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Arctigenic acid, the key substance responsible for the hypoglycemic activity of *Fructus Arctii*

Zhaohui Xu[a,*], Chenchen Gu[a], Kai Wang[b], Jiaxing Ju[a], Haiying Wang[c], Kefeng Ruan[a], Yi Feng[a]

[a] *Engineering Research Center of Modern Preparation Technology of Traditional Chinese Medicine, Ministry of Education, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[b] *Center for Drug Safety Evaluation, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*
[c] *School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China*

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ABSTRACT

We have reported the antidiabetic activity of the total lignans from *Fructus arctii* (TLFA) against alloxan-induced diabetes in mice and rats. In this study, arctigenic acid was found to be the main metabolite in rat plasma detected by UPLC/MS and HPLC/MS/MS after oral administration of TLFA. For the first time, its hypoglycemic activity and acute oral toxicity were evaluated in Goto-Kakizaki (GK) rats, a spontaneous type 2 diabetic animal model, and ICR mice respectively.

GK rats were orally given arctigenic acid (50 mg/kg) twice daily before each meal for 12 weeks. The treatment reduced the elevated plasma glucose, glycosylated hemoglobin and showed significant improvement in glucose tolerance in glucose fed hyperglycemic GK rats. We found that the hypoglycemic effect of arctigenic acid was partly due to the stimulation on insulin secretion, whereas the body weight was not affected by arctigenic acid administration in GK rats. Meanwhile, there was no observable acute toxicity of arctigenic acid treatment at the dosage of 280 mg/kg body weight daily in the acute 14-day toxicity study in mice.

This study demonstrates that arctigenic acid may be the main metabolite in the rat serum after oral administration of TLFA, which showed significant hypoglycemic effect in GK rats, and low acute toxicity in ICR mice. The result prompts us that arctigenic acid is the key substance responsible for *Fructus Arctii* antidiabetic activity and it has a great potential to be further developed as a novel therapeutic agent for diabetes in humans.

Abbreviations

AA,	arctigenic acid
CHOl,	cholesterol
DM,	diabetes mellitus
ELISA,	enzyme-linked immunosorbent assay
FBG,	fasting blood glucose
HbA1c,	glycosylated hemoglobin
HDL-C,	high density lipoprotein cholesterol
LDL-C,	low density lipoprotein cholesterol
OGTT,	oral glucose tolerance test
PBG,	postprandial blood glucose
TG,	triglycerides
T2DM,	type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by defects in insulin secretion, insulin action, or both (Callahan and Mansfield 2000). The global burden of type 2 diabetes mellitus T2DMposes enormous societal costs and has major implications for all healthcare systems (American Diabetes Association 2011).

World Health Organization has suggested the evaluation of potential plants as effective therapeutic agents, especially in areas in which we lack safe modern drugs (Ivorra et al. 1989). Arctigenic acid (AA, CAS Registry Number: 42320-79-6, Fig. 1) was isolated and identified from germinating burdock (*Arctium lappa*) seeds at the first time in 2005 (Keiko et al. 2005). But its pharmacological activity has not been reported yet.

Fructus arctii, called “Niubangzi” in China (Great burdock achene in English), is a well-known Chinese Materia Medica. It is the dried ripe fruit of *Arctium lappa L.* (family Asteraceae) and was included in the Chinese pharmacopoeia for its traditional therapeutic actions. But it has been reported recently that the clinical use of *Fructus arctii*, in a relatively large quantity (15 g/day), resulted in a satisfactory

* Corresponding author. Tel.: +86 021 51323094; fax: +86 021 51323094.
E-mail address: lntcmx@163.com (Z. Xu).

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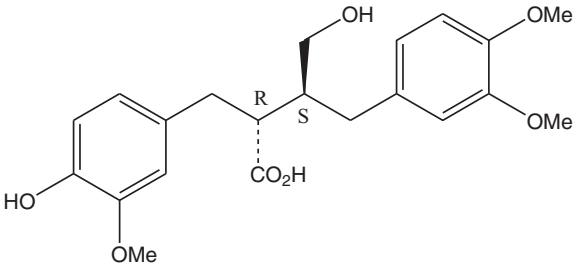

Fig. 1. Structure of arctigenic acid.

hypoglycemic effect in patients T2DM (Chen 1999). Modern pharmacological studies revealed that the powder or extracts of *Fructus arctii* were effective for the treatment of high blood glucose and diabetic nephropathy (Yan and Li 1993; Wang and Chen 2004; Zhang and Piao 2007; Liu et al. 2011).

We have reported (Xu et al. 2008) the antidiabetic activity of the total lignans from the ethanol extract of *Fructus arctii* against alloxan-induced diabetes in mice and rats. In our prior study, TLFA exhibited significant antidiabetic activity, and a notable increase in the serum insulin level was observed at the same time.

In this study, we analyzed the constituents in the serum of the GK rats after TLFA was given orally. The serum samples were detected by UPLC/MS (Waters, US) and HPLC/MS/MS (Finnigan, US), AA was found to be the main ingredient in the serum.

Then we studied acute oral toxicity and hypoglycemic activity of AA, which was prepared with arctigenin by alkaline hydrolysis reaction. The pharmacodynamic study was performed in GK rats, a spontaneous type 2 diabetic animal model, and insulinotropic drug nateglinide was chosen as positive control drug.

