Antidiabetic Effect of Flavones from *Cirsium japonicum DC* in Diabetic Rats

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*Cirsium japonicum DC* is a traditional Chinese herb used along with other herbs to treat hypertension, traumatic hemorrhage, inflammation, and renal cellular injury. Here, we isolated two flavones from *Cirsium japonicum DC*, pectolinarin and 5,7-dihydroxy-6,4'-dimethoxy flavone (DDMF), and investigated their antidiabetic effect in diabetic rats established by intravenous injection with streptozotocin followed by feeding with high-carbohydrate/high-fat diet. Both pectolinarin and DDMF showed antidiabetic effect in diabetic rats. However, FECJ, a mixture of pectolinarin and DDMF, is more effective than pectolinarin and DDMF in improving the plasma glucose, cholesterol and triglycerides levels in diabetic rats. The altered activities of glucose metabolism-related enzymes in diabetic rats were well reversed after flavone treatment. The plasma adiponectin level was greatly increased in diabetic rats treated with FECJ, while no obvious effect of the flavones on the dysregulated plasma insulin level and expressions of leptin and glucose transporter 4 (GLUT4) was observed. Our data indicated that the flavones improved adiponectin expression, accompanied by restoring of the dysregulated activities of the glucose metabolism-related enzymes, ultimately resulting in well improved glucose and lipid homeostasis. Thus, an antidiabetic effect of *Cirsium japonicum DC* was revealed in diabetic rats, suggesting the potential benefit of the *Cirsium japonicum DC* as an alternative in treating diabetes mellitus.

**Key words:** *Cirsium japonicum DC*, Flavone, Antidiabetic, Diabetes

INTRODUCTION

Diabetes mellitus is considered to be a serious endocrine syndrome, affecting more than 200 million people worldwide (Zimmet et al., 2001). Diabetes is associated with severe complications, such as diabetic nephropathy, neuropathy, and retinopathy (Gabir et al., 2000). A complex network of signaling molecules is involved in normal insulin action that leads to increased glucose transport, glycogen synthesis, lipogenesis and decreased gluconeogenesis, glycolysis, and lipolysis (Newsholme and Dimitriadis, 2001). The end result of glucose metabolism regulation by insulin is that hepatic glucose production is reduced, and use of peripheral glucose is increased (Saltiel, 2001). It is now widely believed that insulin acts on glucose and lipid homeostasis mainly through a pathway that involves activation of the insulin receptor, insulin receptor substrates, phosphatidylinositol 3-kinase, protein kinase B (PKB) (Avruch, 1998) and increased GLUT4 activity. PKB activation increases glycogen synthesis, in some way, through inhibition of the PKB substrate glycogen synthase kinase (GSK)-3 (Cross et al., 1995). Inactivation of GSK-3 leads to the activation of glycogen synthase, thus increasing glycogen production in the liver (Lochhead et al., 2001). Activation of both PKB and PI 3-kinase leads to decreased transcription of gluconeogenic enzymes such as glucose-6-phosphatase, thus reducing glucose production in the liver (Cross et al., 1995).

Adipose tissue is an energy-storing organ where bioactive substances are produced and secreted, such as adiponectin and leptin. Leptin plays a key role in
the regulation of appetite and body weight (Zhang et al., 1994). Serum leptin concentrations correlate well with body weight and mutations in the leptin gene cause obesity in rodents and human (Zhang and Scarpace, 2006). Adiponectin which is secreted exclusively by white adipose tissue and is abundant in plasma has been implicated in pharmacological mechanisms for treating diabetes (Lihn et al., 2005), as mice with disrupted adiponectin gene expression are more susceptible to diabetes (Nawrocki et al., 2006). Hypoadiponectinemia has been demonstrated to be independently associated with metabolic syndrome, including type 2 diabetes (Hotta et al., 2000). The plasma adiponectin level is inversely correlated with body weight in spite of its restricted expression in adipose tissue (Arita et al., 1999). Adiponectin down-regulates SREBP-1c which masterly regulates fatty acid synthesis, increasing β-oxidation of free fatty acids and decreasing de novo free fatty acids production within hepatocytes, ultimately preventing triglyceride accumulation (Fruebis et al., 2001).

