Mulberry leaf extract restores arterial pressure in streptozotocin-induced chronic diabetic rats

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Introduction

An overview of diabetes mellitus

Characteristic & Type of Diabetes

- Chronic hyperglycemia
- Disturbance of CHOes, fat and protein metabolism
- Decreased production of insulin by the Beta-cells of the islet of Langerhans in the pancreas or
- Impaired insulin action
THE MAJOR DIABETIC COMPLICATIONS

- Eyes (retinopathy)
- Brain and cerebral circulation (cerebrovascular disease)
- Heart and coronary circulation (coronary heart disease)
- Kidney (nephropathy)
- Lower limbs (peripheral vascular disease)
- Peripheral nervous system (neuropathy)
- Diabetic foot (ulceration and amputation)

Introduction: The pathogenesis of endothelial dysfunction in diabetes
Introduction

*Morus alba* Linn.

**Family:** Moraceae  
**Genus:** *Morus*  
**Species:** *Morus alba* L.
Antioxidant flavonol glycosides in mulberry (Morus alba L.) leaves isolated based on LDL antioxidant activity

Takuya Katsube, Naoto Imawaki, Yasuhiro Kawano, Yoshimitsu Yamazaki, Kuninori Shiwaku, Yosuke Yamane

* Shimane Institute for Industrial Technology, 1 Hiyake-cho, Izumo City, Shimane, 693-8511, Japan
b National Institute of Advanced Industrial Science and Technology, Central 6, 1-1-1 Higashi, Tsukuba City, Ibaraki, 305-8566, Japan
c Department of Environmental and Preventive Medicine, Shimane University School of Medicine, 89-1 Enyocho, Izumo City, Shimane, 693-8511, Japan

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Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits

Chang-Chen Chen, Li-Kaung Liu, Jung-Dong Hsu, Hui-Pei Huang, Mon-Yuan Yang, Chau-Jong Wang

* Institute of Biochemistry, College of Medicine, Chang Gung Medical University, No.1, Sec. 1, Chien Kuo N. Road, Taichung 403, Taichung, Taiwan
b Department of Pathology, College of Medicine, Chang Gung Medical University Hospital, Taichung, Taiwan

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Effects of dietary mulberry, Korean red ginseng, and banaba on glucose homeostasis in relation to PPAR-α, PPAR-γ, and LPL mRNA expressions

Mi-Young Park, Kwang-Seung Lee, Mi-Kyung Sung

*Department of Food and Nutrition, College of Human Ecology, Seoul Women's University, Seoul, 146-742, Korea
b Korea Inum Co., National Agricultural Cooperative Federation, Changcheong-dokdo, 358-811, Korea

Received 1 October 2004; accepted 9 May 2005

Mulberry leaf ameliorates the expression profile of adipocytokines by inhibiting oxidative stress in white adipose tissue in db/db mice

Masayuki Sugimoto, Hidenori Arii, Yukinori Tamura, Toshinori Murayama, Parinda Khangkhan, Takuya Nishio, Koh Ono, Hiroaki Ariyasu, Takashi Akamizu, Yukihiko Ueda, Toru Kita, Shigeo Harada, Kaizo Kamei, Masayuki Yosode

* Department of Clinical Intensive Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan
b Department of CV Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

c Department of Cardiac Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

Received 1 October 2004; accepted 9 May 2005
How *Morus alba* leaf extract reduces the vascular complication of diabetes?
Aims of the study

1. To examine the antihyperglycemic effect of MA in STZ-induced diabetic rats.
2. To examine the antioxidant effect of MA in STZ-induced diabetic rats.
3. To examine the effect of MA on the cardiovascular function in chronic diabetic rats.
Materials & Methods
Plant material & extraction
Leaves of mulberry (Buriram 60 strain) cleaned, shade-dried & minced

Dried leaves were soaked in 50% ethanol (3 days)

The mixtures were filtered through packed cotton & gauze

Ethanol was removed from the supernatant by using the rotary vacuum evaporator at 60 ºC under reduced pressure

The crude extract was freeze-dried by the lyophilizer

The MA was kept at -20 ºC until used (yield is 23.70%)
Determination of gallic acid and quercetin in MA extract

- The ethanolic extract of MA was standardized by HPLC to detect gallic acid and quercetin as biomarkers for quality control of raw materials.
- The extract standardization was generously performed by Assoc. Prof. Dr. Arunporn Itharat (Applied Thai Traditional Medicine Centre, Thammasat University, Bangkok).

