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# Effects of Water Extracts from Mulberry Leaves on Hepatic HMG-CoA Reductase and Acyl-CoA-Cholesterol Acyl Transferase Activity in Rats Fed High Cholesterol Diets

Jeong-Hwa Choi[1], Jung-Hee Hong[2], Jeong-Ah Yang[2], Soon-Jae Rhee[2] and Mo-Ra Park[3†]

[1]School of Food Science, Jinju International University, Jinju 660-759, Korea
[2]Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongsan 712-702, Korea
[3]Department of Food Science and Nutrition, Sangju National University, Sangju 742-711, Korea

## Abstract

**This study investigated the effects of mulberry leaf extract on lipid metabolism in rats fed a high cholesterol diet. Sprague-Dawley male rats weighing 100±10 g were randomly assigned either to one of two normal diet groups, with (NE group) or without (N group) mulberry extract, or one of four high cholesterol groups containing 1% cholesterol and various levels of dietary mulberry leaf extract. The rats fed high cholesterol diets were subdivided into 4 groups according to level of mulberry extract; Mulberry extract free group (HC group), 0.8% mulberry leaf extract group (HCL group), 1.6% mulberry leaf extract (HCM group) and 3.2% mulberry leaf extract (HCH group). The rats were fed their respective diets *ad libitum* for 4 weeks. The levels of serum triglyceride, total cholesterol and LDL-cholesterol of the HC group were higher than mulberry leaf extract supplemented groups. In contrast, the levels of serum HDL-cholesterol in groups supplemented with mulberry leaf extract were significantly lower than that of HC group. Hepatic total lipids, triglycerides, and cholesterol were significantly higher in the high cholesterol groups compared to those of the normal group, but were lower in the HCL, HCM and HCH groups than in the HC group. HMG-CoA reductase activity was significantly decreased in the HC and HCL groups compared to the normal and NE groups. However, the activities in the HCM and HCH group were similar to that of the normal group. The activity of acyl-CoA-cholesterol acyl transferase (ACAT) was increased in high cholesterol groups compared to the normal group. However, the activity was lower for all of the high cholesterol groups fed mulberry leaf extracts, and was lowest for the highest supplemented group (HCH), with no significantly difference from the normal group. In conclusion, the reduction in serum and hepatic lipid composition by mulberry leaf extract may be due to its modulation of HMG-CoA reductase and ACAT activities.**

**Key words:** mulberry leaf extract, HMG-CoA reductase, ACAT, cholesterol, triglyceride

## INTRODUCTION

The recent rapid growth of economic prosperity, increase in the elderly population, and westernized dietary habits have led to increases in various adult diseases such as cancers, arteriosclerosis, hypertension, heart attack, brain diseases, and diabetes, which are now the leading causes of death in Korea, according to the statistics released by the Korea National Statistical Office in 2000 (1). The Framingham Heart Study (2) showed that a 1 percent increase in blood cholesterol level causes a 2 percent increase in the risk of ischemic heart diseases resulting from coronary heart disease. There is a need, therefore, to develop a natural functional dietary supplement that helps prevent or cure hyperlipidemia and cardiovascular diseases. Although recently a variety of synthetic medicines have been widely used to treat these chronic diseases, consumers are shown to prefer a functional health food as an alternative medicine that can effectively treat and prevent these diseases by means of their dietary intake, due to the toxic effects of synthetic medicines.

Mulberry is a well known medicinal plant. About 130 different kinds of mulberries are now cultivated all over the world. A new variety of mulberry tree called "YK-209 Mulberry" was developed in the Sericulture and Insect Division of the Agricultural Promotion Association in 1989, which was suitable for using the leaves for health food rather than for cultivation of silkworm. The YK-209 Mulberry was developed for the production of leaves

†Corresponding author. E-mail: mrpark@sangju.ac.kr
Phone: +82-54-530-5304, Fax: +82-54-530-5309

