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Anti-diabetic activity and potential mechanism of total flavonoids of *Selaginella tamariscina* (Beauv.) Spring in rats induced by high fat diet and low dose STZ

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ABSTRACT

Aim of the study: To evaluate the anti-diabetic effects of the total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST), and to explore the pertinent mechanism.
Materials and methods: High fat diet and STZ (35 mg/kg) induced diabetic rats were administered with TFST at graded oral doses (100, 200 and 400 mg/kg/day, ig.) for 8 weeks. A range of parameters, including blood glucose and lipid, serum insulin and glucagon, glucose tolerance, were tested to evaluate its anti-diabetic effects. The determination of protein expression of peroxisome proliferator activated receptor γ (PPAR-γ) in adipose tissue and insulin receptor substrate 1 (IRS-1) in hepatic and skeletal muscle tissues was used to study the mechanism of TFST. Moreover, the preliminary study of TFST on the antioxidant activity was performed.
Results: The TFST possessed anti-diabetic activities as shown by the decreased serum levels of fast blood glucose (FBG), glycosylated hemoglobulin A1C (HbA1c), triglyceride (TG), total cholesterol (TC), free fatty acid (FFA), low density lipoprotein-cholesterol (LDL-C) and glucagon, as well as increased serum levels of high density lipoprotein-cholesterol (HDL-C), insulin and C-peptide. TFST also improved the oral glucose tolerance test (OGTT) to a certain degree. Furthermore, TFST increased the protein expression of PPAR-γ in adipose tissue, and increased the protein expressions of IRS-1 in hepatic and skeletal muscle tissues. These benefits were associated with increased superoxide dismutase (SOD) and decreased malondialdehyde (MDA) in serum.
Conclusions: TFST exert beneficial effects on hyperglycosemia and hyperlipoidemia in diabetic rats possibly through regulating the levers of PPAR-γ in adipose tissue and IRS-1 in hepatic and skeletal muscle tissues.

1. Introduction

Diabetes mellitus is the world's largest endocrine disease characterized by chronic hyperglycemia associated with abnormalities in carbohydrate, fat, and protein metabolism caused by complete or relative insufficiency of insulin secretion and/or insulin action, and usually accompanied by a variety of microvascular, macrovascular, neurologic and infectious complications. The International Diabetes Federation (IDF) released latest data showing that a staggering 285 million people worldwide suffer from diabetes, or 7% of the population. IDF predicts that, if the current rate of growth continues unchecked, the total number will exceed 435 million in 2030 (International Diabetes Federation, 2009). The increasing worldwide incidence of diabetes mellitus constitutes a global public health burden (Wild et al., 2004).

Treatment of diabetes mellitus involves diet control, exercise and the use of insulin and/or oral hypoglycaemic drugs. However, they usually have decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness (Grover et al., 2002). Because of perceived effectiveness, minimal side effects in clinical experience and relatively low cost, herbal drugs are recognized as a wonderful source for medicines (Bailey and Day, 1989). World Health Organization (WHO) has emphasized strongly on the rational use of traditional

Abbreviations: TFST, total flavonoids of *Selaginella tamariscina* (Beauv.) Spring; IDF, International Diabetes Federation; WHO, World Health Organization; STZ, streptozotocin; HFD, high fat diet; FBG, fasting blood glucose; OGTT, oral glucose tolerance; HbA1c, glycosylated hemoglobulin A1C; TC, total cholesterol; TG, triglyceride; FFA, free fatty acids; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SOD, superoxide dismutase; MDA, malondialdehyde; SDS PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; PPAR-γ, peroxisome proliferators activated receptor γ; IRS-1, insulin receptor substrate 1.

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and natural indigenous medicines, for treating diabetes mellitus (World Health Organization, 1994).

Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for their alleged hypoglycemic activity (Jouad et al., 2001; Grover et al., 2002). A large number of these plants/plant products has been evaluated and confirmed in laboratories. Literature has shown specific chemical constituents of these plants, such as flavonoids to be the active hypoglycemic and hypolipidemic principle in many medicinal plants with blood glucose and lipids-lowering attributes (Oladele et al., 1995).

Selaginella tamariscina (Beauv) Spring belongs to the family *Selaginellaceae*. Since it was first recorded by "Shen Nong Ben Cao Jing" (a classical traditional Chinese medicine book) around 1700 years ago, *Selaginella tamariscina* has been used in oriental medicine to trea tamenorrhea, dysmenorrheal, metrorrhagia, hematuria, prolapse of the anus, chronic hepatitis and hyperglycaemia. Moreover, *Selaginella tamariscina* has been reported to lower blood glucose levels and to facilitate the repair of pancreatic islet B cells injured by alloxan (Miao et al., 1996). The chemical constituents of *Selaginella tamariscina* were studied comprehensively and systematically in our previous research. A number of flavonoids, lignanoids, nucleosides and polyphenols chemical compositions were isolated from *Selaginella tamariscina* (Bi et al., 2004; Wang et al., 2007; Zheng et al., 2008). Data from preliminary research conducted in our laboratory have shown that the *Selaginella tamariscina* has hypoglycemic and hypolipidemic effects but no harmful side effects were observed (Zheng et al., 2011). In addition, previous study determined that the hypoglycemic and hypolipidemic property of *Selaginella tamariscina* appeared to be related to the flavonoid content. Therefore, the aim of the present study is to evaluate the anti-diabetic activity of total flavonoids of *Selaginella tamariscina* (TFST) and to investigate its possible mechanisms of action in diabetic rats induced by high fat diet and low dose STZ.

