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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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
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 (Jouda 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 treat tamaenorrhea, 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 hemoglobin A1C (HbA1C) was purchased from Whittman 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) was 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. Suqing 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 syrup (yield 9.77%, w/w). The syrup was suspended in water and adsorbed topolyamine 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:H2O=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
fasted for 12 h previously every two weeks, and serum glucose levels were estimated. OGTT was performed the day before rats were sacrificed. At the end of the experiment, blood samples were collected from the eyes (venous pool) and centrifuged at 2900 \(\times g\) for 10 min to separate the plasma from the whole blood and stored at \(-80^\circ\text{C}\) until assayed. The adipose in abdomen, hepaties and soleus muscles were harvested, frozen in liquid N\(_2\), and subsequently stored at \(-80^\circ\text{C}\) until required. All experimental animals were observed and approved by the Institutional Animal Care and Use Committee of Henan University of Traditional Chinese Medicine before and during experiments.

### 2.4. Oral glucose tolerance test (OGTT)

The day before sacrificed, rats underwent an oral glucose tolerance test after an overnight fast. Different doses of TFST were administered 60 min prior to oral glucose load (2.0 g/kg). The blood samples were collected from each group just before glucose administration (0 min) and at 30, 60 and 120 min after glucose administration. Plasma glucose concentrations were determined by glucose oxidase method.

### 2.5. Biochemical assays

Glucose levels were estimated by commercially available glucose kits based on glucose oxidase method. HbA1c, FFA, TC, TG, HDL-C and LDL-C were measured using commercial assay kits according to the manufacturer’s directions. Insulin, C-peptide and glucagons were measured by radioimmunoassay using commercial kits. The contents of MDA and the activity of SOD were determined by commercially available kits according to the manufacturer’s directions.

### 2.6. Western blotting

Protein yields from tissues following the commercial kit’s instructions. Protein concentration was determined using the improved lowry protein assay kit. Equal amounts of protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and transferred to the PVDF membrane. The membrane was blocked in 5% (v/v) non-fat milk in TBST (20 mM Tris–HCl, 137 mM NaCl, 0.1% (v/v) Tween-20) for 2 h at room temperature, and then blotted with a primary antibody (PPAR-\(\gamma\) at 1:1000, IRS-1 at 1:8000, \(\beta\)-actin at 1:4000, \(\beta\)-tubulin at 1:10,000) overnight at 4°C. After being washed (6 \(\times\) 5 min) in TBST buffer, the membrane was detected with the second antibody (1:2000) for 1 h at room temperature, followed by additional washes (6 \(\times\) 5 min in TBST). Bound antibody was visualized by enhanced chemiluminescence (eCL). The intensity of target proteins and reference protein were quantified using Image J software and the relative gray levels of target proteins over reference proteins were used to represent the signal strength for each protein.

### 2.7. Statistical analyses

Results are presented as mean ± S.D., and the comparison between groups was performed by one-way ANOVA. \(P < 0.05\) was considered statistically significant.

### 3. Results

#### 3.1. Effects of TFST on blood glucose levels in diabetic rats

The effects of TFST on the fasting blood glucose levels of diabetic rats are summarized in Table 1. After 4 weeks of high fat diet, intraperitoneal injection of STZ (35 mg/kg) led to an over-four fold elevation of the blood glucose level \((P < 0.01)\). Treatment with TFSTI caused significant reduction \((P < 0.01, P < 0.05)\) in blood glucose levels by 18.03, 16.69, 28.08 and 25.15% in the 2nd, 4th, 6th and 8th week, respectively. Treatment with TFSTII and TFSTIII caused a lesser anti-hyperglycemic effect. The percentage fall in the two groups was 10.23, 7.56, 7.62, 12.95% and 15.02, 6.37, 14.17, 9.90%, in the 2nd, 4th, 6th and 8th week, although these did not reach statistical significance \((P > 0.05)\).

#### 3.2. Effects of TFST on glucose tolerance in diabetic rats

Results of the glucose tolerance test conducted on diabetic rats fed with TFST are shown in Table 2. In diabetic control rats, blood glucose reached the highest level at 60 min after oral glucose ingestion, and this hyperglycemia was maintained until 120 min. TFSTI significantly prevented \((P < 0.01)\) the increase in blood glucose levels at 60 min and 120 min. The percentage reduction was 14.38 and 18.84%, respectively. TFSTII significantly decreased \((P < 0.05)\) the blood glucose levels by 9.38% at 60 min.

