Hypoglycemic Effect of Acidic Polysaccharide from *Schisandra Chinensis* on T2D Rats Induced by High-Fat Diet Combined with STZ

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Abstract

Polysaccharide is a key bioactive component of Schisandra chinensis and has significant pharmacological activities. The aim of this study was to evaluate the anti-diabetic effect of acidic polysaccharide from Schisandra chinensis (SCAP). Type 2 diabetic (T2D) rats were developed by giving a high-fat diet (HFD) combined with low-dose streptozotocin (STZ), and administered orally with SCAP (25, 50 mg/kg) for 8 weeks. Fasting blood glucose (FBG), fasting insulin (FINS), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), malondialdehyde (MDA), and superoxide dismutase (SOD) in the rat’s serum were measured. Oral glucose tolerance test (OGTT) and pathological changes of pancreas were observed. Furthermore, expressions of c-Jun N-terminal kinase (JNK), B-cell lymphoma 2-associated X protein (BAX), B-cell lymphoma 2 (Bcl-2), and Cleaved Caspase-3 in pancreatic islet were detected. The results showed that SCAP decreased FBG, TG, TC, LDL-C and MDA levels, increased insulin, HDL-C levels and SOD activity, improved the pathological changes in pancreatic islet. Furthermore, SCAP inhibited the up-regulation of P-JNK, BAX and Cleaved Caspase-3 proteins, and increased Bcl-2 protein expression. These data indicate that SCAP has a therapeutic effect in T2D rats, and the mechanism may be related to its protection against β-cells apoptosis by regulating apoptosis-related proteins expression to alleviate the injury caused by the oxidative stress.

Key words

Schisandra chinensis; polysaccharide; type 2 diabetes mellitus; oxidative stress
INTRODUCTION

According to the World Health Organization (WHO), at least 171 million people worldwide suffer from diabetes, which is likely to be more than double by 2030 to reach 366 million, and type 2 diabetes mellitus (T2DM) accounts for 90-95% of diabetes.\(^1\)\(^-\)\(^3\) It has been proved that the main way of β-cell destruction in diabetes is apoptosis.\(^4\) While, glucose and lipid toxicity were the important reasons for the increase of apoptosis and decline of function of islet β cells in T2DM\(^5\) When diabetic rats were exposed to high glucose and high fat for a long time, they were in a state of oxidative stress, in which the expression of antioxidant enzymes in pancreas was low, resulting in the increase of reactive oxygen species (ROS), the damage of mitochondria caused by oxidative stress and the expression dysregulation of apoptotic factors, finally the apoptosis of β-cells.\(^6\) Therefore, to inhibit the apoptosis of islet β-cells, protect the function of islet β-cells and maintain a certain level of insulin secretion are the key to the treatment of diabetes.

*Schisandra chinensis*, a Chinese medicinal material collected from Changbai Mountain and a famous tonic Chinese herbal medicine, is listed as a superior medicine in “Shen Nong’s Herbal Classic”, with the function of treating thirst due to insufficiency of body fluid and internal heat dispersion-thirst. The polysaccharide is one of its main active ingredient, and previous studies showed that *Schisandra* polysaccharide could significantly alleviate the symptoms of diabetic mice, such as weight loss, polydipsia, polyuria and hyperglycemia, promote the synthesis of hepatic glycogen, inhibit the decomposition of hepatic glycogen, and also improve the lipid metabolism disorders caused by diabetes.\(^7\)-\(^9\) However, the specific anti-diabetic mechanism of *Schisandra* polysaccharide is not clear, and it has not been reported at home and abroad whether *Schisandra* polysaccharide plays an anti-diabetic role by protecting pancreatic cells and preventing islet cells from the apoptosis. In the present study, type 2 diabetic (T2D) rat model was established by giving high-fat diet combined with intraperitoneal injection of low-dose streptozotocin (STZ) to observe the hypoglycemic effect of *Schisandra chinensis* acidic polysaccharide (SCAP) and its protective effect against the damage of pancreatic islet cells, which was expected to provide a new idea and a theoretical basis for the research and development of the SCAP as an anti-diabetic health food.
MATERIALS AND METHODS

