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Comparison of Protective Effects Between Oat β-Glucan and Phenol-Rich Extracts in Hyperlipidemic ICR Mice

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ABSTRACT

β-Glucan in oat is widely considered as the main ingredient for the anti-hypercholesterol effects; however, phenolic compounds in oat are also regarded to possess potential activities against dyslipidemia. In order to understand the underlying mechanisms thoroughly, it is of great significance to evaluate and compare the hypolipidemic properties exerted by oat phenol-rich extract (PE) and β-glucan extract (GE) in hyperlipidemic ICR mice induced by high-fat diet. The study was conducted for 12 weeks. Both PE and GE showed a significant hypocholesterolemic action by reducing the concentration of serum total cholesterol (TC), low density lipoprotein cholesterol and hepatic TC. Only PE reduced the hepatic triacylglycerol (TG), inhibited hepatic 3-hydroxy-3-methylglutary CoA reductase activities and improved the hepatic antioxidant defense system. However, GE significantly increased fecal excretion of neutral cholesterol and bile acids. This study demonstrated that not only β-glucan but also oat phenolic acids contributed to a preventive effect on hyperlipidemia. Based on the cholesterol metabolism and antioxidant defense system, the effects of the two functional components of oat were exerted in different ways.

Key words: Oat, β-glucan, Phenolic acids, Hyperlipidemia, HMG-CoA reductase

INTRODUCTION

Hyperlipidemia, resulted from the metabolic changes of lipid, is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as coronary heart diseases (CHD), ischemic cerebrovascular diseases and peripheral vascular diseases. The control of serum cholesterol or low density lipoprotein (LDL) levels through non-pharmacological treatments or drug administration has been shown to reduce the risk of CHD [(1)].

Results of several studies showed that oat and oat bran contained factors which could reduce the levels of serum total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) both in animals and humans[(2-4)]. The Food and Drug Administration of the USA has approved health claims of the benefit of using foods with oats for CHD[(5)]. It is accepted that the soluble fiber fraction mainly β-glucan, is the primary functional ingredient in oat[(6)]. The most plausible cholesterol-lowering mechanism of oat β-glucan is increasing the viscosity of the gastric and intestinal contents, hence interfering with reabsorption of bile acids and increasing the fecal excretion[(7)]. In addition, oat contains a wide range of phenolic compounds[(8)]. A number of studies have identified the phenolic compounds in oats, such as ferulic acid, caffeic acid, *p*-coumaric acid, sinapic acid, vanillic, and syringic[(9-11)]. Collins isolated and characterized a group of low molecular weight soluble phenolic compounds, named avenanthramides (AVEN), which are not present in other cereal grains[(12)]. These oat phenolics exhibit antioxidant capacity both *in vitro* and *in vivo*[(13-15)]. Chen found oat phenolics inhibited LDL oxidation *in vivo*, so being potential to prevent the important first stage in the development of atherosclerosis[(15)]. Recently studies have reported the antiatherogenic potential of AVEN through inhibition of adhesion of monocytes to human aortic endothelial cell monolayers and serum-induced proliferation of vascular smooth muscle proliferation, both of which are important processes in the

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initiation and development of atherosclerosis[16,17]. Among various plant phenolic compounds, those from plant foods, such as tea, red wine and olive oil were reported to regulate the lipids metabolism *in vivo*[18-20]. Similar to their actions in other foods, simple phenolic acids and polyphenolics from oats may serve as potential functional components on the prevention of hyperlipidemia.

To date, few studies have explored directly the function of oat phenolic compounds for hyperlipidemia and atherosclerosis *in vivo*, and the comparison of the beneficial effects of oat β-glucan and oat antioxidants *in vivo* is still lack. Therefore, to fill this knowledge gap, this study was conducted with the following goals: 1) to investigate the effects of phenolic compounds extracted from oat in high-fat diet-induced hyperlipidemic ICR mice; 2) to compare the prevention function and mechanism of β-glucan and oat phenolic compounds for hyperlipidemia from the aspects of cholesterol metabolism and antioxidant defense system.

MATERIALS AND METHODS

I. *Preparation of Oat β-glucan and Phenol-rich Extracts*

(I) *β-Glucan extract (GE)*

Oat was obtained from Zhangjiakou, Hebei Province, China. β-Glucan was extracted from oat by a process of Aman and Hesselman[21]. In brief, ground oat flour was mixed with boiling water and bacterial α-amylase to extensively hydrolyze starch, solubilize β-glucan and inactivate contaminating β-glucanase activities. Protease (papain) was used to hydrolyze protein in addition to the starch hydrolysis step. Solubilized β-glucan in the clarified liquid fraction was precipitated, washed with ethanol and lyophilized. About 9.53 g GE was obtained from 100 g oat grounds. Analysis of the β-glucan preparation is presented in Table 1. β-Glucan was quantified enzymatically[22] using a Megazyme β-glucan mixed linkage assay. Protein concentration, moisture and fat concentration were determined by AOAC methods 991.20, 926.08, and 933.05, respectively[27]. Ash concentration was determined by Corn Refiners Association Standard Analytical Method A-4[28].

