Singapore Med J 2000; Vol 41(1):9-13

Effects of an Ethanolic Extract of *Gynura procumbens* on Serum Glucose, Cholesterol and Triglyceride Levels in Normal and Streptozotocin-Induced Diabetic Rats

X F Zhang, B K H Tan

ABSTRACT

Aim of Study: The aim was to demonstrate the effects of the leaves of *Gynura procumbens* (Lour.) Merr. on blood sugar and lipid levels in experimental animals.

Methodology: We obtained an ethanolic extract of the leaves of *G. procumbens* and monitored the effects of an oral administration of (i) different single doses of the extract on oral glucose tolerance in streptozotocin-induced diabetic and normal rats and (ii) fourteen doses over 7 days on serum cholesterol and triglyceride levels in streptozotocin-induced diabetic rats. Metformin and glibenclamide were used as positive control drugs.

Results: The extract, at single doses of 50, 150 and 300 mg/kg orally, significantly suppressed the elevated serum glucose levels in diabetic rats; 150 mg/kg was found to be the optimum hypoglycaemic dose. The extract however did not significantly suppress the elevated serum glucose levels in normal rats, unlike glibenclamide. Metformin, but not glibenclamide, improved glucose tolerance in the diabetic rats. When the optimum dose was given to diabetic rats for 7 days, the extract significantly reduced serum cholesterol and triglyceride levels in these rats.

Conclusion: These results indicate that the leaves of *G. procumbens* may have biguanide-like activity.

Keywords: *Gynura procumbens*, diabetic rat, cholesterol, triglyceride, oral glucose tolerance test

Department of Pharmacology
National University of Singapore
10 Kent Ridge Crescent
Singapore 119260

X F Zhang, MBBS (Hunan)
Post-graduate Student

B K H Tan, PhD, MBBS
Associate Professor

Correspondence to:
A/Prof B K H Tan

INTRODUCTION

Gynura procumbens is found in various parts of Southeast Asia. It has been used for the treatment of eruptive fevers, rash and kidney disease[1]. Recently, the leaves of this plant have been used as folk medicine to control diabetes mellitus and hyperlipidemia. However by reviewing the current literature, we know of no previous research on the pharmacological properties of this plant. Therefore experiments were carried out to test the antidiabetic and antihyperlipidemic activities of this plant.

The oral glucose tolerance test (OGTT) is a well-accepted and frequently used assay to screen hypoglycaemic activity[2]. Streptozotocin (STZ) is a valuable agent for the experimental production of diabetes, and it is less toxic than other chemical agents inducing diabetes. The diabetogenic effect of STZ is the direct result of irreversible damage to pancreatic β cells, allowing degranulation and loss of insulin secretion. So the STZ-induced diabetic animal is one of the animal models of human insulin-dependent diabetes mellitus (IDDM)[3,4]. Diabetes mellitus is a disease with profound effects on lipid metabolism. Insulin affects mammalian lipid metabolism in several ways, eg. it inhibits the activity of lipoprotein lipase, therefore it decreases the mobilisation of free fatty acids from the peripheral fat depots. On the other hand, it stimulates the synthesis of fatty acids in the liver, adipose tissue and intestine. The STZ-induced diabetic animal is thus considered as an animal model of hyperlipidemia[5].

The present study was undertaken to evaluate the possible hypoglycaemic activity of the ethanolic extract of *G. procumbens* leaves in the normal and the STZ-induced diabetic rats. The effects of the extract on serum total cholesterol (TC) and triglyceride (TG) levels were also examined in the diabetic rats. To check the safety of taking this plant, acute toxicity and behavioural changes were observed.

MATERIALS AND METHODS

Preparation of the extract

The plant was collected from a private garden and identified as *G. procumbens (Lour.) Merr.* by Dr Ruth Kiew, Keeper of Herbarium and Library, Singapore Botanic Gardens. A dried specimen is deposited in the herbarium (voucher No. BT1).

