Moringa Oleifera Leaf Increases Insulin Secretion after Single Dose Administration: A Preliminary Study in Healthy Subjects

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Background: *Herbal medicine has long been used as an alternative medicine for treatment of type 2 diabetes mellitus (T2DM). Recently, Moringa oleifera (MO or ma-rum in Thai) leaf has been widely used in T2DM patients. Several studies in diabetes rat model have shown that MO had effect on glucose metabolism. However, study in humans is lacking.*
Objective: *Examine effects of MO on plasma glucose and insulin secretion.*
Material and Method: *Ten healthy volunteers were enrolled in this study (mean age 29±5 years; BMI 20.6±1.5 kg/m^2; FPG 81±5 mg/dl). After an overnight fast and every two weeks, subjects received an oral dose of MO at increasing dosages of 0, 1, 2, and 4 g. Plasma glucose (PG) and insulin were collected at baseline and at 0.5, 1, 1.5, 2, 4, and 6 hours after each MO dosage administration. Insulin secretion rate was measured using area under the curve (AUC) of insulin and AUC of insulin/glucose ratio.*
Results: *After doses of 0, 1, 2, and 4 g MO, mean plasma insulin increased (2.3±0.9, 2.7±1.0, 3.3±1.4, and 4.1±1.7 μU/ml, respectively) despite there being no differences in mean PG (77±6, 78±5, 79±6, and 79±5 mg/dl, respectively). AUC of insulin was greater after high-dose MO (4 g) than after baseline or low-dose MO capsule (1 g) (24.0±3.5 vs. 14.5±1.8 or 16.1±2.0, respectively; p = 0.03), while there was no difference in AUC of glucose. Accordingly, insulin secretion rate represented by AUC of insulin/glucose ratio after high-dose MO was significantly increased by 74% (p = 0.041), as compared with that of baseline.*
Conclusion: *We concluded that high-dose (4 g) MO leaf powder capsules significantly increased insulin secretion in healthy subjects. These results suggest that MO leaf may be a potential agent in the treatment of type 2 diabetes. Further studies of MO in patients with T2DM are needed.*

Keywords: *Blood sugar, Herb, Hypoglycemic drug, Moringa oleifera*

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Type 2 diabetes mellitus (T2DM) is a major public health problem. Insulin resistance and impairment of pancreatic insulin secretion characterize the pathogenesis of T2DM[1]. At the present time, there is no curative treatment for T2DM. Poorly controlled glycemia results in microvascular and macrovascular complications that often evolve into morbidity and mortality. Treatment of T2DM and related complications is usually complex and costly. Herbal medicines have long been used as an alternative medicine in the treatment of T2DM[2].

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Moringa oleifera (MO or ma-rum) is a widely known traditional herb in Thailand. MO seed is an important and widely used ingredient in Thai traditional food. In India and Pakistan, MO is used as an herbal medicine for treatment of diarrhea, infections, cancer, hypertension, and hyperthyroidism[3,4]. A recent cross-sectional survey in Senegal found that MO is most commonly used as an herbal medicine treatment for T2DM[5]. Several studies[6,7] in non-diabetic and diabetic rat models demonstrated that MO leaf could decrease plasma and urine glucose and improve results of glucose tolerance status. These hypoglycemic effects have been postulated to be associated with decreased intestinal glucose uptake and slowing of gastric emptying time as a result of fiber content in MO leaf[7]. However, the effects of MO leaf on insulin secretion and insulin resistance have not been studied, most

notably, in humans. Therefore, our preliminary study aimed to evaluate the effect of MO on plasma glucose and insulin secretion in healthy human subjects.

Material and Method

Subjects

Ten healthy subjects (five males and five females) aged 20 to 40 years were enrolled in the study. Study participants had no underlying diseases, took no medications, and had normal body mass index (BMI, 18.5-23 kg/m^2). Exclusion criteria included subjects had first degree relative having diabetes and/or hypertension, fasting plasma glucose (FPG) higher than 100 mg/dl, serum creatinine (Cr) higher than 1.5 mg/dl, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) higher than twice the upper limit of normal, and patients taking any medications known to affect glucose metabolism within the past three months.