(II) *Phenol-rich Extract*

Phenol-rich extract was prepared by the process similar to that of Chen[15] with slight modification. The hulless oats were ground and extracted twice with ethanol : water (4 : 1) up to 1.0 L for 2 h at 40°C with continuous agitation. After cooling, the extraction slurry was centrifuged at 1,250 ×g for 15 min. The supernatant was vacuum rotary evaporated at 45°C and lyophilized to an oat phenol-rich powder and stored at -20°C until use. About 2.41 g PE was obtained from 100 g oat grounds. Folin-Ciocalteu method[23] was used to measure the total phenolic content with a UV-visible spectrometer (GBC scientific equipment Pty Ltd, Australia). The total polyphenolic content in the oat phenol-rich powder was 35.2 mg (207 μmol) gallic acid equivalents/g.

II. *Animals and Treatment*

Forty 6-week-old male ICR mice (Experiment Animal Center of Beijing) were fed for 12 weeks after acclimatization for 7 days. Animals were allocated five per polycarbonate cage in a temperature（23 ± 1°C）and humidity (55 ± 5%) controlled room, with 12-hr light and dark cycles. The ICR mice were randomly divided into four groups: normal diet group (ND), high-fat diet (HFD) group, β-glucan extract (GE) treatment group, and phenol-rich extraction (PE) treatment group. The ND mice received a commercial regular diet, the HFD group and treatment groups were given high-fat diet (HFD) (shown in Table 2, provided by Experiment Animal Center of Beijing). The estimated daily polyphenolic and β-glucan intake for a 70 kg body person is 14 mg/kg[24]

Table 1. Approximate composition of β-glucan concentrates from oats

Composition	Content (%)
Carbohydrates	81.61
β-Glucan	71.68
Protein	9.67
Moisture	5.35
Fat	1.21
Ash	2.16

Table 2. Chemical composition of fed diets (g/100 g d.w.)

Ingredients	Content	
	RD	CED
Carbohydrate	59	48
Protein	21.1	21.1
Fiber	4.9	4.9
Fat	4.2	4.2
Ash	8	8
Sodium cholic acid	–	0.2
Cholesterol	–	1
Lard	–	9.8
P	1	1
Ca	1.8	1.8

Note: RD, regular diet; CED, cholesterol-enriched diet.

and 50 mg/kg[6], respectively, which indicated a suggested daily intake ratio of these two compounds of 14 : 50. The dosage for the animal models should be 10-times greater than the human intake, due to the different metabolic rate between mice and human. Therefore, we chose the dose of 140 mg/kg for PE and 500 mg/kg for GE in present study. Both extracts were delivered in distilled water and administered at a dose of 10 mL/kg by oral gavage. Equal volume of distilled water was given to mice in ND and HFD groups.

The animals were given free access to diets and distilled water throughout the experimental period. Weight gain was measured every three days. At weeks 3, 6, 9, 12, mice were fasted overnight (12 hr), blood samples from mice were obtained by peri-orbital bleeding and centrifuged at 3,000 rpm for 8 min under room temperature and stored at -20°C for future analysis. Feces were collected during the last 3 days using metabolic cages and used for determination of the fecal sterol and bile acids. At the end of the experiment period and blood sampling, the animals were sacrificed by cervical dislocation. The livers were excised, weighed and stored at -80°C until analysis.

III. *Serum and Hepatic Lipids*

Serum lipid parameters, including TC, triacylglycerol (TG), LDL-C, and high density lipoprotein-cholesterol (HDL-C) were assayed individually using the enzymatic kits (Beijing Zhongsheng Hightech Bioengineering Company, Beijing, China) on an Alcyon 300 auto-analyzer (Abbott Laboratories Ltd., US). The atherogenic index (AI) was calculated as (TC-HDL-C) / HDL-C. Hepatic lipids were extracted from 300 mg liver of a mouse with chloroform : methanol (2 : 1, v/v), according to the method of Folch *et al*[25]. After extraction, 1 mL of extract was dried under nitrogen stream. The dried lipid residues were dissolved in 500 μL distilled water by sonication for 5 min. Triton X-100 was added to the dissolved lipid solution to produce final concentration of 5 g/L. The hepatic TC and TG were analyzed with the same enzymatic kits as above in the serum analysis.

IV. *Hepatic Antioxidant Indices*

The total antioxidant capacity (TAC), superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration were determined in liver homogenates with commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TAC was measured by the reaction of phenanthroline and Fe^{2+} in a spectrophotometer at 520 nm and a TAC unit is defined as the amount of antioxidants required to make the absorbance increase 0.01 in 1 mL serum. For SOD measurement, superoxide radicals were generated by NADPH and PMS (phenazine methosulfate) under non-acidic conditions which reduces NBT (nitroblue tetrazolium salt) and forms a blue coloured formazon whose absorbance can be measured at 560 nm. Free radical damage was determined by specially measuring MDA. MDA formed as an end product of lipid peroxidation was treated with thiobarbituric acid to generate a colored product that was measured at 532 nm.

V. *Hepatic 3-hydroxy-3-methylglutary CoA (HMG-CoA) Reductase Activities*

The activity of HMG-CoA reductase in mice was determined by the method of Edwards *et al*[26]. The isolated liver microsomes were mixed with an equal volume of 50% glycerol in phosphate buffer (0.1 M sucrose, 0.05 M KCl, 0.04 M potasium phosphate, 0.03 M potassium EDTA and 10 mM DTT, pH 7.2). The mixture was homogenized and incubated at 37°C for 60 min to solubilize HMG.-CoA reductase. The activity of the solubilized HMG-CoA reductase was assayed in phosphate buffer (0.2 M KCl, 0.16 M potassium phosphate, 0.004 M EDTA, and 0.01 DTT, pH 6.8) together with 0.2 mM NADPH and 0.1 mM *RS*-HMG-CoA at 37°C in a spectrophotometer. The results were expressed as nmol mevalonate synthesized per min per mg protein.