### Table 1

Effects of TFST on glucose levels in diabetic rats \((n = 10) (\bar{x} \pm S)\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose levels (mmol/l)</th>
<th>4 weeks</th>
<th>6 weeks</th>
<th>8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>3.88 ± 0.81</td>
<td>4.50 ± 1.79</td>
<td>4.64 ± 0.31</td>
<td>2.18 ± 0.22</td>
</tr>
<tr>
<td>DC</td>
<td>14.36 ± 2.33*</td>
<td>15.22 ± 3.15*</td>
<td>15.74 ± 2.45*</td>
<td>14.75 ± 3.76*</td>
</tr>
<tr>
<td>RG</td>
<td>14.37 ± 2.53b</td>
<td>13.04 ± 3.84b,c</td>
<td>12.57 ± 1.84b,c</td>
<td>14.02 ± 4.01b</td>
</tr>
<tr>
<td>TFSTI</td>
<td>14.21 ± 2.26b,c</td>
<td>12.68 ± 3.47b,c</td>
<td>11.32 ± 2.48b,d</td>
<td>11.04 ± 3.39b,c</td>
</tr>
<tr>
<td>TFSTII</td>
<td>14.19 ± 2.71b</td>
<td>14.07 ± 1.17b,c</td>
<td>14.54 ± 4.61b</td>
<td>12.84 ± 3.18b</td>
</tr>
<tr>
<td>TFSTIII</td>
<td>14.23 ± 3.36b,c</td>
<td>12.96 ± 2.37b,c</td>
<td>12.45 ± 1.42b</td>
<td>13.51 ± 2.08b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.29 ± 4.58b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* \(P < 0.05\) compared with normal control group at each time point.
* \(P < 0.01\) compared with normal control group at each time point.
* \(P < 0.05\) compared with diabetic control group at each time point.
* \(P < 0.01\) compared with diabetic control group at each time point.

### Table 2

Effects of TFST on glucose tolerance in diabetic rats \((n = 10) (\bar{x} \pm S)\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose levels (mmol/l)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>2.18 ± 0.22</td>
<td>4.72 ± 0.43</td>
<td>5.44 ± 0.46</td>
<td>4.62 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>14.75 ± 3.76b</td>
<td>24.12 ± 2.73b</td>
<td>26.43 ± 2.44b</td>
<td>23.14 ± 1.56b</td>
<td></td>
</tr>
<tr>
<td>RG</td>
<td>14.02 ± 4.01b</td>
<td>21.37 ± 1.44b,c</td>
<td>24.27 ± 0.96b,c</td>
<td>20.06 ± 0.93b</td>
<td></td>
</tr>
<tr>
<td>TFSTI</td>
<td>11.04 ± 3.39b,c</td>
<td>22.80 ± 3.54b</td>
<td>22.63 ± 2.53b,d</td>
<td>18.78 ± 2.60b,d</td>
<td></td>
</tr>
<tr>
<td>TFSTII</td>
<td>12.84 ± 3.18b</td>
<td>23.15 ± 1.43b,c</td>
<td>23.95 ± 2.94b,c</td>
<td>20.81 ± 3.65b,c</td>
<td></td>
</tr>
<tr>
<td>TFSTIII</td>
<td>13.29 ± 4.58b,c</td>
<td>24.36 ± 1.83b</td>
<td>25.90 ± 1.08b</td>
<td>23.23 ± 1.11b</td>
<td></td>
</tr>
</tbody>
</table>

