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Antidiabetic effect of total flavonoids from Sanguis draxonis in type 2 diabetic rats

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ABSTRACT

Ethnopharmacological relevance: Sanguis draxonis (SD) is a kind of red resin obtained from the wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen (*Dracaena cochinchinensis*). It is a Chinese traditional herb that is prescribed for the handling of diabetic disorders, which is also supported by an array of scientific studies published in recent years. Although chemical constituents of this plant material have also been previously evaluated (Tang et al., 1995; Wei et al., 1998), it still remains poorly understood which constituent is the major contributor to its antidiabetic activities. Moreover, very little is known about the molecular mechanisms underlying antidiabetic activities of SD. Flavonoids exist at a high level in SD. The aim of this study is to evaluate the antidiabetic effects of total flavonoids from SD (SDF) in type 2 Diabetes mellitus (T2DM) rats.

Materials and methods: T2DM rats were induced by 4 weeks high-fat diet and a singular injection of streptozotocin (STZ) (35 mg/kg). Then T2DM rats were treated with SDF for 21 days, using normal saline as the negative control. For comparison, a standard antidiabetic drug, metformin (200 mg/kg), was used as a positive control. Three weeks later, relative biochemical indexes were determined and histopathological examinations were performed to assess the antidiabetic activities of SDF.

Results: SDF not only exhibited a significant hypoglycemic activity, but also alleviated dyslipidemia, tissue steatosis, and oxidative stress associated with T2DM. Moreover, considerable pancreatic islet protecting effects could be observed after SDF treatment. Further investigations revealed a potential anti-inflammation activity of SDF by determining serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP).

Conclusions: This study demonstrates both hypoglycemic and hypolipidemic effects of SDF in T2DM rats, suggesting that flavonoids are the major active ingredients accounting for the antidiabetic activity of SD. Alleviating chronic inflammation responses and protecting pancreatic islets are possible mechanisms involved in the antidiabetic activity of SDF.

1. Introduction

Diabetes mellitus is the most prevalent metabolic disorder that is principally characterized by insulin resistance (IR) and elevated blood glucose levels (Mohammadi et al., 2011). Generally, there are two types of Diabetes mellitus, type 1 Diabetes mellitus (T1DM) and type 2 Diabetes mellitus (T2DM). T1DM only accounts for 3–5% of Diabetes mellitus patients. The vast majority of diabetes is of T2DM. There are about 200 million people around the world who are suffering from T2DM and the number is expected to reach 300 million cases by the year 2025 (Waly et al., 2010). T2DM is a heterogeneous disorder characterized by IR, followed by the inability of pancreatic β cells to compensate for IR (pancreatic beta cell dysfunction) (Srinivasan et al., 2005). A main factor contributing to the development of IR is obesity generally resulting from western-style high-fat diet and physical inactivity. An increased accumulation of lipids caused by high-fat diet induces steatosis in livers and skeletal muscles. These events are closely related to the degree of IR in these two tissues (Chapman and Sposito, 2008; Phielix and Mensink, 2008). In the other aspect, IR in itself is an independent and significant risk factor for the pathogenesis of cardiovascular diseases which further attribute to

Abbreviations: SD, sanguis draxonis; *D. cochinchinensis*, *Dracaena cochinchinensis* (Lour.) S. C. Chen; SDF, total flavonoids from SD; T1DM, type 1 Diabetes mellitus; T2DM, type 2 Diabetes mellitus; STZ, streptozotocin; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; CRP, C-reactive protein; IR, insulin resistance; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; TG, triglycerides; TC, total cholesterol; FFA, free fatty acids; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; ELISA, enzyme-linked immunosorbent assay; HE, hematoxylin-eosin; ROS, reactive oxygen species

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the mortalities related to T2DM (Matsumoto et al., 2003). Therefore, besides correcting hyperglycemia, efficient amelioration of dyslipidemia and tissue steatosis constitutes another important factor in clinical T2DM treatment. Searching for new reagents, which could not only control hyperglycemia, but also effectively correct dyslipidemia and tissue steatosis associated with T2DM, is necessary to control this disease.

Sanguis draxonis (SD) is a kind of red resin obtained from the wood of *Dracaena cochinchinensis* (Lour.) S. C. Chen (*Dracaena cochinchinensis*). It has been extensively used in China as a famous folk medicine in the treatment of an array of diseases, including blood stasis syndrome, trauma, inflammation, allergic dermatitis and so on (Chen et al., 2003). In traditional Chinese medicine, diabetes is classified as "Xiao Ke" (consumption and thirst) or "depletion-thirst disease". As documented in an early literature "Synopsis of Golden Chamber" (East Han dynasty, about 2000 years ago), Diabetes mellitus should be treated by promoting blood circulation to remove stasis in body meridians. According to "Bencao Gangmu" (Ming dynasty, about 500 years ago), SD is capable of relieving blood stasis syndrome. Later generations thereby began to use SD in treating Diabetes mellitus-associated diseases, which is supported by a series of recent scientific studies showing that SD has the antidiabetic activity (Hou et al., 2004, 2005). However, the scientific basis, including the active ingredients and possible molecular mechanism, underlying its antidiabetic activities still remain poorly understood. A variety of secondary metabolic products, such as flavonoids, saponins, and phenolic acids, exist abundantly in this plant material (Likhitwitayawuid et al., 2002; González et al., 2004; Fan et al., 2008; Nakashima et al., 2009). Our previous investigations revealed that among all these kinds of chemicals, total flavonoids from SD (SDF) exhibited the most obvious antidiabetic effects (our unpublished data). Therefore, our current study was designed to systemically evaluate the antidiabetic activities of SDF and explore its potential underlying mechanisms in T2DM rats induced by high-fat diet combined with low-dose streptozotocin (STZ).