Study design

The Siriraj Institutional Review Board (SIRB) approved the study protocol and all subjects provided written informed consent. Each subject visited the clinical research unit, Division of Endocrinology and Metabolism, Faculty of Medicine Siriraj Hospital four times (Fig. 1). After an overnight fast and every two weeks, subjects received an oral dose of MO at increasing dosages of 0 g (baseline), 1 g (low dose), 2 g (moderate dose), and 4 g (high dose). Plasma glucose (PG) and insulin level were measured at baseline and 0.5, 1, 1.5, 2, 4, and 6 hours after each dosage of MO. Venous blood samples were also collected for measurements of FPG, blood urea nitrogen (BUN), Cr, AST, ALT, and electrolytes at the first and fourth visit. All subjects continued fasting during the entire 6-hour testing period. If subjects developed symptomatic hypoglycemia (PG of less than 45 mg/dl with hypoglycemic symptoms) during the test, the test was terminated.

Study drug

Preparation of MO leaf powder capsules

A single batch of MO leaf powder capsules was manufactured at the Herbal Medicine and Products Manufacturing Unit, Center of Applied Thai Traditional Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand), according to good manufacturing practice guidelines. Leaves of MO were separated from foreign matter, cleaned, and oven-dried. The dried leaves were then ground and sifted into powder. Gelatin capsules were then filled with MO dried leaf powder to create 500 mg capsules.

Quality assessment of the study drug

MO quality control and assessment consisted of uniformity of weight test (not more than ±15%), disintegration time test (not more than 30 minutes), and qualitative analysis for classes of compounds[8], including alkaloids, cyanogenic glycosides, tannins, phenolic compounds, flavonoids, anthraquinones, saponins, and cardiac glycosides. The results of phytochemical screening are shown in Table 1.

Plasma glucose and insulin measurement

Plasma glucose was measured by enzymatic (hexokinase) method on a Modular P800 system (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Plasma insulin concentration was measured by sandwich immunoassay using electro chemiluminescence immunoassay (ECLIA) on a Modular Elecsys E170 system (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Intra-assay coefficient of variation (CV) 1.5% and inter-assay CV 4.9% apply to insulin detection range of 5.93±0.09 μU/ml. Intra-assay CV 0.9% and inter-assay

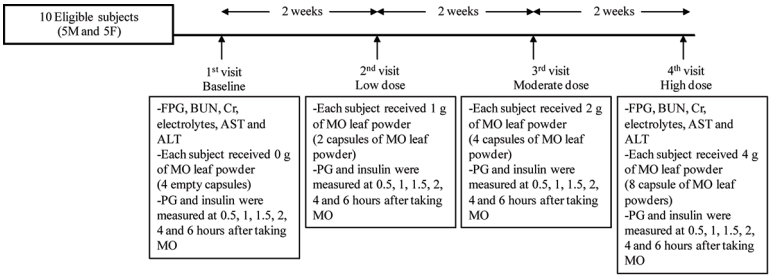

Fig. 1 Study protocol: after an overnight fast and every two weeks, subjects received an oral dose of MO at increasing dosages of 0 g (baseline), 1 g (low dose), 2 g (moderate dose), and 4 g (high dose); plasma glucose and insulin level were measured at baseline and at 0.5, 1, 1.5, 2, 4, and 6 hours after each dose administration of MO.

