Department of Biochemistry and Molecular Biology, University of Madras, Chennai, Tamil Nadu, India

Anti-diabetic effect of *Murraya koenigii* leaves on streptozotocin induced diabetic rats

P. Arulselvan, G. P. Senthilkumar, D. Sathish Kumar, S. Subramanian

Received December 19, 2005, accepted January 23, 2006

Dr. S. Subramanian, Senior Lecturer, Department of Biochemistry and Molecular Biology, University of Madras, Guindy Campus, Chennai – 600 025, Tamil Nadu, India
subbus2020@yahoo.co.in

Pharmazie 61: 874–877 (2006)

The present study was aimed to evaluate the anti-hyperglycemic efficacy of *Murraya koenigii* in STZ-induced diabetic rats. Oral administration of ethanolic extract of *M. koenigii* at a dose of 200 mg/kg/b.w./day for a period of 30 days significantly decreased the levels of blood glucose, glycosylated hemoglobin, urea, uric acid and creatinine in diabetic treated group of animals. Determination of plasma insulin level revealed the insulin stimulatory effect of the extract. The results suggest that *M. koenigii* possesses statistically significant hypoglycemic potential in STZ-induced diabetic rats. The *M. koenigii* extract appeared to be more effective than glibenclamide, a known antidiabetic drug.

1. Introduction

Herbal medicines have been long used for the treatment of diabetic patients and they are currently accepted as an alternative therapy for diabetic treatment. More than 1200 plants have been described in the scientific and popular literature as hypoglycemic agents (Marles and Farnsworth 1995; Wang and Ng 1999). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari 2000).

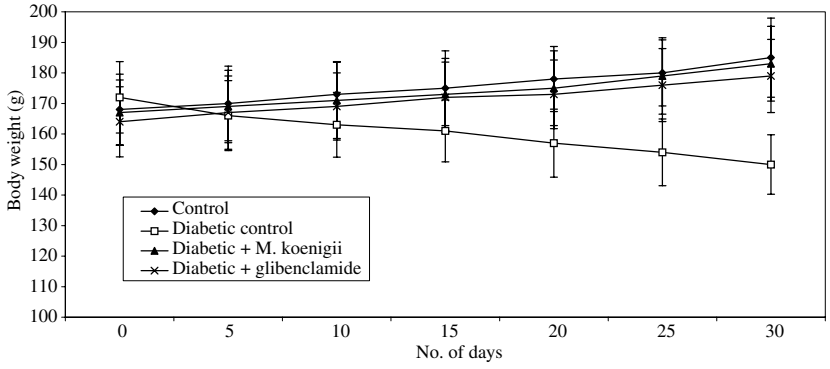
The plant *Murraya koenigii* (L) Spreng belonging to the family Rutaceae, is native to India and now distributed in most of Southern Asia. The leaves of this plant are well-known as "curry leaves" and have been used as one of the important ingredients of Southern India cooking. *M. koenigii* has also been used as a folk medicine (Satyavati et al. 1987). The plant is recognized for its hypoglycemic (Khan et al. 1995) and antifungal properties (Das et al. 1965) as well as a promising agent against colon carcinoma (Khan et al. 1996). Interest in a greater use of curry leaf has been stimulated since its high antioxidant and anticarcinogenic potential was reported (Khan et al. 1997; Khanum et al. 2000). Even though the hypoglycemic activity of the plant has been mentioned in ancient literature, a systematic approach to elucidate its possible therapeutic intervention is still lacking.

The objective of this study was to investigate the hypoglycemic effect of *M. koenigii* in STZ-induced diabetic rats. The possible role of the extract in diabetic rats was compared with glibenclamide, a well-known hypoglycemic drug.

2. Investigations and results

2.1. Changes in body weight of control and experimental groups of rats

The effects of streptozotocin and ethanolic extract of *M. koenigii* leaves on the body weight are shown in the Fig. There was no significant intra-group variation in the



Changes in body weigth of control and experimental groups of rats.
Values are given as mean ± SD for groups of six rats each.