As the number of people worldwide that suffer from diabetes and severe complications is increasing, extensive attention has been raised and much progress witnessed. However, most drugs currently used for treating diabetes, including insulin and other oral hypoglycemic agents, are associated with side effects. Therefore, identification of natural plants as an alternative in treating diabetes is alluring and the antidiabetic properties of some natural plant materials have been established (Raju et al., 2001; Park et al., 2006; Adeneye et al., 2008). Cirsium japonicum DC is a wild annual herbal plant of the Compositae family, and grows in many areas of China as well as Korea and Japan. Its leaves, roots and stalks have long been used along with other traditional Chinese medicines to treat diseases such as hypertension, traumatic hemorrhage, inflammation, and renal cellular injury (Ishida et al., 1987). The water extracts of Cirsium japonicum DC were recently reported to show estrogenic effects and vasorelaxant activity (Park et al., 2008; Kim et al., 2008). The flavone isolated from Cirsium japonicum DC displayed anticancer activity in the S180 and H22 mice (Liu et al., 2007) and improved immunity in mice (Liu et al., 2006). Cirsium japonicum DC has been used to treat diabetes especially in rural regions of China. However, there is still no published report documenting its efficacy. Thus, to validate the use of this plant in diabetes treatment, we performed the present research to study the antidiabetic effect of the flavones from Cirsium japonicum DC in diabetic rats.

MATERIALS AND METHODS

Materials

Cirsium japonicum DC provided by the No.7 Hospital in Xinyu, China and further authenticated by Dr. Xuansheng Liao in this hospital. Male Sprague-Dawley rats used in all the experiments were from the Shanghai Experimental Animal Center, Chinese Academy of Sciences. All animals were housed in climate-controlled room with a 12 h light/12 h dark cycle and cared for according to the Principles of Laboratory Animal Care of the National Institute of Health, USA.

Isolation of flavones

Flavones were isolated from Cirsium japonicum DC as reported (Liu et al., 2006). Briefly, the dried mixture of the 95% ethanol extract from Cirsium japonicum DC with diatomite was put into the glass column and then washed with petroleum ether. After the petroleum ether was evaporated, the mixture was extracted with butanol. The extract and 1% alchlor ethanol solution were dripped on the filter paper and then put under the UV light to observe whether there was Kelly color. The extracted process was continued until there was no flavone reaction. After the butanol was evaporated, the extract was dissolved with 95% ethanol, and then put into polyamide resin column. The column was washed firstly with water and then with 95% ethanol until there was no flavone reaction. Finally, 95% ethanol extracted solution was collected, concentrated and dried in the natural condition and further separately by HPLC. Elutions corresponding to two major peaks were collected and identified to be pectolinarin and DDMF as reported (Liu et al., 2006). The pectolinarin elutions and the DDMF elutions were purified using Sephadex LH20 and silica column chromatography, or mixed first and then purified using the same method. The obtained FECJ in this way was of 62.8% pectolinarin and 36.5% DDMF as analyzed by HPLC (Fig. 1).

Establishment and flavone treatment of diabetic rats

Male Sprague-Dawley rats weighing about 200 g were fasted for 12 h and then intravenously injected with streptozotocin (30 mg/kg body weight) (Sigma) in cold citrate buffer after anesthesia with ethyl ether. The non-diabetic (normal) rats received an injection of the buffer only. All rats continued to be fed with standard diet for 2 weeks. The development of hyperglycemia in rats was confirmed by fasting blood glucose estimation using a drop of blood from the tail.

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Antidiabetic Effect of Flavones from *Cirsium japonicum* DC

vein with a portable glucometer (OneTouch SureStep Meter). The animals with fasting blood glucose level of above 230 mg/dL were considered diabetic and then fed with high-carbohydrate/high-fat diet (70% standard diet, 12% lard, 9% yolk powder, 9% plantation white sugar) to induce diabetic dyslipidemia. Control rats were still fed the standard diet. The diabetic rats were at random divided into groups. Diabetic rats were given pectolinarin, DDMF or FECJ for indicated doses (mg/kg body weight/day) by intragastric administration for three weeks. Control diabetic rats and normal rats were given the same volume of 0.9% saline solution.