interbreeding Yongcheon Mulberry (Y) with an improved mulberry (K). It features more abundant and broader leaves, better cold-resistance, and high concentrations of functional ingredients. As a result, the YK-209 Mulberry was considered to be more useful for the development of mulberry leave-based functional dietary food (3). Among the varied phytochemicals abundantly present in mulberry leaves, guaiacol, eugenol, methyl salicylate, benzaldehyde and phenylacetaldehyde are included in the volatile compounds and flavonoids such as rutin, quercetin, isoquercetin, astragalin, quercetin-3, 7-diglucoside, and quercetin-3-triglucoside are among the non-volatile compounds. Their contents are very abundant and their species is also various. In addition, approximately 50 kinds of inorganic compounds have been identified in substantial quantities, including: Ca, K and Fe, as well as 21 kinds of amino acid including methionine. Thus far, most studies of the physiological activities of mulberries have examined its hypoglycemic effects in diabetic patients (4-6). Various health benefits of mulberry leaves have been reported, such as lipid metabolism improvement (7) and lowering of blood pressure. In addition, Yun and Lee (8) reported in their study on mulberry leave's anti-oxidant action that its methanol extract is more effective in lowering lipid peroxidation than $\alpha$-tocopherol. Hong et al. (9) reported in their study that powdered mulberry leaf helps lower the triglyceride and cholesterol levels in blood and liver tissue of streptozotocin induced diabetic rats, but only a few studies investigated the mechanism of metabolic control. Also, it was observed in a previous study by Kim et al. (7) that when hyperlipidemic rats were fed a water extract of mulberry leaves, the HMG-CoA reductase activity was higher than those given other extracts, but no further systematic study was performed on them.

In this study, accordingly, both changes in lipid concentrations and HMG-Co A reductase activity, among 32 enzymes playing an important role at the early biosynthetic phase of cholesterol, were observed to examine the progressive change in cholesterol metabolism systematically and scientifically. Considering that the enzymatic reaction occurs during the early phase, however, ACAT plays its role during the middle phase of cholesterol metabolism so it was also observed more thoroughly to examine the effects on cholesterol metabolism.

## MATERIALS AND METHODS

### Mulberry leave collection and extracts preparation

The YK-209 mulberry leaves used for this experiment were cultivated by Yeongcheon Silkworms Culture Agriculture Cooperative and Harvested in May 2002. The leaves were thoroughly washed with water before use. After washing, fresh leaves were sorted and dried under a hot blower (8). Dried leaves were again extracted and filtered for 4 hours at 85°C~90°C in distilled water. Then, they were concentrated for 10 hours at 60°C~62°C at 52 degrees of vacuum. As a result, a 60% solid powder was obtained and used to prepare extracts from mulberry leaves for this experiment. As stated above, the hot blower was used to dry fresh mulberry leaves because it minimizes the destruction of functional substances contained in mulberry leaves while preparing extracts from leaves.

### Experimental animals and diet

Sprague-Dawley male rats weighing 100±10 g were purchased from KRITC (Daejeon, Korea). Rats were individually housed in stainless steel cages in a room with controlled temperature (20~23°C) and lighting (alternating 12 h periods of light and dark). They were randomly assigned to either two normal diet groups, with (NE) or without (N) 1.6% mulberry leaf extract, or four high cholesterol diet groups containing 1% cholesterol. The high cholesterol diets group was subdivided into 4 groups according to level of mulberry extract supplementation mulberry extract free group (HC group), 0.8% mulberry leaf extract group (HCL group), 1.6% mulberry leaf extract (HCM group) and 3.2% mulberry leaf extract (HCH group). The rats were fed *ad libitum* for 4 weeks, mulberry leaf extracts were provided as drinking diet and water were freely provided. The experimental design was approved by the Committee of Sangju National University for Care and Use of Laboratory Animals (Table 1).

### Measurement of triglyceride and cholesterol concentrations in serum and liver

A colorimetric kit (Asan Co., Korea) was used to measure serum levels of triglyceride, total cholesterol, and HDL-cholesterol.

Serum LDL-cholesterol was calculated by the Friedewald formula (11) {total cholesterol−(HDL-cholesterol

**Table 1.** Classification of experimental group

| Groups[1)] | | Cholesterol (1%/kg diet) | Mulberry leaves extract (g/kg diet) |
|---|---|---|---|
| Normal diet group | N | - | - |
| | NE | - | 1.6% |
| High cholesterol diet group | HC | + | - |
| | HCL | + | 0.8% |
| | HCM | + | 1.6% |
| | HCH | + | 3.2% |

[1)]N, normal diet; NE, normal diet + 1.6% mulberry extract; HC, high cholesterol diet; HCL, high cholesterol diet + 0.8% mulberry extract; HCM, high cholesterol diet + 1.6% mulberry extract; HCH, high cholesterol diet + 3.2% mulberry extract.

