Pharmacodynamics Study on the Effect of Jerusalem artichoke Compound Granule Particles on type II diabetic mice

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The experiment was performed to study the therapeutic effect of Jerusalem artichoke compound granule particles on streptozotocin-induced type II diabetic model mice. Specific Pathogen Free (SPF) male mice with Streptozotocin (STZ) 120 mg Kg⁻¹ were given intraperitoneal injection disposably to establish type II diabetic mice model; Experimental animals were divided into 5 groups. Among them, the blank group and the model control group were both given distilled water, the positive dose group was given glyburide, the high-dose group, the middle-dose group and the low-dose group were respectively given Jerusalem artichoke compound particles 3000, 1500 and 750 mg/kg. Each mouse was given 2mL/100g corresponding drugs. After 4 weeks, fasting blood glucose (FBG) was measured with glucose meter, total superoxide disambiguation (SOD) and malonic acid (MDA) content in serum were also measured with kits. Following 3000, 1500 and 750 mg/kg Jerusalem artichoke particles treatment for 4 weeks, blood glucose concentrations were decreased by 16.82%, 36.89% and 23.90% in type II diabetic mice respectively. Compared with the model control group, SOD concentrations were increased by 10.01%, 33.28%, 19.51%, and MDA concentrations were decreased by 58.29%, 64.45%, 59.94%. Jerusalem artichoke compound granule particles have a hypoglycemic effect on type II diabetic rats.

Keywords: Jerusalem artichoke compound granule particles; Type II diabetes mellitus; Blood glucose; Total superoxide disambiguation in serum; Total malonic acid in serum

INTRODUCTION

Type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus (NIDDM), is a kind of heterogeneity disease, which is mainly manifested sulin resistance with relatively insufficient sulin and insulin deficiency with insulin resistance. It is also the most common type of diabetes, for which insulin injection has no effect and it can only be controlled by proper diet treatment (Vandam RB et al., 2002). At the same time, some new resource food and healthy food supplements are used to help alleviate the symptoms and discomfts caused by diabetes (Hong Yin et al., 2008). The study has shown that the Jerusalem artichoke particles have a significant effect on the treatment of type I and type II diabetic rats (Bo Zhao et al., 2017). In clinical application, Jerusalem artichoke compound are more significant for the curative effect of diabetes. Therefore, the experiment was conducted in view of the compound to further explore the curative effect of Jerusalem artichoke compound in lowering blood sugar and mechanism of action, and to optimize the preparation process.

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This experiment selected sealwort, Jerusalem artichoke, white mulberry root-bark, fenugreek, purslane, cinnamon the six homology of medicine and food. They were used to compose compound Chinese medicine, by drying, reflux extraction, enrichment of refined, spray drying, preparation of particles. And the mouse model of type II diabetes was established by intraperitoneal injection of streptozotocin after high-fat and high-sugar diets were administered to mice. (Lei Yang et al., 2014), to study the effect of Chinese herbal medicines on reducing blood on type 2 diabetes systematically.

**MATERIALS AND METHODS**

**Medicinal plant**

The plants of sealwort, Jerusalem artichoke, white mulberry root-bark, fenugreek, purslane, cinnamon was derived from Guowei Zhang (Department of Chinese Medicine of Hebei University).

**The optimization process of Jerusalem artichoke composite particles**

On the basis of single factor experiment, we selected distilled water volume fraction, extraction time and extraction times to be the examined factors, with each factor in three levels. According to the orthogonal Table of L₉(3⁴), the polysaccharide yield of the composite particles was determined, the factor level (Table 1), test arrangement and result (Table 2).

**Table 1:** Factors and levels of L₉(3⁴) orthogonal design

<table>
<thead>
<tr>
<th>Level</th>
<th>Water quantities/time</th>
<th>Distillation time/min</th>
<th>Distillation time/times</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>60</td>
<td>(A)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>90</td>
<td>(B)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>120</td>
<td>(C)</td>
</tr>
</tbody>
</table>

**Table 2:** Results of inulin content determination (mg/g)

<table>
<thead>
<tr>
<th>Test Number</th>
<th>Factor</th>
<th>Inulin Content mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Determination of polysaccharides in Jerusalem artichokes**

By orthogonal results (Table 2), the influence degree of each factor on the experiment is A>C>B. For A factor, \( \bar{K}_2 > \bar{K}_1 > \bar{K}_3 \), \( A_2 \) is the optimal level. In the same respect, \( B_1 \) and \( C_2 \) were the best. Therefore, the optimal protocol for the extraction of the Jerusalem artichoke composite (1000g) was \( A_2B_1C_2 \). The best preparation process was extracted twice, with each extraction time 90min, and the water volume 10 times. The content of polysaccharide could be obtained by using anthrone sulfuric acid method (Xu BB et al., 2015). The polysaccharide content in the final preparation was 268.4mg·g⁻¹.

