Mulberry Leaf Extract Inhibits the Oxidative Modification of Rabbit and Human Low Density Lipoprotein

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In a previous study, we demonstrated that the intake of mulberry leaves or their 1-butanol extract (MLBE) reduced the concentration of serum lipids and atheromatous thickening of arterial intima in hypercholesterolemic rabbits. In the present study, we investigated the antioxidative activity of MLBE and isoquercitrin, the main component of MLBE. First, we determined the effect on a stable radical agent, finding that quercetin, isoquercitrin and MLBE scavenged the DPPH radical. We then determined the copper-induced oxidative modification of rabbit and human low-density lipoprotein (LDL). Oxidation of LDL was spectrophotometrically monitored by changes in absorbance at 234 nm accompanied by the formation of conjugated dienes, and measured the formation of thiobarbituric acid reacting substances (TBARS). Quercetin, an aglycone of isoquercitrin, inhibited the formation of conjugated dienes and TBARS by copper-induced oxidative modification of rabbit and human LDLs. MLBE and isoquercitrin also inhibited the oxidation of LDL. These results indicate that mulberry leaves inhibit the oxidative modification of LDL and suggest that mulberry leaves may have prevented atherosclerosis.

Key words mulberry leaf; oxidized low density lipoprotein; quercetin; thiobarbituric acid reacting substance; conjugated diene formation; radical scavenger

Mulberry (*Morus alba* L. Moraceae) has been used to feed silkworms, and its roots have been used as the crude drug "MORI CORTEX". However, mulberry leaves have been rarely used as a drug or food. We previously demonstrated that the intake of mulberry leaves or their 1-butanol extract (MLBE) suppressed increases in serum lipids, hepatic enlargement and atheromatous thickening of the arterial intima in hypercholesterolemic rabbits.^{1,2)} Atherosclerosis is the main risk factor for coronary heart disease and it is widely accepted that oxidative modification of human low density lipoprotein (LDL) may play an important role in atherosclerosis.^{3,4)} The modified LDL shows a diminished affinity for the LDL receptor, and increased affinity for the macrophage scavenger receptor. Hence, the uptake of certain modified LDLs *via* scavenger receptors may be responsible for cholesterol accumulation and foam cell formation since scavenger receptors are not regulated by the cellular cholesterol content. As the intracellular accumulation of the oxidized LDL-derived cholesterol takes place, the presence of foam cell marks the initial onset of atherosclerosis.^{5–8)} Antioxidants such as probucol and α -tocopherol inhibit the oxidation of LDL *in vitro*^{9,10)} and probucol has been shown to reduce the progression of atherosclerotic lesions in Watanabe heritable hyper-lipidemic rabbits *in vivo*.¹¹⁾ This provides additional evidence that oxidized LDL is involved in the early stages of atherosclerosis. On the other hand, flavonoids, phenolic compounds, have also been shown to have antioxidant activity.^{12–16)} Antioxidative effects of quercetin, an aglycone of isoquercitrin, on human LDL have been reported.^{14–16)} Onogi *et al.*¹⁷⁾ identified four flavonoid glycosides, including isoquercitrin, in mulberry leaves, and we have also reported that MLBE contains isoquercitrin and astragalin.²⁾ The present study was designed to investigate the antioxidative effects of MLBE on the oxidative modification of LDL induced by copper on the formation of conjugated dienes and the formation of thiobarbituric acid reacting substances.

MATERIALS AND METHODS

Plant Material and Extract Dried mulberry leaves [ML, *Morus alba* L. (Moraceae)] were obtained from Kanagawa Prefecture Silkworm Center (Ebina, Japan). The cut leaves were extracted twice with 75% methanol at 45 °C for 4 h. After the extract was concentrated, hexane-soluble substances were removed. The precipitated residue was dissolved in water and extracted twice with 1-butanol, then concentrated and dried under reduced pressure (yield: 1.0%).²⁾

Materials Quercetin dihydrate and isoquercitrin were purchased from Extrasynthese (France). Disodium ethylenediaminetetraacetate (EDTA-2Na), butylated hydroxytoluene (BHT), copper sulfate, sodium azide and diisopropyl fluorophosphate (DFP) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Tetramethoxypropane was purchased from Tokyo Chemical Industries (Tokyo, Japan), 2-thiobarbituric acid (TBA) was purchased from Merck (Germany) and β -glucuronidase/sulfatase (Type H-2) was purchased from Sigma Chemicals (St. Louis, MO, U.S.A.).

