

# Dietary *Hibiscus Taiwanensis* Exerts Hypoglycemic in Streptozotocin-Induced Diabetic Rats

Chia-Hsin Huang, Shu-Mei Tsai, Yi-Ru Chen, Ming-Ying Wu, and Juei-Tang Cheng

**Abstract**—The antihyperglycemic effect of *Hibiscus taiwanensis* (HT), was investigated in streptozotocin-induced diabetic rats (STZ-diabetic rats) showing type-1 like diabetes mellitus. A glucose uptake test showed that HT exhibits an increase of glucose uptake activity in a concentration-related manner. HT was isolated a purified biological active compound—syringaldehyde (1). Moreover, an effect by SYR was shown for insulin sensitivity in STZ-diabetic rats. The compound was found to increase insulin sensitivity in STZ-diabetic rats. These results suggest that HT can increase glucose utilization and insulin sensitivity to lower plasma glucose in diabetic rats.

**Index Terms**—*Hibiscus taiwanensis*, syringaldehyde antihyperglycemia, Taiwan's native species.

## I. INTRODUCTION

*Hibiscus taiwanensis* (HT; Fig. 1) Hu, indigenous to Taiwan, is a moderate shrub and widely distributed throughout Taiwan. Species from the genus *Hibiscus* have been used in several applications, such as antidote to poisoning with chemicals and venomous mushrooms in traditional medicine and as a source of fibre to pulp and paper industries. The stem and root of HT have been used as anti-inflammatory, antifungal, antipyretic, and anthelmintic agents in traditional Chinese medicine. From a crude methanol extract of HT, many compounds have been reported, including phenylpropanoid esters, myricic acid, and other known compounds [1]. These active principles have been screened for cytotoxic activity against various carcinoma cell lines [2]. However, the effect on blood sugar of *H. taiwanensis* has not been investigated. Therefore, the chromatographic fractionation of the chemical constituents for their plasma glucose lowering action was carried out. Syringaldehyde (Fig. 1, Fig. 2) was purified from *H. taiwanensis* as an active principle, and its effects on glucose metabolism were further investigated.

## II. MATERIALS AND METHODS

### A. General Experimental Procedure

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The melting point was recorded on a Buchi B-545 melting point apparatus and is uncorrected. polarimeter. <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on a Bruker AM-500 (500 MHz) FT-NMR spectrometer in DMSO-d<sub>6</sub> solution, using the solvent as internal standard. The EIMS was determined on a Finnigan TSQ-700 mass spectrometer. Column chromatography was carried out with Diaion HP 20 (100–200 mesh, Mitsubishi Chemical Industries), MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Industries), and Cosmosil C18-OPN (75 μm, Nacalai Tesque, Inc.). TLC was conducted on silica gel plates (60 F-254, Merck), and 10% sulfuric acid solution was used as a visualizing agent on heating.



Fig. 1. *Hibiscus taiwanensis*.

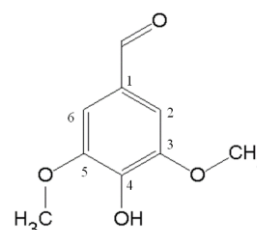


Fig. 2. Syringaldehyde.

### B. Plant Material

The HT were provided by Hercet Co. Ltd. (Kaohsiung, Taiwan). The plant material was identified by Professor M. I. Wu, Kaohsiung Committee of Chinese Medicine (Kaohsiung, Taiwan). A voucher specimen (BT-H-00151) was deposited in the herbarium of the Agricultural Research Institute (Taichung, Taiwan).

### C. Identification of HT DNA from Internal Transcribed Spacer

Region (1) DNA extraction HT DNA in the supernatant was extracted by a genomic DNA extraction kit (Viogene, Taipei, Taiwan). One microliter of the DNA suspension was used for PCR amplification. (2) PCR amplification and DNA sequencing.

Amplification of the ribosomal ITS regions comprising a ITS1 and ITS4 flanked 5.8S ribosomal subunit was performed using a pair of forward primer ITS1primer 5' GAAGGAGAAGTCGTAACAAGG 3' (for-A) ; 5' CTTTTCCTCCGCTTATTGATATG 3'(rev-A) 5'-GCAATTCACAC CAAGTATCGC-3' (rev-B); 5'-CTCTCGGCAACGGATATCTCG-3' (for-B) 5'-CGGCAACGGATATCTCGGCTC-3' (for-C). PCR was performed with 5  $\mu$ l (1 to 5 ng) of template DNA in a total reaction volume of 50  $\mu$ l consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.8 mM deoxyribonucleoside triphosphates (0.2 mM each), 0.7  $\mu$ M primer (each), *Taq* DNA polymerase (1.25 U), and 50  $\mu$ l of a mineral oil overlay. PCR was carried out with an OmniGen thermal cycler (Hybaid Limited, Middlesex, UK) under the following conditions: initial denaturation, 94 °C, 3 min; 35 cycles of denaturation (94 °C, 1 min), annealing (60 °C, 1 min), and extension (72 °C, 1 min), and final extension, 72 °C, 5 min. A negative control was performed with each test run by replacing the template DNA with sterilized water in the PCR mixture. PCR products were purified from 2% agarose gel using the Qiagen Gel Extraction kit (Qiagen, Valencia, Calif.) and were sequenced on a model 377 sequencing system (Applied Biosystems, Taipei, Taiwan).

