

Effect of Enzyme-Treated Radish Leaves on Lipid Metabolism in Rats Fed a High-Fat Diet

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Abstract

The purpose of this study was to investigate the effect of enzyme-treated radish (*Raphanus sativus* L.) leaves on lipid metabolism in rats fed a high fat diet. Rats were divided into four experimental groups which were composed of a high fat diet group (HF group), a high fat diet with 10% radish leaf powder-supplemented group (MA group), a high fat diet with 5% enzyme-treated radish leaf powder-supplemented group (MB group) and a high fat diet with 10% enzyme-treated radish leaf powder-supplemented group (MC group). Total dietary fiber content of enzyme-treated radish leaves were greater than untreated radish leaves. Body weight gain and food efficiency ratio (FER) of the HF group increased compared to the MA, MB and MC groups. The serum total cholesterol, LDL-cholesterol and atherogenic index contents in the radish leaf powder-supplemented groups were lower than that of the HF group, while those values for the MB and MC groups were significantly lower than that of the HF group. The serum HDL-cholesterol contents of the MB and MC groups increased compared to the HF group. The hepatic triglyceride contents of the MA, MB and MC groups decreased compared to the HF group. In fact, the hepatic triglyceride contents of the MB and MC groups were significantly lower than the MA group. The hepatic total cholesterol contents of the MB and MC groups significantly decreased compared to those of the HF group. The fecal total cholesterol contents of the MA, MB and MC groups significantly increased compared to those of the HF group. These results indicate that supplementation with enzyme-treated radish leaves increase the useful fiber contents. Furthermore, it may have a pronounced impact on lipid metabolism of serum and liver in rats fed a high fat diet.

Key words: enzyme-treated radish leaves, cholesterol, triglyceride, blood glucose, high fat diet

INTRODUCTION

The recent increases in standards of living across Asia have led to Westernized dietary life-styles and frequent eating-out, which have increased intakes of animal foods containing high amount of saturated fatty acid and cholesterol. Consequently, metabolic diseases such as obesity, arteriosclerosis, myocardial infarction, hypertension, stroke, and diabetes have become major social concerns (1,2). Domestically, the prevalence of cardiovascular diseases, including hypertension and arteriosclerosis, is trending upwards, accounting for over 30% of all causes of death among Koreans, and resulting in social and medical problems (3,4). These diseases are known to be affected by blood cholesterol and triglyceride concentrations, and thus, interests in the physiological effects associated with functional foods have increased. This increasing interest in the physiological effects of functional foods results from the awareness of factors causing the aforementioned diseases, such as high calorie and fat diets, as well as genetic effects.

Natural substances that help improve lipid metabolism and hyperlipidemia include *Sophorae Fructus* (5), *Gastrodia elata* Blume (6,7), *Zingiber officinale* Roscoe (8) and *Astragalus membranaceus* (9).

Raphanus sativus L., or the radish, is reported to be closely related to reduction in cancer development, belongs to the cruciferous vegetable family (10), and contains a range of digestive enzymes (11). Radishes consist of a root and green leafy parts. The radish leaves contain more vitamins A and C and calcium than the root and are rich in dietary fiber, which is good for diabetes and bowel action. In particular, the soluble dietary fiber (SDF) is reported to influence lipid metabolism, including cholesterol (12-14). In addition, SDF is effective for improving glucose tolerance in diabetics by delaying the absorption of nutrients as foods pass through the intestines as well as by absorbing water, leading to larger volumes that, in turn, form a gel inside the intestine, increasing viscosity (15-17). Many studies have added enzymes to foods or have used enzymatic hydrolysis to

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increase bioactive materials and extraction yields. In addition, Shin (18) reported that bioactive effects, such as the reduction of blood cholesterol, can be gained by completely solubilizing relaxed tissue structures with enzymatic treatment, which increases SDF content. Similarly, Heo and Jeon (19) found that enzymatic hydrolysis serves to decompose fiber, glycoprotein and alginic acid polymeric materials and induces active materials to be extracted seamlessly. In addition, the water-soluble extracts can be used as food ingredients, which are deliverable into the body as safer natural anti-oxidants without chemicals such as organic solvents.