**The preparation of the composite particles**

The purchased medicinal materials were cut and pulverized by pulverizer, according to the best scheme mentioned above, the solution was concentrated into the extract. 10g extract was used for the determination of polysaccharide content, and the rest of the extract was sprayed with SY-6000 small spray dryer to make the composition of traditional Chinese medicine (Pingping Wu et al., 2014).

**Experimental Animals**

Male SPF mice were purchased from the experimental animal center of Hebei Medical University (Beijing, China). All animals were maintained under standard environment conditions (23 ± 2°C, 55 ± 5% humidity and 12-h/12-h light/dark cycle) (Leiria LO et al., 2011).

**The establishment of animal models**

The mice were fed adaptively for 3 days and then randomly grouped by weight, and 9 of them were treated as a blank control group for standard diet, while the remaining mice fed high-fat diet. After 3 weeks, fasted but free access to water for 12 hours, they were given intraperitoneal injection of 125 mg·kg⁻¹ dose STZ (STZ soluble in 0. 1 mol · L⁻¹ pH 4.2 citric acid/sodium citrate buffer, is active, ice bath), fed high-fat diet for 4 weeks, and then fasted but could drink water for 12 hours. A glucometer was used to measure blood sugar, and mice with FBG reaching 11.1mmol/L were regarded as successful models (Li JL et al., 2014, Leiria LO et al., 2011). 45 successful modeling mice were divided into five groups by average weight randomly: the model group, the positive control group, the low-dose group, the medium dose group and the high-dose group, with each group consisting of 9. The following day, the mice were given corresponding dose drugs, with each mouse given 2mL/100g corresponding drugs. The blank control group and the model group were given distilled water, the positive control group for glyburide, and the drug concentrations in the high, middle and low dose groups were respectively 3000 mg/kg, 1500 mg/kg and...
750 mg/kg. They were gavaged once a day for four weeks excluding the mice died in the experiment (Lei Yang et al., 2014).

Specimen collection

After the last lavage administration, each group of mice were fasted but could drink water for 12 hours. Blood was extracted from the eyeball, and heparin was added respectively. The centrifuge was $3000 \text{r} \cdot \text{min}^{-1}$ for 10 minutes (Holvoet, P et al., 1998, He HJ et al., 2012), and then plasma was extracted to determine absorbance at 620 nm and calculate SOD and MDA content.

The blood glucose in mice

During the experiment, the fasting blood glucose was measured by a roche glucometer on the 1st, 10th, 20th, and 30th days of fasting.

Statistical Analysis

The results of the experiment were expressed by $\bar{x} \pm s$, and a single factor variance analysis was performed by SPSS13.0 statistical software. A value of $p<0.05$ was considered statistically significant.

RESULTS

Optimal process of Jerusalem artichoke compound particles

The optimal experimental scheme was obtained from the orthogonal experiment, which was extracted twice, 90 minutes each time, and the water amount was 10 times. The polysaccharide content in the final preparation was 268.4 mg·g$^{-1}$.

The situation of II diabetes mice

A total of 45 mice were successfully modeled. The weight of the mice with the successful modeling were less than those in the blank control group (Figure 1), and the food intake was larger than that of the blank control group (Figure 2). And after the treatment, the weight of the high dose group, the medium dose group, the low dose group and the positive group compared with before has increased, its change was statistically significant ($p < 0.05$). The food intake compared with their previous weight has decreased, and its change was statistically significant ($p < 0.05$). The weight and food intake of the model group didn’t change clearly ($p > 0.05$).

Effects of Jerusalem artichoke compound particles on the fasting glucose (FBG) in diabetic mice

Compared with the blank control group, the blood glucose level of mice after successfully modeling was significantly higher ($p<0.05$). Within 4 weeks after administration, compared with the model group, the high, medium and low dose groups which were given the Jerusalem artichoke compound particles and the positive group which was given glyburide, of which blood sugar levels after 10, 20, 30 days reduced gradually, has significant difference at 30 days (Table 3 and Figure 3).