Chemicals and Reagents

_Schisandra chinensis_ was obtained from Jian Schisandra Seedlings Base of Jilin Province and identified by Professor Li-Hua Zhang at College of Pharmacy, Beihua University, according to the identification standards of the Pharmacopoeia of the People's Republic of China (2015 Edition). SCAP was extracted by the Center of Life Science, Northeast Normal University (Changchun, China). In brief, the water-soluble crude polysaccharide component was isolated from the dried fruits of _Schisandra chinensis_ by water extraction and alcohol precipitation method first, with a yield of 8.55%. Then, _Schisandra _polysaccharide was separated by DEAE-sepharose fast flow column chromatography. Neutral polysaccharide could be obtained by the elution with distilled water and SCAP could be prepared by the elution with 0.5 M NaCl solution, with a yield of 4.02% and 2.8%, respectively (the percentage to the dry weight of raw medicinal material). SCAP was a milk white powder, and the analysis of physicochemical properties and monosaccharide composition results was shown in Table 1.

STZ was bought from Sigma-Aldrich (St. Louis, MO, USA). Kits for measuring levels of malondialdehyde (MDA), superoxide dismutase (SOD), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Blood glucose kit was obtained from Biosing Bio-technology and Science Inc (Beijing, China). Insulin radioimmunoassay kit was obtained from Shang Hai Lengton Bioscience Co. Ltd (Shanghai, China). BCA kit was bought from Beyotime Institute of Biotechnology (Jiangsu, China). Polyclonal antibodies of phosphorylated c-Jun N-terminal kinase (P-JNK), c-Jun N-terminal kinase (JNK), B-cell lymphoma 2-associated X protein (BAX), B-cell lymphoma 2 (Bcl-2), Cleaved Caspase-3 and β-actin were purchased from ABclonal Biotechnology (Wuhan, China). Reagents for Western blotting analysis were acquired from Beijing Dingguo Changsheng Biotechnology Co. Ltd (Beijing, China).
Animals

Wistar rats,♂, were purchased from Changchun Yisi Laboratory Animal Technology Co. Ltd. (Changchun, China) [license number: SCXK (JL) 2016-0003, SPF]. The rats were housed at 18-22 °C room temperature, relative humidity 40-60%, with normal lighting conditions. All experiments were performed in rats weighing 180-230 g. The normal diet (ND, AIN-93) and high-fat diet (HFD, AIN-93 adapted) for the experimental rats were obtained from Changchun City Yisi Laboratory Animal Technology Co. Ltd (Changchun, Jilin, China). The major components of ND and HFD were listed in Table 2. All experiments were approved by the Ethics Committee for Use of Experimental Animals at Beihua University (Jilin, China).

Establishment of DM Model in Rats

Ten rats were randomly selected from seventy Wistar rats as normal control group (CON) and fed with the ND, and the remaining sixty rats were fed with a HFD for 4 weeks. Then a T2D model was established by giving freshly prepared STZ (30 mg/kg) in intraperitoneal injection to the rats fed with HFD, and rats in the CON group were injected intraperitoneally with the equal volume of citrate buffer at the same time. One week after the injection of STZ, the serum fasting blood glucose (FBG) level was measured, in which the rats whose FBG levels were higher than 7.0 mmol/L were considered those with T2DM induced by STZ.

Animal Grouping and Administration

The T2D rats were randomly divided into three groups, model group (MOD), 25 mg/kg SCAP-treated group and 50 mg/kg SCAP-treated group, 10 rats in each group. Rats in SCAP-treated groups were administered intragastrically with 25 mg/kg and 50 mg/kg SCAP, once daily successively for 8 weeks.