Table 1. Phytochemical profile of *Moringa oleifera* (MO) leaf capsule

Phytochemical class	Result
Alkaloids	+
Cyanogenic glycosides	-
Tannins	-
Phenolic compounds	+
Flavonoids	+
Anthraquinones	-
Saponins	-
Cardiac glycosides	-

- = not detected; + = detected

Table 2. Demographic and clinical data of 10 subjects at baseline and after high dose (4 g) of MO leaf powder

	First visit (n = 10)	Fourth visit (n = 10)
Age (year)	29.0±5.0	-
BMI (kg/m^2)	20.6±1.5	-
SBP (mmHg)	110.0±10.0	108.0±10.0
DBP (mmHg)	66.0±8.0	71.0±6.0
FPG (mg/dl)	82.0±5.0	81.0±6.0
BUN (mg/dl)	10.7±2.6	10.5±3.2
Cr (mg/dl)	0.9±0.2	0.8±0.2
AST (u/l)	24.0±4.0	23.0±7.0
ALT (u/l)	17.0±9.0	20.0±9.0

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; BUN = blood urea nitrogen; Cr = creatinine; AST = aspartate aminotransferase; ALT = alanine aminotransferase
Data presented as mean ± SD

CV 3.7% apply to the higher insulin detection range of 14.5±0.13 µU/ml. Minimal detection concentration was 0.20 µU/ml. The assay had no cross-reactivity with proinsulin.

Insulin secretion analysis

Insulin secretion was calculated using area under the curve (AUC) of insulin and AUC of insulin/glucose ratio.

Statistical analysis

All data are expressed as mean ± SD for continuous variables and percentage for qualitative variables. Continuous variables were compared using paired-samples t-test. Differences in insulin secretion between variable dosages of MO were determined using repeated measures ANOVA. For all analyses, a $p<0.05$ was considered to be statistically significant. All statistical analysis was performed using SPSS software version XX (SPSS, Inc., Chicago, IL, USA).

Results

Subject characteristics

Table 2 summarizes the clinical and laboratory characteristics of study subjects. There were no differences in BUN, Cr, AST, and ALT between the first and fourth visit. These findings demonstrated that there were no adverse effects after high dose (4 g) of MO leaf powder.

Effects of MO leaf powder on plasma insulin and glucose

After receiving 0 g, 1 g, 2 g, and 4 g dosages of MO capsules, mean plasma insulin concentrations during the 6-hour study increased (2.3±0.9, 2.7±1.0, 3.3±1.4, and 4.1±1.7 µU/ml, respectively; $p = 0.024$ and $p = 0.011$ for high-dose vs. baseline and high-dose vs. low-dose, respectively) despite there being no differences in mean PG (77±6, 78±5, 79±6, and 79±5 mg/dl, respectively; $p = 1.0$) (Fig. 2A, B).

Effects of MO leaf powder on insulin secretion

AUC of insulin was greater after high dose (4 g) of MO capsule than after baseline or low dose (1 g) of MO capsule (24.0±3.5 vs. 14.5±1.8 or 16.1±2.0, respectively; $p = 0.03$). There was no difference in AUC of glucose (Table 3, Fig. 3A, B). Insulin secretion rate represented by AUC of insulin/glucose ratio after high dose of MO leaf powder was significantly higher than that of baseline and low-dose MO ($p = 0.019$ and $p = 0.016$, respectively). There was no difference in AUC of insulin/glucose ratio after high dose, as compared to moderate dose of MO leaf powder ($p = 0.26$) (Table 3, Fig. 3C).

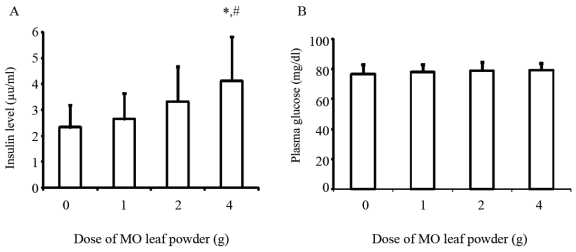

Fig. 2 Mean plasma insulin (A) and mean plasma glucose (B) throughout the 6-hour study after each dose of MO leaf powder. *,# $p<0.05$ for high-dose vs. baseline and high-dose vs. low-dose, respectively.