**Biochemical assay**

The plasma glucose, cholesterol, and triglycerides levels were assayed using commercial kits (Nanjing Jiancheng Biomedical Engineering Co. Ltd.). Plasma insulin, leptin, and adiponectin were measured by rat insulin ELISA kit (Crystal Chem Inc.), rat leptin ELISA kit (Wako Chemicals USA, Inc.), and rat adiponectin ELISA kit (Kamiya Biomedical Company), respectively.

**Assay of enzyme activity**

The liver was dissected out, weighed, washed in saline, and quickly homogenized in cold isotonic sucrose buffer (0.25 M sucrose, 0.02 M triethanolamine, 0.12 mM dithiothreitol, pH 7.4) in a 55 mL Teflon Pesple homogenizer at 4°C. The homogenates were centrifuged at 3,000 × g for 5 min at 4°C and the pellet was discarded. The supernatant was further centrifuged at 100,000 × g for 45 min at 4°C. The final clear supernatant thus contained the cytosolic fraction and was used for the assay of enzyme activities. The activities of phosphofructokinase, pyruvate kinase, Glucose-6-phosphatase and fructose-1,6-bisphosphatase were estimated using previously described methods (Ling et al., 1975; Bucher and Pfleiderer, 1955; Baginsky et al., 1974; Tashima and Yoshimura, 1975). One unit of the enzymes was defined as the amount of Pi liberated per g fresh liver weight per min at 37°C. Assay for the NADP-linked lipogenic enzyme glucose-6-phosphate (G-6-P) dehydrogenase was performed essentially using the method reported previously (Baquer et al., 1975). One unit of glucose-6-phosphate dehydrogenase was defined as 1 µmol of NAD/NADH formed per g fresh tissue weight per min at 25°C.

**Immunoblot analysis**

The subcellular fraction containing GLUT4 was prepared by the method described previously (McKeel and Jarett, 1970). Briefly, the cells were freshly isolated from fat tissue and homogenized at 4°C in buffer containing 20 mM HEPES, 1 mM edetic acid, 255 mM sucrose, pH 7.4. The homogenate was centrifuged at 16,000 × g for 15 min at 4°C. The pellet was suspended in HEPES buffer and layered onto 1.12 M sucrose cushion, centrifuged at 100,000 × g for 60 min. The plasma membrane layer was removed from the sucrose cushion and centrifuged at 40,000 × g for 20 min. The obtained plasma membrane pellet was resuspended in HEPES buffer and protein concentration was determined using Pierce® BCA Protein Assay Kit (Pierce Biotechnology). Equal amount of protein sample was subjected to 10% SDS-PAGE under reducing conditions. GLUT4 was immunodetected using a polyclonal rabbit anti-GLUT4 antibody.

**RT-PCR analysis**

One µg total RNA isolated from liver or fat tissues of the experimental rats using RNAiso™ Plus (TaKaRa) was used to perform the RT reaction. Quantitative PCR using QuantiTect SYBR Green RT-PCR Kit (QIAGEN) was performed with one-tenth (10 µL) of the RT reaction to analyse the mRNA levels of adiponectin, phosphofructokinase, pyruvate kinase, Glucose-6-phosphatase, fructose-1,6-bisphosphatase and glucose-6-phosphate dehydrogenase. The PCR was carried out with an initial denaturation at 94°C for 10 min, followed by 35 cycles (94°C for 15 s, 62°C for 30 s, 72°C...
for 30 s). Fluorescent values were converted into threshold cycle (CT) values using ABI PRISM® 7000 Sequence Detector Program. Quantitative amplification of β-actin was used as the house-keeping gene control to normalize the determined mRNA levels. The primers were listed in Table I.

**Expression and statistical analysis of results**

Results are expressed as means ± S.E.M. The Statistical significance of difference between the data pairs was evaluated by ANOVA followed by Mann-Whitney U test.