VI. *Determination of Fecal Neutral Cholesterol and Bile Acids*

Fecal samples (0.2 g) were homogenized with 2 mL of a mixture of 10 M NaOH and 96% ethanol (1 : 2, v/v) and kept at 70°C for 45 min. The samples were allowed to cool to room temperature followed by centrifugation at 1,000 ×g for 5 min. The supernatant was removed and extracted with n-hexane twice. The combined hexane phases were washed with 70% ethanol until neutral for neutral cholesterol analysis; the lower phases were acidified with HCl and extracted with chloroform:methanol (2 : 1, v/v) for bile acids analysis. The cholesterol and coprostanol was analyzed using a Shimadzu (Shimadzu, Kyoto, Japan) GC-14 gas chromatography system equipped with a flame ionization detector and a 30 m × 0.25 mm DM-5 capillary column maintained at 290°C using nitrogen gas as carrier. The initial column temperature was 220°C and was elevated to 300°C at a rate of 2°C/min. The final temperature was held for 10 min. The flow rate was 1.5 mL/min. Pure cholesterol and coprostanol (Sigma-Aldrich, St. Louis, MO) were used as standards. Total neutral cholesterol was expressed as percent of total fecal cholesterol plus coprostanol. Fecal acidic steroids were determined using an enzymatic kit (Total Bile Acid Kit, Wako, Japan).

VII. *Statistical Analysis*

Data are presented as means ± standard deviation (SD). SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) was used to analyze the variance homogeneity (one-way

ANOVA analysis). Means were compared using the Duncan test at the 95% significance level.

RESULTS

I. *Body Growth and Organ Weights*

Weight gains and relative organ weights are listed in Table 3. Although there was no significant difference among all groups in weight gains, the relative liver weights of PE group were significantly ($P < 0.05$) lower than that of HFD group.

II. *Serum Lipids and Atherogenic Index Profiles*

Serum lipid profiles are listed in Figure 1. It was evident that TC levels in the HFD feeding increased significantly ($p < 0.05$) by 68.95% at week 3 compared with ND group, and this trend sustained until the end of

