Analysis of Effect of Taxus Cuspidata Polysaccharide on Hypoglycemic Effect in Mice

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Abstract

To explore the hypoglycemic effect of active polysaccharides from the branches and leaves of Taxus cuspidata on mice, and provide a scientific basis for the further study of the mechanism of hypoglycemic effect of Taxus cuspidata, in this study, the active polysaccharides from the branches and leaves of Taxus cuspidata were extracted and isolated, and the hypoglycemic mechanism of Taxus cuspidata polysaccharides was studied by establishing a mouse model of Type II diabetes. In this study, the polysaccharides in the branches and leaves of Taxus cuspidata were separated and purified to obtain the polysaccharides of Taxus cuspidata. 10 mice were selected as the blank group. 30 mice were screened as grouped subjects in the established type II diabetic mice. They were randomly divided into model group, high dose Taxus polysaccharide (500 mg/kg) group and low dose Taxus polysaccharide group (125 mg/kg). The hypoglycemic activity of Taxus cuspidata in leaves was confirmed by comparison, and the effect of Taxus polysaccharide on pancreatic tissue of mice was analyzed. The hypoglycemic activity of Taxus polysaccharides was further analyzed by establishing an insulin resistance HepG2 cell model. The experimental results show that the polysaccharides of Taxus cuspidata have consumption obvious effects on glucose consumption, which can reduce blood sugar and may be related to the change of sugar metabolism and insulin-like effects of Taxus polysaccharides. It was also found that the polysaccharide of Taxus can protect the pancreatic tissue of model mice while lowering blood sugar. The experiment found that Taxus polysaccharide could promote the glucose consumption of insulin-resistant HepG2 cells, and the best effect was obtained when the administration concentration was 0.05 mg/mL, which was significantly different from the model group (P<0.05). There are relatively few studies on the polysaccharides of Taxus. Therefore, it is necessary to further study its chemical composition, make its chemical composition clear, and at the same time conduct screening test on bioactive constituents, and clarify the mechanism of action to better provide a basis for clinical medicine use.

Key words: Taxus Cuspidata, Polysaccharide, Hypoglycemic, Hepg2 Cells

1. Introduction

Diabetes Mellitus (DM) is a common metabolic disease caused by endocrine disorders. Type II diabetes is mainly due to the poor effect of insulin in the body, which leads to the relative lack of insulin [1]. Type II diabetes is common in middle-aged and elderly people, and the incidence of obese people is high, often accompanied by high blood pressure, dyslipidemia, arteriosclerosis and other diseases. And studies have shown that most patients have hereditary features. The blood sugar level of diabetic patients is higher than the normal level, and the long-term hyperglycemia symptoms may cause metabolic disorder of blood lipids, proteins and other related substances in the patient, and increase the risk of cardiovascular and cerebrovascular diseases [2]. Currently, Type II diabetes has become one of the major public health problems in the 21st century.

Nowadays, for the pathogenesis of Type II diabetes, the focus of Western medicine targeted drug treatment is mainly divided into the following directions: insulin resistance, inhibition of sugar absorption, insulin secretion defects, increasing sugar excretion and excessive glycogen output [3, 4]. Since the related western medicine still has certain side effects and drug resistance, and the natural extracts have the advantages of mild action and light side effects, the potential for development and utilization is huge. Therefore, actively seeking natural medicine for treating and controlling diabetes has become a focus of medicine research.