2. Materials and methods

2.1. Preparation of SDF

SD, the alcohol extract of the resinous wood of *Dracaena cochinchinensis* (Family Liliaceae), is commercially available. Therefore, we directly purchased SD from Xishuangbanna Resemblance Pharmaceutical Co., LTD for this investigation. The specimen of the alcohol extract (No. EP-100811) has been deposited in the Herbarium of College of Pharmacy, South-Central University for Nationalities. SDF was prepared as previously described (Tu et al., 2011). Briefly, SD (1.0 kg) was ground into powders and extracted with ethyl acetate (solvent: sample=8: 1, v/w) twice, 2 h for each. After filtration, the solution was combined and condensed to obtain a viscous extract (yield 35.5%, w/w). Then, the dried product was extracted twice with 0.34% NaOH (solvent: sample=30: 1, v/w) for 1 h each time. Filtrate was collected and adjusted its pH to 2.0 by 10% HCl. The solution was precipitated for 12 h at room temperature. The precipitation was collected and washed with distilled water. To obtain the total flavonoids of SD, the samples were concentrated under reduced pressure at 40 °C using a vacuum evaporator at room temperature, (SDF, yield 15.9%, w/w). The content of flavonoids was 77.36% determined by the colorimetric method described by China Pharmacopeia (China Pharmacopoeia Committee, 2005). At last, to evenly suspend the gel-like solid SDF in the vehicle, SDF was powdered and sieved for further experiments.

2.2. Animals

Healthy male Sprague-Dawley rats (180–220 g), purchased from the Tongji Medical College of Huazhong University of Science & Technology, were used in this study. Rats were maintained under controlled room humidity (45–75%) and temperature (22 ± 2 °C) with 12 h light/dark cycles. Before the experiments, the rats were acclimatized to the laboratory conditions for 1 week. All the animal experimental procedures were performed in accordance with International Guidelines for Care and Use of Laboratory Animals and approved by the Animal Ethical Committee of the Institute of Health and Epidemic Prevention (Wuhan, PR China).

2.3. Induction of T2DM in rats and drug administration

T2DM rats were prepared as described previously (Zheng et al., 2012). In brief, the rats were fed with high-fat diet containing 24% protein, 41% carbohydrate and 24% fat (Research Diets, Inc., New Brunswick, Canada) for 4 weeks and then injected with 35 mg/kg of STZ (Sigma-Aldrich, St. Louis, USA) intraperitoneally. Three days after injection, rats with a blood glucose level $\geq$ 11.1 mmol/L were considered as T2DM rats and used in further experiments. These T2DM rats were fed with high-fat diet throughout the whole study. To evaluate the antidiabetic activities of SD, 36 rats were divided into 6 groups, with 6 rats for each group. Another 36 rats were used to study the antidiabetic effects of SDF. Thus, totally 72 rats were used in our investigations. The groups for SD or SDF treatment received indicated dosages of SD or SDF. Both SD and SDF were suspended in 0.9% normal saline and administrated via oral gavage. Metformin, a standard antidiabetic drug, was used as a positive control. The vehicle control group received normal saline. A group of health rats were kept as normal controls. At day 0, 7, 14, and 21 of treatment, blood samples were collected by the retro-orbital sinus puncture under diethyl ether anesthesia. The fasted plasma glucose levels were determined using a blood glucose meter (ONETOUCH, Ultra, Lifescan, USA). At day 14 and 18, oral glucose tolerance test and insulin tolerance test were performed respectively.

2.4. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

At day 14, OGTT was performed in overnight fasted rats from all groups. The rats were orally loaded with glucose (2 g/kg) 30 min after the administration of SD or SDF. Blood samples were collected by the retro-orbital sinus puncture under diethyl ether anesthesia, and blood glucose levels were determined at 0, 30, 60 and 120 min after glucose administration. At day 18, ITT was performed in overnight fasted rats from all groups. Half an hour after administrating SD or SDF, the rats were intraperitoneally injected with 0.15 U/kg of insulin (Recombinant Human Insulin Injection, Lilly, France). Blood samples were collected in the same way as indicated in OGTT, and blood glucose levels were determined at 0, 30, 60 and 120 min after insulin injection.

2.5. Biochemical analysis

At the end of the experiment, the animals were fasted overnight and blood samples were collected by retro-orbital sinus puncture using capillary tubes under diethyl ether anesthesia. Then, serums were prepared by centrifuging the blood samples at 4000 rpm for 15 min. The serum levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), triglycerides (TG), total cholesterol (TC), free fatty acids (FFA), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were determined by corresponding assay kits from Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, PR China).