* \(P < 0.05\) compared with normal control group at each time point.
* \(P < 0.01\) compared with normal control group at each time point.
* \(P < 0.05\) compared with diabetic control group at each time point.
* \(P < 0.01\) compared with diabetic control group at each time point.
Table 3
Effects of TFST on HbA1c, C-peptide, insulin and glucagon levels in diabetic rats (n = 10) (± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>HbA1c (%)</th>
<th>C-peptide (nmol/ml)</th>
<th>Insulin (µU/ml)</th>
<th>Glucagon (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>6.96 ± 1.62</td>
<td>0.37 ± 0.04</td>
<td>21.21 ± 5.99</td>
<td>460.16 ± 45.17</td>
</tr>
<tr>
<td>DC</td>
<td>14.05 ± 0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33 ± 0.04</td>
<td>22.02 ± 4.47</td>
<td>512.64 ± 72.88</td>
</tr>
<tr>
<td>RG</td>
<td>11.62 ± 0.92&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.35 ± 0.04</td>
<td>25.78 ± 6.23</td>
<td>493.01 ± 103.16</td>
</tr>
<tr>
<td>TFSTI</td>
<td>11.68 ± 0.86&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.36 ± 0.04</td>
<td>23.40 ± 5.62</td>
<td>381.52 ± 100.27&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTII</td>
<td>11.80 ± 0.74&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.40 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.81 ± 5.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>382.67 ± 76.19&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTIII</td>
<td>11.69 ± 0.94&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>0.41 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29.80 ± 4.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>355.15 ± 36.93&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 compared with normal control group.
<sup>b</sup> P < 0.01 compared with normal control group.
<sup>c</sup> P < 0.05 compared with diabetic control group.
<sup>d</sup> P < 0.01 compared with diabetic control group.

Table 4
Effect of TFST on lipids and lipoprotein in diabetic rats (n = 10) (± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>FFA (µmol/l)</th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>HDL-C (mmol/l)</th>
<th>LDL-C (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>629.30 ± 178.31</td>
<td>1.82 ± 0.32</td>
<td>1.38 ± 0.23</td>
<td>0.77 ± 0.10</td>
<td>0.83 ± 0.08</td>
</tr>
<tr>
<td>DC</td>
<td>1047.83 ± 176.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.36 ± 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.77 ± 0.85&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.52 ± 0.12&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.28 ± 0.62&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>RG</td>
<td>546.59 ± 111.15&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.79 ± 0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.93 ± 0.77&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.66 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.69 ± 0.19&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTI</td>
<td>464.45 ± 164.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.86 ± 0.46</td>
<td>2.33 ± 0.63&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.69 ± 0.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.78 ± 0.24&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTII</td>
<td>403.89 ± 85.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.66 ± 0.38&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.28 ± 0.65&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.68 ± 0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.63 ± 0.16&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTIII</td>
<td>613.35 ± 150.38&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.78 ± 0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.48 ± 0.72&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.55 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.64 ± 0.26&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 compared with normal control group.
<sup>b</sup> P < 0.01 compared with normal control group.
<sup>c</sup> P < 0.05 compared with diabetic control group.
<sup>d</sup> P < 0.01 compared with diabetic control group.

3.3. Effects of TFST on HbA1c, C-peptide, insulin and glucagon levels in diabetic rats

Table 3 shows the levels of HbA1c, C-peptide, insulin and glucagon in serum in each group. The HbA1c level of the diabetic control rats was significantly higher than the normal control rats (P < 0.01). After 8 weeks of administration, the TFST significantly lowered the HbA1c values (P < 0.01). The percentage fall was 16.87, 16.01 and 16.80% in TFSTI, TFSTII and TFSTIII, respectively. The C-peptide level appeared to be a slight downward trend while the insulin and glucagon levels appeared to be a slight upward trend in diabetic control rats, but none of them show significant changes when compared with the diabetic control rats (P > 0.05). When treated for 8 weeks, TFSTI, TFSTII and TFSTIII significantly increased (P < 0.01, P < 0.05) the levels of C-peptide and Insulin, and simultaneously decreased (P < 0.01) the glucagon level.

3.4. Effects of TFST on lipids and lipoprotein in diabetic rats

As shown in Table 4, the increased serum levels of TG, TC, FFA and LDL-C were significantly suppressed (P < 0.01, P < 0.05), whereas the decreased serum HDL-C level was obviously elevated (P < 0.01, P < 0.05) by TFST treatment in diabetic rats. In the rats treated with TFSTI, TFSTII and TFSTIII, the FFA level were reduced by 55.68, 61.45 and 41.46%, the TC level were reduced by 21.19, 29.66 and 24.58%, the TG level were reduced by 38.20, 39.52 and 34.22%, the LDL-C level were reduced by 39.06, 50.78 and 50.00%, the HDL-C level were increased by 32.69, 30.77 and 5.77%, when compared with the diabetic control rats. After 8 weeks administration, TFST tended to bring the lipids and lipoprotein values closer to normal range.