Oral Glucose Tolerance Test (OGTT)

The glucose tolerance of rats in the different groups was evaluated by a simple OGTT. OGTT was performed on the 8th week after administration, all rats that fasted overnight were
orally fed with glucose solution (2.5 g/kg). Blood samples were collected from the caudal vein by means of a small incision at the end of the tail at 0, 30, 60 and 120 min after the glucose administration. Subsequently, the level of blood glucose was measured by glucose oxidase method. The results were expressed as integrated area under the curve for glucose (AUC glucose), which was calculated by trapezoid rule.

\[
AUC_{\text{glucose}} = \frac{(C_1+C_2)(t_1+t_2)}{2}
\]

Where \(C_1\) and \(C_2\) are concentration of glucose at the different time points, and \(t_1\) and \(t_2\) are the different tested time points.

**Blood and Tissue Sample Collection**

After the last administration, all rats fasted for 12 h, with a free access to water, and then the blood samples were collected through the abdominal aorta after they were anesthetized with ether. The serum was separated by centrifugation at 3500 \(\times\) g for 10 min and stored at \(-80^\circ\)C. The pancreas tissue was carefully isolated and divided into two parts: the first part was fixed with 10% neutral formaldehyde for the histopathological examination, and the second part was preserved at \(-80^\circ\)C for analyzing the mechanism of drug action.

**Detection of Biochemical Indexes in the Serum of Rats**

The FBG in the serum of rats was estimated by a commercially available glucose kit based on the glucose oxidase method. The insulin level was estimated by radio immunoassay. The SOD activity, and MDA, TG, TC, LDL-C and HDL-C levels in the rat’s serum were determined by microplate reader analysis according to manufacturer’s instructions.

**Histomorphological Analysis of Pancreatic Islet**

The pancreatic islet samples were fixed in 10% neutral formalin solution, paraffin-embedded and sectioned. The pancreatic islet sections were stained with hematoxylin-eosin (H&E) for the histomorphological analysis. All sections were observed and imaged under alight microscope (Olympus, Tokyo, Japan).
Western Blotting Analysis

Protein samples from the pancreatic islet were extracted though homogenization with ice-cold lysis buffer containing protease inhibitor cocktail and phosphatase inhibitor for 1 h, and the lysate samples were centrifuged at 12000×g, 4 °C, for 20 min. The supernatants were taken and the protein concentrations were determined by BCA assay (BCA protein assay kit).

P-JNK, JNK, Bcl-2, BAX, Cleaved Caspase-3 and β-actin protein expressions in the supernatants were analyzed by western blot. The denatured protein samples (30-60 μg) were separated by 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and then transferred to Polyvinylidene Flouride (PVDF) membranes (Millipore, USA) at 4 °C. After transfer, the membrane was blocked in 5% non-fat milk for 2 h at room temperature. Subsequently, the blocked membranes were incubated with 1:1000 dilution of P-JNK, JNK, Bcl-2, BAX, Cleaved Caspase-3 and β-actin primary rabbit polyclonal antibodies overnight at 4 °C. The membranes were washed and incubated with the second antibody (1:5000) for 1 h. The protein expression was observed by chemiluminescent imaging system (Protein simple, FluorChem, CA, USA).

Statistical Analysis

Data are presented as mean±S.D. The differences in various groups were analyzed with one-way ANOVA using the GraphPad Prism program. \( P < 0.05 \) was considered statistically significant.

RESULTS

Effects of SCAP on Body Weight, FBG and INS Levels in T2D Rats

As shown in Fig. 1A, body weights of the rats in the CON group and SCAP-treated groups slowly increased from the starting administration, while those in the MOD group did not changed significantly; those in the CON group and SCAP-treated groups were higher than those in the MOD group from the second week after the administration \( (P < 0.05) \).

As shown in Fig. 1B-1C, compared to CON group, the FBG level was elevated and the
INS level was lowered significantly \((P < 0.01)\) in rats’ serum in the MOD group, suggesting that the STZ-induced rat T2D model is successfully established. After the administration of SCAP for 8 weeks, the FBG level decreased and the INS level increased significantly in the T2D rats \((P < 0.01)\), indicating that SCAP should have a therapeutic effect on T2DM in rats (Fig. 1).