The present study attempted to identify the influence of different concentrations of *Raphanus sativus* L. on lipid metabolism by feeding white rats a high fat diet supplemented with enzyme-treated radish leaves.

MATERIALS AND METHODS

Preparation of radish leaf powder

The leaves used in this experiment were from Gwan-Dong Radishes harvested in the summer and fall. After investigating changes in quality, Minongdanbaek radish leaves were excluded. Pre-treatment was done by washing, drying and salting. The leaves were then freeze-dried and ground into particles of an identical size before use.

Preparation of enzyme-treated radish leaves powder

The washed radish leaves (100 kg) were blanched by steam for 10 minutes at 100°C, chopped, and triturated. Next, viscozyme was added at 0.05% to breakdown the mixed fibers, followed by incubation for 24 hours at 30°C, drying, and crushing. Viscozyme is a carbohy-

drate-decomposing enzyme complex containing arabinase, β -glucanase, hemicellulase, and xylanase and was prepared from selected strains of *Aspergillus*. Its optimal pH is 3.5~5.5, optimal reaction temperature is 40~50°C, and the enzyme activation is 120 FBG (fungal β -glucanase) unit/mL (20).

Analysis of dietary fiber

Dietary fiber concentration was measured by the method of Prosky et al. (21) and the total dietary fiber assay kit (Sigma Chemical Co. USA) was used.

Experimental animals and diet

Sprague-Dawley male rats weighing 100 ± 10 g were purchased from Bio Genomics (Bio Genomics., Seoul Korea). Rats were individually housed in stainless steel cages in a room with controlled humidity of $50 \pm 10\%$, temperature of 20~23°C and alternating 12 hr periods of light and dark. Rats were divided into four experimental groups, which were composed of a high fat diet group, a high fat diet with 10% radish leaf powder-supplemented group (MA group), a high fat diet with 5% enzyme-treated radish leaf powder-supplemented group (MB group) and a high fat diet with 10% enzyme-treated radish leaf powder supplemented group (MC group) (Table 1). The rats were fed with the experimental diets for a period of 4 weeks. The experimental design was approved by the committee of Korea University for the care and use of laboratory animals.

Body weight gain, food intake and food efficiency ratio

Over the 4 week experimental period, food-intake was monitored daily at the same time, while body weight was tested every week at the same time. FER (food effi-

Table 1. Compositions of diets in experiment groups (g/kg diet)

Ingredients	Groups ¹⁾			
	HF	MA	MB	MC
Corn starch	392	292	342	292
Casein	200	200	200	200
Sucrose	100	100	100	100
Corn oil	60	60	60	60
Mineral mixture ²⁾	40	40	40	40
Vitamin mixture ³⁾	10	10	10	10
Cellulose	50	50	50	50
DL-methionine	3	3	3	3
Choline chloride	3	3	3	3
Cholesterol	2	2	2	2
Lard	140	140	140	140
Radish leaves of nonenzyme	—	100	—	—
Radish leaves of enzymic treated	—	—	50	100
Total	1000	1000	1000	1000

¹⁾HF: high fat diet, MA: high fat + 100 g radish leaves diet (10%), MB: high fat + 50 g extract of enzyme-treated radish leaves diet (5%), MC: high fat + 100 g extract of enzyme-treated radish leaves diet (10%).

²⁾AIN-76 mineral mixture (g/kg mixture).

³⁾AIN-76 vitamin mixture (g/kg mixture).

Table 2. The contents of dietary fiber in the enzyme-treated radish leaves

Sample	Dietary fiber (%)		
	IDF (insoluble dietary fiber)	SDF (soluble dietary fiber)	TDF (total dietary fiber)
Non-enzyme treated	36.75 ± 0.88	0.01 ± 0.37	36.75 ± 0.51
Enzyme treated	37.97 ± 6.12	0.39 ± 0.15	38.36 ± 6.26

ciency ratio) was calculated by total body weight gain (g)/ total food intake amount (g).