Fig.:
Changes in body weight of control and experimental groups of rats

Table 1: Effect of ethanolic extract of *Murraya koenigii* leaves and glibenclamide on blood sugar level in glucose-loaded diabetic rats

Group	Blood sugar level (mg/dl)				
	Fasting	30 min	60 min	90 min	120 min
Control	81.42 ± 6.10	147.23 ± 10.30	183.12 ± 13.18	131.82 ± 9.09	86.25 ± 6.12
Diabetic control	266.90 ± 18.94*[a]	322.51 ± 24.18*[a]	393.14 ± 29.48*[a]	346.28 ± 24.93*[a]	311.35 ± 22.10*[a]
Diabetic + *Murraya koenigii*	83.21 ± 5.65*[b]	147.44 ± 10.46*[b]	180.32 ± 11.72*[b]	127.13 ± 8.64*[b]	91.36 ± 6.57*[b]
Diabetic + glibenclamide	86.24 ± 5.78*[c]	148.80 ± 10.86*[c]	184.64 ± 12.55*[c]	134.89 ± 9.17*[c]	97.25 ± 6.42*[c]

Values are given as mean ± SD for groups of six rats in each.
Values are statistically significant at * $p < 0.05$.
Statistical significance was compared within the groups as follows:
[a] Diabetic rats were compared with control rats; [b] *Murraya koenigii* treated diabetic rats were compared with diabetic rats; [c] Glibenclamide treated diabetic rats were compared with diabetic control rats

basal body-weights on the 0 day of the experiment. While control rats gained significant weight in the experimental period, body weight decreased in the diabetic controls over the same period. On the other hand, *M. koenigii* and glibenclamide treated diabetic rats gained significant weight compared to the diabetic control rats.

2.2. Glucose tolerance test (GTT) in control and experimental groups of rats

The effect of oral administration of *M. koenigii* extract on glucose tolerance is presented in Table 1. The blood glucose value in the control rats rose to a peak value at 60 min after glucose load and decreased to near normal levels after 120 min. In diabetic control rats the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. *M. koenigii* leaves extract and glibenclamide treated diabetic rats showed a significant decrease in blood glucose concentration at 60 and 120 min when compared with diabetic control.

2.3. Levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin and urine sugar of control and experimental groups of rats

The levels of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin and urine sugar of control and experimental groups of rats are shown in Table 2. Control rats did not show any significant variation in the blood glucose throughout the experimental period. Administration of STZ led to over 3-fold elevation of blood glucose levels, which was maintained over the experimental period. In experimental rats there was a significant elevation in the levels of blood glucose and glycosylated hemoglobin during diabetes, while the levels of hemoglobin and insulin were decreased when compared with the control group of rats. Upon oral administration of *M. koenigii* leaves extract and glibenclamide, these levels were found to be similar to those of normal rats and the effect was more pronounced in the group of rats treated with *M. koenigii*.
Urine sugar present in diabetic rats were drastically controlled by the extract as well as by the glibenclamide treatment.

2.4. The levels of total proteins, blood urea, serum uric acid and plasma creatinine of control and experimental groups of rats

Table 3 shows the levels of total proteins, blood urea, serum uric acid and plasma creatinine of control and experimental groups of rats. These biochemical variables were significantly altered in STZ-induced diabetic rats when compared to control rats. Upon oral administration of *M. koenigii* leaves extract and glibenclamide, these levels were found to be similar to those in normal rats and the effect was more pronounced in the group of rats treated with *M. koenigii* leaf extract.

3. Discussion

The dose (200 mg/kg b.w) was selected after preliminary behavioral and acute toxicity tests. The extract of the drug was found to be safe for biological studies as no lethality was observed at 1000 mg/kg, in rats. Assay of pathophysiological enzymes such as AST, ALT and alkaline phosphatase revealed the non-toxic nature of the plant extract.
Further, the preliminary phytochemical screening of the ethanolic extract of *M. koenigii* revealed the presence of biologically active ingredients such as alkaloids, flavonoids, glycosides, triterpenoids, phenols etc. (data not shown).