Besides common evaluation indexes of hypoglycemic activity such as fasting blood glucose (FBG), postprandial glucose (PBG), oral glucose tolerance test (OGTT), as well as glycated hemoglobin, particular attention was paid to the effects of AA on insulin secretion and pancreas tissue sections, with a view to investigate its mechanism of hypoglycemic activity and providing a pharmacological rationale for the future development of it for diabetes.

Materials and methods

Plant material

The *Fructus arctii* purchased from Xuzhou Pharmaceutical Co., Ltd. was collected from Xuzhou, Jiangshu Province. The plant material was identified by Botanical Professor Zhong Liu at the School of Pharmacy, Shanghai Jiao Tong University. The voucher specimen is deposited in the Engineering Research Center of Modern Preparation Technology of Traditional Chinese Medicine, Ministry of Education, Shanghai University of TCM, under the number 20121211. The whole plant was then dried under air, insolation and mechanically powdered separately to obtain a coarse powder, which was then subjected to extraction.

Preparation of TLFA and AA

Simply comminuted dried *Fructus arctii* (10 kg) was extracted circumfluently with 95% aqueous ethanol (2 × 80 l) at 80 °C for 6 h each time. The combined extracts were filtered and concentrated to yield liquid extract (EtOH-extract, 2.0 l, density 1.05 g/cm^3) in a rotatory evaporator at 55 °C under reduced pressure, and then defatted with petroleum ether (60–90 °C) followed by extraction with ethyl acetate. The ethyl acetate extract was concentrated and dried in vacuum at 50 °C, with a yield of 8.6% (w/w). Thus, the extracts containing total lignans (TLFA) were obtained.

Purity of TLFA was determined by ultraviolet spectrometry (Zhai et al. 2009). In detail, the precisely weighed 10.0 mg TLFA powder was dissolved with 25 ml MeOH, and 1.0 ml of dissolution was transferred into a 10 ml of volumetric flask to prepare sample solution. Arctigenin (purity > 99%, Lot number: A1854, Sigma–Aldrich Chemical Company) was dissolved in MeOH to 30 mg/ml as the control solution. Absorbance of the sample and the control were determined at 280 nm using ultraviolet spectrometry with MeOH as a blank. Finally, the purity of TLFA was calculated to be 86.0%.

The TLFA (200 g) was fractionated by column chromatography at atmospheric pressure over silica gel G using petroleum ether, chloroform, ethyl acetate, acetone and methanol successively, as a result, five fractions were obtained. The fraction eluted by chloroform was concentrated to one-tenth of the original volume in a rotatory evaporator at 40 °C under reduced pressure, then the concentrate was crystallized at 4 °C, followed by recrystallization with methanol to obtain purified arctigenin (yield 4.3%, w/w), which was identified by comparing the physical and chemical parameters with reference substance arctigenin.

The arctigenin was hydrolyzed in 12-fold (w/v) of 60% aqueous ethanol containing 4% NaOH (w/v) at 60 °C for 3 h, then the reaction product was adjusted to neutral pH with 1 M of HCl. After standing overnight at room temperature, the reaction product was centrifuged, and the precipitate was washed by deionized water until washings become colorless, then it was dried in vacuum at 37 °C to give a white amorphous powder with a yield of 96.6% (w/w). The chemical structure of this arctigenin hydrolysis product was further identified by spectral and elemental analysis.

Animals

The acute toxicity test was carried out on ICR mice of either sex weighing between 18 and 23 g. Male GK rats and Wistar rats of both sexes aged 9 weeks were used for serum pharmacochemistry study and hypoglycemic activity assessment.

All animals were obtained from Shanghai Laboratorial Animal Center, Chinese Academy of Science. All animals were housed in polycarbonate cages (five rats or mice per cage) with a wooden chip mat on the floor and tap water was available ad libitum. High fat diet (Suzhou Shuangshi Laboratory Animal Feed Science Co., Ltd, China) was provided to GK rats and standard chow was provided to mice and Wistar rats. The animal room was kept on a 12-h light/dark cycle, with a temperature range of 24 ± 1 °C and a relative humidity of 55% ± 5% throughout the experimental period. All the procedures were in strict accordance with the PR China legislation on the use and care of laboratory animals and with the guidelines established by Institute for Experimental Animals of Shanghai University of TCM and were approved by the university ethical committee for animal experiments.

Analysis of the constituents in the rat serum after oral administration of TLFA

Sample collection

After 7 days acclimation, six experiment GK rats were fasted overnight before the experiments. Two milliliters of blood was taken from orbital sinus of every rat. Then vacuum dried powder of TLFA was dissolved with the solvent composed by ethanol:Tween 80:distilled water (1:1:8) as a stock solution (35 mg/ml) and was orally administrated to rats (1 ml/100 g body weight). 60 min after drug administration, the blood samples were collected from orbital sinus and immediately centrifuged at 13,000 rpm for 10 min at 4 °C, the supernatant (serum) obtained were frozen immediately and stored at −40 °C before analysis.

HPLC grade ethanol (300 μl) was added to 100 μl of the above serum and vortexed for 1.5 min, centrifuged (13,000 rpm) at 4 °C for