The fresh leaves of *G. procumbens* (1 kg) were

blended and extracted with 95% alcohol (1.5L) until exhaustion. After filtration with cotton wool, the mixture was centrifuged at 10,000 g for 20 min. The supernatant was concentrated to 0.5L at 38°C by a rotavapor (Buchi Labortechnik AG, Switzerland). This solution was then freeze-dried, yielding 16.38 g of light green powder. The extract was suspended in distilled water before use.

Animals

Male Sprague-Dawley (SD) rats, aged 7 weeks (150 – 210 g), and male BALB/c mice (20 – 22 g) were obtained from The Laboratory Animal Center, National University of Singapore. The rats were housed in individual cages and maintained on standard laboratory rat chow with water *ad libitum*.

Streptozotocin-induced diabetic rats

Diabetic rats were induced by a single intraperitoneal injection of freshly dissolved STZ (Sigma Chemical Co., MO, USA) (60 mg/kg body weight in citrate buffer 0.01 M, pH4.5) to overnight fasted rats.

Diabetes was identified by polydipsia, polyuria and by measuring non-fasting serum glucose concentration 48 hours after injection of STZ. Rats with a blood glucose level above 300 mg/dL were considered to be diabetic and were used in the experiment.

Treatment schedules

1) For the OGT test, the diabetic rats were randomly divided into groups of 4 – 5 rats each. The groups treated with metformin (500 mg/kg) (Pharmacy, National University Hospital, Singapore) and glibenclamide (5 mg/kg) (RBI Research Biochemical International, MA, USA) were taken as the positive control. Metformin and glibenclamide were finely powdered and dispersed in distilled water. The group treated with an equal volume of distilled water was used as the negative control. The rest were treated with 50, 150 and 300 mg/kg body weight of extract of *G. procumbens* leaves. The normal rats were divided into three groups of 4 rats each: negative control (distilled water), treatment (150 mg/kg extract), positive control (5 mg/kg glibenclamide).

2) Repeated administration of the extract in diabetic rats: 6 diabetic rats were treated orally with 150 mg/kg extract twice daily (at 9.00 am and 6.00 pm) for 7 days. Another 6 diabetic rats were given orally an equal volume of distilled water and served as controls. These diabetic rats were used for the determination of serum total cholesterol and triglyceride as well as liver P_{450} content. Food and water were given *ad libitum*; the amount consumed as well as the body weight were recorded daily.

3) Acute toxicity: The BALB/c mice were used to investigate the possible toxic effect of the extract. The extract was given orally to two different groups of BALB/c mice (n = 4) at a dose of 1 g/kg and 5 g/kg of body weight, respectively. All mice were observed for 8 hours after administration of the extract to check for toxic symptoms. They were kept under observation for 7 days.

The extract, antidiabetic drugs and their respective vehicles were administered orally by gavage to rats.

Collection of blood samples

Blood samples were collected from the tail vein in centrifuge tubes, and were centrifuged at 1,000 g for 15 min to obtain the serum.

OGTT

All the diabetic rats were fasted overnight (at least 12h) prior to the test. Thirty minutes following the various treatment schedules, each rat was given an oral glucose load, 3 g/kg body weight according to Al-Awadi et al[6]. Blood samples were collected from the tail vein at -30 minutes (just before the administration of the extract), time 0 (prior to the glucose load), 30, 60 and 120 minutes after the glucose load. Serum glucose concentrations were measured by the glucose oxidase method[7], using glucose analyser (Sigma Chemical Co., MO, USA).

Serum TC and TG

The fasting blood samples were collected from the tail vein before treatment and after the 7-day treatment. Serum TC and TG concentrations were analysed by the colorimetric method, using wet reagent diagnostic kits (Boehringer Mannheim GmbH).