Table 2: Levels of blood glucose, hemoglobin, glycosylated, plasma insulin, hemoglobin and urine sugar in control and experimental groups of rats

	Fasting blood glucose (mg/dl)	Hemoglobin (g/100ml)	Glycosylated hemoglobin (% Hb)	Plasma insulin (μU mL^{-1})	Urine sugar
Control	83.41 ± 5.33	13.68 ± 0.84	5.9 ± 0.29	17.25 ± 1.06	Nil
Diabetic control	252.15 ± 17.90*[a]	10.07 ± 0.60*[a]	12.8 ± 0.76*[a]	4.86 ± 0.30*[a]	+ + +
Diabetic + *Murraya koenigii*	89.32 ± 6.34*[b]	12.16 ± 0.76*[b]	7.0 ± 0.39*[b]	15.04 ± 0.95*[b]	Nil
Diabetic + glibenclamide	95.50 ± 6.97*[c]	12.04 ± 0.74*[c]	7.4 ± 0.43*[c]	13.82 ± 0.74*[c]	Nil

Values are given as mean ± SD for groups of six rats in each.
Values are statistically significant at * $p < 0.05$.
Statistical significance was compared within the groups as follows:
[a] Diabetic rats were compared with control rats; [b] *Murraya koenigii* treated diabetic rats were compared with diabetic rats; [c] Glibenclamide treated diabetic rats were compared with diabetic control rats.
+ + + indicates more than 2% of sugar

Table 3: Level of total proteins, blood urea, serum uric acid and plasma creatinine in control and experimental groups of rats

Groups	Total proteins (g/dl)	Blood urea (mg/dl)	Serum uric acid (mg/dl)	Plasma creatinine (mg/dl)
Control	8.11 ± 0.50	20.12 ± 1.18	2.62 ± 0.15	0.69 ± 0.02
Diabetic control	5.92 ± 0.34*[a]	37.60 ± 2.44*[a]	4.99 ± 0.28*[a]	0.99 ± 0.04*[a]
Diabetic + *Murraya koenigii*	7.87 ± 0.44*[b]	21.78 ± 1.32*[b]	2.80 ± 0.15*[b]	0.71 ± 0.03*[b]
Diabetic + glibenclamide	7.26 ± 0.42*[c]	24.13 ± 1.40*[c]	2.98 ± 0.16*[c]	0.75 ± 0.03*[c]

Values are given as mean ± SD for groups of six rats in each.
Values are statistically significant at * $p < 0.05$.
Statistical significance was compared within the groups as follows:
[a] Diabetic rats were compared with control rats; [b] *Murraya koenigii* treated diabetic rats were compared with diabetic rats; [c] Glibenclamide treated diabetic rats were compared with diabetic control rats.

Streptozotocin induced experimental diabetes was scientifically accepted as a precious tool to study both pathophysiological mechanisms of diabetes mellitus and hypoglycemic activity of plants (EL Fiky et al. 1996). Streptozotocin was recognised as a specific toxic agent for β-cells of the islets of Langerhans (Rakieten et al. 1963) and has since then been widely used for the induction of diabetes with concomitant insulin deficiency (Serradas et al. 1989).

Glibenclamide is often used as a standard diabetic drug in STZ-induced moderate diabetes to be compared with a variety of hypoglycemic compounds and its effectiveness in insulin stimulation is known (Andrade-Cetto and Widenfield 2001).

The present study was carried out to assess the antihyperglycemic effect of *M. koenigii* leaves extract on STZ-induced diabetic rats, an experimental model for type-I diabetes mellitus. STZ effectively induced diabetes in control rats as reflected by glycosuria, hyperglycemia and body weight loss when compared with control rats. STZ-induced diabetes is characterised by a severe loss of body weight (Al-Shamaony et al. 1994) and this was also seen in the present study.