Measurement of triglyceride, cholesterol concentrations in serum, liver and fecal

A colorimetric kit (Asan Co., Gyeonggi, Korea) was used to measure serum levels of triglyceride (TG), total cholesterol, and HDL-cholesterol. Serum LDL-cholesterol was calculated by the Friedewald formula (22) {total cholesterol - (HDL-cholesterol + TG/5)} and the atherogenic index (AI) was calculated using the following formula: {(total cholesterol - HDL-cholesterol)/HDL-cholesterol} (23). Liver and fecal lipids were extracted by the Folch methods (24) and the triglyceride and cholesterol concentrations were analyzed by the same methods as serum. This study measured absorbance at 550 nm and 500 nm to determine triglyceride and cholesterol levels, respectively, after removing the turbidity that may occur at the time of the color reaction in the course of combining 0.5% triton X-100 and 3 mM sodium cholate in the fixed quantity of lipid in liver tissue, using the modified methods of Sale et al. (25).

Measurement of blood glucose

Blood glucose concentrations in the rats were measured using the enzymatic kit AM 102K (Asan Co.) and absorbance was read at 500 nm.

Statistical analysis

Results were analyzed by ANOVA and Tukey's honestly significant difference test (26). Statistical significance was defined as $p < 0.05$.

RESULTS

Fiber contents of enzyme-treated radish leaves

The contents of dietary fiber in the radish leaves are

shown in Table 2. The results indicate that the insoluble dietary fiber (IDF) content of the enzyme-treated radish leaves is greater than untreated radish leaves. SDF content in the untreated radish leaves and enzyme-treated radish leaves were 0.01% and 0.39%, respectively. In addition, the total dietary fiber (TDF) content of the enzyme-treated radish leaves was greater than untreated radish leaves.

Weight gain, food intake and food efficiency

Body weight gain, food intake and food efficiency ratio (FER) are shown in Table 3. The body weight gain of the radish leaf supplemented groups was lower than that of the HF group. Notably, the MB group and MC group were lower than the MA group. In addition, food intake and food efficiency showed similar trends.

Serum on total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and atherogenic index

The serum total cholesterol contents (Table 4) in the radish leaf-supplemented groups were lower than that of the HF group. The values of the MB group and MC groups were significantly lower than that of the HF group. The serum triglyceride (TG) contents (Table 4)

Table 3. Effect of extracts from enzyme-treated radish leaves on body weight gain, food intake and food efficiency ratio (FER) in rats fed high fat diets

Groups ¹⁾	Body weight gains (g)	Food intake (g/day)	FER
HF	158.33 ± 10.01 ^a	25.94 ± 1.081 ^a	0.332 ± 0.038 ^{ab}
MA	148.25 ± 4.172 ^a	25.89 ± 0.807 ^a	0.298 ± 0.005 ^a
MB	136.00 ± 13.70 ^{ab}	23.63 ± 0.345 ^c	0.293 ± 0.003 ^a
MC	134.50 ± 6.768 ^b	23.09 ± 0.707 ^{bc}	0.264 ± 0.008 ^b

¹⁾Groups are the same as in Table 1.

All values are mean ± SE (n = 10). Those with different superscripts in the same column are significantly different at $p < 0.05$ by Tukey's test.

