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

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Abstract

Free radical-induced vascular dysfunction plays a key role in the pathogenesis of vascular disease found in chronic diabetic patients. *Morus alba* (MA) leaf extract is promoted for good health especially in diabetic patients. Interestingly, antidiabetic and antioxidant activities of MA have been reported in experimental animals. Thus, the hypothesis of this study was that the long-term treatment with MA could improve vascular reactivity of chronic diabetic rats. To test this hypothesis, we examined the effect of long-term treatment with MA on the vascular responses to vasoactive agents in streptozotocin-induced chronic diabetic rats. The diabetic rats were either orally administered with distilled water, MA (0.25, 0.5 and 1 g/kg per day) or subcutaneously injected with insulin (4 U/kg per day) for 8 weeks. After each treatment, the fasting blood glucose, blood pressure, vascular responses to vasoactive agents and tissue malondialdehyde were examined. *Morus alba* at the doses of 0.5 and 1 g/kg, which significantly reduced blood glucose level, also significantly decreased the high blood pressure in diabetic rats. Vascular responses of the chronic diabetic rats to vasodilators, acetylcholine (3-30 nmol/kg) and sodium nitroprusside (1-10 nmol/kg) were significantly suppressed by 26% to 44% and 45% to 77% respectively, whereas those to vasoconstrictor, phenylephrine (0.01-0.1 μmol/kg) were significantly increased by 23% to 38% as compared to normal rats. Interestingly, the administration of 0.5 and 1 g/kg MA or 4 U/kg insulin significantly restored the vascular reactivities of diabetic rats. Moreover, 8 weeks of diabetes resulted in the elevation of malondialdehyde content in tissues (liver, kidney, heart, and aorta), and MA treatment significantly lessened this increase. These results provide the first evidence for the efficacy of MA in restoring the vascular reactivity of diabetic rats, the mechanism of which may associate with the alleviation of oxidative stress.

Keywords: *Morus alba*; Mulberry; Diabetes mellitus; Endothelial dysfunction; Vascular reactivity; Lipid peroxidation; Rat

Abbreviations: ACh, acetylcholine; AGEs, advanced glycation end products; ANOVA, analysis of variance; DBP, diastolic blood pressure; HR, heart rate; MA, *Morus alba* leaf extract; MAP, mean arterial pressure; MDA, malondialdehyde; NO, nitric oxide; PE, phenylephrine; ROS, reactive oxygen species; SBP, systolic blood pressure; SNP, sodium nitroprusside; STZ, streptozotocin.

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1. Introduction

The main etiology of morbidity and mortality in patients with chronic diabetes is vascular complication [1]. Previous studies suggested that the increased reactive oxygen species (ROS) generation might be associated with the development of vascular complications in diabetes [2]. The decreased endothelium-dependent vasodilatation and increased vasoconstriction have been demonstrated in various vascular beds of different animal models with diabetes [3,4] and in humans [5] with type 1 diabetes. The biochemical pathways resulting from hyperglycemia including glucose oxidation, the formation of advanced glycation end-products (AGEs), and the activation of polyol pathways are associated with the generation of ROS and ultimately leading to lipid peroxidation and cellular damage in a vascular tissues [6] and also in various body tissues [7]. Malondialdehyde levels are the indicative of the extent of lipid peroxidation as a result of oxidative degeneration of polyunsaturated fatty acids.

In traditional medicine, several medicinal plants or their extracts are widely used in many countries for the treatment of diabetes such as *Momordica charantia* and *Tinospora cordifolia* [8]. Mulberry tree (*Morus alba* L. [MA]) is widely grown throughout Thailand. Mulberry leaves are commonly used as silkworm diet, and mulberry leaf extract has been promoted for good health, especially in diabetic patients. An antidiabetic activity of mulberry leaf extract in experimental animals has been reported [9-11]. In addition, mulberry leaf extract has been demonstrated to contain several substances that can act as potent antioxidants or free radical scavengers such as flavonoids and moracins [12,13]. Importantly, there has been scientific evidence showing that the antioxidant compounds found in mulberry leaf ethanol extract are absorbed from the small intestine into the blood circulation and retain their antioxidant activity in the animal [14]. Therefore, we hypothesized that by hypoglycemic and antioxidant activities of mulberry leaf extract, the consumption of the extract might decrease blood glucose and improve vascular reactivity of chronic diabetics. This potential would be beneficial for the treatment and alleviation of vascular complication in diabetic patients.

To verify our hypothesis, the objective of this study was to evaluate the effect of mulberry leaf extract consumption on the blood glucose and on the vascular function in streptozotocin-induced chronic diabetic rats by determining blood pressure and vascular responses to vasoactive agents. To clarify antioxidant activity of the extract in chronic diabetes, the oxidative damage of relevant organs was also examined.

2. Methods and materials

2.1. Drugs and chemicals

EDTA; thiobarbituric acid (TBA); sodium dodecyl sulfate; butylated hydroxyluene; 1,1,3,3-tetraethoxypropane; and phenylephrine hydrochloride were purchased from Sigma Chemical Co Ltd (St Louis, Mo). Trichloroacetic acid, acetylcholine chloride, and sodium nitroprusside were obtained from Fluka Chemika Co Ltd (Buchs, Switzerland). All other chemicals used were of analytical grade quality.

2.2. Plant extraction

Leaves of mulberry were collected from a demonstration plot at the Department of Entomology, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, between March and June 2007. A voucher specimen (KKU 2513) is deposited at the Pharmaceutical Science Herbarium, Khon Kaen University, Thailand.

Dried leaves were extracted with 50% ethanol. The mixture was filtered, evaporated in vacuum evaporator, and then lyophilized in order to obtain the dry extract. Using this procedure, the yield was 23.7% of the starting dry weight of the leaves. The obtained MA leaf extract was kept in air-tight container at −20°C until it was used. The extract was standardized using high-performance liquid chromatography to examine the amount of gallic acid and quercetin which were 65 ± 7 and 79 ± 1 μg/g, respectively.

2.3. Animals

Male Sprague-Dawley rats (200-250 g) were obtained from the Experimental Animal Unit, Faculty of Medicine, Khon Kaen University, Thailand. They were maintained in an air-conditioned room (25 ± 1°C), a 12-hour light/dark cycle and fed with standard diet (CP Mouse Feed, Bangkok, Thailand) containing all essential nutrients recommended for growing rat by the National Laboratory Animal Center, Bangkok, Thailand, and water ad libitum. All procedures complied with national standards for the care and use of experimental animals and were approved by the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (Rec. No: AEKKU0019/05). At the end of the experiments, the animals were euthanized by an overdose of anesthetic drug.

2.4. Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of 45 mg/kg body weight streptozotocin (STZ) dissolved in 0.1 M citrate buffer (pH 4.5). Seven days after STZ injection, the blood was collected from tail vein to determine fasting blood glucose level. Only rats with fasting blood glucose over 200 mg/dl were considered diabetes and included in the experiments.

2.5. Experimental design

The rats were divided into 6 groups with 6 rats in each group and treated as follows: group 1: nondiabetic control rats with distilled water; group 2: diabetic rats with distilled water; group 3 to 5: Diabetic rats with MA at doses of 0.25, 0.5, and 1.0 g/kg per day, respectively, and group 6: diabetic rats with 4 U/kg per day insulin (Monotard). All treatments were continued for 8 weeks. Distilled water and MA were