The decrease in body weight observed in uncontrolled diabetes might be the result of protein wasting due to the unavailability of carbohydrates for energy utilization (Virdi et al. 2003). The enhancement of body weight in the extract treated diabetic groups of rats indicates the *M. koenigii* leaves extract increases glucose metabolism and thus enhances body weight in STZ-induced diabetic rats.

The possible mechanism by which *M. koenigii* leaves extract brings about a decrease in blood glucose and an increased level of insulin may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the remnant β-cells of the islets of Langerhans or its responsiveness. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effects (Pari and Latha 2002).

The observed decrease in hemoglobin content during diabetes may due to the formation of glycosylated hemoglobin. Increase in the level of hemoglobin in rats given *M. koenigii* may be due to decreased levels of blood glucose and glycosylated hemoglobin.

STZ-induced diabetic rats manifest a negative nitrogen balance related to enhanced proteolysis in muscles and tissues coupled with lowered protein synthesis.

Which readily accounts for the observed decrease in the total plasma protein level. A reduction in the level of body protein in the absence of insulin has been reported, as insulin is required for protein synthesis (McNurlan and Garlick 1981). The antihyperglycemic property of *M. koenigii* leaves may have been the factor responsible for enhancing the levels of proteins in diabetic rats treated with *M. koenigii* leaves extract in this study.

Increased urea nitrogen production in diabetes may be due to the enhanced catabolism of both liver and plasma proteins (Morris and Leon 1960). Almdal and Vilstrup (1988) have reported that insulin therapy in diabetes leads to normalization of nitrogen contents through urea synthesis. Impaired balance of nitrogen coupled with lowered protein synthesis leads to increased concentration of urea in blood (Asayama 1994). Administration of *M. koenigii* leaves extract to diabetic rats significantly decreased the level of blood urea.

The diabetic hyperglycemia induces elevation of the plasma levels of urea, uric acid and creatinine, which are considered as significant markers of renal dysfunction (Almdal and Vilstrup 1988). The levels of these parameters were reverted back to near normal after treatment with *M. koenigii* extract and glibenclamide, which provides an additional evidence to prove the anti-diabetogenic property of *M. koenigii* leaf extract.

In our study uric acid levels were increased in diabetic rats. This may be due to metabolic disturbances in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation and increased triglyceride and cholesterol (Madianov et al. 2000). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of perinea and it is the main source of uric acid as well as in activity of xanthine oxidase (Anwar and Meki 2003). Restoration of uric acid levels by the administration of *M. koenigii* leaves extract implies its control over purine catabolism.

Creatinine concentration is the variable used not only to assess impairment of kidney function but also as clinical chemistry end point to detect treatment related toxic effects of compounds on the kidney in experimental rats. In the present investigation we have found significant elevation in the levels of creatinine in STZ-induced diabetic rats. Treatment with *M. koenigii* leaves extract significantly prevented the elevation of creatinine levels. To conclude, the present data suggests that, treatment with *M. koenigii* leaves extract exerts protective effects on kidney in STZ-induced diabetic rats.

It is therefore, conceivable that the biologically active ingredients present in the ethanolic extract of *M. koenigii* leaves exert their hypoglycemic effect by potentiating the β-cells of the pancreas. The extract at a dose 200 mg/kg was more effective than the standard drug, glibenclamide. Thus, it may be postulated that *M. koenigii* leaves can serve as a good adjuvant in the present armamentarium of antidiabetic drugs. Further biochemical and pharmacological investigations are in progress in our laboratory to elucidate its therapeutic potency at the molecular level.

4. Experimental

4.1. Plant material

Fresh, mature *M. koenigii* leaves (Rutaceae) were collected from a plant in Attur, Tamil Nadu, India. The plant was identified and authenticated

by Dr. V. Kaviyarasan, Centre for Advanced Studies in Botany, University of Madras, and a voucher specimen was deposited at the herbarium of Botany.