#### D. Extraction and Isolation

Dried HT (500 g) stems were extracted with 60% aqueous acetone (ratio of solvent volume/dry weight about 2 mL/g) three times, each for 2 days, at room temperature. After evaporating the solvents under vacuum at 45 °C, a residue was obtained. This residue was dissolved in H<sub>2</sub>O (1.5 L) and then extracted successively with CH<sub>2</sub>Cl<sub>2</sub> (1 L  $\times$  3) and n-BuOH (1 L  $\times$  3). The n-BuOH extract (22% dry weight) was subjected to column chromatography over Diaion HP20 (15  $\times$  120 cm) and eluted with a step gradient system (H<sub>2</sub>O–MeOH, 0–100%) to give fractions A–E. Syringaldehyde (1, 1.27 g) was obtained as colorless needles (methanol) from fraction B (0.28% dry weight) by MCI-gel CHP 20P column (8  $\times$  100 cm) chromatography, using MeOH–H<sub>2</sub>O (from 0% to 40%) as the solvent system.

#### E. Animal Model

Male Wistar rats between the ages of 8 and 10 weeks (200–250 g body weight) were obtained from the Animal Center of National Cheng Kung University Medical College. Diabetic rats were prepared by giving an intravenous injection of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) (60 mg/kg) to the fasting rats [4]. Rats with plasma glucose concentrations of 20 mmol/L or greater in addition to polyuria and other diabetic features were considered as having type-1-like diabetes mellitus. All studies were carried out two weeks after the injection of STZ. Animal procedures were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University and were performed according to the Guide for the Care and Use of Laboratory Animals of the U.S. National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

#### F. Effect of HT or Syringaldehyde (1) on Plasma Glucose

A solution of HT (1 g/kg BW) or syringaldehyde (1, purity

>98%, with normal saline to a concentration of 5 mg/mL. The fasting STZ diabetic rats received an intravenous injection of HT or 1 at the desired doses, and blood samples (0.1 mL) were collected under sodium pentobarbital anesthesia (30.0 mg/kg, ip) from the tail vein for measurement of plasma glucose. In preliminary experiments, HT or 1 was found to produce a maximal plasma glucose lowering action in STZ-diabetic rats 60 min after intravenous injection. Control rats received a similar injection of vehicle at the same volume. The antihyperglycemic activity was calculated as a decreased percentage of the initial value according to the formula  $[(Gi/Gt) \times 100\%]$ , where Gi is the initial glucose level and Gt is the plasma glucose concentration after treatment. Uptake of 2-NBDG into Rat L6 Myocytes.

The glucose uptake test was carried out using 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) as a fluorescence indicator according to previous reports,<sup>20,21</sup> with some modifications used. The L6 cells were cultured in a 10 cm dish for 48 h. The number of L6 cells in each assay was  $1 \times 10^6$  cells/mL. The medium was removed, and the cells were washed gently with phosphate buffer solution (PBS). Cells were detached from the dish by using a trypsin treatment, suspended in 0.2 mM 2-NBDG and 1 at the indicated concentration in PBS, and then incubated in a 37 °C water bath for 60 min in the dark. The cells were centrifuged (4 °C, 5000  $\times$ g, 10 min) to discard the supernatant. The pellet was washed three times with cold PBS and subjected to ice cooling. The pellet was suspended in 1 mL of PBS. The fluorescence intensity in cell suspension was evaluated using a fluorescence spectrofluorometer (Hitachi F-2000, Tokyo, Japan), with excitation and emission wavelengths of 488 and 520 nm, respectively. The intensity of fluorescence reflected the uptake of 2-NBDG in the cells [3].

### III. RESULTS AND DISCUSSION

The rDNA sequence of HT was shown in Fig. 3. The HT sources have been identified 600 and 800 bp region rDNA electroporesis.

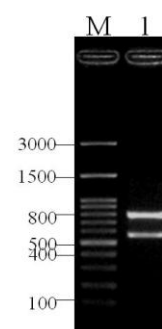


Fig. 3. Identification of HT was confirmed by ITS region of the ribosomal RNA gene (rDNA). PCR products were separated by electrophoresis in a 2.0% agarose gel and then stained with ethidium bromide. The gel was observed and photographed under ultraviolet light. The 100-bp ladder (M) as molecular weight standards is shown in intervals.

