Effects of Polygonum Cuspidatum Total Anthraquinone on Diabetic Mice Model

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Abstract. Objective: Effects of Polygonum cuspidatum total anthraquinone on streptozotocin induced diabetic mouse model, To investigate the hypoglycemic mechanism and characteristics of Polygonum cuspidatum anthraquinone. Method: By intravenous injecting streptozotocin induced diabetic mice model, detecting the serum glucose, glycosylated serum protein content, observing the pancreas pathological changes, Studying Polygonum cuspidatum on blood glucose in the mice pancreas. Result: Successful established diabetic mice model. Compared with model group, The metformin group, high, medium and low doses Polygonum cuspidatum total anthraquinone can reduce the blood sugar and glycosylated serum protein level in diabetic model mice at different levels( \( P < 0.01, P < 0.05 \)), and increased liver glycogen, To inhibit the atrophy, necrosis, dissolution and degeneration of pancreatic islet cells in mice. Conclusion: Polygonum cuspidatum total anthraquinone has the effects of reducing blood sugar, glycosylated serum protein level and reducing blood fat, Promoting insulin secretion and hepatic glycogen formation, improve insulin resistance, Improving the pathological changes of pancreas in diabetic mice, It has a good hypoglycemic effect, delayed the process of diabetes to some extent.

Introduction

Polygonum cuspidatum also known as "Yin and Yang lotus.", slightly warm in nature, bitter in taste, Liver, gall bladder, lung, It is the dry rhizome and root of Polygonum cuspidatum. The main function of dispelling wind and dampness stasis, pain, relieving a cough and reducing phlegm[1]. Modern pharmacological studies showed that Polygonum cuspidatum can hypoglycemicing, antioxidantig, anti-shock, improve microcirculation, inhibit platelet aggregation, lowering blood lipid, antitussive, antiasthmatic, enhancing immunity, anti-pathogenic microorganism, relaxing intestinal muscle, anti-tumor, increased white blood cells and platelets[2-4]. Clinical treatment for hyperlipidemia, upper gastrointestinal bleeding, hepatitis, neonatal jaundice, neutropenia, pneumonia, arthritis, burns, vaginitis and other diseases[5]. As a kind of traditional Chinese medicine for activating blood circulation to dissipate blood stasis and reducing blood sugar, Polygonum cuspidatum has long been used in the folk, In recent years, there are a few reports of hypoglycemic effect of Polygonum cuspidatum. But the research is not deep enough, and lacks the mechanism of hypoglycemic effect. As a hypoglycemic drug, Polygonum cuspidatum is widely used in addition to the compound, There is little modern research on single use and its hypoglycemic components and hypoglycemic effects. The main purpose of this study is to observe the intervention effect
of Polygonum cuspidatum total anthraquinone(PCTA) on diabetic mice, and to provide experimental basis and research ideas for further research on clinical application and follow-up experiments of Polygonum cuspidatum for the prevention and treatment of diabetes.

Materials

Drugs and Reagents

Polygonum cuspidatum total anthraquinone(PCTA, Total anthraquinone content was 64.5%, batch number: ZY2015-13, provided by the Henan University of traditional Chinese Medicine), Metformin hydrochloride, Shanghai Pharmaceutical (Group) Co., Ltd. Xinyi pharmaceutical factory, 160310; Streptozotocin, sigma, inc, 20160382; Blood glucose kit, Zhejiang Dongou Biological Engineering Co Ltd, 2016060350; GSP kit, the first branch of Nanjing Institute of biological engineering, 20161007; Liver glycogen kit, Nanjing Jiancheng Biological Engineering Institute, 20160907.

Instruments

Uv-2000 UV-Vis spectrophotometer: Unico (Shanghai) Instrument Co., ltd.; Constant temperature water bath, Beijing Guangming instrument factory; Centrifuge, Beijing medical instrument repair factory; FA (n) /ja (n) series electronic balance, Shanghai min bridge Precision Instrument Co., ltd.; Adjustable pipette analysis instrument Shanghai Co., ltd.; PH analyzer, Olympus optical microscope.

Animals

KM mice, male, Weight: 18 ~ 20g; By the Hebei experimental animal center, the certificate number: 611040

Method

Male mice, 18 ~ 20g, fed normally for 3 days and fasted after 12h, Caudal vein was injected with streptozotocin (80mg/kg, 0.02ml/10g), fasted after 12h when the eleventh days after injection, used the tail blood sampling to measure the fasting blood glucose, Select 55 mice with >11.1mmol/l, with obvious polydipsia, polydipsia and polyuria, They were randomly divided into 5 groups, High, medium and low dose PCTA groups, Positive control group and model group, Take high, medium and low dose PCTA respectively(400mg/kg, 200mg/kg, 100mg/kg, 0.2ml/10g), Metformin (0.5g/kg, 25mg/ml, 0.2ml/10g), model group received the same volume of normal saline, Another 11 mice were taken as blank control group, and received the same volume of normal saline, Continuous administration for 30 days. At the end of 10,20,30 days, blood was taken from the tail to measure blood glucose, Thirtieth days after fasting 12h, and administered at 1h, Take blood, measure blood sugar and glycosylated serum protein(the results are shown in Table 1, table 2), The measurement of liver glycogen in liver homogenate, taking the pancreas and 10% formalin fixed for pathological section(The result was shown in Table 3) 1.