**RESULTS**

**Antidiabetic effect of flavones in diabetic rats**

Two flavones, pectolinarin and DDMF, were isolated from *Cirsium japonicum DC* and identified by HPLC and MS as reported (Liu et al., 2006). The FECJ was analyzed by HPLC to be 62.8% pectolinarin and 36.5% DDMF. Diabetes was induced in the rats by an intravenous injection of streptozotocin (30 mg/kg body weight) in cold citrate buffer and confirmed by plasma glucose levels of above 230 mg/dL. Diabetic dyslipidemia was induced by feeding the diabetic rats with a high-carbohydrate/high-fat diet. Diabetic rats, divided into groups, were treated with or without different doses of pectolinarin, DDMF or FECJ. The plasma glucose, cholesterol and triglyceride levels were assayed in all experimental rats. Hyperglycemia was observed, particularly for FECJ. As shown in Table II, the increased plasma glucose level in diabetic rats was decreased in 24.5% and 19.6%, respectively, after treatment with pectolinarin or DDMF (50 mg/kg body weight/day). However, it was decreased in 44.7% to be 167.3 ± 13.5 mg/dL in the FECJ-treated diabetic rats (50 mg/kg body weight/day). The plasma cholesterol and triglyceride levels were 1.81 ± 0.20 mmol/L and 1.47 ± 0.10 mmol/L in the normal rats, respectively, and were increased to be 2.96 ± 0.32 mM and 2.31 ± 0.13 mM in the diabetic rats (Table II). The plasma cholesterol level was decreased to be 2.46 ± 0.10 mM in the pectolinarin-treated diabetic rats and 2.55 ± 0.14 mM in the DDMF-treated diabetic rats. However, it was decreased in 29.1% to be 2.11 ± 0.12 mM in the FECJ-treated diabetic rats, lower than those in the pectolinarin- or DDMF-treated diabetic rats. Similarly, the plasma triglyceride levels were 2.01 ± 0.07 mM in the pectolinarin-treated diabetic rats and 1.71 ± 0.08 mM in the FECJ-treated diabetic rats. Meanwhile, we assayed the plasma insulin and leptin levels in all experimental rats. ELISA data showed that non-treated diabetic rats displayed a dysregulated plasma insulin level of 0.58 ± 0.09 ng/mL (p < 0.01) and a dysregulated plasma leptin level of 3.67 ± 0.14 ng/mL (p < 0.05), which were not significantly affected after treatment with single flavone compound or FECJ even at a dose of 50 mg/kg body weight/day. In addition, a body weight loss of 11.5% and a liver weight gain of 25.6% (p < 0.05) were observed in the FECJ-treated diabetic rats (50 mg/kg body weight/day), as compared to the non-treated diabetic rat. Similarly, both the loss of body weight and the gain of liver weight in diabetic rats were less when treated with pectolinarin or DDMF. These results demonstrated an