Colorimetric Determination of the Scavenging Effect of Substances on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical The scavenging effect of each substance on the DPPH radical was measured by monitoring the decrease in absorbance at 517 nm.¹⁸⁾ To 0.5 ml of a 200 nmol/ml ethanol solution of DPPH (final concentration of DPPH: 40 nmol/ml) was added 1 ml ethanol, quercetin ethanol solution or MLBE ethanol solution and 1 ml water. In the case of isoquercitrin, to 0.5 ml of a 200 nmol/ml ethanol solution of DPPH was added 1 ml ethanol and 1 ml isoquercitrin aqueous solution. After mixing for 10 s on a vortex mixer, the solution was left to stand for 30 min and the absorbance of the resulting solution at 517 nm was measured. The scavenging activity on the DPPH radical was expressed as EC₅₀.

Isolation of Human LDL Plasma was obtained after low-speed centrifugation of fresh blood collected in the presence of EDTA-2Na (0.2 M) and stored at –20 °C before use.

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LDL was isolated from plasma by preparative ultracentrifugation.¹⁹⁾ Aliquots of 6.4 ml of plasma mixed with 1 mM EDTA-2Na were measured into Beckman ultra-clear centrifuge tubes, and 3.2 ml of solution of density 1.006 was layered over the surface. Tubes were centrifuged (Beckman model L-70) in a Beckman type 70.1Ti rotor at 22500 rpm ($31000\times g$) at 16 °C for 30 min. The bottom 6.4 ml layer was again overlaid with 3.2 ml of solution of density 1.006 and centrifuged at 41000 rpm ($115000\times g$) for 6 h. The bottom 6.4 ml layer was mixed with 3.2 ml of solution of density 1.182, and the mixture was centrifuged at 43000 rpm ($125000\times g$) for 8 h. The top 3.2 ml layers from the 2 tubes were combined and mixed with 1 mM DFP and 0.01% sodium azide, and finally, with 3.2 ml of solution of density 1.182. Solution of density 1.006 (3.2 ml) was layered over the surface and the tube was centrifuged at 43000 rpm ($125000\times g$) for 6 h. The 3–4 ml layer at the top of the higher density solution was collected as the LDL layer.

The protein content of the LDL layer was measured using a BCA protein kit (Pierce, IL, U.S.A.)²⁰⁾ and the LDL concentration expressed as a protein content. The LDL layer was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a single protein band corresponding to apoprotein B-100 following Coomassie brilliant blue R-250 staining. The LDL layer was stored at 4 °C for later use. Human LDL was dialyzed three times in 24 h at 4 °C by a 100-fold volume of 10 mM deoxygenated phosphate buffer containing 0.16 M sodium chloride (PBS, pH 7.4) to remove EDTA-2Na.

Isolation of Rabbit LDL Male Japanese white rabbits weighing 2.5–3.0 kg (SLC Inc. Hamamatsu, Japan) were anesthetized with pentobarbital (Nembutal, 50 mg/kg i.p.). Blood from rabbits was collected into tubes containing EDTA-2Na (1 g/ml). Plasma was obtained after low-speed centrifugation of fresh blood collected in the presence of EDTA-2Na and stored at –80 °C before use. Rabbit LDL was isolated and dialyzed by the same methods used for human LDL.

Continuous Monitoring of Conjugated Diene Formation of LDL Dialyzed LDL was diluted to a 2.05 mg protein/ml with 10 mM PBS. Conjugated diene formation during oxidation of LDL was continuously monitored by the spectrophotometric method of Esterbauer *et al.*,²¹⁾ which is based on measuring the change in absorbance at 234 nm. The concentrations of LDL and copper were 0.05 mg/ml and 1.66 μ M, respectively. Oxidation was initiated by addition of freshly prepared aqueous CuSO₄ solution at 37 °C (final concentration 1.66 μ M) in the presence or absence of MLBE, quercetin and isoquercitrin, and the absorbance at 234 nm continuously monitored by a Hitachi spectrophotometer (Tokyo, Japan). LDL not treated with test substances was used as the control.

Oxidative Modification of LDL Oxidation of LDL was performed according to the method of Parthasarathy *et al.*¹⁰⁾ Dialyzed LDL was diluted to a 0.25 mg protein/ml with PBS, and mixed with 10 mM CuSO₄ and then incubated at 37 °C for 4 h in the presence or absence of test substance. The reaction was stopped by addition of 50 μ M BHT.