Assay of liver microsomal P_{450} content

1) *Preparation of liver microsomes.* The diabetic rats given the extract at a dose of 150 mg/kg twice daily for 7 days were decapitated; their livers were quickly removed, rinsed in ice-chilled normal saline and weighed. Liver microsomes were prepared, using the ultra-centrifugation method[8]. Briefly, rat liver (2 g) was homogenised in ice 1.15% KCI (20 mL) using a glass Potter-Elvejhem homogeniser. The homogenate obtained was centrifuged at 10,000 g for 10 min at 4°C and the supernatant was recentrifuged at 100,000 g for 45 min at 4°C. The microsomal pellet was resuspended in a volume of glycerol buffer [200 mM potassium phosphate buffer, pH 7.4; 50% (v/v) glycerol and 1.15% (w/v) potassium chloride (5:8:7)] equivalent to twice the liver weight. The pellet was further homogenised with 7 strokes of the pestle after which aliquots of the microsomal suspension were stored at -70°C.

2) *Measurement of protein content in microsomes.* Protein content in each microsomal suspension was determined by Lowry's method[9].

3) *Spectrophotometric measurement of liver microsomal P_{450} content.* An aliquot of microsomal preparation of 1 mg protein/mL was obtained by adding 0.5 mL of 1 M potassium phosphate buffer and the required volume of 1.15% KC1. The modified technique of Omura and Sato[10] was adopted in this assay to eliminate the absorption peak at 420 nm due to contamination by haemoglobin in the sample. The microsomal preparation was placed in two cuvettes and initially saturated with carbon monoxide. A small amount of sodium dithionite (not more than 2 mg) was added to the sample cuvette only. The microsomal

P_{450} content was then determined from the difference in absorbance values between the dithionite-reduced and control microsomal preparations using a Shimadzu UV-2100 dual-beam spectrophotometer. The molar extinction coefficient of microsomal P_{450} at the γ_{max} of 450 nm was 91 mM^{-1} cm^{-1}.

Statistical analysis

All values were expressed as the mean ± SE obtained from a number of experiments (n). The serum glucose levels of extract-treated animals, the reference drug-treated and vehicle-treated controls were compared by ANOVA followed by Duncan's Multiple Range test[11]. Data on serum TC and TG values were analysed by the Student's *t*-test. Differences with $p < 0.05$ were considered to be statistically significant.

RESULTS

Body weight, food and water intake

During the 7-day period of study, no significant difference was found in the food and water intakes of extract-treated STZ-induced diabetic SD rats when compared to their respective vehicle-treated controls (data not shown). The body weight increase of the extract-treated rats was slightly more than that of the control rats, being 20.4% ± 1.7 and 17.6% ± 1.6, respectively, but the difference was not significant.