### Table I. Specific forward (F) and reverse (R) primers for quantitative real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer (5’ → 3’)</th>
<th>Size (bp)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1,6-bis</td>
<td>(F) TGCTGAAGTCGTCCTATGCTAC</td>
<td>330</td>
<td>NM_012558.3</td>
</tr>
<tr>
<td>G-6-P</td>
<td>(F) CTGTGATCGCTGACCTCACAGAAG</td>
<td>320</td>
<td>L37333.1</td>
</tr>
<tr>
<td>PFK</td>
<td>(F) TGCGTCAGTGTCTCCAAACAC</td>
<td>330</td>
<td>BC061791.1</td>
</tr>
<tr>
<td>PK</td>
<td>(F) CATTGCAGCTACAACTTCCTC</td>
<td>350</td>
<td>NM_012624.3</td>
</tr>
<tr>
<td>G-6-P dehydrogenase</td>
<td>(F) CATTGTAGGCTATGCCGGCT</td>
<td>315</td>
<td>NM_017006.2</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>(F) GTGCAGGTGGATGCAGGCG</td>
<td>320</td>
<td>NM_144744.2</td>
</tr>
<tr>
<td>Actin</td>
<td>(F) AGAAGATTGCGACACCACACCCTTCTC</td>
<td>320</td>
<td>NM_031144.2</td>
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Table II. General parameters in all experimental rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group Value</th>
<th>Normal1</th>
<th>Diabetic2</th>
<th>Diabetic+pectolinarin3 (mg/kg body weight/day)</th>
<th>Diabetic+DDMF4 (mg/kg body weight/day)</th>
<th>Diabetic+FECJ (mg/kg body weight/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td>245</td>
<td>312</td>
<td>± 7.0</td>
<td>±15.3</td>
<td>± 21.1</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td></td>
<td>6.6</td>
<td>4.3</td>
<td>± 0.5</td>
<td>±0.8</td>
<td>± 0.6</td>
</tr>
<tr>
<td>Plasma cholesterol (mM)</td>
<td></td>
<td>1.81</td>
<td>2.96</td>
<td>± 0.20</td>
<td>±0.32</td>
<td>± 2.95</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td></td>
<td>90.8</td>
<td>302.5</td>
<td>± 6.7</td>
<td>±16.3</td>
<td>± 278.2</td>
</tr>
<tr>
<td>Plasma insulin (ng/mL)</td>
<td></td>
<td>1.32</td>
<td>0.58</td>
<td>± 0.11</td>
<td>±0.09</td>
<td>± 0.54</td>
</tr>
<tr>
<td>Plasma leptin (ng/mL)</td>
<td></td>
<td>1.18</td>
<td>3.67</td>
<td>± 0.07</td>
<td>±0.14</td>
<td>± 3.39</td>
</tr>
<tr>
<td>Plasma triglycerides (mM)</td>
<td></td>
<td>1.47</td>
<td>2.31</td>
<td>± 0.10</td>
<td>±0.13</td>
<td>± 2.11</td>
</tr>
<tr>
<td>Plasma adiponectin (µg/mL)</td>
<td></td>
<td>13.2</td>
<td>6.8</td>
<td>± 1.3</td>
<td>±0.5</td>
<td>± 0.17</td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

Administration of pectolinarin, DDMF or FECJ to diabetic rats for three weeks restored the altered biochemical parameters in diabetic rats, except for the plasma insulin and leptin levels. Values are means ± S.E.M. The number of animals is 1n = 15, 2n = 12, 3n = 10, 4n = 9, 5n = 14.

- Not assayed

**Antidiabetic Effect of Flavones from Cirsium japonicum DC**

Antidiabetic effect for both pectolinarin and DDMF, but a stronger antidiabetic effect for FECJ.

**Effects of flavones on the metabolic enzymes in liver**

The activities of several important glycolytic, gluconeogenic and NADP-linked lipogenic enzymes were assayed in the liver of all experimental rats (p < 0.05). Pectolinarin, DDMF and FECJ, in general, all improved the altered enzyme activities in a dose-dependent manner. The activities of glycolytic enzymes in the non-treated diabetic rats were 1.97 ± 0.15 units/g/min.

Table III. Effects of pectolinarin, DDMF and FECJ on glycolytic, gluconeogenic and NADP-linked lipogenic enzyme activities in diabetic rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group Value</th>
<th>Normal1</th>
<th>Diabetic2</th>
<th>Diabetic+pectolinarin3 (mg/kg body weight/day)</th>
<th>Diabetic+DDMF4 (mg/kg body weight/day)</th>
<th>Diabetic+FECJ (mg/kg body weight/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>F-1,6-bis</td>
<td></td>
<td>3.78</td>
<td>10.11</td>
<td>± 0.24</td>
<td>±0.87</td>
<td>± 9.62</td>
</tr>
<tr>
<td>G-6-P</td>
<td></td>
<td>12.8</td>
<td>25.6</td>
<td>± 1.1</td>
<td>±3.1</td>
<td>± 24.0</td>
</tr>
<tr>
<td>PFK</td>
<td></td>
<td>3.30</td>
<td>1.97</td>
<td>± 0.11</td>
<td>±0.15</td>
<td>± 1.95</td>
</tr>
<tr>
<td>PK</td>
<td></td>
<td>34.7</td>
<td>21.5</td>
<td>± 4.1</td>
<td>±1.3</td>
<td>± 22.2</td>
</tr>
<tr>
<td>G-6-P dehydrogenase</td>
<td></td>
<td>1.75</td>
<td>1.23</td>
<td>± 0.12</td>
<td>±0.08</td>
<td>± 1.26</td>
</tr>
</tbody>
</table>

