Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2017, **15**(3): 0168–0177
Chinese Journal of Natural Medicines

Antihyperglycemic, antihyperlipidemic and antioxidant effects of standard ethanol extract of *Bombax ceiba* leaves in high-fat-diet- and streptozotocin-induced Type 2 diabetic rats

XU Guang-Kai [1], QIN Xiao-Ying [1Δ], WANG Guo-Kai [2], XIE Guo-Yong [1], LI Xu-Sen [1], SUN Chen-Yu [1], LIU Bao-Lin [3*], QIN Min-Jian [1*]

[1] *State Key Laboratory of Natural Medicines, Department of Resources Science of Traditional Chinese Medicines, China Pharmaceutical University, Nanjing 210009, China;*
[2] *Anhui Key Laboratory of Modernized Chinese Materia Medica, Anhui University of Traditional Chinese Medicine, Hefei 230012, China;*
[3] *Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University, Nanjing 210009, China*

Available online 20 Mar., 2017

[ABSTRACT] The present study aimed at exploring the therapeutic potential of standard extract of *Bombax ceiba* L. leaves (BCE) in type 2 diabetic mellitus (T2DM). Oral administration of BCE at doses of 70, 140, and 280 mg·kg^{-1}, to the normal rats and the high-fat-diet- and streptozotocin-induced T2DM rats were carried out. Effects of BCE on blood glucose, body weight, and a range of serum biochemical parameters were tested, and histopathological observation of pancreatic tissues was also performed. HPLC-ESI-Q/TOF-MS/MS analysis indicated that the chemical composition of BCE mainly contained mangiferin, isoorientin, vitexin, isomangiferin, isovitexin, quercetin hexoside, 2'-trans-*O*-cumaroyl mangiferin, and nigricanside. BCE caused a significant decrease in the concentrations of fasting blood glucose, glycosylated hemoglobin, total cholesterol, triglyceride, low density lipoprotein-cholesterol, serum insulin, and malondialdehyde, and increases in oral glucose tolerance, high density lipoprotein-cholesterol, and superoxide dismutase in the T2DM model rats. Moreover, considerable pancreatic *β*-cells protection effect and stimulation of insulin secretion from the remaining pancreatic *β*-cells could be observed after BCE treatment. The results indicated that BCE exhibited an excellent hypoglycemic activity, and alleviated dyslipidemia which is associated with T2DM. Antioxidant activity and protecting pancreatic *β*-cells are the possible mechanisms involved in anti-diabetic activity of BCE.

KEY WORDS] *Bombax ceiba* ; Type 2 diabetic mellitus; Antihyperglycemic; Antihyperlipidemic; Antioxidant

CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2017)03-0168-10

Introduction

Diabetes mellitus, an extensive chronic metabolic disease, is characterized by hyperglycemia and carbohydrate, protein, and fat metabolism disturbances. Persistent hyperglycemia often induces complications affecting patient's visual, nervous, renal, and other systems. There are many issues to be addressed to cure diabetes, in addition to glycemic control. Currently available synthetic oral antihyperglycaemic agents have not shown to alter the progressive *β* cell failure and may be associated with an increased risk of unwanted effects after prolonged use. As traditional herbal medicines may have multiple effects on diabetes with greater tolerability and fewer side effects, there is an increasing need to search for more natural therapeutic agents from natural plants.

Bombax ceiba L. (Bombacaceae), also known as "Hero Tree" or "Panzhihua" in China, is widely distributed and cultivated in temperate Asia, tropical Asia, Africa, and Australia [1]. The plant contains health-promoting phytopharmaceuticals such as phenolics, flavonoids, sesquiterpenoids, steroids, naphthoquinones, and neolignans [2-6], possessing medicinal properties and a strong ethnobotanical background [7]. It has been extensively used in southern China, India, and rural area of northern Pakistan as a famous folk medicine

[Received on] 15-Apr.-2016
[*Corresponding author] Tel: 86-25-86185127, Fax: 86-25-86185292, E-mail: zhongyao440@163.com (LIU Bao-Lin); Tel: 86-25-86185130, Fax: 86-25-85301528, E-mail: minjianqin@163.com (QIN Min-Jian).
[Δ]Co-first author
These authors have no conflict of interest to declare.

in the treatment of a range of diseases, including edema, hepatotoxicity [3, 8-10], ulcer [11], pyrexia [6], hypertension [2, 12], and diabetic mellitus [13-14]. In addition, the flower, leave, stem bark, and root of *Bombax ceiba* are all shown to have antimicrobial and antioxidant activities [13, 15-17].

Through bioassay screening for beneficial biological agents for diabetes from ethnomedicines, we found that ethanol extract of *Bombax ceiba* leaves (BCE) possessed antihyperglycemic effects. Therefore, in the present study, standard BCE was prepared and high-performance liquid chromatography-electrospray ionization quadrupole time-of-flight tandem mass spectrometry (HPLC-ESI-Q/TOF-MS/MS) analysis method was used to reveal the phytopharmaceuticals of BCE. It was assumed that herbal medicines can only be effective as an alternative to oral hypoglycaemic agents in type-2 diabetes mellitus (T2DM) when pancreatic islets are not totally destroyed. Therefore, a series of systematic pharmacological experiments of BCE on high-fatdiet (HFD) and streptozotocin (STZ) induced T2DM rats were carried out. The results from the present study could provide scientific evidences for the utilizing of *Bombax ceiba* leaves to treat T2DM.