4.2. Preparation of leaf extract

The *M. koenigii* leaves were dried at room temperature and powdered in an electrical grinder and stored at 5 °C until further use. The *M. koenigii* powder was extracted with petroleum ether (60–80 °C) to remove lipids. It was then filtered, the filtrate was discarded and the residue was extracted with 95% ethanol by in a soxhlet extractor. The ethanol was evaporated in a rotary evaporator at 40–50 °C under reduced pressure. The phytochemical screening of solvent-free extract was qualitatively carried out by the method of Harborne (1973).

4.3. Animals

Male albino Wistar rats weighing about 160–180 g obtained from the Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, were selected as the animal model. They were acclimatized to animal house conditions. The rats were fed standard rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water until treatment or time of sacrifice. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No.01/029/04).

4.4. Experimental induction of diabetes

The animals were fasted for 18 h, and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of STZ (55 mg/kg of body weight) in 0.1 M cold citrate buffer (pH 4.5) (Rakieten et al. 1963). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hyperglycemia. Control rats were injected with equal volume of citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the 4th day after STZ administration. The treatment was started on the 5th day after STZ injection and this was considered as 1st day of treatment which was continued for 30 days.

4.5. Experimental set up

The animals were divided into two sets, one for the evaluation of the glucose tolerance test and a second one for the analysis of biochemical parameters. Each set has four groups with six rats in each group.
Group I: Control rats receiving 0.1 M cold citrate buffer (pH 4.5)
Group II: Diabetic control rats.
Group III: Diabetic rats were given a single dose of *M. koenigii* leaves extract (200 mg/kg b.w./day) in aqueous solution orally.
Group IV: Diabetic rats were given a single dose of glibenclamide (600 µg/kg b.w./day) in aqueous solution orally (Pari and Umamaheswari 2000).
The body weight changes all the grouped rats were recorded at regular intervals.

4.6. Glucose tolerance test

After 30 days of treatment, a fasting blood sample was taken from all the groups of rats: Four more blood samples were collected at 30, 60, 90 and 120 min intervals after administration of glucose at a concentration of 2 g/kg of body weight (Joy and Kuttan 1999). All the blood samples were collected with EDTA solution for the estimation of glucose.

4.7 Biochemical assays

After 30 days of treatment, the rats were fasted overnight and sacrificed by cervical decapitation, and the blood was collected using EDTA as anticoagulant. The whole blood was used for the estimation of glucose (Sasaki et al. 1972) and urea (Natelson et al. 1951). The levels of hemoglobin and glycosylated hemoglobin were estimated according to methods of Drabkin and Austin (1932) and Sudhakar Nayak and Pattabiraman (1981). The plasma was separated and used for the assay of insulin which was estimated using RIA assay kit (for rats) supplied by Linco Research Inc., USA, proteins (Lowry et al. 1951), uric acid (Caraway 1963) and creatinine (Brod and Sirota, 1948).

4.8. Statistical analysis

All the grouped data were statistically evaluated with SPSS/10.00 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significant. All the results were expressed as mean ± S.d. for six rats in each group.

References

Almdal TP, Vilstrup H (1988) Strict insulin treatment normalizes the organic nitrogen contents and the capacity of urea nitrogen synthesis in experimental diabetes in rats. Diabetologia 31: 114–118.

Al-Shamaony L, Al-Khazraji SM, Twaiji HA (1994) Hypoglycemic effect of *Artemisia herba* alba. II: Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol 43: 167–171.

Andrade-Cetto A, Wiedenfeld H (2001) Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. J Ethnopharmacol 78: 145–149.

Anwar MM, Meki MA (2003) Oxidative stress in streptozotocin-induced diabetic rats: effects of garlic oil and melatonin. Comp Biochem Physiol Part A 135: 539–547.

Asayama K, Nakana T, Uchida N, Hayashibe H, Dobashi K, Nakazawa S (1994) Serum antioxidant status in streptozotocin-induced diabetic rat. Horm Metab Res 26: 313–315.

Brod J, Sirota JH (1948) Renal clearance of endogenous creatinine in man. J Clin Invest 27: 645.