2. Materials and methods

2.1. Chemicals and reagents

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Rosiglitazone was purchased from Taiji Group, Chongqing Fuling Pharmaceutical Factory (Chongqing, China). The kit for blood glucose was purchased from Biosino Bio-technology and Science Inc. (Beijing, China). The kit for glycosylated hemoglobulin A1C (HbA1c) was purchased from Whitman Biotech Co. (Jiangsu, China). The kits for total cholesterol (TC) and triglyceride (TG) were purchased from Zhejiang Audit Biotechnology Corp. (Zhejiang, China). The kit for high density lipoprotein-cholesterol (HDL-C) and low density lipoprotein-cholesterol (LDL-C) were purchased from Yantai Ausbio Biology Engineering Corp. (Shanghai China). The kits for free fatty acid (FFA), malondialdehyde (MDA) and superoxide dismutase (SOD) were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). The kits for Insulin, C-peptide and glucagon were purchased from Beijing North Institute of Biological Technology (Beijing, China). The total protein extraction kit and protease inhibitor were purchased from Applygen Technologies Ins. (Beijing, China). IRS-1 rabbit polyclonal antibody was purchased from Cell Signaling Technology (Beverly, MA, USA). PPAR-γ, β-actin and β-tubulin rabbit polyclonal antibodies were purchased from Abcam (Cambridge, UK). Anti-rabbit IgG HRP-conjugated antibody, eECL western kit and improved lowry protein assay kit were from Cowin Biotech Co. (Beijing, China). Protein ladder was purchased from Fermentas Life Sciences (Burlington, ON, Canada). Polyvinylidene fluoride (PVDF) membranes were from Millipore Corporation (Bedford, MA, USA). Organic solvents and other chemicals were of the highest analytical grade.

2.2. Preparation of TFST

Selaginella tamariscina was purchased from Henan Shunkang Pharmaceutical Co. LTD (Henan, China) in May, 2009, and was authenticated by Prof. Chengming Dong and Prof. Suiqing Chen in Department of Medicinal Plant, School of Pharmacy, Henan University of Traditional Chinese Medicine, Henan, China. The voucher specimen (no. XX20090518001) was deposited in our laboratory.

Air-dried whole *Selaginella tamariscina* was refluxed with 70% ethanol twice (1:10, w/v) for 2 h each time. After filtration, the solution was combined and condensed to obtain a syrupy (yield 9.77%, w/w). The syrupy was suspended in water and adsorbed topolyamide column, and then eluted with distilled water, 50%, 80%, 95% ethanol successively. The 80% fraction was concentrated under reduced pressure at 40 °C using the vacuum evaporator, and vacuum dried at room temperature, to obtain the total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST, yield 12.81%, w/w).

TFST appeared as yellow spots on TLC plate (mobile phase, EtOAC:EtOH:H_2O = 40:2:1). The flavonoids content in TFST was determined to be 59.05% by spectrophotometric method, using amentoflavone as the reference compound. Based on separation and structure elucidation, TFST mainly consisted of eight flavonoids, identified as amentoflavone, 2,3-dihydroamentoflavone, hinokiflavone, neocryptomerin, podocarpusflavone, quercetin, apigenin, luteolin. All doses given are the gram-weight of the administered TFST powder in double distilled water.

2.3. Experimental model and drug treatment

Male adult Wistar rats, initially weighing 180–220 g, were obtained from Laboratory Animal Center of Zhengzhou University [Certificate no. SCXK(YU) 2005-0001]. Animals were maintained under standard laboratory conditions (temperature: 25 ± 2 °C, humidity: 60 ± 5%, 12 h dark/light cycle), and fed a standard laboratory diet and water.

After a 1-week acclimation period, rats were randomly divided into two groups. The normal control group (10 rats) was fed a basic diet, whereas the experimental group was fed a high fat diet (consisting of 18% fat, 20% carbohydrate, 3% egg and 59% basic diet (w/w), made by the Animal Experimental Center of Zhengzhou University) for a period of 4 weeks. After 4 weeks of dietary manipulation, the experimental rats were fasted overnight and were intraperitoneally injected with a freshly prepared solution of STZ (35 mg/kg) in 0.1 M citrate buffer (pH 4.21) to induce type 2 diabetic model, while the normal control rats were given the 0.1 M citrate buffer in a dose volume of 1 ml/kg respectively. The rats with fasting plasma glucose level of above 11.1 mmol/l 72 h post STZ injection were considered diabetic and only uniformly diabetic rats were induced in the study.

The rats were divided into five groups: Group NC – normal control rats; Group DC – diabetic control rats; Group RG – diabetic rats treated with rosiglitazone (2 mg/kg, ig.); Group TFSTI – diabetic rats treated with TFST (100 mg/kg, ig.); Group TFSTII – diabetic rats treated with TFST (200 mg/kg, ig.). Group TFSTIII – diabetic rats treated with TFST (400 mg/kg, ig.). The doses of TFST (100, 200 and 400 mg/kg/day, ig.) were equivalent to 0.5, 1 and 2 times of the crude drug amount recommended in traditional medicine when calculated according to the yields. The rats were treated for 8 weeks. Blood samples were collected 2 h after administration from the rats