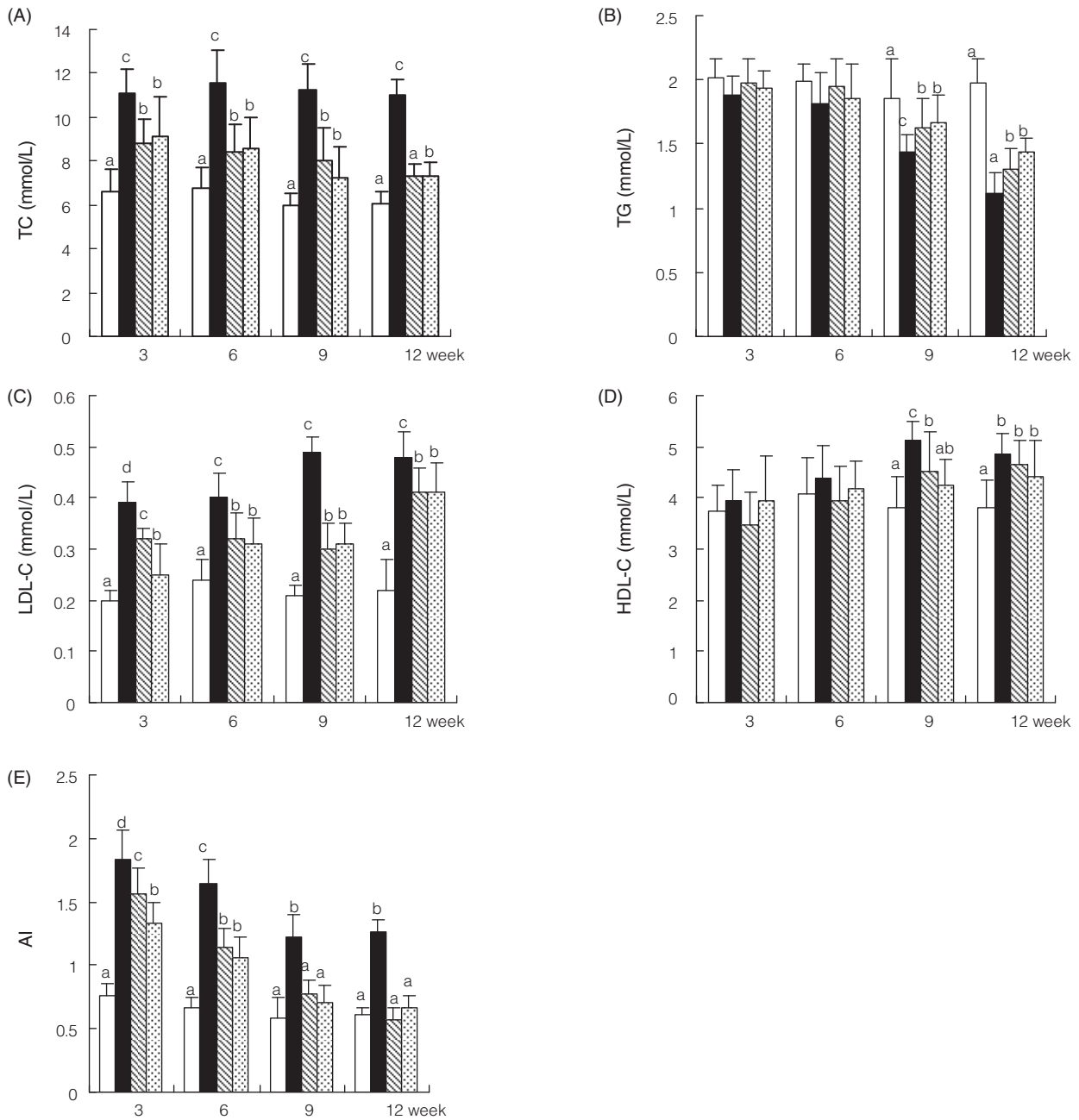

Figure 1. Serum levels of lipids in each group. (A) total cholesterol (TC), (B) triacylglycerol (TG), (C) low density cholesterol (LDL-C), (D) high density cholesterol, (E) atherogenic index(AI, calculated by: (TCHDL-C)/HDL-C). □, normal diet group; ■, high-fat diet group; ▨, β-glucan extract (GE) treated group; ⊡, phenol-rich extract (PE) treatment groups. Values are expressed as mean ± SD (n = 10). Values not sharing the same letter determined at the same period are significantly different at $p < 0.05$.

Table 3. Body weight gain and liver weight (g/body weight) in control and treatment groups

Treatment	Body weight			Liver weight	
	Initial (g)	Final (g)	Weight gain (g)	Total (g)	g/100 g body weight
ND	26.38 ± 2.00	35.61 ± 5.69	9.65 ± 5.16	1.41 ± 0.25^{a}	3.95 ± 0.18^{a}
HFD	26.30 ± 1.83	38.26 ± 2.25	11.81 ± 1.93	1.62 ± 0.10^{b}	4.23 ± 0.08^{b}
GE	26.31 ± 1.52	38.82 ± 4.95	12.51 ± 3.72	1.59 ± 0.21^{ab}	4.10 ± 0.22^{ab}
PE	26.31 ± 1.48	37.49 ± 2.80	11.47 ± 2.68	1.48 ± 0.15^{ab}	3.96 ± 0.22^{a}

* ND, normal diet group; HFD, high-fat diet group; GE, β-glucan extract treatment group; PE, phenol-rich extract treatment group. Data are presented as mean ± SD (n = 10). Values in a line with different superscript are significant different at $p < 0.05$.

Table 4. Hepatic lipid profile in each group

Hepatic lipid	ND	HFD	GE	PE
Cholesterol (μmol/g liver)	5.50 ± 1.23^{a}	18.6 ± 1.59^{c}	12.90 ± 1.34^{b}	13.53 ± 2.27^{b}
Triacylglycerol (μmol/g liver)	44.97 ± 3.25^{a}	71.77 ± 6.93^{c}	72.57 ± 6.58^{c}	52.93 ± 7.36^{b}

* ND, normal diet group; HFD, high-fat diet group; GE, β-glucan extract treatment group; PE, phenol-rich extract treatment group. Data are presented as mean ± SD (n = 10). Values in a row with different superscript are significant different at $p < 0.05$.

this experiment. Compared with HFD group, serum TC content in GE and PE significantly ($p < 0.05$) reduced by 33.9% and 33.5% at the end of experiment (week 12), respectively; yet no significant difference was observed between GE and PE group.

No difference was observed in serum TG levels across all groups at week 3 and 6. Subsequently, at week 9 and 12, serum TG levels in HFD group significantly ($p < 0.05$) decreased to 77.42% and 56.35% of ND group, respectively. The serum TG levels in both GE and PE group decreased significantly ($p < 0.05$), though mild, as compared with HFD group. TG levels for GE and PE groups increased to 87.63%, 89.25% and 65.99%, 73.10% of ND group at week 9 and 12, respectively. A significant increase of serum LDL-C levels as 118.18% was detected in HFD group compared with ND group. Dramatic decreases of LDL-C levels were observed in both GE and PE group compared with HFD group. PE group had a better ($p < 0.05$) LDL-C lowering effect than GE group at the beginning of the experiment, with reduction to 82.05% vs. 64.1% of HFD group at week 3. However, at the end of the experiment, PE and GE reduced LDL-C to the similar level. Although the HDL-C level did not differ significantly across groups during the first 6 weeks, 28.16% increase of serum HDL-C was detected at the end of the experiment (week 12) in HFD group compared with ND group ($p < 0.05$). Neither GE nor PE group showed HDL-C-elevating effects compared with HFD group. GE and PE group significantly ($p < 0.05$) decreased AI by 55.1% and 48.0% respectively compared with HFD group.

III. *Hepatic lipid profiles*

Data are listed in Table 4. Hepatic TC and TG levels were significantly ($p < 0.05$) elevated by HFD. However, TC contents in GE and PE group significantly ($p < 0.05$) decreased in the whole liver by 30.65% and 27.30%, respectively. TG levels did not differ significantly between GE and HFD group, but it decreased significantly ($p < 0.05$) in PE group by 26.3% compared with HFD group.

IV. *Hepatic TAC, SOD Activity and MDA Levels*

The profile of antioxidative status is presented in Table 5. There was a decrease in the activities of hepatic TAC and SOD ($p < 0.05$) in HFD group compared with ND group. Administration of PE brought about a significant improvement ($p < 0.05$) in antioxidant defenses of HFD-fed mice, and GE seemed to have no effect on TAC and SOD. Hepatic MDA levels increased significantly in the HFD group compared with ND group. Administration of PE restored MDA concentration to ND levels significantly ($p < 0.05$), while GE group failed to diminish lipid peroxidation, showing MDA values similar to that of HFD group.