Taxus cuspidata grows in Heilongjiang, Jilin, Liaoning. Taxus contains a wide variety of chemical components, mainly taxine, taxinine, steroids, glycosides, fatty acids and polysaccharides [5-7]. Polysaccharides are widely found in natural plants and are a large class of polymers composed of more than 10 monosaccharides linked by glycosidic bonds [8]. Polysaccharide has immunomodulatory activity, can also be used for the prevention and treatment of diabetes [9], promote the healing and repair of gastric ulcer, and has various effects
such as anti-radiation and hypolipidemic [10-12]. Polysaccharides and their derivatives have become one of the research hotspots of natural medicines in recent years. Therefore, the research on the pharmacological effects of polysaccharides has important practical significance and clinical application value. In this study, the effective part of hypoglycemic effect of Taxus cuspidata branches and leaves was screened out, and the mechanism of hypoglycemic effect was studied by mouse experiment, in order to systematically clarify the material basis of hypoglycemic effect, and lay a foundation for the research of polysaccharides of Taxus cuspidata.

2. Materials and Methods

2.1. Extraction and Purification of Crude Polysaccharides in Taxus cuspidata

Extraction of Taxus crude polysaccharide: Take 10g of Taxus cuspidata bark residue, add distilled water according to liquid to material ratio 1:30, extract by reflux extraction in hot water bath for 3h at 90 °C, filter, re-extract the filter residue once again in the same way, combine two filtrates, and extract a part of the liquid as the test sample for the content determination, and the rest is evaporated by a rotary evaporator (manufacturer: BUCHI(Switzerland) Co., Ltd.; model: Buchi R-100), and concentrated under vacuum at 50 °C to a certain volume, add 3 times volume of 95% ethanol to the concentrate to 80% ethanol volume, stir well and place it for 24h, using low speed large capacity multi-tube centrifuge (manufacturer: Shanghai Precision Instrument Co., Ltd.; model: LXJ-IIB) to centrifuge at 3000 r/min for 20 min and discard the filtrate. The ethanol precipitation was washed successively with absolute ethanol, acetone and diethyl ether, and placed in a freeze dryer (manufacturer: Beijing Songyuan Huaxing Technology Development Co., Ltd.; model: FD-A10N-50) for lyophilization, then the refined polysaccharide of the Taxus cuspidata branch and leaves is obtained.

Purification of crude polysaccharide: take 1g of crude polysaccharide in 250ml conical flask with stopper, add 100ml distilled water to ultrasonically dissolve it, then transfer it to the separating funnel, add 1/4 volume of Savage reagent to the solution and vibrate for 30min, then place it for 60 min, the agglomerates of the lower organic phase and the intermediate layer of denatured protein and the Savage reagent were discarded, the supernatant was retained, and the Savage reagent was added. The above deproteinization step was repeated until the lower organic phase became clear and the intermediate layer disappeared. Take the upper layer of clear solution, recover the solvent to dryness under reduced pressure, dissolve it with a small amount of distilled water, add 95% ethanol to ethanol reaches a volume fraction of 80%, stir well, then place it at 4 °C for 24 h, then use low-speed large-capacity multi-tube centrifugal machine to centrifuge at 3000 r/min for 15 min and discard the filtrate. The ethanol precipitation was washed successively with absolute ethanol, acetone and diethyl ether, and then lyophilized in a freeze dryer to obtain refined polysaccharides of Taxus cuspidata branches and leaves. In Savage deproteinization, it should be noted that: firstly, sample solution and chloroform-n-butanol volume ratio of 3:1; secondly, vibrate for 30min, centrifugate for 1min, then separate the two phases. The volume of 1/3 of chloroform-n-butanol was added to the aqueous phase, and the above operation was repeated.

2.2. Test Subject

Construction of a mouse model of type II diabetes: 60 healthy clean male mice were selected, weighing approximately 13-15 g, and experimental animal license number: SCXK (liao) 2015-0001(Liaoning Changsheng biotechnology co., Ltd). The mice were housed in a cage at room temperature of 20 °C to 24 °C and a relative humidity of 50% to 70%, and fed normal for one week. 10 of them were randomly selected as a blank group, and the blank group was fed normal feed until the end of the experiment, and the remaining 50 mice were given a high-glucose and high-fat diet for 4 weeks. After 4 weeks of feeding, all the groups except the blank group were fasted for 12 h, and intraperitoneal injection of STZ 35 mg/kg for 3 consecutive days, further enhancing the metabolic disorder of the mice, continuing to give high-fat diet for one week, and fasting for 24 h after one week. The fasting blood glucose was measured, and the mice with the fasting blood glucose level above 16.7 mmol/L and the symptoms of polydipsia and polyuria were selected as type II diabetes model mice, and the unmodeled mice were excluded.