Table 4. Effects of extracts from enzyme-treated radish leaves on serum total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol and AI (atherogenic index) of rats fed high fat diets

Groups ¹⁾	Total-cholesterol	Triglyceride	HDL-cholesterol	LDL-cholesterol	AI
	(mg/dL)				
HF	44.59 ± 8.06 ^a	93.49 ± 12.32 ^{NS}	1.43 ± 0.416 ^b	94.43 ± 13.85 ^a	47.79 ± 12.15 ^{ab}
MA	31.05 ± 3.50 ^{ab}	75.01 ± 6.549	2.14 ± 0.468 ^{ab}	78.69 ± 10.06 ^{ab}	38.76 ± 6.024 ^a
MB	27.63 ± 2.97 ^b	72.74 ± 7.612	2.67 ± 0.338 ^{ab}	66.91 ± 7.150 ^{ab}	29.65 ± 5.633 ^{ab}
MC	26.91 ± 0.08 ^b	74.85 ± 3.609	3.16 ± 0.440 ^a	62.12 ± 6.622 ^b	26.31 ± 2.154 ^b

¹⁾Groups are the same as in Table 1.

All values are mean ± SE (n = 10). Those with different superscripts in the same column are significantly different at $p < 0.05$ by Tukey's test. NS: not significant.

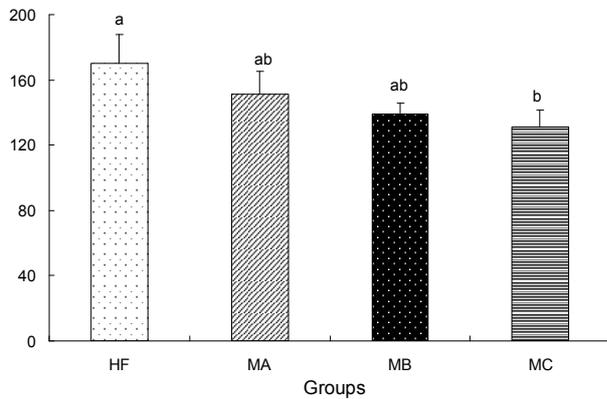


Fig. 1. Effects of extracts from enzyme-treated of radish leaves on serum blood glucose levels of rats fed high fat diets. Groups are the same as in Table 1. All values are mean \pm SE (n=10). Values with different letters are significantly different at $p < 0.05$ by Tukey's test.

of the MA, MB and MC groups were lower than that of the HF group. However, these differences were not significant among the experimental groups. The serum HDL-cholesterol contents (Table 4) in radish leaf-supplemented groups were greater than that of the HF group. The serum HDL-cholesterol content of MC group was significantly greater than that of the HF group. Likewise, the serum LDL-cholesterol contents (Table 4) in radish leaf-supplemented groups were lower than that of the HF group. Notably, the MC group was significantly lower than that of the HF group. The serum atherogenic index (Table 4) in radish leaf-supplemented groups were lower than that of the HF group.

Blood glucose

The serum blood glucose content after 12 hours of fasting is shown in Fig. 1. The blood glucose contents in the radish leaf-supplemented groups were lower than that of the HF group, with the 10% enzyme-treated radish leaves supplemented group (MC group) being significantly lower than HF group.

Hepatic total lipid and triglyceride concentrations

The hepatic total lipid contents (Table 5) in the radish leaves supplemented groups were lower than that of the HF group. Especially, MB and MC groups were reduced by 19% and 25%, respectively, as compared with MA group. However, these differences were not significant among the experimental groups. The hepatic TG contents (Table 5) in radish leaves supplemented groups were lower than that of the HF group. Especially, MB group and MC group were significantly lower than that of the HF group.

Fecal total lipid and triglyceride concentrations

The fecal total lipid contents (Table 6) in radish leaf-supplemented groups were greater than that of the HF

Table 5. Effects of extracts from enzyme-treated of radish leaves on liver total lipid and triglyceride levels of rats fed high fat diets

Groups ¹⁾	Total lipid (mg/g)	Triglyceride (mg/g)
HF	0.017 \pm 0.003 ^{NS}	286.9 \pm 28.42 ^a
MA	0.016 \pm 0.003	245.0 \pm 23.68 ^{ab}
MB	0.013 \pm 0.003	181.4 \pm 31.56 ^{bc}
MC	0.012 \pm 0.002	175.8 \pm 18.42 ^c

¹⁾Groups are the same as in Table 1.

All values are mean \pm SE (n=10). Those with different superscripts in the same column are significantly different at $p < 0.05$ by Tukey's test.