Table 3. Area under the curve (AUC) of insulin, glucose, and insulin/glucose after each dosage of MO leaf powder

AUC	MO leaf power (g)			
	0	1	2	4
Insulin	14.46±1.76*	16.11±2.01*	19.47±2.48	24.07±3.46
Glucose	478.8±13.7	468.4±10.5	489.3±11.6	491.7±9.9
Insulin/glucose	2.99±0.32*	3.30±0.38*	3.95±0.44	4.85±0.62

Data presented as mean ± SEM
* $p<0.05$ compared to high dose (4 g) of MO leaf powder

Discussion

In this study, we demonstrated significant increase in insulin secretion after administration of a single 4 g dose of MO leaf powder, despite there being no change in plasma glucose, without any adverse effect in healthy subjects. This is the first study in humans showing the effect of MO leaf on insulin secretion, one of two important components in the pathogenesis of T2DM. This data suggests that MO leaf powder may be useful in the treatment of T2DM.

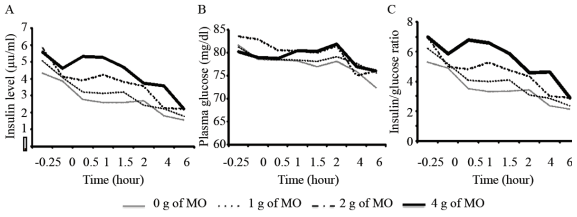

Fig. 3 Plasma insulin (A), glucose (B), and insulin/glucose ratio (C) at different time points after each dose of MO leaf powder. AUC of insulin/glucose ratio after high-dose MO (4 g) was significantly increased by 74% (p = 0.041), as compared with that of baseline (Fig. 4).

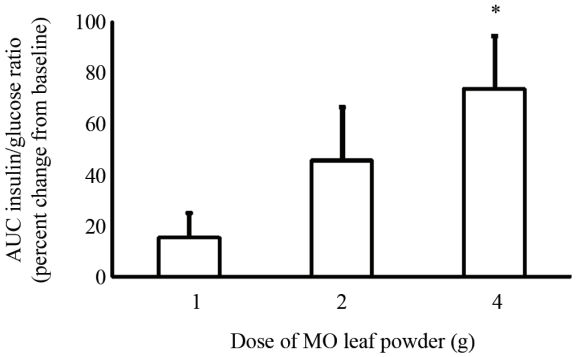

Fig. 4 Percent change in AUC of insulin/glucose ratio compared to baseline. * $p<0.05$ compared to baseline dose (0 g) of MO leaf powder. MO = *Moringa oleifera*; AUC = area under the curve.

Interestingly, a recent in vitro study[9] demonstrated that an extraction from MO leaf could inhibit alpha-glucosidase enzyme activity comparable to the effect of an alpha-glucosidase inhibitor (an oral hypoglycemic agent). These results suggest another effect of MO leaf that should be investigated and verified, especially in T2DM subjects.

Kar et al studied the hypoglycemic activity of 30 medicinal plants in alloxan diabetic rats and showed that treatment with MO stem bark once a day for one week decreased blood glucose from 203±22 to 108±13 mg/dl and urine sugar from one plus to negative, respectively[6]. Another study[7] compared the effect of a single dose (200 mg/kg) of MO leaf powder to placebo on glucose tolerance in Wistar rats (normal rat) and Goto-Kakizaki rats (GK) (model of T2DM rat) and showed that MO significantly decreased AUC of glucose in both normal and T2DM rats, as compared to placebo. A study in streptozotocin (STZ) diabetic rats (model of T1DM rat) demonstrated that aqueous extract of MO leaf reduced FPG and improved glucose tolerance[10]. Moreover, a recent study has shown that 8 g of MO leaf powder for 14 days decreased fasting plasma glucose and 2-hour postprandial glucose level in human T2DM[11]. In contrast to the studies described above, our study showed no difference in plasma glucose levels after each dose of MO leaf powder. This discrepancy probably occurred due to subjects in this study being healthy and having normal compensatory mechanism during fasting to prevent hypoglycemia. As a result, there was no effect of MO leaf on plasma glucose. As such, further studies are needed to clarify the long-term effects of MO leaf on plasma glucose and the mechanism by which MO leaf powder decreases plasma glucose in subjects with T2DM.