Effects of compound composition of Jerusalem artisan compound on SOD and MDA in serum

After administration, compared with the model group, the levels of MDA in the positive group, the middle-dose group and the low-dose group significantly decreased ($p<0.05$), and the SOD level increased significantly ($p<0.05$). There was no significant difference in the high-dose group ($p>0.05$) (Figure 4 and 5).

Figure 1: Effects on the weight (g) of mice after administration
Figure 2: Effect on food intake(g) of mice after administration

Figure 3: Effect on blood glucose(mmol/L) of mice after administration

Table 3: Effect on blood glucose(mmol/L) of mice after administration (x + s)

<table>
<thead>
<tr>
<th>group</th>
<th>Number</th>
<th>FBG/ mmol·L⁻¹</th>
<th>The 1st day</th>
<th>The 10th day</th>
<th>The 20th day</th>
<th>The 30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-dose group</td>
<td>9</td>
<td>11.231</td>
<td>10.987</td>
<td>9.964</td>
<td>9.342</td>
<td></td>
</tr>
<tr>
<td>Middle-dose group</td>
<td>9</td>
<td>11.861</td>
<td>10.966</td>
<td>8.652</td>
<td>7.486</td>
<td></td>
</tr>
<tr>
<td>Low-dose group</td>
<td>9</td>
<td>11.473</td>
<td>11.044</td>
<td>9.755</td>
<td>8.731</td>
<td></td>
</tr>
<tr>
<td>Model control group</td>
<td>9</td>
<td>11.215</td>
<td>11.177</td>
<td>11.760</td>
<td>11.445</td>
<td></td>
</tr>
<tr>
<td>Positive control group</td>
<td>9</td>
<td>11.631</td>
<td>10.889</td>
<td>8.611</td>
<td>8.328</td>
<td></td>
</tr>
<tr>
<td>Blank group</td>
<td>9</td>
<td>7.652</td>
<td>7.677</td>
<td>7.728</td>
<td>7.536</td>
<td></td>
</tr>
</tbody>
</table>
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DISCUSSION

In the experiment, streptozotocin is a free radical activator, which can selectively destroy islet cells and cause glucose metabolism disorder, so it can cause hyperglycemia and diabetic symptom (KIHO T et al., 2002). In this experiment, the mice models of type II diabetes were successfully established by intraperitoneal injection of STZ in mice fed with high-fat and high-sugar diets.

Studies have shown that oxidative stress is closely related to the course of diabetes (Young-Min Lee et al., 2009). SOD and MDA are markers of oxidative stress (Fujita H et al., 2012). On the one hand, diabetic hyperglycemia had antioxidants proteins in defense enzyme for scavenging of bodily free radicals inactivated, reduced activity, and eventually destroyed, the normal oxidation-oxidation system balance, so the body enhanced oxidative stress level, inhibiting energy metabolism and reducing membrane island element synthesis and secretion. On the other hand, oxidative stress signaling pathways activated could decrease the peripheral tissue sensitivity to rancid inolin and the membrane island effect to further strengthen the resistance of membrane island element. Therefore, oxidative stress can affect the body's antioxidant enzyme activity, increase the level of free radicals, and further aggravate the patient's sugar and lipid metabolism (Liu YL et al., 2016). Under normal circumstances, the body's production and elimination can maintain dynamic balance without causing damage to the body. In diabetes, the oxidative stress induced by hyperglycemia increases, and the scavenging ability of the body decreases, resulting in damage to the tissue cells. It has been reported that the MDA content of type II diabetes population on the average is higher than that of the general population (Yin SL et al., 2010. Du XX et al., 2013). SOD activity was significantly lower than that in healthy people, so the peroxide stress significantly higher than that in healthy people. Oxidative stress can affect the body's antioxidant enzyme activity, and increase the level of free radicals, further aggravating patient sugar and lipid metabolism (Shen BQ et al., 2014).

In this experiment, it was found that by lavage Jerusalem artichoke compound combination, the SOD level in the high-dose, middle-dose and low-dose groups significantly increased, and the MDA level decreased significantly. Especially the middle dose group is the most obvious, and it was similar to the positive group.
CONCLUSIONS

The Jerusalem artichoke compound granule particles have retained the full and powerful hypoglycemic effect, which is strongly supported using in folk medicine and promoting the development of new therapeutics based on natural products.

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REFERENCES