NS: not significant.

Table 6. Effects of extracts from enzyme-treated of radish leaves on fecal total lipid and triglyceride levels of rats fed high fat diets

Groups ¹⁾	Total lipid (mg/day)	Triglyceride (mg/day)
HF	61.27 \pm 6.261 ^b	14.09 \pm 2.814 ^b
MA	83.70 \pm 5.585 ^a	18.04 \pm 1.630 ^{ab}
MB	70.41 \pm 13.52 ^{ab}	20.22 \pm 1.418 ^a
MC	66.58 \pm 9.561 ^{ab}	14.85 \pm 0.815 ^b

¹⁾Groups are the same as in Table 1.

All values are mean \pm SE (n=10). Those with different superscripts in the same column are significantly different at $p < 0.05$ by Tukey's test.

group. The fecal TG contents (Table 6) in radish leaf-supplemented groups were greater than that of the HF group.

Hepatic total cholesterol and fecal total cholesterol concentrations

The hepatic total cholesterol contents (Fig. 2) in radish leaf-supplemented groups were lower than that of the HF group. Especially, the hepatic total cholesterol contents of the MB group and MC group were significantly lower than that of the HF group. The fecal total cholesterol contents (Fig. 2) in radish leaf-supplemented groups were significantly greater than that of the HF group. Indeed, the enzyme-treated radish leaf-supplemented groups tended to increase total lipid of fecal and triglyceride levels in rats fed high fat diets, according to the amount of extracts added.

DISCUSSION

Regarding the lipid metabolism effect, the radish leaves have a significant triglyceride and cholesterol reducing effect. It was found that effects of the enzyme-treated radish leaves were lower than the radish leaves.

Dietary fiber is divided into insoluble fibers such as cellulose, hemicellulose and lignin, and soluble fiber such as pectin, gums and algal polysaccharides (19), and

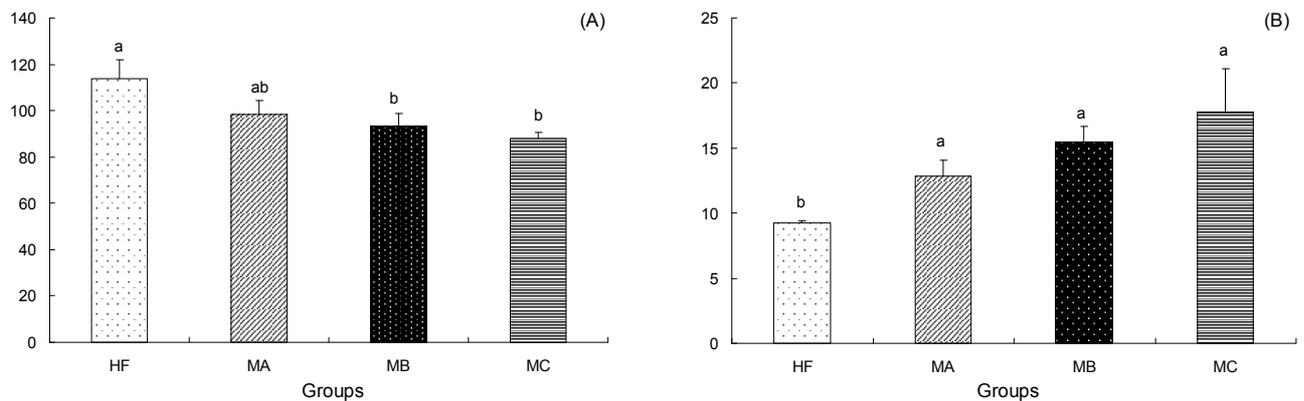


Fig. 2. Effects of extracts from enzyme-treated of radish leaves on liver (A) and fecal (B) total cholesterol levels of rats fed high fat diets. Groups are the same as in Table 1. All values are mean \pm SE (n=10). Values with different letters are significantly different at $p < 0.05$ by Tukey's test.