- Gallic acid = 65 ug/g
- Quercetin = 79 ug/g
Induction of STZ-induced diabetic rats

Male Sprague-Dawley rats
(200-250 g)

A single dose of STZ (45 mg/kgBW, i.p.)

After 7 days, overnight fasted

Blood glucose level was determined by glucometer
(200 mg/dl were considered diabetic)
Investigation of MA extract on blood pressure and vascular reactivity
STZ-induced diabetic rats

• Diabetic control (distilled water)
• Positive control (insulin 4 U/kgBW)
• MA treatments (0.25, 0.5 or 1.0 g/kgBW)

8 weeks

Fasting blood glucose level

Rats were anesthetized & femoral artery was cannulated

SBP, DBP, MAP & HR

Ach (3-30 nmol/kg, i.v.) or
SNP (1-10 nmol/kg, i.v.) or
PE (0.01-0.1 umol/kg, i.v.)

Mean arterial blood pressure

Rats were euthanized (pentobarbital over dose)

Heart, liver, kidney and thoracic aorta were isolated, homogenized and centrifuged
(Clear supernatant was collected for assay of MDA)
Results & Discussion
Table 1 Effects of long-term treatment with mulberry leaf extract (MA) on fasting blood glucose and body weight in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Fasting blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Normal control</td>
<td>217.2 ± 2.0</td>
<td>289.6 ± 6.4*</td>
</tr>
<tr>
<td>DM control</td>
<td>215.6 ± 3.7</td>
<td>178.6 ± 1.7**</td>
</tr>
<tr>
<td>DM + Insulin 4 U/kg</td>
<td>216.6 ± 5.9</td>
<td>263.0 ± 5.0*</td>
</tr>
<tr>
<td>DM + MA 0.25 g/kg</td>
<td>214.8 ± 4.2</td>
<td>181.2 ± 3.7**</td>
</tr>
<tr>
<td>DM + MA 0.5 g/kg</td>
<td>215.4 ± 1.2</td>
<td>248.4 ± 2.6*</td>
</tr>
<tr>
<td>DM + MA 1.0 g/kg</td>
<td>216.5 ± 4.2</td>
<td>254.0 ± 5.3*</td>
</tr>
</tbody>
</table>

* $P<0.05$, increases as compared to their initial values (before)
** $P<0.05$, decreases as compared to their initial values (before)
Table 2 Effect of mulberry leaf extract (MA) on systolic (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) in chronic diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Arterial blood pressure (mmHg)</th>
<th>Heart rate (beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SBP</td>
<td>DBP</td>
</tr>
<tr>
<td>Normal control</td>
<td>110.0 ± 2.7</td>
<td>79.8 ± 2.0</td>
</tr>
<tr>
<td>DM control</td>
<td>132.0 ± 7.3*</td>
<td>92.2 ± 3.21*</td>
</tr>
<tr>
<td>DM + Insulin 4 U/kg</td>
<td>108.4 ± 5.4**</td>
<td>75.2 ± 5.1**</td>
</tr>
<tr>
<td>DM + MA 0.25 g/kg</td>
<td>123.5 ± 5.3*</td>
<td>88.8 ± 3.1*</td>
</tr>
<tr>
<td>DM + MA 0.5 g/kg</td>
<td>117.0 ± 5.4**</td>
<td>77.6 ± 6.2**</td>
</tr>
<tr>
<td>DM + MA 1.0 g/kg</td>
<td>112.7 ± 2.1**</td>
<td>77.4 ± 3.3**</td>
</tr>
</tbody>
</table>

* \( P<0.05 \) as compared to normal control
** \( P<0.05 \) as compared to diabetic control
Figure 1 Effect of MA on changes in mean arterial pressure (MAP) in response to acetylcholine (ACh 3, 10 and 30 nmol/kg), sodium nitroprusside (SNP 1, 3 and 10 nmol/kg) and phenylephrine (PE 0.01, 0.03 and 0.1 µmol/kg) in diabetic rats.