Administration of pectolinarin, DDMF or FECJ to diabetic rats for three weeks improved the dysregulated activities of the enzymes. Enzyme units are expressed as units/g/min. Each value is a mean ± S.E.M. The number of animals is 1n=15, 2n=12, 3n=10, 4n=9, 5n=14.
for PFK and 21.5 ± 1.3 units/g/min for PK (Table III). They were increased to be 2.33 ± 0.11 units/g/min for PFK and 25.1 ± 1.5 units/g/min for PK in the pectolinarin-treated diabetic rats (50 mg/kg body weight/day), 2.21 ± 0.13 units/g/min for PFK and 24.2 ± 2.6 units/g/min for PK in the DDMF-treated diabetic rats (50 mg/kg body weight/day). Interestingly, FECJ treatment (50 mg/kg body weight/day) improved these activities to be 2.85 ± 0.13 units/g/min for PFK and 30.1 ± 3.5 units/g/min for PK, representing an increase of 44.7% and 40.0% respectively, as compared to the non-treated diabetic rats (Table III). In accordance, the dysregulated transcriptions of PFK and PK in diabetic rats were well improved. As shown in Fig. 2, the mRNA levels for PFK and PK in the FECJ-treated diabetic rats were 33.8% and 32.9% higher than those in the non-treated diabetic rats (p < 0.01), respectively. The pattern of change in the activity of NADP-linked lipogenic enzyme was similar to that of the glycolytic enzymes (Table III). The activity of G-6-P dehydrogenase was 1.23 ± 0.08 units/g/min in the non-treated diabetic rats and increased when treated with pectolinarin or DDMF, being highest in the FECJ-treated diabetic rats (50 mg/kg body weight/day). As shown in Fig. 2, the G-6-P dehydrogenase mRNA level in the non-treated diabetic rats was 27.0% lower than that in the normal rats, but was not affected by FECJ during diabetes. The activities of the gluconeogenic enzymes were 25.6 ± 3.1 units/g/min for G-6-P and 10.11 ± 0.87 unit/g/min for F-1,6-bis in the non-treated diabetic rats. They were 22.4 ± 1.1 units/g/min for G-6-P and 7.71 ± 0.92 unit/g/min for F-1,6-bis in the pectolinarin-treated diabetic rats (50 mg/kg body weight/day), 21.6 ± 1.5 units/g/min for G-6-P and 8.12 ± 1.21 unit/g/min for F-1,6-bis in the DDMF-treated diabetic rats (50 mg/kg body weight/day). FECJ treatment lowered the activities of both enzymes be 17.2 ± 2.8 units/g/min for G-6-P and 6.89 ± 0.51 units/g/min for F-1,6-bis (Table III). It was further supported by the RT-PCR analysis (p < 0.05) showing that the increased mRNA levels for G-6-P and F-1,6-bis in the FECJ-treated diabetic rats were decreased in 20.1% and 19.3% respectively, as compared to the non-treated diabetic rats.

**FECJ improved circulating plasma adiponectin level**

Diabetic rats exhibited not only elevated plasma glucose and abnormal metabolic functions, but also altered protein levels. The plasma adiponectin concentration is inversely correlated with the severity of insulin resistance (Hotta et al., 2001). As FECJ treatment with a dose of 50 mg/kg body weight/day produced a stronger antidiabetic effect in the diabetic rats, so we next selectively analysed the plasma adiponectin levels in the normal, non-treated diabetic and FECJ-treated diabetic rats to find out insight into the molecular mechanism through which FECJ functions in the diabetic rats. As shown in Table II, the plasma adiponectin level in the non-treated diabetic rats was dysregulated and decreased in 48.5% to be 6.8 ± 0.5 µg/mL (p < 0.05), as compared to the normal rats. Accordingly, the adiponectin mRNA level in fat tissue from the non-treated diabetic rats was 59.3% of that in normal rats (Fig. 2). FECJ treatment significantly restored the altered circulating plasma adiponectin level, which was increased by 63.2% to be 11.1 ± 0.8 µg/mL (p < 0.01) (Table II). Supporting this, the