Modeling mice were divided into groups: 30 mice were selected as type II mice, and were randomly divided into three groups: model group, high dose group and low dose group, with 10 in each group. The high dose group (500mg/kg) and the low dose group (125mg/kg) were prepared with 5% sodium carboxymethylcellulose solution to prepare the solution for gavage; the model group and the blank group were given the same volume of 5% sodium carboxymethylcellulose solution for gavage once a day for four weeks.

2.3. Serum Index Measurement

Measurement of blood sugar level: Take the blood at the end of the rat tail, and measure the blood sugar level with a blood glucose meter (manufacturer: Beijing Yicheng Bioelectronics Technology Co., Ltd.; model: JPS-7 type).
Measurement of serum index: after the last dose, the mice were fasted for 12h, after ether anesthesia, the eyeball was taken for blood, the blood was placed at 4 °C for 20 min, centrifuged at 12000 r / min for 15 min, the supernatant was drained, and the fasting insulin (FINS) and fasting blood glucose (FBG) were measured by enzyme-linked immune sorbent assay (ELISA), and the homeostasis model assessment of insulin resistance (HOMA-IR) index of the steady state model was calculated according to the following equation.

\[ \text{HOMA-IR} = \frac{A \times B}{22.5} \]  

In the equation, A represents FIN, mU/L, and B represents FBG, mmol/L.

The morphological changes of pancreatic tissue were observed in the 4 groups of mice. After the fourth week of administration, the mice were fasted for 12 hours and then sacrificed. The pancreatic tissue was quickly removed. After routine formalin solution was fixed, the pancreatic tissue was embedded with paraffin, sectioned, stained with HE, and the morphological changes were observed under light microscope.

2.4. Experiment on Glucose Consumption of Taxus Polysaccharide in Hepg2 Cell Insulin Resistance Model

HepG2 cell line was obtained from Chuan Qiu Biotechnology, and the polysaccharide of Taxus cuspidata was prepared in the previous experiment. HepG2 cells were subculture and an insulin resistance model was established. After digesting the cells in the logarithmic growth phase, a single cell suspension was prepared using DMEM glycoside medium containing 10% FBS, adjusted to a cell density of 5×10^4 /mL, and inoculated in a 96-well culture plate, 100 μL of cell suspension was added to each well.

In this experiment, normal control group, insulin resistance model group and Taxus polysaccharide group were set up. Normal control group and model group were added with single culture solution. Taxus polysaccharide group was added with different concentrations of serum-free medium of Taxus polysaccharide. All three groups included the physiological insulin group and the physiological insulin-free group. The glucose content in the medium was measured using a glucose assay kit, and the blank wells were used as a control group, and the glucose consumption of each group of cells was calculated.

2.5. Statistical Method

The experimental results of this study were expressed as mean ± standard deviation ( x ± s), using SPSS19.0 for windows statistical software for statistical processing, the comparison between groups was performed by rank sum test, the results between the two groups were compared using t test, P <0.05 was considered statistically significant, and P < 0.01 was considered to be remarkable statistically significant.

3. Result

3.1. Standard Curve Drawing of Crude Polysaccharide Extraction

It has been reported in the literature that Taxus chinensis polysaccharides have hypoglycemic activity in diabetic mice, but there are few studies on the physicochemical properties and pharmacological activities of Taxus polysaccharides. In this study, 1.0 ml of the reference solution was accurately taken, and 1.0 ml of distilled water was used as a blank control, and then 1.0 ml of 5% phenol solution was added, mixed thoroughly, and 5.0 ml of sulfuric acid was quickly added dropwise, and shaken up gently. After placed for 5 min at room temperature, it was heated in a boiling water bath for 15 min. The mixture was quickly taken out and placed in an ice water bath to cool to room temperature, and the absorbance was measured at 487 nm. The concentration of the glucose standard solution is plotted on the abscissa and the absorbance is plotted on the ordinate. A standard curve is drawn and the regression equation is obtained.

