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Hypoglycemic and anti-hyperglycemic study of *Gynura procumbens* leaf extracts

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PEER REVIEW

Peer Reviewer

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Comments

The work is of high quality. Sufficient data has been generated on a time line basis which has allowed relevant metabolic changes of the diabetic state to be monitored. Structural activity relationship based on different phytochemical ingredient solubilised by the different ethanol-water solvent system in relation to their anti-diabetic effects is relevant in drug development.

(Details on Page 365)

ABSTRACT

Objective: To study the antidiabetic activity of *Gynura procumbens* (*G. procumbens*) used in the traditional management of diabetes in Southern Asia. **Methods:** *G. procumbens* leaves were extracted sequentially with graded percentage of ethanol in water (95%, 75%, 50%, 25% and 0%), and the extracts were tested for antidiabetic activity using acute (7 h), subcutaneous glucose tolerance test and sub-chronic (14 d) test in non-diabetic and streptozotocin-induced diabetic rats. The extracts were further subjected to phytochemical studies. **Results:** In acute dose (1 g/kg), the extracts significantly lowered fasting blood glucose (FBG) in streptozotocin-induced diabetic rats ($P<0.05$). However, the FBG-lowering effect of the 25% extract compared to the other extracts, was rapid (47% after 2 h) and the highest: 53%, 53% and 60% in the 3rd, 5th, and 7th h, respectively ($P<0.05$), comparable only to the effect of metformin. Furthermore, the extracts suppressed peak FBG in subcutaneous glucose tolerance test, but only the 0% and 25% extracts, and metformin sustained the decrease until the 90th min ($P<0.05$). Moreover, in the 14 days study, the 25% extract exerted the highest FBG-lowering effect, namely 49.38% and 65.43% on days 7 and 14, respectively ($P<0.05$), similar to the effect of metformin (46.26% and 65.42%). Total flavanoid and phenolic contents in the extracts were found to decrease with increase in polarity of extraction solvents. The composition of reference compounds (chlorogenic acid, rutin, astragalin and kaempferol-3-O-rutinoside) followed a similar trend. **Conclusions:** *G. procumbens* contains antidiabetic principles, most extracted in 25% ethanol. Interaction among active components appears to determine the antidiabetic efficacy, achieved likely by a metformin-like mechanism.

KEYWORDS

Antidiabetic, *Gynura procumbens*, Fasting blood glucose, Subcutaneous glucose tolerance test, Streptozotocin-induced diabetes, Flavanoids, Phenolics

1. Introduction

Gynura procumbens (Lour) Merr (*G. procumbens*), a composite known locally in Malaysia as Sambung Nyawa, is an annual evergreen shrub that grows extensively in Southeast Asia, particularly in Indonesia, Malaysia, and Thailand, where it is traditionally used for treatment of eruptive fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus, and cancer[1]. Some of these traditional claims have been validated in scientific and pharmacological studies, including, anti-herpes simplex virus, anti-inflammatory, and anti-hyperlipidemic and anti-hypertensive activities[2].

G. procumbens has recently received particular attention in the pharmacology of antidiabetic medicinal plants, probably because of its avowed empirical evidence and efficacy in the traditional management of diabetes mellitus. However, the scientific reports on the antidiabetic activity

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of this plant have been conflicting and inconsistent. For instance, Zhang and Tan had reported that 95% ethanol extract improved glucose tolerance in STZ-induced diabetic rats, but not in normal rats[3]. Its aqueous extract was also reported by these authors to exert significant anti hyperglycemic action in STZ-induced diabetic rats. Later on, Akowuah *et al.* on the contrary indicated its glucose lowering effect in normal rats[4]. In the most recent study, the extract of *G. procumbens* was reported to produce significant elevation in the fasting blood glucose (FBG) levels of normal rats, but a decrease in diabetic rats[5]. There is a basic need to stream line these reports, given the widespread traditional use of *G. procumbens*.

Moreover, these study designs are not targeted at natural product discovery or production of standardized herbal forms. Adequate research on medicinal plants beyond screening for biological activity should be conducted with the aim to systematically standardize and develop them into natural products or dosage forms which should effectively complement or supplement existing conventional measures[6].