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**Fig. 2.** Quantitative real-time RT-PCR analysis. Total RNA was isolated from liver or fat tissues and used for RT-PCR. Transcriptions of key metabolic enzymes and adiponectin were dysregulated during diabetes and well improved after FECJ treatment. After being normalized against house-keeping gene control (β-actin), values are calculated as percent of normal and expressed as means ± S.E.M. *p < 0.05, #p < 0.01.
adiponectin mRNA level ($p < 0.05$) in fat tissue from the FECJ-treated diabetic rats was increased in 43.5%, as compared to the non-treated diabetic rats (Fig. 2). We analysed the expression of GLUT4 which is responsible for transporting glucose from the blood into the cell when stimulated by external factors, such as hormones, exercise and oxidation pressure. The total GLUT4 level in the non-treated diabetic rats was significantly lower than in the normal rats, but was not obviously affected by FECJ treatment during diabetes (Fig. 3). Furthermore, the plasma membrane GLUT4 level in the non-treated diabetic rats was nearly the same with that in the FECJ-treated diabetic rats, although both were a little lower than that in the normal rats (Fig. 3).

**DISCUSSION**

Diabetes mellitus is a metabolic disorder that affects the metabolism of carbohydrates, fat, and protein. Owing to the increasing worldwide incidence of diabetes mellitus and particularly the adverse side effects associated with the therapeutic agents used for treating diabetes mellitus, there has been a growing interest in herbal remedies. In the present study, we firstly isolated two flavones from *Cirsium japonicum DC* which were analysed to be pectolinarin and 5,7-dihydroxy-6,4'-dimethoxy flavone (DDMF) as reported (Liu et al., 2006). We investigated the hypoglycemic and hypolipidemic effects of the two flavones and their mixture FECJ in the diabetic rats induced by a combination of intravenous injection with streptozotocin and feeding with high energy diet. Administration of pectolinarin, DDMF or FECJ to diabetic rats for three weeks in general decreased the plasma glucose level in a dose-dependent manner, producing a more significant decrease at a dose of 50 mg/kg body weight/day. As reported, the total plasma cholesterol and triglycerides levels got elevated during diabetes. Similarly, pectolinarin, DDMF or FECJ administration to the diabetic rats improved the abnormal plasma cholesterol and plasma triglyceride levels, indicating a hypolipidemic effect for the flavones. In addition to hyperglycemia, hypoinsulinemia is also a major cause for the metabolic disorders in diabetes mellitus. However, neither single flavone nor FECJ showed obvious effect on the dysregulated plasma insulin level in the diabetic rats, suggesting that the flavones might function independent of insulin. These results demonstrated a significant antidiabetic effect for the flavones isolated from the *Cirsium japonicum DC*, producing improved glucose and lipid homeostasis. Meanwhile, our data showed that the mixture of pectolinarin and DDMF had a much stronger antidiabetic effect than pectolinarin and DDMF, indicating a systemic regulation involved in diabetes treatment.