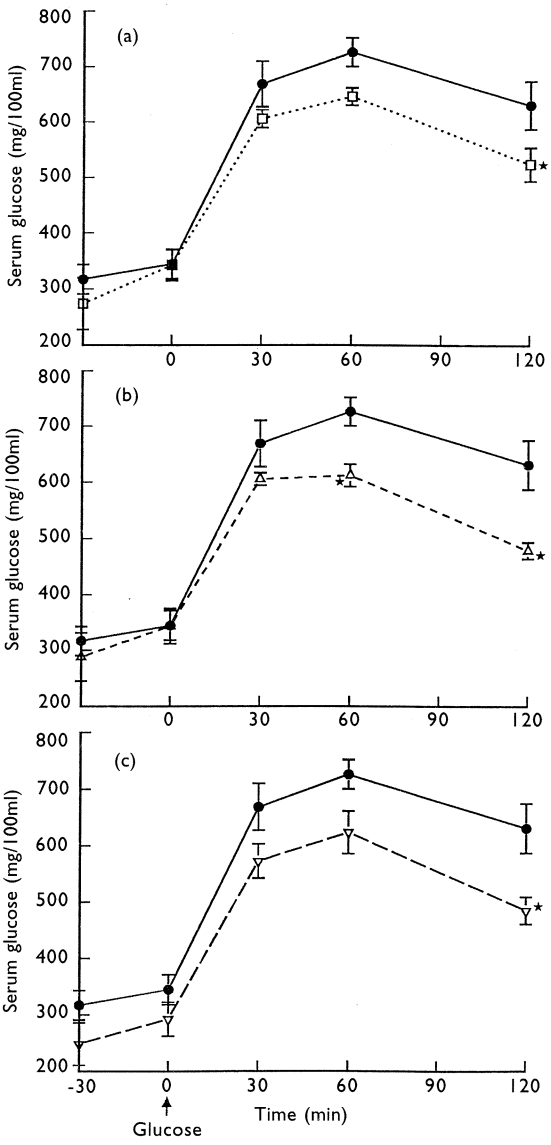
OGTT in diabetic rats

The effects of oral administration of three different doses of the plant extract and the positive controls on serum glucose levels of STZ-induced diabetic rats challenged with a glucose load are presented in Fig 1 and 2 respectively.

At 60 mins after glucose load, serum glucose levels in all the animals reached a peak. At 120 mins, the three doses of the extract produced significantly lower serum glucose levels compared to the vehicle. A maximum decrease of 15.8% was observed with 150 mg/kg of extract; this dose also produced a significant decrease in serum glucose at 60 mins. The reference drug, metformin (500 mg/kg) caused a significant decrease in serum glucose levels at 30, 60 and 120 mins compared with the vehicle. However the serum glucose levels in the glibenclamide (5 mg/kg)-treated group throughout the study were similar to those of the vehicle-treated group.

OGTT in normal rats

The results showed that 150 mg/kg was the optimum dose producing significant lowering of serum glucose levels in diabetic rats. This dose was chosen for the OGTT in normal rats. The change of serum glucose levels in normal rats is shown in Fig 3. The extract (150 mg/kg) did not produce any significant decrease in serum glucose level in these rats when compared to the vehicle. However, with the reference drug, ie. glibenclamide, the serum glucose levels were significantly reduced at 60 mins and 120 mins after the oral glucose load, compared to the vehicle.



Vertical bars indicate standard errors of the means.
★ $P < 0.05$ compared with the corresponding negative control
- ● - vehicle (distilled water), n = 5
- □ - *G. procumbens* (50 mg/kg), n = 5
- △ - *G. procumbens* (150 mg/kg), n = 5
- ▽ - *G. procumbens* (300 mg/kg), n = 5

Fig 1 – Effects of different doses of ethanolic extract of *G. procumbens* (a) 50 mg/kg (b) 150 mg/kg and (c) 300 mg/kg on OGTT in STZ-induced diabetic rats.

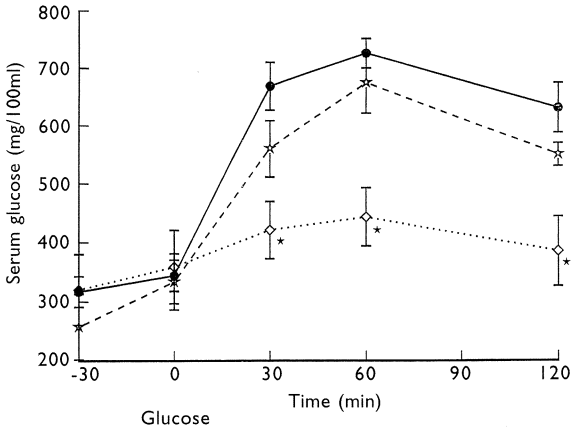
Serum TC And TG

As shown in Table I, serum TC was significantly decreased in diabetic rats treated with the extract of *G. procumbens* twice daily for 7 days. However, it was slightly increased in diabetic rats treated with the vehicle. The 72.6% increase in serum TG of the vehicle-treated rats was 2.7 times higher than that noted in the extract-treated rats (26.7%).