3.5. Effects of TFST on MDA level and SOD activity in diabetic rats

Table 5 indicates that the MDA level has significantly increased (P < 0.05) whereas the SOD activity has significantly decreased (P < 0.05) in diabetic rats compared with normal control rats. TFSTI, TFSTII and TFSTIII significantly increased (P < 0.01, P < 0.05) the SOD activity in serum in comparison to diabetic control rats. TFSTIII significantly decreased (P < 0.05) the MDA level.

3.6. Effects of TFST on protein expression of PPAR-γ in adipose tissue of diabetic rats

The PPAR-γ protein levels in adipose tissue examined using western blotting analysis are shown in Fig. 1. The protein levels of PPAR-γ were slightly lower by 12.41% in diabetic control rats than that in the normal control rats, but no significant difference was observed between them (P > 0.05). TFSTII significantly increased PPAR-γ protein expression by 56.28% above diabetic control rats and 35.56% above normal control rats (P < 0.01). TFSTI and TFSTII increased PPAR-γ protein expression by 23.99% and 30.38% above that of diabetic control rats, however, none of them show significant differences compared with the diabetic control rats or normal control rats (P > 0.05).

3.7. Effects of TFST on protein expressions of IRS-1 in hepatic tissue of diabetic rats

Fig. 2 shows the changes of the IRS-1 expression in hepatic tissue examined using a western blotting analysis. The basal level of IRS-1 in diabetic control rats was depressed to 65.82% of that in normal control rats, but no significant difference was observed between them (P > 0.05). TFSTII resulted in only 46.67% increase against the diabetic controls (P > 0.05). TFSTII and TFSTIII significantly increased IRS-1 protein expression by 98.33% and 85.00%

Table 5
Effect of TFST on MDA level and SOD activity in diabetic rats (n = 10) (± s).

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/ml)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>206.09 ± 7.59</td>
<td>9.91 ± 1.90</td>
</tr>
<tr>
<td>DC</td>
<td>189.98 ± 19.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.86 ± 1.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>RG</td>
<td>192.84 ± 9.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.16 ± 3.30&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTI</td>
<td>205.76 ± 2.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.28 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TFSTII</td>
<td>214.41 ± 5.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.41 ± 0.93</td>
</tr>
<tr>
<td>TFSTIII</td>
<td>208.60 ± 10.48&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.62 ± 1.42&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 compared with normal control group.
<sup>b</sup> P < 0.01 compared with normal control group.
<sup>c</sup> P < 0.05 compared with diabetic control group.
<sup>d</sup> P < 0.01 compared with diabetic control group.
3.8. Effects of TFST on protein expressions of IRS-1 in skeletal muscle tissue of diabetic rats

Fig. 3 shows the changes of the IRS-1 expression in skeletal muscle tissue examined using a western blotting analysis. In the diabetic control rats, IRS-1 protein expression of skeletal muscle decreased to 68.64% of that in normal control rats, but no significant difference was observed between them (P > 0.05). At the termination of 8-week treatment with TFSTI, TFSTII and TFSTIII, the IRS-1 levels in skeletal muscle were significantly elevated by about 108.62%, 191.37% and 117.24% as compared to diabetic control rats, respectively (P < 0.01, P < 0.05).

4. Discussion

Type 2 diabetes is a complex, heterogeneous, and polygenic disease. There are many underlying factors that contribute to the high blood glucose levels in these type 2 diabetes patients. An important factor is the body’s resistance to insulin, and the second factor is the failing production of insulin by the β cells of the pancreas. Currently, studies have reported that the HFD feeding rats develop insulin resistance (Schrauwen, 2007). At the same time, streptozotocin has been known to selectively target and destroy the pancreatic β-cell by necrosis (Mythili et al., 2004). Therefore, the rat model by high-fat diet following low-dose STZ, which closely mimic the natural history and the metabolic characteristics of the type 2 diabetes in humans, was selected in the present study to evaluate the effect of TFST on the treatment of type 2 diabetes.