Statistical Treatment

Data analysis uses SPSS 17 for windows medical statistical package for statistical processing of data, The measurement data is expressed by the mean + standard deviation,
Single factor analysis of variance was used in each group, Together with ANOVA least significant difference (LSD) method, games-howell method was used to test the heterogeneity of variance.

**Experimental Results**

**Effects of PCTA on Blood Glucose in Streptozotocin Induced Mice Model**

From Table 1 shows that, Compared to control group, The blood glucose levels of model group significantly higher than those of the control group \((P<0.01)\) at the beginning and the 10th,20th,30th day, illustrate the success of streptozotocin diabetes mellitus model. Compared with model group, After 20th days of administration, HDG and MDG PCTA, metformin group significantly lower the blood glucose level \((P<0.01)\), LDG PCTA obviously lower the blood sugar level \((P<0.05)\), HDG, MDG and LDG, PCTA, and metformin significantly reduced blood sugar levels \((P<0.01)\).

Table 1. Effects of PCTA on blood glucose in streptozotocin induced mice model( \(X \pm s)\).

<table>
<thead>
<tr>
<th>group</th>
<th>Number of animals</th>
<th>Dosage (g/kg)</th>
<th>blood sugar(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>The First Day</td>
</tr>
<tr>
<td>control group</td>
<td>10</td>
<td>-</td>
<td>4.561±0.675</td>
</tr>
<tr>
<td>metformin group</td>
<td>10</td>
<td>0.208</td>
<td>15.424±2.273</td>
</tr>
<tr>
<td>HDG PCTA</td>
<td>10</td>
<td>0.4</td>
<td>15.682±2.162</td>
</tr>
<tr>
<td>MDG PCTA</td>
<td>10</td>
<td>0.2</td>
<td>15.758±2.311</td>
</tr>
<tr>
<td>LDG PCTA</td>
<td>10</td>
<td>0.1</td>
<td>15.582±2.414</td>
</tr>
</tbody>
</table>

Notes: Compared to control group, ## indicates \(P<0.05\), # indicates \(P<0.01\); compared to model groups, * indicates \(P<0.05\), ** indicates \(P<0.01\)

**Effects of PCTA on Liver Glycogen and Glycosylated Serum Proteins in Streptozotocin Induced Mice Model**

From Table 2 shows that, Compared to control group, The level of liver glycogen in the model group decreased significantly \((P<0.01)\), and the level of Gsp increased significantly \((P<0.01)\). Compared with model group, HDG, MDG PCTA and metformin significantly increased the liver glycogen level \((P<0.01)\), LDG PCTA significantly increased the liver glycogen level \((P<0.05)\). HDG, LDG PCTA and metformin group significantly reduced serum Gsp levels \((P<0.01)\), MDG PCTA significantly decreased serum Gsp levels \((P<0.05)\).
Table 2. Effects of PCTA on liver glycogen and glycosylated serum proteins in streptozotocin induced mice model (x ± s).

<table>
<thead>
<tr>
<th>group</th>
<th>Number of animals</th>
<th>Dosage (g/kg)</th>
<th>hepatic glycogen (mmol/L)</th>
<th>Gsp (HbA1c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>10</td>
<td>-</td>
<td>28.754±5.251**</td>
<td>1.514±0.297##</td>
</tr>
<tr>
<td>model group</td>
<td>10</td>
<td>-</td>
<td>11.477±1.982</td>
<td>2.648±0.542</td>
</tr>
<tr>
<td>metformin group</td>
<td>10</td>
<td>0.208</td>
<td>16.920±2.307**</td>
<td>1.729±0.204**</td>
</tr>
<tr>
<td>HDG PCTA</td>
<td>10</td>
<td>0.4</td>
<td>20.411±3.554**</td>
<td>1.793±0.309**</td>
</tr>
<tr>
<td>MDG PCTA</td>
<td>10</td>
<td>0.2</td>
<td>18.853±2.927**</td>
<td>2.170±0.546*</td>
</tr>
<tr>
<td>LDG PCTA</td>
<td>10</td>
<td>0.1</td>
<td>14.978±3.219*</td>
<td>2.133±0.453**</td>
</tr>
</tbody>
</table>

Notes: Compared to control group, ## indicates P<0.05, # indicates P<0.01; compared to model groups, * indicates P<0.05, ** indicates P<0.01

Effects of PCTA on Pancreatic Tissue in Streptozotocin Induced Mice Model

From Table 3 shows that, in the control group, the pancreatic islet cells were rich in cytoplasm, no atrophy and edema and vacuolar degeneration, Compared to control group, Most of the pancreatic islet cells were atrophic in the model group, at the same time, edema and vacuolar degeneration were observed, After Ridit test, P<0.01. Compared with model group, HDG, MDG and LDG PCTA and metformin group, most pancreatic islet cells are rich in cytoplasm, increased islet volume, Partial cell atrophy, The cytoplasm of cells decreased obviously, The nucleus is dense, After Ridit test, P<0.01. The results showed that PCTA had a protective effect on pancreatic islet injury caused by streptozotocin, and the effect was stronger than that of metformin group.