Hepatic microsomal P_{450} content and acute toxicity study

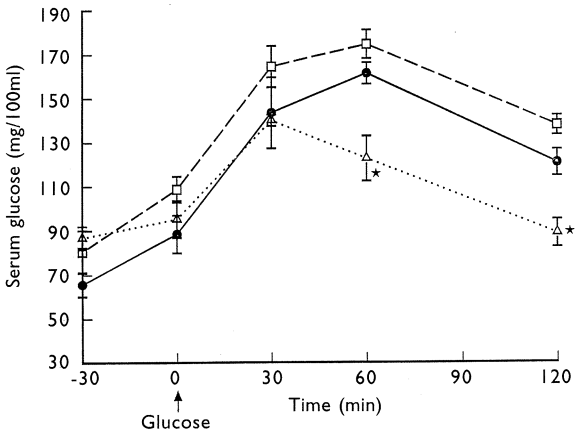
There was no significant difference in the liver content of microsomal P_{450} between the extract-treated and vehicle-treated diabetic rats (data not shown).

BALB/c mice given 1 g/kg and 5 g/kg body weight of the extract of *G.procumbens* did not show any visible signs of toxicity, eg. excitement, restlessness,



Vertical bars indicate standard errors of the means.
★ $P < 0.05$ compared with the corresponding negative control
- ● - vehicle (distilled water), n = 5
- □ - metformin (500 mg/kg), n = 4
- ☆ - glibenclamide (5 mg/kg), n = 4

Fig 2 – Effects of metformin and glibenclamide on OGTT in STZ-induced diabetic rats.



Vertical bars indicate standard errors of the means.
★ $P < 0.05$ compared with the corresponding negative control
- ● - vehicle (distilled water), n = 4
- □ - *G. procumbens* (150 mg/kg), n = 4
- △ - glibenclamide (5 mg/kg), n = 4

Fig 3 – Effects of ethanolic extract of *G. procumbens* and glibenclamide on OGTT in normal male SD rats.

Table I – Serum cholesterol and triglyceride levels in diabetic rats treated twice a day orally with 150 mg/kg extract of *G. procumbens*

	Cholesterol (mg/100ml)		Triglyceride (mg/100ml)	
	Vehicle	**Extract-treated**	**Vehicle**	**Extract-treated**
Day 0	92.3 ± 2.1	95.4 ± 2.8	79.6 ± 3.8	78.4 ± 4.9
Day 7	97.1 ± 4.3	82.1 ± 0.9[a,b]	137.6 ± 8.6[b]	99.3 ± 10.9[a]
Mean % changes after 7 days	+ 5.2%	-13.9%	+72.9%	+26.7%

Values are expressed as mean ± SEM (n = 6).

[a] Indicates statistically significant difference ($P < 0.05$) compared to the vehicle;

[b] Indicates statistically significant difference ($P < 0.01$) compared to the corresponding value on day 0.

respiratory distress, convulsion, or coma. Moreover, they remained alive and well for up to 7 days. They also maintained their body weights during this period. The mean body weights of BALB/c mice given 1 g/kg and 5 g/kg extract were 22.1 g and 20.2 g on day 0, and 22.8 g and 20.2 g on day 7, respectively.

DISCUSSION

In the present study, the hypoglycaemic activity of the ethanol extract of *G.procumbens* was evaluated in the normal and STZ-induced diabetic rats, a model of human insulin-dependent diabetes, using the OGTT. The extract improved the glucose tolerance in STZ-induced diabetic rats, but not in normal rats. Under the same conditions, metformin produced a significant glucose clearance in STZ-induced diabetic rats, but glibenclamide did not. Furthermore, after 7 days administration of the extract, the serum TC and TG levels of the extract-treated diabetic rats were significantly lower than those of the control rats.