* $P<0.05$ vs. normal control
# $P<0.05$ vs. diabetic control
### Table 3 Effect of mulberry leaf extract (MA) on malondialdehyde (MDA) content in liver, kidney, heart and thoracic aorta of diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Malondialdehyde (nmol/g protein)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Heart</td>
<td>Aorta</td>
</tr>
<tr>
<td>Normal control</td>
<td>42.5 ± 2.9</td>
<td>62.7 ± 3.9</td>
<td>77.9 ± 10.6</td>
<td>98.2 ± 4.8</td>
</tr>
<tr>
<td>DM control</td>
<td>97.1 ± 14.7*</td>
<td>120.7 ± 6.9*</td>
<td>107.0 ± 1.0*</td>
<td>185.4 ± 4.3*</td>
</tr>
<tr>
<td>DM + Insulin 4 U/kg</td>
<td>46.7 ± 5.9**</td>
<td>53.8 ± 2.8**</td>
<td>78.4 ± 6.0**</td>
<td>102.0 ± 6.5**</td>
</tr>
<tr>
<td>DM + MA 0.25 g/kg</td>
<td>75.6 ± 2.5*</td>
<td>103.9 ± 22.6*</td>
<td>101.7 ± 5.9*</td>
<td>173.7 ± 3.4*</td>
</tr>
<tr>
<td>DM + MA 0.5 g/kg</td>
<td>60.7 ± 4.4**</td>
<td>87.4 ± 13.6**</td>
<td>88.9 ± 6.3**</td>
<td>139.0 ± 11.7*, **</td>
</tr>
<tr>
<td>DM + MA 1.0 g/kg</td>
<td>58.3 ± 7.6**</td>
<td>77.5 ± 13.7**</td>
<td>79.3 ± 5.7**</td>
<td>120.7 ± 1.5**</td>
</tr>
</tbody>
</table>

* $P<0.05$ vs. normal control  
# $P<0.05$ vs. diabetic control
Discussion
Diabetes: ↓ relaxation in response to the endothelium-dependent and endothelium-independent vasodilators (ACh & SNP) 

↑ constriction in response to the α₁-adrenoceptor agonist (PE)

(Dresner et al., 1997; Kamata et al., 1992; Laight et al., 2000)
Discussion

• Mulberry leaf extract components
  – Quercetin, rutin & isoquercitrin
    • Free radical scavenging effect (antioxidant activity) (Katsube et al., 2006)
• Synthetic quercetin : restored the vascular function
  (Anjaneyulu et al., 2004; Machha et al., 2007)
• Clinical effectiveness of a flavonoid-rich dietary supplement in alleviating the vascular complication in diabetic patients. (Zibadi et al., 2008)
Morus alba Linn.

• Restoration of vascular response: vasodilator and vasoconstrictor
  – Hypoglycemic activity
  – Antioxidant activity: ↓ MDA content
Thank you for your attention
Actions of insulin in the vascular wall. The vascular wall responds to insulin stimulation by producing both nitric oxide (via tetrahydrobiopterin stimulation) and endothelin. The balance of insulin action on endothelin and nitric oxide is maintained in insulin-sensitive states. EC = Endothelial cells; ET-1 = endothelin-1; NO = nitric oxide; VSMC = vascular smooth muscle cells; BH4 = tetrahydrobiopterin.
Normal Endothelium

- Vasodilation
- Fibrinolysis
- Antiaggregation

Diabetic Endothelium

- ↑ Vascular tone
- ↑ Adhesion Molecules
- ↑ Platelet aggregation
- Collagen exposure
- Altered Extracellular
- ↓ NO bioavailability
- ↑ PKC-MAPK activation
- ↑ ROS production
- ↓ PI3-K/Akt activation
- ↑ AGE accumulation