SDS-PAGE of the LDL Layer Modifications of LDL were verified by SDS-PAGE using Atto Pagel SPG-520L (Atto, Tokyo, Japan). Each band was observed on SDS-

PAGE stained by Coomassie brilliant blue R-250. This method was also used to evaluate changes in the electrophoretic mobility of LDL after oxidative modification with quercetin, isoquercitrin or MLBE.

Assay of Thiobarbituric Acid Reactive Substances (TBARS) TBARS levels were determined spectrophotometrically.²²⁾ A mixture of 0.75 ml 20% glacial acetic acid, 0.85 ml, 0.71% TBA, 0.4 ml of water (or aliquots of post-incubation mixture and also tetramethoxypropane standards (0–4 nmol)) and 25 μ l BHT solution in glacial acetic acid was placed in a test-tube with a screw cap. The tube was kept at 5 °C for 60 min and then placed in a boiling water bath for 60 min. After cooling, the reaction mixture was extracted with 2.0 ml 1-butanol, and then the tube was centrifuged at 3000 rpm for 15 min. The absorbance of the supernatant was measured at 532 nm.

HPLC Analysis of Flavonoid Digestive Absorption in Mice Twelve male ddy mice weighing 42–53 g (SLC Inc., Hamamatsu, Japan) were randomly divided into four groups. Each group was orally administered water, quercetin–water solution (50 μ mol/ml), isoquercitrin–water solution (50 μ mol/ml) or MLBE (50 mg/ml), at 0.2 ml/10 g, once a day for 3 d. Two hours after administration on the third day, the mice were anesthetized with diethyl ether. Urine was then obtained from the bladder, and blood was obtained from the abdominal aorta by heparinized syringe. Plasma was obtained by centrifugation of the blood at 3000 rpm for 15 min. Metabolites in plasma or urine were treated with β -glucuronidase and sulfatase.^{14,23)} Plasma and urine from mice were acidified (to pH 4.9) with 0.1 volume of 0.583 μ mol/l acetic acid solution. The solutions were treated for 40 min at 37 °C in the presence of 2.5×10^6 units/l β -glucuronidase and 1.2×10^5 units/l sulfatase, then treated with 8.5 volumes of acetone and centrifuged. Supernatants were evaporated and spiked with 50 μ mol/l fisetin and diluted with water/methanol (1:1). For analysis, 20 μ l of each preparation was injected into a 4.6 mm i.d. $\times$ 150 mm Inertsil ODS-3 column (GL Science, Tokyo, Japan) and elution was performed using 0.5% phosphoric acid/acetonitrile (70:30). The flow rate was 1 ml/min and chromatograms were recorded by monitoring the absorbance at 370 nm.

RESULTS

The scavenging effect of polyphenols such as flavonoid and tannin, have been reported^{24–26)} and so we compared the effects of MLBE and isoquercitrin on the DPPH radical. The absorbance of the DPPH radical at 517 nm is reduced by scavenger. As shown in Table 1, addition of 2.3 nmol/ml quercetin, 4.4 nmol/ml isoquercitrin or 17.3 μ g/ml MLBE produced a 50% scavenging effect.

Next, we estimated the effect of test substances on rabbit plasma LDL. The incubation of rabbit LDL with copper showed a lag-phase of 35 min during which the lipophilic antioxidants protect the polyunsaturated fatty acids against oxidation, before onset of the propagation phase during which polyunsaturated fatty acids undergo conversion to conjugated lipid hydroperoxides. The photometric changes in rabbit LDL in oxidative modification are shown in Fig.1. In the presence of quercetin at a final concentration of 0.5 or 1.5 nmol/ml, the lag-phase increased 3.8-fold and 11.2-fold

Fig. 1. Inhibitory Effects of Isoquercitrin and a 1-Butanol Extract of Mulberry Leaves on Copper-Induced Oxidation of Rabbit LDL Determined by Continuous Monitoring of Absorbance at 234 nm by Conjugate Dienes

Rabbit LDL (0.05 mg protein/ml) was incubated at 37°C with cupric sulfate (2 nmol/ml) in the absence (—) or presence of quercetin (0.5 nmol/ml, —○—), quercetin (1.5 nmol/ml, —○—), isoquercitrin (0.6 nmol/ml, —▲—), isoquercitrin (1.5 nmol/ml, —▲—) and (0.4 µg/ml, —●—), (1.0 µg/ml, —●—), (1.5 µg/ml, —●—), (2.0 µg/ml, —●—) a 1-butanol extract of mulberry leaves in the total incubation volume.