Materials and Methods

Chemicals and reagents

The kits for measurement of superoxide dismutase (SOD), fasting blood glucose (FBG), glycosylated hemoglobin (HbA_{1c}), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and malondialdehyde (MDA) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). The ELISA kit for insulin detection was purchased from R&D Systems (Minneapolis, MN, USA). Streptozotocin was purchased from Sigma Co. (St Louis, MO, USA). The standard antidiabetic drug glimepiride was obtained from Wanbang Pharmaceutical GmbH (Xuzhou, Jiangsu, China). Mangiferin (purity > 98%) was purchased from Guangrun Pharmaceutical Technology Co. Ltd. (Nanjing, China). Rutin (purity > 92.6%) was purchased from National Institutes for Food and Drug Control (Beijing, China). All solvents used in the present study were of analytical reagent grade. Column chromatography was carried out with macroreticular resin AB-8 (Chemical Plant of Baoen, Hebei, China).

Plant materials and preparation of BCE

The leaves of *Bombax ceiba* L. were collected from Ledong, Hainan, China, in August, 2013, and identified by Prof. QIN Min-Jian (China Pharmaceutical University, Nanjing, China). A voucher specimen (No. BM-201301) was deposited in the Herbarium of Medicinal Plants of China Pharmaceutical University, Nanjing, China.

Air-dried *Bombax ceiba* leaves (2.5 kg) were refluxed with 80% ethanol at the ratio of 1 : 10 (solvent and sample ratio, *V/W*) for 2 h. The extracted solutions were combined, filtered, and then concentrated by a rotary evaporator under reduced pressure to remove the ethanol solvent. The powdered extract was then dissolved with deionized water at appropriate concentrations, adsorbed to macroporous resin column, and then eluted with distilled water and 20%, 65%, and 95% ethanol successively. The 65% fraction was concentrated using a vacuum evaporator, and vacuum dried at room temperature, to obtain the standard *Bombax ceiba* leaves ethanol powder extract (BCE, yield 12.81%, *W/W*).

Phytochemical profiling

The HPLC-ESI-Q/TOF-MS/MS analysis was conducted using an Agilent Technologies Series 1290 Infinity HPLC instrument (Agilent, Waldbronn, Germany) coupled with an Agilent 6530 Q-TOF mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) equipped with an automatic degasser, an autosampler and a column compartment. Chromatographic separation was performed at 30 °C on a C_{18} column (210 mm × 4.6 mm, 5 μm; Hanbang Company, China) at a flow rate of 1.0 mL·min^{-1}, and the injection volume was 10 μL. The mobile phase was consisted of 0.1% formic acid aqueous solution (A) and acetonitrile (B) using a gradient elution as follows: 0−15 min, 14% B; 15−20 min, 14%−17% B; 20−45min, and 17%−18% B. The mass spectra were acquired across the range of *m/z* 150−950 in a negative mode. The operating parameters of mass spectrometer were as follows: drying gas (N_2) flow rate, 10 L·min^{-1}; drying gas temperature, 320 °C; nebulizer, 35 psig; capillary voltage, 3 000 V; fragment voltage 120 V; skimmer voltage, 60 V, and Oct RFV, 750 V. The collision energy was set at 15 V. All the MS data were controlled by MassHunter software B.02.00 ChemStation (Agilent Technologies, Santa Clara, CA, USA).

Determination of total phenolic compounds

The determination of total phenolic compounds in BCE was accomplished using the method described in Chinese Pharmacopoeia [18]. 2-mL extract appropriately diluted was mixed with 2 mL $AlCl_3$ (0.1 mol·L^{-1}) and 3 mL of KAc (1 mol·L^{-1}), andthe mixture was adjusted to 10 mL with 60% ethanol and let it rest for 30 min. Its absorbance (A) was measured at 421 nm, and 60% ethanol was used as a blank control. Rutin was used as a reference standard and the content of total phenolics was expressed as rutin equivalents (RE, μg·mg^{-1} extract) through the calibration curve with rutin.

HPLC-DAD analysis

High performance liquid chromatography (HPLC) analysis was conducted on an Agilent Series 1260 LC instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with an on-line degasser, a quaternary pump, a diode-array detector (DAD), a thermostated column compartment, and an auto-sampler. The analytes separation was performed on a C_{18} analysis column (210 mm × 4.6 mm, 5 μm; Hanbang Company, China). The column temperature was maintained at 30 °C. The mobile phase was consisted of 0.1% formic acid aqueous solution (A) and acetonitrile (B), and the gradient program was optimized as follows: 10%−16% B at 0−1 min, 16%−18% B at 1−11 min and