Normally, diabetes is detected by measuring glucose blood levels. However, due to wide variations in the circulating glucose concentrations, a randomized glucose measurement does not give clear data for overall glycemic control. A better approach to assess the level of control is the measurement of the HbA1c value, which is a commonly used laboratory test for assessing long-term diabetic control (Andrade-Cetto et al., 2008). In addition, diabetic rats have impaired glucose tolerance. Additional load of glucose is found to impair the tolerance further. Therefore, our research investigated both short-term (i.e., FBG and OGTT) and long-term (i.e., HbA1c) metabolic parameters during TFST treatment. From the results obtained, it was obvious that fasting blood glucose concentrations and HbA1c values were dramatically elevated together with corresponding deteriorating oral glucose tolerance in diabetic rats. TFST at dose of 100 mg/kg produced a statistically significant decrease in FBG beginning from the 2nd week and progressing till the end of 8th week in comparison to the diabetic control group. All the three doses of TFST reduced the HbA1c values significantly, which indicated TFST were more beneficial to the long term glucose blood control. From the data obtained in the OGTT, it was clear that TFST at dose of 100 mg/kg blocked an increase in blood glucose levels after 60 min and 120 min of glucose administration, while TFST at dose of 200 mg/kg prevented an increase in blood glucose levels after 60 min of glucose administration, compared with the diabetic controls. The test suggested that TBST acts by increasing peripheral utilization of glucose.

Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile. In uncontrolled type 2 diabetes mellitus, there will be an increase in TC, LDL-C and VLDL-C and TC with decrease in HDL-C which contributes to the coronary artery disease (Arvind et al., 2002). Raised plasma FFA level plays a major role in the pathogenesis of insulin resistance and type 2 diabetes (Kovacs and Stumvoll, 2005). In addition, hypertriglyceridermia is also an important maker of insulin resistance (Schwartz, 2006). In the present study, a rise in blood glucose was accompanied with the lipid metabolism disorder. A marked increase in serum lipids and lipoprotein in diabetic rats was observed in diabetic rats. A regular administration of the TFST for 8 weeks lowered the TC, TG, FFA, and LDL-C values while elevate the HDL-C value. The alterations in lipid profiles during experimental diabetes were restored to near normal levels. Results revealed that TFST was more effective in showing hypolipidemic activities than hypoglycemic activities.
and it is encouraging that the 8-weeks treatment of TFST attenuated the disorder of lipid metabolism.

Insulin is the leading hormone that regulates blood glucose homeostasis by stimulating the utilization of glucose by liver, muscle and adipose tissue, coupled with stimulation of anabolic processes such as glucose, protein and lipid synthesis. Glucagon has opposing effects, causing release of glucose from glycogen, release of fatty acids from stored triglycerides and stimulation of gluconeogenesis. The balance between insulin and glucagon are responsible for regulates blood glucose and fat metabolism, promoting a stable inner metabolic milieu (or homeostasis). Insulin is initially synthesized in the form of pro-insulin. In this form the alpha and beta chains of active insulin are linked by a third polypeptide chain called the connecting peptide, or C-peptide, which are assumed to primarily serve as a facilitator for the formation of active insulin by helping insulin to fold and be cleaved as necessary (Steiner et al., 1967). C-peptide appears to be inactive but of interest because it has a much longer half-life than insulin and is released simultaneously with insulin at the molar ratio of 1:1. One can estimate the rate of insulin secretion by measuring the level of C-peptide. So the three parameters were detected in the research for evaluating the metabolic control on diabetics. In the diabetic group, the C-peptide level was weekly reduced, while the levels of insulin and glucagon were weekly increased, but none of them showed any statistical significance. However, after the administration of TFST, we observed a significant increase in insulin and C-peptide, and a dramatic decrease in glucagon. It is suggested that regulation of insulin and C-peptide secretion might be one of the possible factors responsible for the anti-diabetic activity of TBST in diabetic rats.

