Effects of Flavonoids from Sophora Japonica Thunb on Diabetes Mellitus in Mice

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Abstract. Objective: To investigate the hypoglycemic effect of flavonoids from Sophora japonica L. and its mechanism. Method: The model of hyperglycemia induced by streptozotocin was injected into the tail vein of the mice. The rats were fasted for 12 hours on the 11th day of injection and the fasting blood glucose. The total flavonoids of Sophora japonicus were continuously fed for 30 days. Blood glucose and glycogen content were measured at the 10th, 20th and 30th day after administration. Take the formalin fixed pancreas, do histopathology. Result: The total flavonoids of Sophora japonica significantly reduced the hyperglycemia of streptozotocin-induced diabetic mice, improved the shape and function of pancreatic islet cells, and played a good role in lowering blood glucose. Conclusion: Flavonoids of Sophora japonica L. have obvious hypoglycemic effect, which can improve hyperlipidemia and kidney damage of diabetes mellitus.

Introduction

Sophora japonica for the leguminous plants Huai dry flowers and buds. The former known as the "Sophora japonica", the latter known as the "Huaimi".Was contained in the "Japan and China Materia Medica", bitter taste, slightly cold, to the liver, large intestine[1]. 2015 version of "Pharmacopoeia of the People's Republic of China," said the cooling blood to stop bleeding, Liver purging fire. For blood in the stool, hemorrhoids, bloody diarrhea, uterine bleeding, hematemesis, Nvxue, liver heat red eyes, headache, vertigo, etc. card[2]. Mainly contains flavonoids and their glycosides, saponins and aglycones, steroids and other ingredients. Flavonoids which mainly rutin, as the main component of Sophora japonica[3]. It has anti-inflammatory, antiviral, antifungal, anti-tumor, hypolipidemic, inhibiting aldose reductase, anti-oxidation, cardiovascular and capillary effects, analgesia and so on in modern research[4]. Pre-study also shows that the total flavonoids of Sophora japonica has a good hypoglycemic effect, prevention and treatment of complications of diabetes, at the same time have better lipid-lowering, regulating cytokines, inhibition of protein kinase C, α-glucosidase in vitro inhibition effect[5]. In this study, the hypoglycemic effect of total flavonoids of Sophora japonica L. and the mechanism of improving diabetic complications were studied in order to provide a theoretical basis for the clinical treatment of Diabetes.
Materials and Methods

Materials

Instruments and reagents drugs: Sophora japonica total flavonoids (by the Henan Provincial Development of Engineering Research Center of Traditional Chinese Medicine); metformin hydrochloride, Shanghai Pharmaceutical (Group) Co., Ltd. Xinyi Pharmaceutical Factory production; Streptozotocin, sigma production; saline, Zhengzhou Yonghe Pharmaceutical Ltd; production of citric acid (analytical grade), the Hubei Provincial Pharmaceutical Company of the glass; sodium citrate (analytical grade), Tianjin Chemical Reagent Wholesale; blood glucose kit, Zhejiang Dongou Biological Engineering Co., Kit, built in Nanjing Biological Engineering Institute production.


Animals: Mice: Kunming species, II grade, male, by the Hebei Medical Experimental Animal Center, the certificate number: 608155.

Methods

Effect of Streptozotocin - Induced Diabetes Mellitus in Mice[6]. In male mice, normal feeding 3 days, 12 h after fasting, tail intravenous chain urea with bacteria, 80 (10 mg/kg, 0.02 ml/g).After fasting for 12 hours on the 11th day of injection, the fasting blood glucose was measured in the tail. Select mouse blood sugar value > 11.1 tendency for L, has obvious drinking, eating, 50 urinary symptoms of mice. The rats were randomly divided into 5 groups, namely large, medium and small dosage of total flavonoids of Sophora japonica, positive control group and model group. The mice were fed with large, medium and small doses of total flavonoids (600mg/kg, 300mg / (0.5g/kg, 25mg / ml, 0.2ml /10g) and the same volume of physiological saline (0.2g/10g).Another take 10 mice as blank control group and physiological saline irrigation served the same volume. The drug was administered once daily for 30 days. The blood glucose level was measured at the 10th day, the 20th day and the 30th day after the administration. On the 30th day, fasting 12h after the last administration 1h, mice orbital blood, serum separation for blood glucose measurement. Then the mice were sacrificed, weighed the liver, homogenate, for measuring liver glycogen with; take the pancreas, 10% formalin solution for pathological sections, for light microscopy observation of pancreatic tissue. The results are shown in Table 1 and Table 2.

Data analysis SPSS 10.0 for windows statistical software, measurement data between groups using single factor analysis of variance, grade data using Ridit analysis.

Results

Effects of streptozotocin on blood glucose and pancreatic tissue morphology in mice with diabetes mellitus.
Table 1. Effects of total flavonoids of Sophora Japonica on blood glucose level in streptozotocin-induced diabetic mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial</th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
<th>HG (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank</td>
<td>4.771±0.686**</td>
<td>4.929±0.847**</td>
<td>4.758±0.703**</td>
<td>4.760±0.637**</td>
<td>28.754±5.251**</td>
</tr>
<tr>
<td>Metformin</td>
<td>15.424±2.273</td>
<td>18.724±2.511</td>
<td>15.035±3.008**</td>
<td>12.582±2.499**</td>
<td>17.089±2.275**</td>
</tr>
<tr>
<td>Middle dose</td>
<td>15.574±2.535</td>
<td>18.666±2.486</td>
<td>16.947±2.696*</td>
<td>15.573±2.629**</td>
<td>18.187±2.946**</td>
</tr>
</tbody>
</table>