is known to affect the internal absorption and digestion of several nutrients such as fat, protein and inorganic matters. SDF contents in the radish leaves and enzyme-treated radish leaves were 0.01% and 0.39%, respectively. Thus, SDF content of enzyme-treated radish leaves is greater than untreated radish leaves. Jang et al. (27) reported that ginger contained nearly 6.0% crude fiber, which increased to 15.7% after zymolysis. Radish leaves were also found to have higher levels of IDF and SDF through enzyme treatment, indicating that TDF content increased. In addition, consistent with a report stating that extraction yields differed depending on the enzymes that were used (28), the present study also observed an increase in dietary fiber by adding a fiber-dissolution enzyme mix containing arabinase, cellulase and glucanase.

The body weight gains of the groups supplemented with the enzyme-treated radish leaf were lower than those of the HF group. This result is similar to that reported by Han and Han (29) that body weight decreased when dietary fibers pectin and cellulose were added to a high fat diet. These results were also in agreement with previous reports by Ku et al. (10), who show that radishes are rich in cellulose and pectin which work to slow digestion, suggesting a potential effect on weight loss. In addition, food intake and food efficiency showed similar trends, such that ingestion of enzyme-treated radishes with increased water-SDF may provide post-prandial satiety and reduce food intake and body weight by continuous reduction of food consumption (30).

Similar to a report that describes how enzyme treatments increased SDF and thus reduced blood cholesterol (18), these studies found that feeding with enzyme-treated radish leaves appears to be more effective than feeding with untreated radish leaves. SDF was found to be excellent at decreasing plasma cholesterol, and indigestible dextrin, which is a starch-derived low-vis-

cosity SDF, was reported to lower total lipid and cholesterol levels in plasma (30). Nishina et al. (31) showed that white rats fed 8% pectin had decreased plasma cholesterol concentrations and all the lipoproteins concentrations, and Yukiko et al. (32) found lowering effects on blood cholesterol with levan, one of the SDFs. It was reported that a high cholesterol diet with ingested water-SDF lowered the concentration of serum LDL-cholesterol and raised HDL-cholesterol concentration. Therefore, the enzyme-treated radish leaves may effectively prevent hardening of the arteries and be useful as dietary supplement.

Song and Hong (33) showed the phenomenon of blood glucose levels decreasing when supplying Psyllium and water SDF in their study. They also reported that the intake of water soluble fibers enhances glucose metabolism in the body and lowers blood sugar levels. The greater reduction of differences between diet groups in the liver than in the blood is similar with the report that water SDF has a significant influence, mostly on plasma cholesterol concentration than on hepatic cholesterol concentrations (34). The water-SDF levels increased by treated-enzyme radish were found to reduce the hepatic cholesterol concentrations, furthermore the inhibitory effect of obesity can be considered. These results were in agreement with the previous reports made by Kang and Song (35).

The fecal total lipid, triglyceride (TG) and total cholesterol contents in radish leaf-supplemented groups were greater than those of the HF group. The findings of the current study agree with the result of Jang et al. (27) in which the feces, total lipid, total cholesterol, and TG contents were increased by feeding with untreated radish leaves, and the physiological effects of dietary fiber and flavonoids were reported by Kang et al. (2) The enzymatic treatment of radish leaves increases the

useful dietary fiber contents and the previous results can indicate that enzyme-treated radish leaves are more effective in increasing water-SDF levels than non enzyme-treated radish leaves. The findings of the current study also agree with the result of Yim et al. (36) where glucomannan, an SDF, reduced weight while significantly decreasing dry weight water and fat contents, helping excretion with no side effects. Also, glucomannan inhibited fat absorption in the intestines and significantly lowered plasma cholesterol and triglyceride levels, which are considered desirable body-fat loss effects. These findings indicate that the intake of dietary fiber contained in radish leaves lowers plasma cholesterol, and enzyme-treated radish leaves contain a larger amount of SDF, which can work effectively to prevent diseases associated with lipid metabolism including arteriosclerosis.

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