V. *Hepatic HMG-CoA Reductase Activities*

Compared with ND group, hepatic HMG-CoA reductase activity was decreased in HFD group (Table 5). Administration of PE slightly ($p < 0.05$) decreased

Table 5. Hepatic antioxidant status and HMG-CoA reductase activit

	ND	HFD	GE	PE
Hepatic antioxidant status				
TAC (U/mg protein)	1.91 ± 0.18[a]	1.03 ± 0.19[c]	1.00 ± 0.11[c]	1.44 ± 0.08[b]
SOD (U/mg protein)	175.09 ± 0.53[a]	77.89 ± 5.90[c]	94.03 ± 6.50[c]	118.36 ± 6.90[b]
MDA (nmol/mg protein)	0.75 ± 0.07[a]	1.54 ± 0.09[c]	1.52 ± 0.06[c]	0.96 ± 0.06[b]
Hepatic HMG-CoA reductase (nmol/min/mg protein)	6.71 ± 0.18[a]	5.37 ± 0.33[c]	5.05 ± 0.33[bc]	4.77 ± 0.28[b]

* ND, normal diet group; HFD, high-fat diet group; GE, β-glucan extract treatment group; PE, phenol-rich extract (PE) treatment group; TAC, total antioxidant capacity; SOD, superoxide dismutase; MDA, malondialdehyde; HMG-CoA, 3-hydroxy-3-methylglutary CoA reductase. Data are presented as mean ± SD (n = 10). Values in a row with different superscript are significant different at $p < 0.05$.

Table 6. Fecal neutral sterol and total bile acids concentrations in each group

Components	ND	HFD	GE	PE
Neutral steroids (mg/d)				
Cholesterol	0.20 ± 0.03[a]	0.97 ± 0.05[b]	1.24 ± 0.10[c]	0.95 ± 0.08[b]
Coprostanol	0.52 ± 0.07[a]	1.30 ± 0.25[b]	2.62 ± 0.35[c]	1.41 ± 0.24[b]
Total	0.72 ± 0.06[a]	2.27 ± 0.26[b]	3.86 ± 0.40[c]	2.36 ± 0.35[b]
Total bile acids (mg/d)	0.48 ± 0.03[a]	1.51 ± 0.10[b]	1.89 ± 0.12[c]	1.54 ± 0.10[b]

* ND, normal diet group; HFD, high-fat diet control group; GE, β-glucan extract treatment group; PE, phenol-rich extract treatment group. Data are presented as mean ± SD (n = 10). Means within the same row without a common letter differ significantly at $p < 0.05$.

HMG-CoA reductase activity compared with HFD group, but GE group did not modify its activity significantly.

VI. *Fecal Excretion of Neutral Cholesterol and Bile Acids*

Fecal neutral sterol and total bile acids excretion data are shown in Table 6. HFD group excreted significantly more fecal neutral sterols and total bile acids than ND group. Compared with HFD group, GE group significantly increased fecal sterol and bile acids excretion by 58.0% and 25.2%, but PE group did not show such an effect.

DISCUSSION

High serum TC and LDL-C are the main risk factors in the pathogenesis of CHD[1]. The results of this investigation showed that HFD intake led to an increase of cholesterol content both in serum and liver. In our study, serum HDL-C levels increased significantly in HFD group compared with ND group too. This HDL-C elevating effect in hyperlipidemic ICR mice may result from the stress reaction caused by high-fat diet *in vivo*, and similar observations were also reported in other researches[30-35]. Both PE and GE had a distinct cholesterol-lowering effect by decreasing serum TC, serum LDL-C and hepatic TC, accordingly reducing the risk of CHD. Similar cholesterol-lowering results have been previously reported in β-glucan[3,4] and many polyphenols from different plant foods[18-20]. For serum LDL-C at the beginning of the experiment, oat phenolic compounds may have a better LDL-C-lowering effect than β-glucan in the initial stage of hyperlipidemia; but for long term, their modification on LDL-C are similar. AI (TC-HDL)/HDL) has direct correlation with the cardiovascular disease. Both PE and GE were able to decrease AI significantly, contributing to the low risk in pathology of hyperlipidemia and atherosclerosis.

The rise in cholesterol in liver and serum may be due to increased uptake of exogenous cholesterol and subsequent deposition and decreased catabolism of cholesterol to bile acids[36]. To elucidate the mechanism of the TC-lowering action of PE and GE, we examined the hepatic HMG-CoA reductase activity and fecal cholesterol and bile acids excretion. HMG-CoA reductase is the rate-limiting enzyme in the cholesterol biosynthetic pathway. The inhibition of HMG-CoA reductase suppresses cholesterol synthesis and is very effective

in lowering serum cholesterol[37]. High cholesterol diet itself inhibited HMG-CoA reductase activity in our study for the feedback regulation of cholesterol, which was also illustrated by Hayashi *et al*[38]. Previous studies demonstrated that polyphenols extract from virgin olive oils[20] and naringenin[39], a kind of phenolic compound, suppresses HMG-CoA reductase activity in liver microsome. Similarly, PE group significantly reduced the activity of HMG-CoA reductase compared with HFD group, suggesting the hypocholesterolemic effects of oat phenolic compounds by suppressing the rate of cholesterol synthesis. Although β-glucan might be subjected to microbial degradation in the lower gut to propionic acid, an inhibitor of HMG-CoA reductase[40], GE group was observed to have no obvious effect on HMG-CoA reductase. However, GE group enhanced the excretion of neutral steroids and bile acids, indicating the hypocholesterolemic effect of GE by decreasing intestinal cholesterol absorption and bile acids reabsorption for its viscosity property as reported in oat bran and dietary fiber[7,41]. Accelerated rate of catabolism of cholesterol to bile acids is also related to enhance fecal bile acids excretion, which would be another mechanism of the hypocholesterolemic effect of GE, but further study is needed to prove it.

