

Anti-hyperglycemic Properties of *Moringa oleifera* Lam. Aqueous Leaf Extract in Normal and Mildly Diabetic Mice

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Abstract: Aqueous leaves extract of *Moringa oleifera* Lam. was studied for its anti-hyperglycemic properties in normal and mildly diabetic mice by the Oral Glucose Tolerance Test (OGTT). The glibenclamide (1 mg/100 g BW) was used as reference drug. And mildly diabetes was induced by a single intraperitoneal injection of streptozotocin (6 mg/100 g BW). The blood glucose tolerance efficiency of the extract at doses of 100, 200 and 300 mg/100 g BW in normal group were 25.99, 31.25 and 43.19% and in mildly diabetic mice were 45.17, 53.31 and 59.16% of glibenclamide, respectively. In conclusion, the aqueous leaves extract of *Moringa oleifera* Lam. revealed anti-hyperglycemic activity in normal mice and improved the glucose tolerance impairment in mildly diabetic mice. Thus, *Moringa oleifera* Lam may be introduced as an anti-diabetic herb.

Keywords: Diabetes, glibenclamide, glucose tolerance, *Moringa oleifera* Lam., OGTT, streptozotocin

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder. Diabetic patients exhibit a state of chronic hyperglycemia and glucose tolerance impairment (Tiwari and Roa, 2002). Furthermore, oxidative stress concordantly occurs with hyperglycemia (Vijayalingam *et al.*, 1996) and causes pathogenesis in many organs, such as vasculopathies, neuropathies, nephropathies and ophthalmopathies (Atkinson and Maclaren, 1994). Thus, throughout their lives patients need to use a synthetic drug to control their blood glucose level and to improve blood glucose tolerance. Recently, the use of herbal products has gained more interest for remedy of diabetes and other ailments. One source is *Moringa oleifera* Lam., which has the common name drumstick tree and belongs to the family of Moringaceae. It is called the miracle tree, since all of its parts are edible and valued in nutrition and medicine. It is used as a traditional medicine for abortion (Nath *et al.*, 1992), pyretherapy (Shigh and Kuma, 1999) and for diabetes (Gupta and Mishra, 2002). Especially, the hypoglycemic action of *M. oleifera* aqueous leaf extract has been found in normal and sub, mildly and severely diabetic rats (Jaiswal *et al.*, 2009). Moreover, the beneficial effects of ethanolic extracts of *M. oleifera* leaves were found in diabetic rat only (Tende *et al.*, 2011). The aim of this study was to determine the anti-hyperglycemic activity of *Moringa oleifera* Lam. leaf extract in the normal and mildly diabetic mice.

MATERIALS AND METHODS

Extract preparation: *Moringa oleifera* Lam. was obtained from gardeners in Loei province, northeast Thailand and authenticated by a plant taxonomist from the Department of Biology, Faculty of Science, Khon Kaen University, Thailand. Voucher specimens (reference No. Yithaya -1) are preserved in KKU Herbarium, Thailand. The leaves (480 g) were cleaned, extracted by boiling for 1 h, filtered with cotton mesh and then evaporated in a hot air oven at 45°C. This dried extract weighed 75.85 g and was diluted with distilled water at concentrations of 200, 400 and 600 mg/mL for oral administration at 0.5 mL/100 g BW of animal by feeding needle.

Animals: Adult male mice (ICR strain, 8-week old, weighing 35-40 g) were obtained from the National Laboratory Animal Center, Nakornprathom province, Thailand. They were housed in a room under light: dark = 12:12 h, 25±1°C and were fed on a standard pellet diet (Chareanpogapan Ltd., Bangkok, Thailand) with water *ad libitum*. The experiments were performed after approval by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand (Reference No. 0514.1.12.2/28).

Diabetic induction: Male mice had diabetes induced by a single intraperitoneal injection of streptozotocin (6 mg/100 g BW). Diabetes was confirmed by fasting

blood glucose determination on the third day after induction and blood glucose levels greater than 140 mg/dL but less than 200 mg/dL were considered as mildly diabetic animals (Latha and Pari, 2004).