HT or syringaldehyde (1) was studied by p.o. or intravenous injection in animals. It was found that bolus injection of 1 can lower plasma glucose concentrations effectively in streptozotocin-diabetic (STZ-diabetic) rats. As

shown in Fig. 4 or Fig. 5, a dose-dependent increase of antihyperglycemic activity was observed in STZ-diabetic rats upon intravenous injection of 1 at the dose range increase in dosage beyond 7.2 mg/kg. The minimal and maximal plasma glucose-lowering activities of 1 in STZ-diabetic rats were  $11.08 \pm 1.0\%$  at 1.8 mg/kg and  $31.29 \pm 2.07\%$  at 7.2 mg/kg, respectively. In a previous study using the same conditions, it was demonstrated that the maximal glucose-lowering effect of metformin, a clinically used antidiabetic agent, was  $32 \pm 5\%$  at an oral dose of 100 mg/kg in STZ diabetic rats.<sup>7</sup> By comparison with metformin, this indicated that 7.2 mg/kg of 1 is an effective dose for subsequent experiments. In preliminary screening, it was determined that the plasma glucose lowering activity at the same dose of 1 in normal Wistar rats (number size = 8) is  $25.93 \pm 5.63\%$ , which seems slightly less than in STZ-diabetic rats. Thus, syringaldehyde (1) can be considered as a potential effective plasma glucose lowering agent.

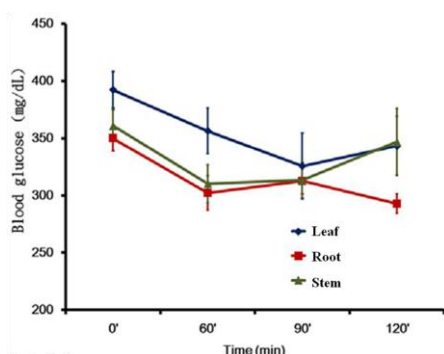


Fig. 4. Antihyperglycemic action of HT (1 g/kg) in STZ diabetic rats.

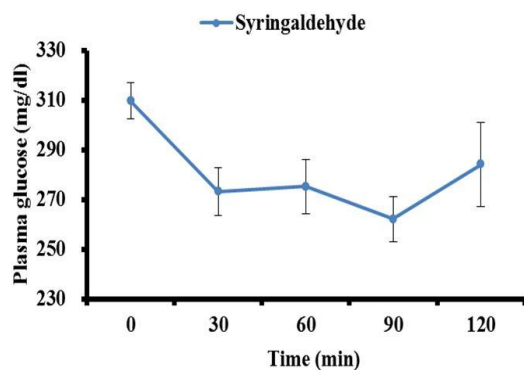


Fig. 5. Antihyperglycemic action of syringaldehyde (1) in STZ diabetic rats.

Skeletal muscle is the major site of glucose disposal.<sup>9</sup> Glucose transport, which depends on insulin-stimulated translocation of glucose carriers to the cell membrane, is the rate-limiting step in the carbohydrate metabolism of skeletal muscle [5]. Insulin stimulated glucose uptake into skeletal muscle is the major site for the regulation of plasma glucose concentrations [6]. In the present study, cultured L6 myoblasts were used to identify the effect of 1 on glucose uptake as described previously [7]. It was found that 1 can increase glucose uptake in a concentration dependent manner (Fig. 6). Thus, an increase in glucose uptake may be considered as a potential mechanism for the observed glucose-lowering action of 1.

In addition, the possible mechanism for 1 to increase glucose uptake may be related to the regulation of GLUT4 in

skeletal muscle. However, more experimentation is needed to find out the possible mechanism(s) for the increase of glucose uptake by 1.

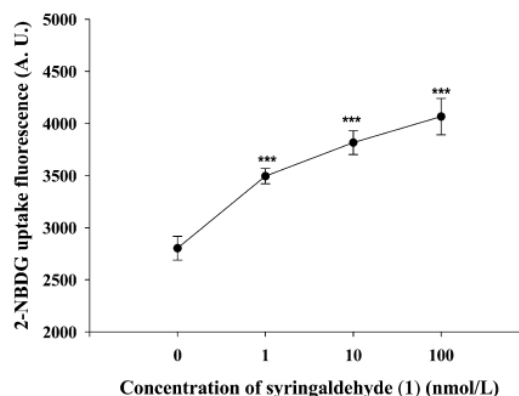


Fig. 6. Effect of syringaldehyde (1) on glucose uptake in rat L6 myocytes. Cells were suspended in 0.2 mM 2-NBDG to incubate with syringaldehyde (1) at the indicated concentration or the same volume of vehicle (as 0 nmol/L) for 60 min. Values (means  $\pm$  SEM) of absorbance units (AU) were obtained from each group of eight experiments; \*\*\* $p < 0.001$  as compared with vehicle-treated group (0 nmol/L).

#### IV. CONCLUSION

In conclusion, the data obtained suggest that HT and its active principle 1 can lower plasma glucose in STZ-diabetic rats through an increase of glucose utilization. Thus, HT or 1 might be suitable as an adjuvant for the treatment of diabetic patients in the future.

#### ACKNOWLEDGMENT

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