Several reports have demonstrated increased serum TG by HFD[29]; however, in this study HFD reduced serum TG content in the end of the experiment, associating with an increase in hepatic TG. This observation was accordant with the previous research by Pan *et al*.[42], who proved that high-fat diet containing cholesterol and bile salt (called as cholic acid in current study) could contribute to the imbalanced serum TG level in ICR mice. In current study, the high-fat diet contained both cholic acid and cholesterol (Table 2). Also, our lab has recently tested the hypothesis that the reduction of serum TG is associated with the elevation of hepatic TG, resulting in ectopic accumulation in liver and leading to the progression of fatter liver and hepatic steatosis[32,34]. Hepatic TG content is regulated by TG biosynthesis, lipoprotein secretion to the bloodstream, and degradation of fatty acid by β-oxidation[43]. It was reported that dietary cholesterol reduced the rate of fatty acid oxidation and increased the rate of TG biosynthesis in rats[44]. In some hyperlipidemic animal models with elevated serum TG, phenol-rich extracts[45] and β-glucan from barley[46] can regulate the serum TG level by decreasing it. Our research suggested that oat PE and GE might have the potential to dual regulate the imbalanced serum TG level by increasing serum TG levels approaching the normal levels. PE was also beneficial to decrease TG level in liver with lower relative liver weights. However, GE showed no effect on hepatic TG. It has been reported that many plant phenolic compounds such as sesamin[47], tea catechin[48], and citrus flavonoids[49] increased the hepatic fatty acid oxidation in experiment animals, the alteration of which is a crucial factor to the modification of serum and hepatic lipid levels[50]. It is possible that oat phenolic compounds could regulate the hepatic fatty acid oxidation and thus significantly affect the hepatic TG level. Further research on this mechanism is still in progress.

Oxidative stress is one of the causative factors that link hyperlipidemia with the pathogenesis of atherosclerosis[51]. Thereby it is of great importance to evaluate the antioxidant defense system *in vivo*. Although early studies demonstrated the *in vivo* antioxidant effects of oat products in different animal models, they all focused on β-glucan of the oat product[3,4,52,53] instead of other functional components. Oat phenolics have been proved to scavenge free radicals and reduce LDL oxidation *in vitro*[13,14]. Based on this study, administration of PE prevented buildup of oxidative stress by restoring enzymatic antioxidant SOD activities and TAC in liver, as well as inhibiting the augmentation of hepatic MDA. It is evident that PE exerts great antioxidant effects *in vivo*. Whereas, GE had no effect on liver TAC, SOD activity and MDA level, suggesting that oat phenolic compounds might contribute to enhance the oxidation resistance in hyperlipidemic mice.

CONCLUSIONS

Both oat β-glucan and phenolic compounds have positive effects on the prevention of hyperlipidemia by improving serum and hepatic lipids profile. Oat phenolic compounds possess a more significant effect on attenuating hepatic oxidative stress than β-glucan. β-glucan and phenolic compounds demonstrate different effects on cholesterol metabolism pathway, i.e., promoting cholesterol catabolism rate and suppressing biosynthesis rate, respectively.

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REFERENCES

1. Grundy, S. M. 1986. Cholesterol and coronary heart disease: a new era. J. Am. Med. Assoc. 256: 2849-2858.
2. Anderson, J. W., Spencer, D. B., Hamilton, C. C., Smith, S. F., Tietyen, J., Bryant, C. A. and Oeltgen, P. 1990. Oat-bran cereal lowers serum total and LDL cholesterol in hypercholesterolemic men. Am. J. Clin. Nutr. 52: 495-459.
3. Kerckhoffs, D. A. J. M., Hornstra, G. and Mensink, R. P. 2003. Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects

may decrease when beta-glucan is incorporated into bread and cookies. Am. J. Clin. Nutr. 78: 221-217.