Evidence suggests that complex alterations in the activities of antioxidant enzymes as well as the oxidative stress marker MDA were observed in diabetic rats (Scott and King, 2004). The imbalance between oxidative stress and anti-oxidative defense mechanisms in diabetics may result in cell and tissue damage and play an important role in the pathogenesis of complications associated with the chronic diabetic state. Some anti-diabetic drugs may have antioxidant properties independently of their main role on glycaemia control, which can provides protection against free radical induced damage in diabetes mellitus and its complications. In the anti-oxidative defense system, SOD is the first enzyme of the scavenger enzyme series to protect tissues against oxygen free radicals by catalyzing the removal of superoxide radicals, which damages the membrane and biological structures (Arivazhagan et al., 2000). MDA reflected the degree of lipid peroxidation and the increased malonaldehyde production played an important role in the progression of diabetic pancreas damage (Ilhan et al., 2001). Therefore, we determined the effects of TBST on the SOD and MDA levels in diabetic rats. In the present study, an increased level of MDA and a decrease activity of SOD were noticed in diabetic rats. Results showed that administration of TBST for 8 weeks nearly normalized the SOD and MDA levels. Flavonoid antioxidant, which is among the most powerful antioxidants existing in nature, has been reported to effectively ameliorate diabetes and its complications (Okezie et al., 2007). Our results corroborate these observations. It is indicated that the anti-diabetic effect of TFST could be related to the anti-oxidative stress effect by flavonoids.

The peroxisome proliferator activated receptors (PPARs) are members of a nuclear receptor superfamily, which are ligand modulated transcription factors that regulate gene expression of many important biological processes (Vergees, 2004). Three PPAR subtypes have been identified as PPAR-α, PPAR-γ, and PPAR-δ. PPAR-γ is mainly present in adipose tissue. Studies have shown that activation of PPAR-γ can increase the number of small adipocytes, which are more sensitive to insulin, and decrease the number of large adipocytes in white adipose tissue (Okuno et al., 1998). It is also reported that PPAR-γ is the central regulator of insulin and glucose metabolism leading to improved insulin sensitivity in type 2 diabetic patients as well as in diabetic rodent models (Hallakou et al., 1997). Thus, PPAR-γ in white adipose tissue may play an essential role in insulin sensitivity. Several flavonoids act as a ligand of PPAR-γ and activate its expression (Liang et al., 2001). Here, it was shown that TFST regulated the PPAR-γ expression in the adipose tissue, which may exert potent insulin sensitizing effect and improved insulin resistance in a rat model of type 2 diabetes mellitus.

Insulin resistance is a condition in which circulating insulin decreases in responsiveness to target tissues, due to defects in insulin signaling cascade (Schinner et al., 2005). Although insulin signaling is transducted intracellularly via an extensive signaling network with multiple alternative pathways, insulin receptor substrate-1 (IRS-1) is believed to be the first basic cytosolic mediator (Cheatham and Kahn, 1995). IRS-1 appears to play a central role in the insulin-stimulated signal transduction pathway and links the insulin receptor to its final biological actions via a series of intermediate effectors. Studies have shown that differential regulation of IRS-1 in liver and muscle were observed in diabetic animals, which may produce differential alterations in insulin signaling in these two tissues and contribute to the hepatic and muscular insulin resistance (Saad et al., 1992). We focused on the expression of IRS-1 in the hepatic and skeletal muscle tissue. Data showed that decreased IRS-1 expression in skeletal muscle and liver were observed in diabetic rats. From the results obtained, it was obvious that 8 weeks of TFST treatment reversed the defect in expression of IRS-1 of diabetic rats, which was a desirable feature. These results suggested that the favorable impact of TFST on hyperlipidemia was probably associated with the improvement of insulin signal transduction in target tissues, which plays an important role in regulating glucose metabolism.

In conclusion, this research revealed that total flavonoids of Selaginella tamariscina (Beauv.) Spring, which is the main component extracted from Selaginella tamariscina, had the potential to attenuate the glucose metabolism disorder and nearly normalized the lipid metabolism. These changes may be related to the elevation of the levels of PPAR-γ in adipose tissue and IRS-1 in skeletal muscle and hepatic tissue, increasing of insulin sensitivity and improvement of insulin signal transduction in target tissues, as well as regulation of the insulin and glucagon secretion by TFST treatment. These benefits were associated with attenuation of oxidative stress. Our results suggest that TFST has the potential to be a suitable candidate for further investigations as an anti-diabetic agent in humans. Further pharmacological and biochemical investigations are in progress confirm our results and to elucidate the detailed mechanisms. Furthermore, the anti-diabetic activities of the TFST may be attributed to one or more of the identified or as-yet-unknown compounds. Studies are in progress to isolate and identify the active principle(s) of TFST, which may be valuable in the treatment of dyslipidemia and atherosclerosis in diabetic patients.

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References