**Table 2.** Effects of mulberry leaf extracts on body weight gains, food intake, and food efficiency ratio (FER) in rats fed high cholesterol diets

| Group[1)] | Body weight gains (g) | Food intake (g/day) | Drink intake (g/day) | FER |
|---|---|---|---|---|
| N | 108.80±10.33[2)NS3)] | 19.47±0.60[NS] | 31.90±5.63[NS] | 0.20±0.02[NS] |
| NE | 118.17±13.4 | 19.74±1.3 | 26.07±2.2 | 0.21±0.0 |
| HC | 99.00±6.5 | 20.59±2.3 | 31.64±8.7 | 0.17±0.02 |
| HCL | 98.38±17.6 | 20.35±4.8 | 24.53±4.4 | 0.18±0.05 |
| HCM | 113.88±9.5 | 20.01±0.9 | 25.71±2.4 | 0.20±0.0 |
| HCH | 121.25±14.5 | 21.31±0.6 | 26.96±3.8 | 0.20±0.0 |

[1)]Groups are the same as in Table 1.
[2)]All values are mean±SE (n=10).
[3)]Not significant.

+TG/5)} and the atherogenic index was calculated as {(total cholesterol−HDL-cholesterol) / HDL-cholesterol} (12).

Liver lipids were extracted by the Folch methods (13) and the triglyceride and cholesterol concentrations were analyzed by the same methods as for serum.

This study measured absorbance at 550 nm and 500 nm 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, as the emulsifier to the sample enzyme liquid for measuring triglyceride and cholesterol in the modified methods of Sale et al. (14).

### Measurement of HMG-CoA reductase and ACAT activities

Microsomes were separated from hepatic tissue and measured by the method of Hulcher and Oleson (15) with slight modification. The HMG-CoA reductase activities were determined as described by Shipiro et al. (16) with the slight modification of using freshly prepared hepatic microsomes. The ACAT activities were determined using freshly prepared hepatic microsomes according to the method developed by Erickson et al. (17) as modified by Gillies et al. (18).

### Protein determination

Protein concentration was measured by the method of Lowry et al. (19), with bovine serum albumin as the standard.

### Statistical analysis

All data are expressed as the mean±the standard error. Results were assessed by ANOVA and Tukey's Honestly Significant Difference test. Differences were considered significant at $p<0.05$.

## RESULTS AND DISCUSSION

### Weight gains, dietary intakes, extract intakes, and food efficiency ratio

The results of body weight gain, food intake and food efficiency ratio during the experimental period are shown in Table 2.While there was a significant increase in the weight gains of the cholesterol-fed HC group, the group fed extracts from mulberry leaves showed weight gains similar to those of the normal group. There was no significant difference in weight gains between groups fed mulberry leaf extracts. In addition, there was no significant difference in dietary intakes, extracts intakes or dietary efficiency among experimental groups. These results were consistent with the report of Kim et al. (7) that rats on high cholesterol diets lost weight when

**Table 3.** Effects of different supplementation levels of mulberry leaf extract on serum triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol and A.I in rats fed high cholesterol diets

| Group[1)] | TG (mg/dL) | Total-C (mg/dL) | HDL-C (mg/dL) | LDL-C (mg/dL) | A.I |
|---|---|---|---|---|---|
| N | 39.61±7.00[2)c3)] | 127.63±40.31[c] | 60.96±1.75[a] | 48.27±6.36[c] | 1.25±0.57[c] |
| NE | 39.98±5.41[c] | 129.22±27.80[c] | 66.85±3.75[a] | 48.68±5.60[c] | 1.25±0.72[c] |
| HC | 70.42±3.11[a] | 396.45±26.84[a] | 16.85±0.84[c] | 324.16±17.38[a] | 22.45±2.55[a] |
| HCL | 54.10±3.26[b] | 259.78±32.82[b] | 21.40±2.43[b] | 257.15±11.80[b] | 13.61±2.53[b] |
| HCM | 55.75±3.44[b] | 265.74±53.10[b] | 24.44±2.23[b] | 264.84±19.51[b] | 11.14±2.13[b] |
| HCH | 53.30±2.93[b] | 294.50±27.91[b] | 24.44±1.58[b] | 264.84±19.51[b] | 14.78±2.26[b] |

[1)]Groups are the same as in Table 1.
[2)]All values are mean±SE (n=10).
[3)]Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test.

fedmethanolic mulberry leaf extracts for 2 weeks.