Note: * P <0.05 compared with the model group, ** compared with the model group P <0.01

As can be seen from Table 1, compared with the blank group, during the administration period, the blood glucose level of model group was significantly increased (P<0.01), liver glycogen level was significantly lower (P<0.01). There were no significant differences in the initial blood glucose between the groups. On the 10th day after administration, the blood glucose level of each group decreased, but there was no significant difference. Compared with the model group, the blood glucose level of the metformin group and high dose group decreased significantly (P<0.01) and the middle dose group also decreased (P<0.05) on the 20th day of administration. Compared with the model group, the high - dose group, middle - dose group, low - dose group and metformin group decreased the blood glucose level significantly (P<0.01) at the 30th day of administration, and the low - dose Sophorae flavescens group significantly. Lower blood glucose levels(P<0.05). High-dose group, middle-dose group, low-dose group and metformin group can significantly increase the level of liver glycogen (P<0.01).

Table 2. Sophora japonica flavonoids on streptozotocin-induced diabetic mouse pancreatic tissue.

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses (mg/kg)</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>model</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Metformin</td>
<td>208</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>High dose</td>
<td>600</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>middle dose</td>
<td>300</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>small dose</td>
<td>150</td>
<td>0</td>
<td>7</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: “-”Islet cell cytoplasm rich, no atrophy, edema and vacuolar degeneration, were normal.”+”Islet cells showed atrophy, without edema and vacuolar degeneration.”++”Most of the islet cells atrophy, a small number of cells without edema and vacuolar degeneration.”+++”Islet cell atrophy, edema and vacuolar degeneration at the same time obvious.

As can be seen from the table, by Ridit test, compared with the blank group, the model group of pancreatic pathological changes significantly (P<0.01), indicating that the success of the mouse model of diabetes. Compared with the model group, the high dose group, the
middle dose group and the low dose Sophora flavescens flavone significantly alleviated the pathological changes of the pancreas ($P<0.01$). The pancreatic tissue of mice in each group was observed by light microscopy: the islets of the control group were rich in cytoplasm, large cell body, large islet volume. In the model group, the islet cell cytoplasm of the pancreatic islets was significantly reduced, the cell body was shrunk, the cells appeared to be dense, the islet cell part appeared vacuole. In the metformin group, the cytoplasm of the pancreatic islets in the islets was abundant, the cytoplasm of some cells was obviously reduced. Most of the cells in the large dose of Sophora japonica total flavonoids group were recovered, the cytoplasm was abundant, the individual cells were edematous and the surrounding cells showed atrophy. In the medium-dose Sophora japonica total flavonoids group, some of the cells in the pancreatic tissue showed edema, some of them recovered, the cytoplasm appeared abundant. In the small dose of Sophorae japonicus total flavonoids group, the islet cells in the pancreas of the mice showed atrophic state, the cell volume decreased, the nuclei were denser, and a few cells appeared atrophy, but some cytoplasm.

**Discussion**

Sophora japonica was contained in the "Japan and China Materia Medica", history of Materia Medica have documented, is commonly used traditional Chinese medicine, bitter, cool, the main liver, large intestine. Sophora japonica has a cooling blood to stop bleeding, Qinggan Xiehuo role, mainly for blood in the stool, hemorrhoids, blood dysentery, uterine bleeding, hematemesis, Nvxue, liver heat red headache, dizziness and other card[7]. Modern studies suggest that the total flavonoids of Sophora japonica is the main active site, and its flavonoids are mainly rutin[8]. In this study, streptozotocin-induced animal model of diabetes mellitus, is used in pharmacological study of the classic method. As the drug induced diabetes model has a high incidence of modeling time is short, neat onset time and severity of the disease than the uniform characteristics of[9]. In recent years has successfully induced a similar animal model of human diabetes, improve the practical value of the results.

Diabetes is a disorder of endocrine and metabolic diseases, can cause a variety of pathogenesis of elevated blood sugar[10]. The aim of this study was to observe the hypoglycemic effect of total flavonoids from Sophora Japonica on streptozotocin-induced diabetic mice from the point of recovery of islet β-cells. The results showed that the total flavonoids of Sophora Japonica significantly reduced the hyperglycemia of streptozotocin-induced diabetic mice, and the intensity of action increased with the dose. At the same time to promote blood glucose into glycogen, significantly increased the amount of glycogen in the mouse model[11]. The mechanism of action may be to promote the recovery of animal pancreatic islet β-cells.

The most prominent feature of diabetes is a long course of disease and complications, complications and more[12]. So far the cause has not yet fully elucidated, and no drugs can be completely cured. At present, the leading drug in clinical hypoglycemia is still western medicine[13]. Chinese medicine treatment of diabetes has its unique, such as hypoglycemic effect of mild and lasting, side effects, with a comprehensive therapeutic effect, can effectively delay the occurrence and development of complications[14]. However, traditional Chinese medicine and its compound preparation is ineffective, poor reproducibility and so on. Therefore, how to carry out the treatment and health care of diabetes, to find effective new drugs to prevent and treat diabetes, especially from the natural drug to screen and study hypoglycemic ingredients, has become the focus of domestic and foreign medical
workers[15]. In this study, the effective part of the hypoglycemic effect of Sophora japonica L. is the total flavonoids of Sophora japonica L., which shows that the hypoglycemic effect of Sophora japonica L. is exact and opens up a new way for the research of hypoglycemic drugs, thus laying the foundation for the further development and utilization of Sophora japonica.

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References