4. Delaney, B., Nicolosi, R. J., Wilson, T. A., Carlson, T., Frazer, S., Zheng, G. H., Hess, R., Ostergren, K., Haworth, J. and Knutson, N. 2003. β-Glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters. J. Nutr. 133: 468-475.
5. FDA. 1996. Food labeling: health claims. Oats and coronary heart disease. Fed. Regist. 61: 296-337.
6. FDA. 1997. Food labeling: health claims. Soluble fiber from whole oats and risk of coronary heart disease. Fed. Regist. 62: 15343-15344.
7. Andersson, J. W. and Bridges, S. R. 1993. Hypocholesterolemic effects of oat bran in humans. In "Oat Bran". pp. 139-157. Wood, P. J. ed. American Association of Cereal Chemists Inc. St Paul, Minnesota, U. S. A.
8. Peterson, D. M. 2001. Oat antioxidants. J. Cereal Sci. 33: 115-129.
9. Daniels, D. G. H. and Martin, H. F. 1961. Isolation of a new antioxidant from oats. Nature 191: 1302.
10. Xing, Y. M. and White, P. J. 1997. Identification and function of antioxidants from oat groats and hulls. J. Am. Oil Chem. Soc. 74: 303-307.
11. Durkee, A. B. and Thivierge, P. A. 1997. Ferulic acid and other phenolic acids in oat seeds (Avena sativa L. var Hinoat). J. Food Sci. 42: 551-552.
12. Collins, F. W. 1989. Oat phenolics: avenanthramides, novel substituted N-cinnamoylanthranilate alkaloids from oat groats and hulls. J. Agric. Food Chem. 37: 60-66.
13. Gray, D. A., Clarke, M. J., Baux, C., Bunting, J. P. and Salter, A. M. 2002. Antioxidant activity of oat extracts added to human LDL particles and in free radical trapping assays. J. Cereal Sci. 36: 209-218.
14. Peterson, D. M., Emmons, C. L. and Hibbs, A. H. 2001. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. J. Cereal Sci. 33: 97-103.
15. Chen, C. Y., Milbury, P. E., Kwak, H. K., Collins, F. W., Samuel, P. and Blumberg, J. B. 2004. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. J. Nutr. 134: 1459-1466.
16. Lin, N., Mitchell, L. W., David, M. P., Mohsen, M. 2006. Avenanthramide, a polyphenol from oats, inhibits vascular smooth muscle cell proliferation and enhances nitric oxide production. Atherosclerosis 186: 260-266.
17. Liu, L. P., Zubik, L., Collins, F. W., Marko, M. and Meydani, M. 2004. The antiatherogenic potential of oat phenolic compounds. Atherosclerosis 17: 39-49.
18. Suzuki, Y., Kobayashi, M., Unno, T., Nozawa, A., Sagesaka, Y. M. and Kakuda, T. 2005. Hypolipidemic effect of tea catechins with a galloyl moiety in hamsters fed a high fat diet. J. Japan Soc. Food Sci. Technol. 52: 167-171.
19. Auger. C., Teissedre, P. L., Gerain, P., Lequeux, N., Bornet, A., Serisier, S., Besançon, P., Caporiccio, B., Cristol, J. P. and Rouanet, J. M. 2005. Dietary wine phenolics catechin, quercetin, and resveratrol efficiently protect hypercholesterolemic hamsters against aortic fatty streak accumulation. J. Agric. Food Chem. 53: 2015-2021.
20. Benkhalti, F., Prost, J., Paz, E., Perez-Jimenez, F., El-Modafar, C. and El-Boustani, E. 2002. Effects of feeding virgin olive oil or their polyphenols on lipid of rat liver. Nutr. Res. 22: 1067-1075.
21. Aman, P. and Hesselman, K. 1985. An enzymatic method for analysis of total mixed-linkage β-glucans in cereal grains. J. Cereal Sci. 3: 231-217.
22. McCleary, B. V. and Codd, R. 1991. Measurement of (1-3)(1-4)-b-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric. 55: 303-312.
22. Singleton, V. L., Joseph, A. and Rosso, J. R. 1965. Colorimetry of total phenolics with phosphomolyBdic-phosphotungstic acid Reagents. J. Sci. Food Agric. 10: 144-148.
24. Kühnau, J. 1976. The flavonoids. A class of semi-essential food components: their role in human nutrition. World Rev. Nutr. Diet 24: 117-191.
25. Folch, J., Lees, M. and Sloan-Stanley, G. H. 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.
26. Edwards, P. A., Lemongello, D. and Fogelman, A.M. 1979. Improved methods for the solubilization and assay of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A reductase. J. Lipid Res. 20: 40-46.
27. AOAC. 2000. Official Methods of Analysis of AOAC Iinternational. 17th ed. Association of Official Analytical Chemists. Gainthersburg, Maryland, U. S. A.
28. CRA. 1997. Standard Analytical Methods of the Member Companies of Corn Refiners Association. 6th ed. Corn Refiners Association Inc. Washington, District of Columbia, U. S. A.
29. Zhao, H. L., Cho, K. H., Ha, Y. W., Jeong, T. S., Lee, W. S. and Kim, Y. S. 2006. Cholesterol-lowering effect of platcodin D in hypercholesterolemic ICR mice. Eur. J. Pharmacol. 537: 166-173.
30. Chen, G., Luo, Y. C., Ji, B. P., Li, B., Guo, Y., Li, Y., Su, W. and Xiao, Z. L. 2008. Effect of polysaccharide from *auricularia auricula* on blood lipid metabolism and lipoprotein lipase activity of ICR mice fed a cholesterol-enriched diet. J. Food Sci. 73: H103-H108.
31. Luo, Y. C., Chen, G., Li, B., Ji, B. P., Guo, Y. and Tian, F. 2009. Evaluation of antioxidative and hypolipidemic properties of a novel functional diet formulation of *auricularia auricula* and hawthorn. Innov. Food Sci. Emerg. Tech. 10: 215-221.
32. Luo, Y. C., Chen, G., Li, B., Ji, B. P., Xiao, Z. L., Guo, Y. and Tian, F. 2009. Dietary intervention with AHP, a functional formula diet, improves both serum and hepatic lipids profile in dyslipidemia mice. J. Food Sci. 74: H189-H195.
33. Yang, Z. W., Ji, B. P., Zhou, F., Li, B., Luo, Y. C.,

Yang, L. and Li, T. 2009. Hypocholesterolaemic and antioxidant effects of kombucha tea in high-cholesterol fed mice. J. Sci. Food Agric. 89: 150-156.