In conclusion, high-dose MO leaf powder capsules significantly increased insulin secretion in healthy subjects. These results suggest that MO leaf may be a potential agent for treating T2DM. Further studies in patients with T2DM are needed.

What is already known on this topic?

Several studies showed that MO had effects on glucose metabolism in diabetes rat model but the study in human is lacking. These hypoglycemic effects have been postulated to be associated with decreased intestinal glucose uptake and slowing gastric emptying time by fiber in MO leaf. The effects of MO leaf on insulin secretion and insulin resistance have not been studied.

What this study adds?

MO leaf powder capsules significantly increased insulin secretion in healthy subjects. These results suggested that MO leaf might be a potential agent to treat type 2 diabetes patients.

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Funding disclosure

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Potential conflicts of interest

None.

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ผลของใบมะรุมอบแห้งต่อการหลั่งอินซูลินในอาสาสมัครที่ไม่เป็นเบาหวาน

พิมพ์ใจ อันทานนท์, ณัชกร ล้ำเลิศกิจ, ประวิทย์ อัครเสรีนนท์, สาธิต วรรณแสง, อภิรดี ศรีวิจิตรกมล

ภูมิหลัง: *มะรุมเป็นพืชพื้นบ้านที่ใช้เป็นส่วนประกอบของอาหารไทยมานาน ในประเทศไทยมีผู้เป็นเบาหวานจำนวนมากที่ใช้ใบมะรุมตากแห้งเพื่อรักษาเบาหวาน จากการศึกษาในสัตว์ทดลองพบว่าใบมะรุมมีผลต่อเมตะบอลิสมของกลูโคส แต่อย่างไรก็ตามยังไม่มีการศึกษาผลของใบมะรุมตากแห้งในคน*

วัตถุประสงค์: *เพื่อศึกษาผลของใบมะรุมอบแห้งต่อการหลั่งอินซูลิน*

วัสดุและวิธีการ: *โดยการศึกษานี้คัดเลือกผู้เข้าโครงการศึกษาที่ไม่เป็นเบาหวานทั้งสิ้น 10 คน เพื่อทดสอบการหลั่งของอินซูลินก่อนรับประทานแคปซูลใบมะรุมตากแห้ง และหลังจากรับประทานแคปซูลใบมะรุมขนาด 1, 2 และ 4 กรัมต่อครั้ง โดยแต่ละครั้งห่างกันอย่างน้อย 2 สัปดาห์ โดยแคปซูลใบมะรุมตากแห้งนี้ผลิตจากสถานการแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ศิริราชพยาบาล*

ผลการศึกษา: *จากการศึกษาพบว่าหลังจากอาสาสมัครรับประทานแคปซูลใบมะรุมอบแห้งในขนาดที่สูงขึ้นจะมีระดับอินซูลินในเลือดเพิ่มขึ้น โดยที่ระดับน้ำตาลในเลือดไม่แตกต่างกัน โดยการรับประทานแคปซูลมะรุมขนาด 4 กรัม ซึ่งเป็นขนาดสูงสุดที่ทำการศึกษาจะทำให้มีการหลั่งอินซูลินออกมาเพิ่มขึ้นมากกว่าตอนที่ไม่ได้รับประทานแคปซูลใบมะรุมถึงร้อยละ 74 โดยไม่มีผลของข้างเคียงทั้งอาการและหน้าที่การทำงานของตับและไตจากการรับประทานแคปซูลใบมะรุม*

สรุป: *ดังนั้นการศึกษานี้จึงแสดงให้เห็นว่าแคปซูลใบมะรุมตากแห้งนี้สามารถเพิ่มการหลั่งอินซูลินได้ในอาสาสมัครที่ไม่เป็นเบาหวาน*