Caraway WI (1963) Uric acid. In: Standard methods of clinical chemistry. Seligson D. (ed.), Vol.4, Academic Press, New York, p. 239–247

Das KC, Chakraborty DP, Bose PK (1965) Antifungal activity of some constituents of *Murraya koenigii*. Spreng. Experientia 21: 340–345.

Drabkin DL, Austin JM (1932) Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood. J Biol Chem 98: 719–733.

El-Fiky FK, Abou-Karam MA, Afify EA (1996) Effect of *Luffa aegyptiaca* (seeds) and *Carissa edulis* (leaves) extracts on blood glucose level of normal and streptozotocin diabetic rats. J Ethnopharmacol 50: 43–47.

Harborne JB (1973) Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd., London.

Joy KL, Kuttan R (1999) Antidiabetic activity of *Picrorrhiza kurroa* extract. J Ethnopharmacol 167: 143–148.

Khan BA, Abraham A, Leelamma S (1995) Hypoglycemic action of *Murraya koenigii* (Curry leaf) and *Brassica juncea* (mustrad): mechanism of action. Indian J Biochem Biophys 32: 106–108.

Khan BA, Abraham A, Leelamma S (1996) *Murraya koenigii* and *Brassica juncea* alterations on lipid profile in 1-2 dimethyl hydrazine induced colon carcinogenesis. Invest New Drugs 14: 365–369.

Khan BA, Abraham A, Leelamma S (1997) Antioxidant effects of curry leaf, *Murraya koenigii* and mustard seeds, *Brassica juncea* in rats fed with high fat diet. Indian J Exp Biol 35(2): 148–150.

Khanum F, Anilakumar KA, Sudarshana KKR, Viswanathan KR, Santhanam K (2000) Anticarcinogenic effects of curry leaves in dimethylhydrazine-treated rats. Plant Foods Hum Nutr 55: 347–355.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein determination using the folin phenol reagent. J Biol Chem 193: 265–275.

Madianov IV, Balabolkin M, Markov DS, Markova TN (2000) Main causes of hyperuricemia in diabetes mellitus. Ter Arkh 72: 55–58.

Marles RJ, Farnsworh NR (1995) Antidiabetic plants and their active constituents. Phytomedicine 2(2): 137–189.

McNurlan MA, Garlick PJ (1981) Protein synthesis in liver and small intestine in protein deprivation and diabetes. Am J Physiol 241: E238–E245.

Morris G, Leon M (1960) Protein metabolism and protein synthesis is perfused livers of normal and alloxan diabetic rats. J Biol Chem 235: 3202–3208.

Natelson S, Scott ML, Begga E (1951) A rapid method for the estimation of urea in biological fluids. Am J Clin Pathol 21: 275–281.

Pari L, Latha M (2002) Effect of *Cassia auriculata* flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. Singapore Med J 43: 617–621.

Pari L, Umamaheswari J (2000) Antihyperglycemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. J Ethnopharmacol 14: 136–138.

Rakieten N, Rakieten M, Nadkarni M (1963) Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemother Rep 29: 91–98.

Sasaki T, Matsy S, Sonae A (1972) Effect of acetic acid concentration on the colour reaction in the o-toluidine boric acid method for blood glucose estimation. Rinsh Kagaku 1: 346–353.

Satyavati GU, Gupta AK, Tandon N (1987) Medicinal plants of India, Vol.2, Indian Council of Medicinal Research, New Delhi, Indian, p. 289–299

Serradas P, Giroix MH, Portha B (1989) Evaluation of the pancreatic B-cell function in the rat after prenatal exposure to streptozotocin or N-nitrosomethylurea. Diabete Metab 15: 30–37.

Sudhakar Nayak S, Pattabiraman TN (1981) A new colorimetric method for the estimation of glycosylated hemoglobin. Clin Chem Acta 109: 267–274.

Virdi J, Sivakami S, Shahani S, Suthar AC, Banavalikar MM, Biyani MK (2003) Antihyperglycemic effects of three extracts from *Momordica charantia*. J Ethnopharmacol 88: 107–111.

Wang HX, Ng TB (1999) Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci 65: 2663–2677.