34. Zhang, H. J., Zhou, F., Ji, B. P., Li, B., Luo, Y. C., Yu, H. Q. and Zhang, Z. G. 2009. Effects of fructose and/or fat in the diet on developing the type 2 diabetic-like syndrome in CD-1 mice. Horm. Metab. Res. 41: 40-45.
35. Lee, H. S., Ahn, H. C. and Ku, S. K. 2006. Hypolipemic effect of water extracts of Picrorrhiza rhizoma in PX-407 induced hyperlipemic ICR mouse model with hepatoprotective effects: A prevention study. J. Ethnopharmacol. 105: 380-386.
36. Jaganathan, S. N., Conor, W. E., Baker, W. and Bhattacharya, A. K. 1974. The turnover of cholesterol in human atherosclerotic arteries. J. Clinl. Invest. 54: 366-370.
37. Goldstein, J. L. and Brown, M. S. 1990. Regulation of the mevalonate pathway. Nature 343: 425-430.
38. Hayashi, T., Rani, J. A., Fukatsu, A., Matsui-Hirai, H., Osawa, M. and Miyazaki, A. 2004. A new HMG-CoA reductase inhibitor, pitavastatin remarkably retards the progression of high cholesterol induced atherosclerosis in rabbits. Atherosclerosis 176: 255-263.
39. Lee, M. K., Moon, S. S., Lee, S. E., Bok, S. H., Jeong, T. S., Park, Y. B. and Choi, M. S. 2003. Naringenin 7-O-cetyl ether as inhibitor of HMG-CoA reductase and modulator of plasma and hepatic lipids in high cholesterol-fed rats. Bioorg. Med. Chem. 11: 393-398.
40. Bartnikowska, E. and Rakowska, M. 1994. Effect of dietary fibre from oat and barley on lipid metabolism in animals and man. Biuletyn. Instytutu. Hodowli. i. Aklimatyzacji. Roslin. 190: 67-76.
41 Fernandez, M. L. 1995. Distinct mechanisms of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum and psyllium. J. Lipid Res. 36: 2394-2404.
42. Pan, S. Y., Yang R, Dong, H., Yu, Z. L. and Ko, K. M. 2006. Bifendate treatment attenuates hepatic steatosis in cholesterol/bile salt-and high-fat diet-induced hypercholesterolemia in mice. Eur. J. Pharmacol. 552: 170-175.
43. Gibbsons, G. F., Islam, K. and Pease, R. J. 2000. Mobilisation of triacylglycerol stores. Biochim. Biophys. Acta 1483: 37.
44. Fungwe, T. V., Cagen, L. M., Cook ,G. A., Wilcox, H. G. and Heimberg, M. 1993. Dietary cholesterol stimulates hepatic biosynthesis of triglyceride and reduces oxidation of fatty acids in the rat. J. Lipid Res. 34: 933-941.
45. Skottova. N., Kazdova, L., Oliyarnyk, O., Vecera, R., Sobolova, L. and Ulrichova, J. 2004. Phenolics-rich extracts from Silybum marianum and Prunella vulgaris reduce a high-sucrose diet induced oxidative stress in hereditary hypertriglyceridemic rats. Pharmacol. Res. 50: 123-130.
46. Wilson, T. A., Nicolosi, R. J., Delaney, B., Chadwell, K., Moolchandani, V., Kotyla, T., Ponduru, S., Zheng, G. H., Hess, R., Knutson, N., Curry, L., Kolberg, L., Goulson, M. and Ostergren, K. 2004. Reduced and high molecular weight barley beta-glucans decrease plasma total and non-HDL-cholesterol in hypercholesterolemic syrian golden hamsters. J. Nutr. 134: 2617-2622.
47. Ashakumary, L., Rouyer, I., Takahashi, Y., Ide, T., Fukuda, N., Aoyama, T., Hashimoto, T., Mizugaki, M. and Sugano, M. 1999. Sesamin, a sesame lignan, is a potent inducer of hepatic fatty acid oxidation in the rat. Metabolism 48: 1303-1313.
48. Murase, T., Nagasawa, A., Suzuki, J., Hase, T. and Tokimitsu, I. 2002. Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int. J. Obes. Relat. Metab. Disord. 26: 1459-1464.
49. Huong, D. T. T., Takahashi, Y. and Ide, T. 2006. Activity and mRNA levels of enzymes involved in hepatic fatty acid oxidation in mice fed citrus flavonoids. Nutrition 22: 546-452.
50. Ide, T. and Ontko, J. A. 1981. Increased secretion of very low density lipoprotein triglyceride following inhibition of long chain fatty acid oxidation in isolated rat liver. J. Biolog. Chem. 256: 10247-10255.
51. Young, I. S. and McEneny, J. 2001. Lipoprotein oxidation and atherosclerosis. Biochem. Soc. Trans. 29: 358-362.
52. Lopez-Bote, C. J., Sanz, M., Rey, A., Castano, A. and Thos, J. 1998. Lower lipid oxidation in the muscle of rabbits fed diets containing oats. Anim. Feed Sci. Technol. 70: 1-9.
53. Hallfrisch, J., Scholfield, D. J. and Behall, K. M. 1997. Diets containing soluble oat extracts reduce urinary malondialdehyde in moderately hypercholesterolemic men and women. J. Nutr. Biochem. 8: 497-501.