3.2. Precision and Stability Experiment

Precision test: accurately take five parts of test solution 1.0ml into a 10 ml test tube with stopper, and use 1.0 ml of distilled water as a blank control to measure the absorbance. As a result, the average absorbance was 0.428, and the RSD value was 1.21%, indicating that the precision meets the requirements. The specific data is shown in Table 1.

<table>
<thead>
<tr>
<th>Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Average value</th>
<th>RSD (%)</th>
</tr>
</thead>
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<td>Absorbance</td>
<td>0.424</td>
<td>0.430</td>
<td>0.419</td>
<td>0.422</td>
<td>0.428</td>
<td>0.425</td>
<td>1.21</td>
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Stability test: in this study, the content of polysaccharides in the leaves of Taxus chinensis was measured by sulfuric acid-phenol colorimetry, which is sensitive and stable. Accurately take 1.0 ml of the test solution in a 10 ml test tube with stopper, 1.0 ml distilled water as a blank control, measure the absorbance according to the method under the standard curve preparation, measure once every 30 min, and the average absorbance is measured to be 0.417, the RSD value is 0.50%. The results showed that after the coloration of phenol-sulfuric acid, the test solution remained stable within 2.5 h. The specific data are shown in Table 2.

<table>
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<tr>
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<th>1</th>
<th>1.5</th>
<th>2</th>
<th>2.5</th>
<th>Average value</th>
<th>RSD (%)</th>
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<tr>
<td>Absorbance</td>
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<td>0.418</td>
<td>0.416</td>
<td>0.417</td>
<td>0.416</td>
<td>0.417</td>
<td>0.50</td>
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3.3. The Dose-Effect Relationship of Taxus Polysaccharides in Improving Glucose Metabolism in Type II Diabetic Mice

In vivo experiments using mice as a model carrier, analysis and comparison of the dose-effect relationship of glucose metabolism in four groups of mice are performed, further verifying the glucose consumption effect of Taxus polysaccharides, in order to achieve the purpose of lowering blood sugar. In Figure 2, the abscissa is the polysaccharide of Taxus at different doses; the doses are 125 mg/kg and 500 mg/kg respectively. The ordinate is fasting blood glucose (FBG), fasting insulin (FINS), and insulin resistance (IR). It can be seen from the figure that the optimal dose for glucose metabolism can be obtained from the effect of the intervention of Taxus polysaccharides in different dose groups on the glucose metabolism index. The serum indexes of the model group, 125 mg/kg dose polysaccharide group and 500 mg/kg dose polysaccharide group were significantly different from the normal group (P<0.05).

![Figure 2. Relationship between different doses of Taxus polysaccharides and blood glucose index](image)
At present, there is little research on the mechanism of hypoglycemic effect of Taxus polysaccharides. The mechanism and target spot of Taxus polysaccharides in regulating Type II diabetes are not clear. At present, studies have shown that the mechanism of polysaccharides in Taxus may be to increase insulin sensitivity in insulin resistance model cells by releasing insulin regulation or altering glucose metabolism and Para-insulin, thereby increasing the utilization of glucose by cells, increasing the activity of glycogen synthase in the liver, and reducing gluconeogenesis in the liver, which in turn increases glucose consumption [13].

