemic effect. Further studies are needed to isolate the active components in Rhodiola extract having this effect.

The relationship between dietary oxidized fatty acids and atherogenesis focused on the oxidized LDL (low density lipoprotein). It has been shown that oxidized fatty acids can be absorbed by the intestine and incorporated into lipoproteins and could potentially impose an oxidative

stress and exacerbate atherogenesis.[39]

This experiment was conducted in order to elucidate the effect of Rhodiola extract on antioxidant properties and cholesterol-lowering action in rats fed highly oxidized linoleic acid. The antioxidant capacity of Rhodiola extract was shown in the decreased TBARS concentrations and increased activities of catalase in the microsomal fraction of liver. It has been shown that plasma cholesterol has been decreased by Rhodiola possibly through the increase in fecal cholesterol excretion.

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