**Serum triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, and artherogenic index**

Serum lipids and the A.I are shown in Table 3. The high cholesterol group (HC group) exhibited significantly higher serum triglycerides compared to the normal group, while all the groups fed mulberry extract had significantly lower triglycerides than HC but higher than the normal group. Compared to the normal group, blood cholesterol was 310% higher in the high cholesterol-fed group (HC group), but in the groups fed mulberry extract it tended to be lower than in the HC group and was significantly lower than HC in the group fed the highest dose (HCH). The LDL-cholesterol levels tended to be similar to the total blood cholesterol levels, but the HDL-cholesterol level was significantly higher in the groups fed extracts from mulberry leaves compared with the HC group. The atherogenic index of the HC group was 17.96 times higher compared to the normal group, however, it was significantly lower in the groups fed mulberry extracts than the HC group, with intermediate values between the normal and HC groups. The hypolipidemic effects were similar to the report of Kim et al. (7) that experimental white rats with hyperlipidemia fed extracts from mulberry leaves had lower blood triglyceride and total cholesterol levels. It has been demonstrated that insoluble cellulose contained abundantly in mulberry leaves helps eliminate toxic matters from the body and stimulate intestinal function, and that in addition to β -sitosterol and other plant sterol, the unsaturated fatty acids including oleic acid, linoleic acid and linolenic acid help reduce blood cholesterol levels (20). Further studies are needed to further elucidate the mechanism of the hypolipidemic effects of mulberry leaves.

**Hepatic triglyceride and cholesterol concentrations**

Hepatic total lipid, triglyceride and total cholesterol were measured, as shown in Table 4. Liver total lipids were 382 percent ($p<0.05$) higher in the HC group compared to the normal group, but adding mulberry extract to the high cholesterol diet resulted in a significantly lower total lipids than with high cholesterol alone, though still higher than with a normal diet. Similarly, liver triglycerides were also reduced in the groups fed mulberry leaf extracts compared to the high cholesterol diet-fed group, but there were no significant differences among the groups fed mulberry leaf extracts. Cholesterol levels in liver tissue tended to be similar to the triglyceride contents. Compared to the HC group, in particular, it was significantly decreased in the HCH group, fed the highest levels of mulberry leaf extract ($p<0.05$). These results implied that supplementing with extracts from mulberry leaves helped reduce the triglyceride content and cholesterol levels in the liver of high cholesterol diet-fed white rats sufficiently to prevent fatty liver. Based on the report that the extracts from Yeongji mushroom (21) safflower seed powder (22) and ginseng saponin (23) are effective in controlling both triglyceride and cholesterol in the liver, it seems probable that the extracts from mulberry leaves contain some functional substance that helps reduce the triglyceride and cholesterol in the liver. Therefore, further studies are needed to confirm these findings.

**HMG-CoA reductase activity**

HMG-CoA reductase activity, the rate-limiting enzyme of cholesterol biosynthesis in the liver tissue, was measured, as shown in Fig. 1. Liver HMG-CoA reductase activity was significantly decreased in the high cholesterol diet-fed control group and the group fed 0.8 mulberry leaf extract percent extract (HC group and HLC groups) ($p<0.05$) compared to the normal group (N group) and the normal diet group fed the extracts from mulberry leaves (NE group). However, the activities of the high cholesterol diet-fed group fed 1.6 percent and 3.2 percent extracts from mulberry leaves (HCM group and HCH group) were similar to that of the normal group. When high cholesterol diet-fed rats were fed the

**Table 4.** Effects of different supplementation levels of mulberry leaf extract on hepatic total-lipid, triglyceride and cholesterol in rats fed high cholesterol diets

| Groups[1)] | Total-lipid (mg/g Liver) | Triglyceride (mg/dL) | Total-cholesterol (mg/dL) |
|---|---|---|---|
| N | $67.00 \pm 11.51^{2)c3)}$ | $11.11 \pm 1.27^{c}$ | $1.80 \pm 0.09^{c}$ |
| NE | $70.00 \pm 12.75^{c}$ | $11.07 \pm 0.74^{c}$ | $1.99 \pm 0.17^{c}$ |
| HC | $256.25 \pm 13.77^{a}$ | $18.99 \pm 1.59^{a}$ | $4.96 \pm 0.10^{a}$ |
| HCL | $197.14 \pm 26.12^{b}$ | $17.56 \pm 1.13^{ab}$ | $4.57 \pm 0.20^{ab}$ |
| HCM | $197.14 \pm 16.04^{b}$ | $16.74 \pm 1.36^{ab}$ | $4.49 \pm 0.29^{ab}$ |
| HCH | $184.00 \pm 20.43^{b}$ | $15.54 \pm 0.87^{ab}$ | $4.46 \pm 0.15^{b}$ |

[1)]Groups are the same as in Table 1.
[2)]All values are mean ± SE (n=10).
[3)]Values within a column with different superscripts are significantly different at $p<0.05$ by Tukey's test.