It is well known that sulfonylureas and biguanides are the major oral hypoglycaemic agents used worldwide. Glibenclamide, a sulfonylurea derivative, causes hypoglycaemia by stimulating pancreatic β cells to release more insulin, and inhibiting glucagon secretion. As these effects require a functional pancreas, it can lower blood sugar levels in non-diabetic subjects[12]. In contrast to sulfonylureas, metformin, a biguanide derivative, has no such stimulating activity. Its blood glucose-lowering action dose not depend on functional pancreatic β cells, being due to its extrapancreatic actions, such as 1) increase of glucose utilisation and 2) reduced hepatic gluconeogenesis[12]. Metformin can also ameliorate abnormalities in lipid levels, causing a decrease in circulating triglyceride and total cholesterol concentrations[13].

In view of the similarity between the effects of *G. procumbens* and metformin rather than glibenclamide, it seems that the hypoglycaemic effect of *G. procumbens* may be due to a biguanide-like activity.

P_{450} is an enzyme that is responsible for drug metabolism and detoxification in the liver[14]. As P_{450} was not altered by extract treatment in the present experiment, *G. procumbens* is unlikely to produce a pharmacokinetic interaction with other drugs taken together. The extract was also non-toxic to the laboratory mice.

In conclusion, our studies show that the ethanolic extract of *G. procumbens* leaves has antihyperglycaemic and antihyperlipidaemic activities in diabetic rats. Further pharmacological and phytochemical investigations are being done to identify the active compound(s), and also to elucidate the mechanism(s) of action.

ACKNOWLEDGEMENTS

The authors wish to thank the National University of Singapore for the research grant (RP 60329) and a research scholarship awarded to Dr X F Zhang.

REFERENCES

1. Perry LM. Medicinal plants of East and Southeast Asia: attributed properties and uses. England: The MIT Press, 1980:94-5.
2. Niyonzima G, Laekeman GM, Metz T, Scharpe S, Van Beeck L, Vlietinck AJ. Hypoglycaemic activity of Spathodea campanulata stem bark decoction in mice. PhytotherapyResearch 1993; 7:64-7.
3. Arison RN, Ciaccio EI, Glitzer MS, Casaro JA, Pruss MP. Light and electron microscopy of lesions in rats rendered diabetic with streptozotocin. Diabetes 1967; 16:51-6.
4. Junod A, Lambert AE, Atauffacher W, Renold AE. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J Clin Investigation 1969; 48:2129-39.
5. Suckling KE, Jackson B. Animal models of human lipid metabolism. Progress in Lipid Research 1993; 32(1):1-24.
6. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry 1969; 6:24.
7. Al-Awadi FM, Khattar MA, Gumaa KA. On the mechanism of the hypoglycemic effect of a plant extract. Diabetologia 1985; 28:432-4.
8. El Defrawy, El Masry S, Cohen GM, Mannering GJ. Sex-dependent differences in drug metabolism in the rat. I: Temporal changes in the microsomal drug-metabolizing system of the liver during sexual maturation. Drug Metabolism and Disposition 1974; 2:267-78.
9. Lowry OH, Rosenbrough NJ, Farr AL, Randall JL. Protein measurement with the folin phenol reagent. J Bio Chem 1951; 193:265-75.
10. Omur T, Sato R. The carbon monoxide binding pigment of liver microsomes, I: Evidence for its hemoprotein nature. J Bio Chem 1964; 239:2370-8.
11. Armitage P. Interim Anaglyses in Clinical Trials. In: Hoppe FM, editor. Multiple comparisons, selection, and applications in Biometry. New York: Marcel Dekker Inc. 1993;391-402.
12. Karam JH. Pancreatic hormones and antidiabetic drugs. In: Katzung BG, editor. Basic and Clinical Pharmacology. 7th ed. Stamford: Simon and Schuster Company, 1998:684-705.
13. Bailey CJ. Biguanides and NIDDM. Diabetes Care 1992; 15:755-72.
14. Correia MA. Drug biotransformation. In: Katzung BG, editor. Basic and Clinical Pharmacology. 7th ed. Stamford: Simon and Schuster Company, 1998:50-61.