Table 3. Effects of PCTA on Pancreatic tissue in streptozotocin induced mice model.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of animals</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>control group</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>model group</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>metformin group</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>HDG PCTA</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MDG PCTA</td>
<td>10</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>LDG PCTA</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

Notes: ‘’-’’The pancreatic islet cells are rich in cytoplasm, no atrophy, edema and vacuolar degeneration were normal; ‘’+’’: The pancreatic islet cells showed atrophy, No edema and vacuolar degeneration; ‘’++’’: Most Pancreatic islet cells atrophy, A small number of cells are edematous without vacuolar degeneration; ‘’+++’’: Pancreatic islet cells atrophy, At the same time, edema and vacuolar degeneration were obvious

Discussion

Diabetes mellitus belongs to the Chinese medicine "Xiaoke" category, It is characterized by polydipsia, overeating, polyuria, weight loss, or urine turbidity and urine sweetness[6]. Western medicine believes that diabetes mellitus is a chronic metabolic disease caused by a variety of causes, It is caused by the lack of insulin in the body or by an increase in insulin resistance or an abnormal combination of glucose, protein, and lipid metabolism that insulin
does not play a normal physiological role in target cells[7-8]. The current clinical treatment of diabetes medicine can be divided into insulin, insulin secretion, insulin sensitizing agents, nitric oxide synthase inhibitor, glycogen gluconeogenesis inhibitor etc.[9-11]. However, these drugs still suffer from liver damage, hypoglycemia, gastrointestinal reactions and other side effects, And with the prolonged use of drugs, also needs to increased dosage, so scholars will turn their attention to the development of natural drugs at home and abroad.

Chinese medicine treatment of diabetes has a long history, The pathogenesis of Diabetes mellitus in TCM is mainly Yin deficiency, dryness-heat, Qi and yin injuries, Deficiency of yin and Yang, Dryness heat and blood stasis. For DM alone Chinese herbal medicine can be divided into about water swelling drugs, Qi regulating drugs, promoting blood circulation and removing stasis drugs, heat clearing drugs in several categories[12-13]. The study on screening and mechanism of hypoglycemic components of Chinese herbal medicine is more, It is found that the main components of hypoglycemic include polysaccharides, saponins, anthraquinones, flavonoids, alkaloids and other chemical structure types, Some of them have obvious anti platelet aggregation and adhesion, They can cause beta cell regeneration, repaired and elevation of insulin, Inhibiting gluconeogenesis, promote glycolysis, So as to play a role in reducing blood sugar and lipid-lowering[14-15]. In recent years, promoting blood circulation to dissipate blood stasis has become a hot spot in the treatment of diabetes mellitus, Further screening and further research on Chinese medicine for activating blood circulation to dissipate blood stasis is imperative, Polygonum cuspidatum can dispel the wind, remove dampness, eliminating stasis to activate blood circulation, analgesic therapy, relieve a cough and phlegm. It mainly contains free anthraquione and anthraquinone glycosides, tannins, flavonoids, a small amount of polysaccharides and amino acids and other ingredients[16]. Anthraquinone is the main active component of Polygonum cuspidatum, Recent studies have shown that anthraquinones also have hypoglycemic effects.

Summary
Disorder of glucose metabolism is the most important and basic pathological change in diabetes mellitus, Therefore, the determination of blood glucose is the most important index to evaluate the success of the model and the effectiveness of the drug. Glycogen is the body to satisfy the demands of the energy of body, excess glucose into the first polymerization, Glucose metabolism disturbance in patients with diabetes, it is very meaningful.to adjust reasonable glycogen synthesis, maintain normal blood glucose and improve glucose tolerance. Glycated serum proteins can cause a series of pathological changes, which is one of the key indicators of chronic complications of diabetes mellitus. The results of this experiment show that PCTA can reduce the blood sugar and glycosylated serum protein level in diabetic model mice, Increased liver glycogen level, The hypoglycemic mechanism of PCTA may be related to the promotion of liver glycogen synthesis and insulin secretion have a certain relationship. Through the pathological changes of pancreas, it found that PCTA could protect islet cells, It can enhance the energy metabolism and promote protein synthesis of pancreatic islet cells, Thereby promoting the secretory activity of secretory cells.

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References