Table 1. Scavenging Effects of Quercetin, Isoquercitrin and a 1-Butanol Extract of Mulberry Leaves on the DPPH Radical

Sample	EC ₅₀
Quercetin	2.29 ± 0.05 nmol/ml
Isoquercitrin	4.41 ± 0.14 nmol/ml
1-Butanol extract of mulberry leaves	17.3 ± 1.4 µg/ml

Values are means ± S.D. of triplicate measurements. To 40 nmol/ml ethanol solution of DPPH was added quercetin, isoquercitrin and a 1-butanol extract of mulberry leaves. The solution was left to stand for 30 min and the absorbance of the resulting solution at 517 nm was measured. The scavenging activity on the DPPH radical was expressed as EC₅₀, the concentration of the test compound required to give a 50% reduction in the absorbance compared with 40 nmol/ml DPPH in ethanol.

respectively compared with that without quercetin. These results show that quercetin inhibits the oxidation of rabbit plasma LDL. In the presence of 1.5 nmol/ml isoquercitrin, the lag-phase increased 6.9-fold so that isoquercitrin inhibits oxidation, but more weakly than quercetin. In the presence of 0.4 to 2.0 µg/ml MLBE, the lag-phase increased from 1.5- to 8.3-fold with the increase in concentration. The TBARS levels are shown in Fig. 2. Incubation of rabbit plasma LDL (0.25 mg/ml) with 10 nmol/ml copper for 4 h resulted in the formation of approximately 130 nmol TBARS/mg LDL, without the test substance. However, when 6 nmol/ml quercetin was present during incubation of rabbit LDL with copper, the formation of TBARS was completely inhibited, and 1.5 nmol/ml quercetin inhibited TBARS formation by 10%. Isoquercitrin gave similar results to quercetin. MLBE also completely inhibited TBARS formation following incubation with 20 µg/ml (Fig. 2).

The effect on human plasma LDL was examined in the same manner as for rabbit plasma LDL. The effect of the formation of conjugated dienes on the oxidation of human LDL is shown in Fig. 3. Incubation of 50 µg/ml human LDL with 1.66 nmol/ml copper produced a lag-phase of 78 min before onset of the propagation phase. On the other hand, in the presence of 0.5 nmol/ml quercetin the lag-phase was increased to about 110 min. In the presence of 0.5 nmol/ml and 1.0 mol/ml isoquercitrin, the lag-phase was increased to

Fig. 2. Inhibitory Effects of Isoquercitrin and a 1-Butanol Extract of Mulberry Leaves on Copper-Induced Oxidation of Rabbit LDL Measured by Determining TBARS Formation

Rabbit LDL (0.25 mg/ml) was incubated at 37°C with cupric sulfate (10 nmol/ml) for 4 h in the presence of quercetin, isoquercitrin or a 1-butanol extract of mulberry leaves. The LDL oxidation was measured by determining the formation of TBARS and the results are expressed as the percentage inhibition for the amount of TBARS without test substance.

about 100 and 165 min respectively. In the presence of 0.5 to 2.0 µg/ml MLBE, the lag-phase was increased from about 92 to 170 min, depending on the concentration. The relative lag-times for each concentration of isoquercitrin or MLBE for the lag-time in 0.5 nmol/ml quercetin are shown in Table 2. The effect of 0.5 nmol/ml isoquercitrin was weaker than that of quercetin at the same concentration, and the effect of 1.0 nmol/ml isoquercitrin was prolonged with a lag-time of about 1.5 times that of 0.5 nmol/ml quercetin. The effects of MLBE were equivalent to that of 0.5 nmol/ml quercetin at concentrations from 1.0 to 2.0 µg/ml. The inhibitory activity of mulberry components on the oxidation of LDL was further demonstrated by SDS-PAGE. While native LDL migrated as a single lipoprotein band on SDS-PAGE, as shown by Coomassie brilliant blue R-250 staining, the copper treated LDL showed multiple smaller bands as well as the original band on SDS-PAGE. No small molecular weight bands were seen in the case of LDL treated with copper in the presence of quercetin. Isoquercitrin showed a slight inhibitory effect on LDL decomposition while MLBE had a