Blood glucose determination: After overnight fasting for 16 h with free access to water, Fasting Blood Glucose (FBG) was determined via a drop of blood from the tail artery by a blood glucometer (ONE TOUCH® Horizon™, LifeScan, Inc. 2006, Johnson and Johnson Company, Bangkok, Thailand).

Experiments:

Anti-hyperglycemic properties of *M. oleifera* Lam. leaf extract in normal mice by OGTT: Thirty male mice were used. Body weight and fasting blood glucose levels of all mice were determined before the start of the experiment. They were assigned into 5 groups of 6 animals each.

Group I: Was given distilled water 0.5 mL/100 g BW

Group II: Was given glibenclamide (reference drug) 1 mg/100 g BW

Groups III-V: Were given the extract at doses of 100, 200 and 300 mg/100 g BW, respectively (following the method of Jaiswal *et al.* (2009)).

After 30 min of administration, all groups were orally fed with 2 g/100 g BW of glucose and the blood glucose levels were determined at 0, 1, 2 and 3 h after glucose uptake.

Anti-hyperglycemic properties of *M. oleifera* Lam. leaf extract in mildly diabetic mice by OGTT: Thirty mildly diabetic male mice were used. Body weight and fasting blood glucose levels of all mice were measured before the start of the experiment. They were divided into 5 groups of 6 animals each:

Group I: Received distilled water at 0.5 mL/100 g BW

Group II: Received glibenclamide (reference drug) at 1 mg/100 g BW

Groups III-V: Received the extract at doses of 100, 200 and 300 mg/100 g BW, respectively

After 30 min of treatment, all groups were gavaged with 2 g/100 g BW of glucose and the blood glucose levels were determined at 0, 1, 2 and 3 h after glucose uptake.

Statistical analysis: Blood glucose levels were expressed in mg/dL as mean±standard deviation ($\bar{x} \pm S.D.$), then the results were converted to glucose tolerant efficiency. One-way ANOVA and Duncan multiple test were used to analyze the different results among groups. Values of $p < 0.05$ were considered to be statistically significant (Zarr, 1999).

RESULTS

Anti-hyperglycemic properties of *M. oleifera* Lam. Extract (MoE) in normal mice: Blood glucose levels of normal mice were detected at 0, 1, 2 and 3 h (data not shown) and then the results were converted to glucose tolerant efficiency (Table 1).

Anti-hyperglycemic properties of *M. oleifera* Lam. Extract (MoE) in mildly diabetic mice: Blood glucose levels of diabetic mice detected at 0, 1, 2 and 3 h (data not shown) and then the results were converted to glucose tolerance efficiency (Table 2). However, the blood glucose tolerance efficiency of the extract in the normal mice was less potent than those in the diabetic mice (Table 1 and 2).

DISCUSSION

Oral administration of *M. oleifera* aqueous leaf extract at doses of 100, 200 and 300 mg/100 g BW has been found to have anti-hyperglycemic properties by increasing blood glucose tolerance in the normal mice, which was less potent than those of streptozotocin induced mildly diabetic mice. The streptozotocin was used to induce type 2 diabetes in adult animals. It caused DNA damage and generated superoxide radicals to destroy the beta-cells (Szkudelski, 2001). This experiment used glibenclamide as a reference drug,

Table 1: Efficiency of blood glucose tolerance of *M. oleifera* Lam. leaf Extract (MoE) in normal mice

Treatment (mg/100 g BW) N = 6	Increase of blood glucose (%) ^A (blood glucose tolerance) ^B				Efficiency of blood glucose tolerance
	1 h	2 h	3 h	/hours	
0	89.93±45.00 ^a	20.02±26.70 ^a	-11.04±6.17 ^a	32.97±21.60 ^a	-
Glibenclamide 1	-9.65±31.20 ^c (99.58)	-63.03±6.54 ^c (83.05)	-70.81±5.76 ^b (59.77)	-47.83±9.81 ^b (80.80)	100
MoE 100	52.43±10.39 ^b (37.50)	4.09±22.91 ^{ab} (15.93)	20.59±16.80 ^c (9.55)	11.97±14.42 ^c (21.00)	25.99
MoE 200	50.42±13.22 ^b (39.51)	-6.51±16.43 ^b (26.53)	20.84±16.73 ^c (9.80)	7.69±13.69 ^c (25.28)	31.29
MoE 300	29.76±22.13 ^b (60.17)	10.35±23.04 ^b (30.37)	-25.19±9.10 ^a (14.15)	-1.93±9.99 ^c (34.90)	43.19