Consequently, the present investigation using an ethnomedical drug discovery program, evaluated the antidiabetic activity of *G. procumbens* used in the traditional health system of the Southeast Asia, as an effective remedy and management for diabetes mellitus and other ailments. This systematic screening is a fundamental requirement for natural product exploration and development of therapeutic agents from medicinal plants.

2. Materials and methods

2.1. Plant material

Fresh leaves of *G. procumbens* collected from Herbagus Sdn Bhd, Kepala Batas, Penang, Malaysia, were authenticated by Mr. V. Shunmugam a/l Vellosamy of the herbarium unit, School of Biology, Universiti Sains Malaysia (USM), and a voucher specimen (No. 11432) was deposited in the herbarium for future reference. The leaves were washed with water, then dried in an oven at 45 °C and milled into powder (1 200 g).

2.2. Preparation of plant extracts

The powdered plant material (1 200 g) was first extracted via maceration (45 °C) in 2 L of 95% ethanol, with solvent replenished every 6 h for 3 d. These were pooled together, and then filtered using Whatman No.1 filter paper. The filtrate was concentrated in vacuo in a rotary evaporator (Buchi Labortechnik AG, Switzerland) at 60 °C to about 10% of original volume and thereafter freeze-dried (Lebconco Corporation, Missouri USA) yielding 5 g (0.42%) of dried 95% ethanol extract. The residue of the plant material from the above was dried and re-extracted with 75% ethanol using the same procedure as for 95% (v/v) ethanol, then repeated for 50%, 25% and 0% ethanol (100% water). The respective yields for these subsequent extracts were 8.0 g (0.67%), 17.3 g (1.44%), 25.3 g (2.12%), and 30.1 g (2.51%). The solvents used for the extraction were prepared according to ratios shown in Table 1.

Table 1
Graded ratios of ethanol to water used in preparation of the different extracts.

Stock solvents (v/v)	Ethanol 95% (mL)	Water (mL)
95%	1 000	10
75%	789	211
50%	526	474
25%	263	737
0%	0	1 000

2.3. Experimental animal

Sprague Dawley rats (200-250 g) obtained from the Animal Research and Service Centre, Universiti Sains Malaysia (USM) were used in this study. The rats were acclimatized for a period of 7 d in the Animal Transit Room, School of Pharmaceutical Sciences, USM where the experiments were carried out. They were allowed access to food (Gold Moher, Lipton India, Ltd.) and tap water *ad libitium*. Temperature of facility was (22±3) °C and light/darkness alternated 12 h apart. The experimental procedures were approved by the Animal Ethics Committee, Universiti Sains Malaysia Penang, Malaysia and the National Institutes of Health Principles of Laboratory Animal Care (1985) were observed.

2.4. Experimental protocol

Diabetes were induced in rats by intraperitoneal injection of 55 mg/kg of streptozotocin (STZ, Sigma Aldrich Chemical Co., USA) reconstituted in 0.1 mol/L cold citrate buffer (pH 4.5) after an overnight fast. After 72 h of STZ administration, blood glucose level was measured in blood collected from tail vein puncture using Accu-check Advantage II clinical glucose meter (Roche Diagnostics Co., USA). Rats with FBG≥15 mmol/L (270 mg/dL) were considered diabetic and included in the study.

The percentage change in blood glucose was calculated thus:

Percentage of clycaemic change=(Gx- Gi)/Gx×100

where Gx is the glycaemia at time x and Gi is the glycaemia at the initial time (i). Prior to diabetes induction, an optimum STZ dose selection study was carried out to determine the appropriate dose that will produce the needed chronic hyperglycemia, but with moderate mortality. To 6 groups (n=4) of overnight fasted rats (200-250 g), varying doses of STZ (65, 60, 55, 50, 45 and 40 mg/kg) reconstituted in freshly prepared buffer (0.1 mol/L cold citrate buffer of pH 4.5) were administered intra-peritoneal. These rats were monitored for

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