Fig. 3. Inhibitory Effects of Isoquercitrin and a 1-Butanol Extract of Mulberry Leaves on Copper-Induced Oxidation of Human LDL Determined by Continuous Monitoring of Absorbance at 234 nm by Conjugate Dienes

Human LDL (0.05 mg protein/ml) was incubated at 37°C with cupric sulfate (1.66 nmol/ml) in the absence (—) or presence of quercetin (0.5 nmol/ml, —○—), quercetin (1.0 nmol/ml, —○—), isoquercitrin (0.5 nmol/ml, —▲—), isoquercitrin (1.0 nmol/ml, —▲—) and (0.5 µg/ml, —●—), (1.0 µg/ml, —●—), (2.0 µg/ml, —●—) a 1-butanol extract of mulberry leaves in the total incubation volume.

Table 2. Inhibitory Effects of a 1-Butanol Extract of Mulberry Leaves on the Lag-Time in the CuSO₄-Induced Oxidation of Human LDL

Samples	Conc.	n	Relative lag time ^{a)}
Quercetin	0.5 nmol/ml	6	1
Isoquercitrin	0.5 nmol/ml	6	0.71±0.21
	1.0 nmol/ml	6	1.55±0.43
1-Butanol extract of mulberry leaves	0.5 µg/ml	6	0.77±0.09
	1.0 µg/ml	5	0.86±0.05
	2.0 µg/ml	5	1.34±0.21

Values are means±S.D. of triplicate measurements. Human LDL (0.05 mg protein/ml) was incubated at 37°C with cupric sulfate (1.66 nmol/ml) in the absence or presence of quercetin, isoquercitrin and a 1-butanol extract of mulberry leaves in the total incubation volume. ^{a)} Values are relative to the lag-time of 0.5 nmol/ml quercetin assigned an arbitrary value of 1.0.

Table 3. Inhibitory Effects of a 1-Butanol Extract of Mulberry Leaves on CuSO₄-Induced Oxidation of Human LDL Determined by Measuring TBARS Formation

Samples	Conc.	TBARS (nmol/mg protein)	Rate of inhibition (%)
Control		29.8±0.8	
Quercetin	10 nmol/ml	0	100
	5 nmol/ml	9.1±2.8	68±12
	2.5 nmol/ml	31.0±0.2	0
Isoquercitrin	10 nmol/ml	0	100
	5 nmol/ml	26.7±1.2	11±4
1-Butanol extract of mulberry leaves	12.5 µg/ml	1.1±0.6	99±6
	10 µg/ml	10.4±1.6	61±6
	5 µg/ml	28.1±0.4	6±1

Values are means±S.D. of triplicate measurements. Human LDL (0.25 mg/ml) was incubated at 37°C with cupric sulfate (10 nmol/ml) for 4 h in the presence of quercetin, isoquercitrin or a 1-butanol extract of mulberry leaves. The extent of LDL oxidation was measured by determining the formation of TBARS and the results expressed as the percentage inhibition of the amount of TBARS without the test substance.

stronger inhibitory effect than isoquercitrin. The results of TBARS formation in human LDL are shown in Table 3. Incubation of 0.25 mg/ml human plasma LDL with 10 nmol/ml copper for 4 h resulted in the formation of approximately 30 nmol TBARS/mg LDL. However, when 10 nmol/ml

quercetin was added to the incubation mixture, the formation of TBARS was completely inhibited, and 2.5 nmol/ml quercetin had no effect. When 10 nmol/ml isoquercitrin was added to the incubation mixture, the formation of TBARS was completely inhibited. On the other hand, in the presence from 12.5 to 5 mg/ml MLBE, the formation of TBARS was inhibited by 99 to 6%.

Finally, we examined the hypothesis that isoquercitrin and MLBE may be hydrolyzed by the β -glycosidase of intestinal bacteria and isoquercitrin metabolites may be absorbed from the intestine. Quercetin, isoquercitrin and MLBE were given to mice for three days, and then plasma and urine were collected from the mice and treated with β -glucuronidase/sulfatase before HPLC analysis. As shown in Fig. 4, a peak corresponding to quercetin was seen in the plasma of mice given quercetin or isoquercitrin, but no peak in control mice. On the other hand, the chromatogram of the plasma from mice given MLBE showed peaks corresponding to quercetin and kaempferol. Analysis of the urine gave clearer results than plasma (data not shown).