Carbohydrate and lipid homeostasis depends on the balance between their formation and utilization by major peripheral tissues, such as liver. This process is usually severely altered during diabetes. Glucose over- or under-utilization is of critical importance during diabetes, since it leads to the accumulation of cellular glucose and glucose toxicity, ultimately contributing to the severe complications associated with diabetes. We assayed the activities of enzymes involved in glucose and lipid metabolism and found that pectolinarin, DDMF or FECJ treatment to diabetic rats modulated several key metabolic enzymes in the liver, probably thus resulting in improved plasma glucose, cholesterol and triglyceride levels. Pectolinarin, DDMF or FECJ treatment improved the decreased activities of two glycolytic enzymes (PFK and PK) and the increased activities of two gluconeogenic enzymes (G-6-P and F-1,6-bis) in the diabetic rats, as compared to the non-treated diabetic rats. The activity of the lipogenic enzyme (G-6-P dehydrogenase) was decreased in the diabetic rats and then increased by pectolinarin, DDMF or FECJ treatment, which to some degree inversely correlated with what pectolinarin, DDMF and FECJ did to the plasma cholesterol and triglyceride. Glucose is the major energy source for many mammalian cells in various tissues and its level is maintained carefully in these tissues. Normal glucose metabolism in the peripheral tissues, such as the liver, plays a pivotal role in achieving and maintaining normoglycemia. Glucose under- or over-utilization by peripheral tissues thus plays a key role in the disordered glucose metabolism that contributes to the unusual plasma glucose levels in diabetes. Our experimental data supported the possibility that FECJ potentiates the reversal of the activities of the glycolytic, gluconeogenic and lipogenic enzymes which were dysregulated during diabetes, producing the improved...
hepatic glucose metabolism and control of the plasma glucose, cholesterol and triglycerides levels. There are usually many factors directly affecting the activity of one enzyme, such as the enzyme concentration. We selectively analysed the expression of these five metabolic enzymes by quantitative real-time RT-PCR. In consistence with what FECJ brought to the activities of PFK, PK, G-6-P and F-1,6-bis in the diabetic rats, FECJ well restored the altered transcriptions of the enzyme-encoded genes in the diabetic rats. Unexpectedly, no effect of FECJ on the glucose-6-phosphatase dehydrogenase expression was observed in the diabetic rats. It thus pointed out that the improved activities of the glycolytic enzymes and gluconeogenic enzymes in the diabetic rats might come from their improved expressions, while it was a different thing for the NADP-linked lipogenic enzyme, suggesting a complex regulating mechanism for FECJ in the diabetic rats.

Adipose tissue is a massive source of bioactive substances and plays an important role in insulin resistance syndrome through the dysregulated production and secretion of adipose-derived proteins, such as leptin and adiponectin. Leptin is a key factor in the regulation of body weight and energy balance. Serum leptin concentrations correlate well with body weight. Here our data showed that the non-treated diabetic rats had a body weight gain and treatment with pectolinarin, DDMF or FECJ produced a body weight loss. However, pectolinarin, DDMF and FECJ did not obviously affect the plasma leptin level elevated during diabetes. Adiponectin is an adipocyte-derived protein present in the circulating plasma with concentrations range 5-30 µg/mL in humans (Scherer et al., 1995). The plasma adiponectin concentration is inversely correlated with the severity of insulin resistance (Hotta et al., 2001). It was demonstrated that administration of adiponectin increased fatty acid oxidation in muscles and decreased hepatic glucose production, resulting in amelioration of insulin resistance and improved glucose metabolism in diabetic mice (Yamauchi et al., 2001; Berg et al., 2001). Our study showed that FECJ treatment significantly restored the dysregulated plasma adiponectin level in diabetic rats, which was further confirmed by mRNA analysis, suggesting that FECJ improves the activities of the glucose metabolizing enzymes in diabetic rats most possibly through directly or indirectly restoring the dysregulated plasma adiponectin level. GLUT4 is an insulin-sensitive membrane protein, responsible for the transportation of glucose from the blood into the cell. Although FECJ treatment significantly decreased the plasma glucose level in diabetic rats, the total or plasma membrane GLUT4 level in the FECJ-treated diabetic rats remained nearly the same as in nontreated diabetic rats. These results further suggested that it was not the insulin/insulin receptor signaling pathway through which FECJ exerted its antidiabetic effect.

In conclusion, we have biochemically demonstrated the antidiabetic effect of two flavones from Cirsium japonicum DC in diabetic rats, particularly when used as a mixture of the two flavones, confirming the potential benefit of Cirsium japonicum DC as an alternative in diabetes treatment. Meanwhile, we investigated the possible mechanism through which the flavones exerted their antidiabetic effect. Our data suggested that the flavones improved the severely dysregulated adiponectin expression in diabetic rats, probably thus leading to a reversal of the altered activities of key metabolic enzymes in liver and ultimately producing an improved glucose homeostasis. Additionally, the present study excluded the possibility that the typical insulin/insulin receptor signaling pathway was involved in the antidiabetic function of the flavones, indicating that further investigation on how the flavones regulate the expressions of adiponectin and the key metabolic enzymes will be a valuable perspective.

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Antidiabetic Effect of Flavonoids from Cirsium japonicum DC


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