N: Number of experimental animals; BGL: Blood glucose level; A: BGL at n h (mg/dL) - BGL at 0 h (mg/dL) /BGL at 0 h×100; B: Increase of BGL in control group (%) - increase of BGL in treated group (%); Same letter in column means non-significant difference ($p > 0.05$); Different letter in column means significant difference ($p < 0.05$)

Table 2: Efficiency of blood glucose tolerance of *M. oleifera* Lam. leaf Extract (MoE) in diabetic mice

Treatment (mg/100 g BW) N = 6	Increase of blood glucose (%) ^A (blood glucose tolerance) ^B				Efficiency of blood glucose tolerance
	1 h	2 h	3 h	/hour	
0	76.22±16.49 ^a	50.98±9.27 ^a	23.48±21.61 ^a	50.23±11.95 ^a	-
Glibenclamide 1	31.35±18.60 ^b (44.87)	-42.57±7.19 ^b (93.55)	-52.84±4.61 ^b (76.32)	-21.35±9.25 ^b (71.58)	100
MoE 100	75.42±22.53 ^a (0.80)	0.59±13.72 ^c (51.57)	22.30±12.32 ^c (45.78)	17.90±6.45 ^c (32.33)	45.17
MoE 200	56.09±11.52 ^{ab} (20.13)	1.09±17.94 ^c (49.08)	20.98±14.89 ^c (44.46)	12.07±9.95 ^c (38.16)	53.31
MoE 300	55.03±31.93 ^{ab} (21.19)	13.90±12.10 ^c (64.88)	17.50±13.72 ^c (40.98)	7.88±11.77 ^c (42.35)	59.16

N: Number of experimental animals; BGL: Blood glucose level; A: BGL at n h (mg/dL) - BGL at 0 h (mg/dL) /BGL at 0 h×100; B: Increase of BGL in control group (%) - increase of BGL in treated group (%); Same letter in column means non-significant difference (p>0.05); Different letter in column means significant difference (p<0.05)

which is a synthetic hypoglycemic agent and has been used as an antidiabetic drug in type 2 diabetic patients from 1973 until now (WHO, 2007). This drug acts by stimulation of insulin release (Serranto-Martin *et al.*, 2006). *M. oleifera* leaves contain many powerful antioxidant phytochemicals, especially quercetin and kaempferol (Fuglie, 1999). There are many reports about hypoglycemic activities of kaempferol derivatives from many medicinal plants such as *Sterculia rupestris* (Desoky and Youssef, 1997) and *Equisetum myriochaetum* (Andrade-Cetto *et al.*, 2000). Furthermore, they also improved chronic hyperglycemia impaired pancreatic beta cells viability and insulin secretion in vitro (Zhang and Liu, 2011). Quercetin, a strong antioxidant flavonoid revealed a protective effect against streptozotocin induced diabetes in rats by intraperitoneal injection of quercetin 15 mg/kg BW for 3 days prior to streptozotocin administration (Coskun *et al.*, 2005) and protected an Insulin Secreting cell line (INS-1) against oxidative damage (Youl *et al.*, 2010). It also exhibited hypoglycemic properties in diabetic rats (Shetty *et al.*, 2004). Vessal *et al.* (2003) reported that quercetin significantly increased hepatic glucokinase activities as an insulin-like effect.

Our results found that aqueous leaf extract of *M. oleifera* Lam. exhibited anti-hyperglycemic activities in normal mice and improved glucose tolerance impairment in mildly diabetic mice. These properties may be due to the kaempferol present, which involves by stimulating insulin secretion and quercetin, which increases hepatic glucokinase as insulin like effect. It may be suggest the *M. oleifera* Lam. as an anti-diabetic herb.

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