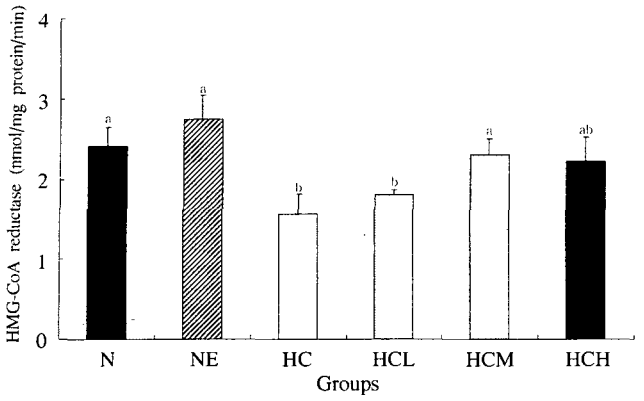


**Fig. 1.** Effects of water extracts from mulberry leaves on liver 3-hydroxy-3-methylglutaryl CoA reductase activity in rats fed high cholesterol diets. Groups are the same as in Table 1. Bars with different letters are significantly different at $p<0.05$ by Tukey's test.

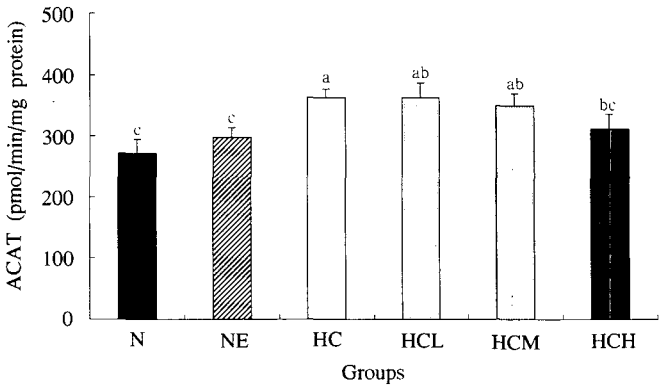


**Fig. 2.** Effects of water extracts from mulberry leaves on liver acyl-CoA-cholesterol acyltransferase (ACAT) in rats fed high cholesterol diets. Groups are the same as in Table 1. Bars with different letters are significantly different at $p<0.05$ by Tukey's test.

extracts from mulberry leaves, blood cholesterol levels in were lower, probably because HMG-Co A reductase activity during the early phase of cholesterol biosynthesis was reduced.

**Acyl-CoA-cholesterol acyltransferase (ACAT) activity**

Liver ACAT activities are shown in Fig. 2. Compared to the normal diet groups (N and NE groups), the high cholesterol diet-fed groups (HC group, HLC group and HCM group) exhibited a significantly higher ACAT activity ($p<0.05$). However, the activities tended to be lower for all of the groups fed high cholesterol diets plus extracts from mulberry leaves (HCL group, HCM group and HCH group) than the HC group, but significantly so only for the HCH group, which was not significantly different from the normal diet groups. ACAT is an enzyme used for synthesis of cholesteryl ester (CE) from free cholesterol. ACAT is believed to be one of the factors causing cardiovascular diseases since the risk of atherosclerosis appeared to be reduced in ACAT transgenic rats compared to the control group (24). A wide variety of ACAT inhibitors, therefore, have been developed to treat or prevent arteriosclerosis. As a result of this experiment, it seems that the ACAT is reduced by feeding the high cholesterol diet-fed rats the extracts from mulberry leaves. When it is taken into consideration that mulberry leaf extract normalizes the activity of HMG CoA reductase and ACAT, significantly decreases cholesterol levels in the liver tissue and blood, and reduces triglyceride concentrations, it may help treat and prevent arteriosclerosis, as well as hyperlipidemia. In conclusion, this research suggests that mulberry leaves may be a safe and effective functional dietary supplement for the prevention and treatment of diseases, such as hyperlipidemia and arteriosclerosis.

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