3.4. Effect of Taxus Polysaccharide on Pancreas of Type II Diabetic Mice

The liver slices of each group of mice after four weeks of administration are shown in the figures below. The pancreatic tissue of the blank group had clear structure and leafy composition. The exocrine pancreatic epithelial cells had no degeneration or necrosis. The pancreas islet of the endocrine is oval cell clusters. The morphology is regular, the boundaries are clear, the quantity of pancreas islet is relatively high, and the cells in the pancreas islet are evenly distributed. The number is large, as shown in Figure 3(a). In the model group, the pancreas islet structure was disordered and loose, the shape was irregular, and there were different degrees of atrophy. The islet area was obviously smaller and the edge was not neat. The cells in the islets were disordered, the number was significantly reduced, and some cell cytoplasm was vacuolated, as shown in Fig. 3. (b).

(a) Normal group

(b) Model group

(c) 500mg/kg dose polysaccharide group
The pancreas islet structure of the 500mg/kg dose polysaccharide group was disordered and atrophied to different degrees. The pancreas islet area was significantly smaller and the edge was not regular. The cells in the pancreas islets were disordered, the number was significantly reduced, some cells were vacuolated and intercellular space some in areas were increased, as shown in Figure 3(c). In the 125mg/kg dose polysaccharide group, the pancreas islet structure was disordered, irregular in shape, atrophy in different degrees, the pancreas islet area was significantly smaller and the edges were not regular, the cells in the pancreas islets were disordered, the number was significantly reduced, and some cells were vacuolated, as shown in Figure 3 (d).

3.5. In Vitro Hypoglycemic Activity of Taxus Polysaccharides

The results of glucose tolerance test in mice indicate that Taxus polysaccharides can reduce postprandial blood sugar. Taxus polysaccharides promoted the glucose consumption of insulin-resistant HepG2 cells, and the best effect was obtained when the concentration was 0.05 mg/mL. At the same time, according to the MTT results, the doses administered have different degrees of inhibition on HepG2 cells, which means that the glucose consumption of HepG2 cells is not caused by its promotion of cell proliferation. The Taxus polysaccharide still showed good promotion of sugar consumption when the effect of MTT was removed. At the same time, the data in the table also showed that the cells proliferated after establishing the insulin resistance model, and the MTT value was larger than the blank group. The addition of physiological insulin promotes the mild proliferation of cells. After removing the effect of MTT, it is found that physiological insulin has a certain synergistic effect with Taxus polysaccharide.

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>Medici ne (mg/mL)</th>
<th>Add physiologic al insulin (U/mL)</th>
<th>MTT (A490)</th>
<th>GC (mmol/L)</th>
<th>GC/MTT (mmol/L)</th>
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<tr>
<td>Normal group</td>
<td>-</td>
<td>-</td>
<td>0.830±0.1057</td>
<td>1.667±0.0155</td>
<td>2.008±0.0105</td>
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<td>-</td>
<td>0.001</td>
<td>0.0889±0.200</td>
<td>1.918±0.0046</td>
<td>2.152±0.0122</td>
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<tr>
<td>-</td>
<td>0.001</td>
<td>0.910±0.1090</td>
<td>1.402±0.0311*</td>
<td>1.683±0.0107*</td>
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</tr>
<tr>
<td>-</td>
<td>0.001</td>
<td>0.910±0.1090</td>
<td>1.577±0.0217*</td>
<td>1.735±0.0116*</td>
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<tr>
<td>Model group</td>
<td>0.5</td>
<td>-</td>
<td>0.405±0.0170#/∧</td>
<td>1.056±0.0100</td>
<td>2.605±0.0120# ∧</td>
</tr>
<tr>
<td>0.5</td>
<td>0.001</td>
<td>0.508±0.0230#/∧</td>
<td>1.429±0.0217</td>
<td>2.087±0.0302# ∧</td>
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<tr>
<td>0.1</td>
<td>-</td>
<td>0.638±0.0207#/∧</td>
<td>1.740±0.0210</td>
<td>2.728±0.0107# ∧</td>
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<tr>
<td>0.1</td>
<td>0.001</td>
<td>0.676±0.0310#/∧</td>
<td>2.042±0.0260</td>
<td>3.043±0.0188# ∧</td>
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<tr>
<td>Taxus polysaccharide group</td>
<td>0.05</td>
<td>-</td>
<td>0.778±0.0207#/∧</td>
<td>2.153±0.0167# ∧</td>
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<td>0.05</td>
<td>0.001</td>
<td>0.840±0.0117 ∧</td>
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<tr>
<td>0.01</td>
<td>-</td>
<td>0.818±0.0191# ∧</td>
<td>1.980±0.0232# ∧</td>
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<tr>
<td>0.01</td>
<td>0.001</td>
<td>0.874±0.0210</td>
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<tr>
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<td>0.001</td>
<td>0.890±0.0203</td>
<td>4.195±0.125#</td>
<td>2.402±0.0244# ∧</td>
<td></td>
</tr>
</tbody>
</table>