DISCUSSION

It has been reported that atherosclerosis is accelerated by macrophages that are eventually transformed into foam cells, accompanied by cellular accumulation of oxidatively modified LDL-derived cholesterol.³⁻⁷⁾ On the other hand, we reported that mulberry leaves and MLBE exert hypolipidemic effects in hypercholesterolemic rabbits, such as suppression of the increase in levels of serum lipids and hepatic enlargement, and prevented atherosclerosis.^{1,2)} Also, it has been reported that mulberry leaves contain flavonoid glycosides, such as isoquercitrin, and the mulberry leaves and MLBE used in this study contained 1.35 or 5.18% isoquercitrin and 0.83 and 2.81% astragalin, respectively.²⁾

Quercetin, an aglycone of isoquercitrin, has been reported to have a variety of biological effects. There have been many reports of its hypolipidemic effects, such as reducing serum lipid levels, suppression of oxidation of LDL and formation of lipid hydroperoxide. The antioxidative effects of fla-

Fig. 4. HPLC Chromatograms of Plasma Obtained from Mice after β -Glucuronidase and Sulfatase Treatment (as Described in the Experimental Section)

A) Fisetin (50 μ mol/l, F), quercetin (50 μ mol/l, Q), kaempferol (50 μ mol/l, K). B) Plasma from mice given water orally. C) Plasma from mice given quercetin orally. D) Plasma from mice given isoquercitrin orally. E) Plasma from mice given a 1-butanol extract of mulberry leaves orally.

vonoids such as quercetin are mediated by the chelating action of the phenolic hydroxyl group with metal cations as oxidative inducers¹³⁾ or by free radical scavenging effects.^{24–27)} We investigated the scavenging activity of mulberry leaves on the DPPH radical. Quercetin, isoquercitrin and MLBE all exhibited a scavenging action on DPPH radicals. Quercetin had double the activity of isoquercitrin, and the effect of 17.3 μ g/ml MLBE was equivalent to that of 4.41 nmol/ml isoquercitrin.

We examined the effects of isoquercitrin and MLBE on the oxidative modification of LDL by determining the formation of TBARS and conjugated dienes. After incubation of human or rabbit LDL with copper, the production of conjugated dienes and TBARS increased. In contrast, when LDL was incubated with copper in the presence of quercetin, isoquercitrin or MLBE, oxidative modifications were inhibited in a dose-dependent manner, and the effect of isoquercitrin was only slightly weaker than that of quercetin. SDS-PAGE indicated that quercetin, isoquercitrin and MLBE inhibited physical and chemical changes involving oxidation of LDL. These findings indicate that MLBE suppresses the progression of atherosclerosis by scavenging hydrogen oxide radicals.

Quercetin, an aglycone, showed a higher antioxidative effect on the oxidation of LDL than its glycoside, isoquercitrin, in this study. It had been reported that flavonoid glycosides are hydrolyzed by mouth or intestinal bacterial flora to produce the biologically active aglycone,^{28–30)} and quercetin and quercetin metabolites have been found in plasma, bile acid and urine as glucuronide or sulfate conjugates, when rutin, a glycoside of quercetin, was orally administered to mice.¹⁴⁾ Therefore, we examined the metabolism and absorption of isoquercitrin and MLBE *in vivo*. When isoquercitrin or MLBE was orally administered to mice, isoquercitrin was metabolized to quercetin and MLBE was metabolized to quercetin and kaempferol, an aglycone of astragalin. The latter has also been reported to have antioxidative effects³⁰⁾ although these are considerably weaker than those of quercetin.

MLBE contains 5.2% isoquercitrin and 2.8% astragalin. If both of these compounds undergo complete hydrolysis, the amount of quercetin and kaempferol in 1.2 μ g MLBE would be 0.2 nmol and 0.1 nmol, respectively. However, 1.2 μ g/ml MLBE showed an inhibitory activity corresponding to that of 0.5 nmol quercetin in the formation of conjugated dienes. Thus, MLBE must contain other antioxidative compounds besides isoquercitrin and astragalin.

Our results show that isoquercitrin and astragalin in mulberry leaves prevent atherosclerosis in hypercholesterolemic rabbits. Also, these observations suggest that extracts of mulberry leaves contain other compounds that may be useful for the treatment of atherosclerosis.

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