Note: Compared with the normal control group, *P<0.01, compared with the model group without insulin, **P<0.05, compared with the model group with insulin, &P<0.05.
It is currently believed that the two main features of Type II diabetes are insulin resistance and secretion defects of HepG2 cells [14]. Insulin resistance refers to a metabolic state in which the sensitivity and reactivity of peripheral tissues and target organs to the internal and/or exogenous insulin are reduced. It affects insulin signaling throughout the reduction process and can therefore be described as a defect in insulin signaling [15]. The liver is an important metabolic organ in the body. It can not only use glucose, but also produce glucose. Hepatocytes are one of the densest organ cells in the distribution of insulin receptors in the body, and they are extremely sensitive to insulin-promoting glucose consumption. The main use of glucose in the liver is through the synthesis of glycogen, followed by oxidation energy supply. HepG2 cells retain substantially normal hepatocyte activity, and high-affinity insulin receptors on their surface meet the criteria required for typical insulin receptors, including glucose uptake, glycogen synthase activity, lipid production, and RNA synthesis. Insulin resistance is a fundamental problem in the development of Type II diabetes, and it is also closely related to the emergence of other complications.

4. Conclusions

Diabetes is currently recognized as one of the most serious diseases that can be controlled but not curable, affecting global human health. It is characterized by glucose insufficiency and chronic hyperglycemia due to insufficient or relative lack of insulin secretion and insulin resistance, with a group of metabolic disorders such as fat, electrolytes, and proteins. The morbidity and mortality of Type II diabetes are also increasing year by year, and also cause a series of complications: damage to the eyes, kidneys and nerves, as well as micro vessels and great vessels, which promote cardiovascular and cerebrovascular diseases. Epidemiological studies and clinical studies have shown that hyperglycemia is the main cause of complications, and effective control of blood glucose is a key factor in reducing complications and improving patients’ quality of life. Long-term hyperglycemia seriously affects lipid metabolism in HepG2 cells, causing lipid metabolism disorders, so controlling hyperglycemia is a key step in the treatment of diabetes.

In recent years, polysaccharides have been found to be a very natural extract with extremely mild side effects, and have unique curative effects in many diseases, especially for stubborn diseases such as tumors, low immunity, high blood sugar, and aging. Up to now, more than 300 polysaccharide compounds have been isolated from natural products, of which water-soluble polysaccharides extracted from plants, especially from traditional Chinese medicines, are of the most importance. This study found that polysaccharides have hypoglycemic effects in normal mice or mice with hyperglycemia caused by medicine. Previous studies on the mechanism of hypoglycemic effect of polysaccharides have shown that polysaccharides can lower blood sugar by reducing hepatic glycogen, promoting the utilization of sugar by peripheral tissues and organs, regulating the enzymatic activity and hormone activity related to glucose metabolism, and protecting islet cells. The composition of Taxus polysaccharides is relatively complex, and contains not only polysaccharides, but also including proteins, amino acids, etc. It remains to be further studied on the hypoglycemic activity of fine polysaccharides after protein removal.

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