**Effects of SCAP on the MDA Level and SOD Activity in the Serum of T2D Rats**

As shown in Fig. 2, the serum MDA level was increased significantly, while the SOD activity was decreased markedly \((P < 0.01)\) in rats of MOD group compared to CON group; the serum MDA level decreased \((P < 0.01)\) and the SOD activity elevated significantly in SCAP-treated groups \((P < 0.01)\).

**Effects of SCAP on TG, TC, HDL-C and LDL-C Levels in the Serum of T2D Rats**

The lipid profiles of each group are shown in Fig. 3. Compared to CON group, the TC, TG and LDL-C levels were higher, while the HDL-C levels were lower significantly in the serum of rats in the MOD group. The TG, TC and LDL-C levels decreased \((P < 0.05 \text{ or } P < 0.01)\), and the HDL-C level increased \((P < 0.05 \text{ or } P < 0.01)\) significantly in the serum of T2D rats in SCAP-treated groups. These data indicated that SCAP could effectively ameliorate the lipid metabolism in T2D rats.

**Effects of SCAP on the Oral Glucose Tolerance in T2D Rats**

As shown in Fig. 4, the blood glucose of rats in the CON group and SCAP group reached the peak at 30 min after the glucose administration. During the following 90 min, the blood glucose level decreased gradually and returned to normal level of rats in the CON group at 120 min after the injection, while the blood glucose level decreased slowly, and was maintained at a higher level in the MOD group (Fig. 4A) than that in the CON group. Furthermore, the AUC glucose value in the MOD group was significantly higher than that in the CON group, and the AUC glucose value in the SCAP-treated groups was significantly lower than that in the MOD group (Fig. 4B). The results showed that the glucose tolerance of
rats decreased and the insulin resistance could be found in the MOD group, while SCAP treatment partially restored the glucose tolerance and improved the insulin resistance in T2D rats.

**Effects of SCAP on the Pathomorphology of Pancreatic Tissue in T2D Rats**

The pathological results showed that the pancreatic islets were regular and round or elliptical in shape, with clear boundary, the β-cells were uniformly distributed closely arranged in the islets, and the cytoplasm was abundant in the CON group. While in the MOD group, the number of islets decreased, the area of islets became smaller, the islets shrank significantly, the structures were not clear and the edges were irregular, and the number of islet β-cells in the islets decreased significantly and the β-cells exhibited a sparse arrangement. After SCAP treatment, the islet boundary became clear, the area increased, the number of β-cells increased significantly, and the cell morphology tended to be intact in pancreatic tissue of T2D rats (Fig. 5).

**Effects of SCAP on the Expression of Proteins**

To further confirm whether the SCAP-mediated increase in FINS in T2D rats was due to the protective effects of SCAP on islet tissue survival, the expression levels of pro-apoptotic factor BAX, anti-apoptotic factor Bcl-2, Cleaved Caspase-3, JNK and phosphorylated JNK (P-JNK) were examined in pancreatic islets isolated from rats in various group. As shown in Fig. 6, compared to CON group, the ratio of BAX/Bcl-2 and Cleaved Caspase-3 protein expression were significantly increased in islets from T2D rats ($P < 0.01$). The treatment with SCAP significantly reduced the ratio of BAX/Bcl-2 and protein expression of Cleaved Caspase-3 compared to MOD group ($P < 0.05$ or $P < 0.01$). Moreover, the SCAP treatment inhibited P-JNK protein expression in the islets of T2D rats (Fig. 6B) ($P < 0.05$ or $P < 0.01$). The results above indicated that SCAP treatment might alleviate the apoptosis of islet tissue in T2D rats through inhibiting the JNK signal pathway.
DISCUSSION

*Schisandra chinensis*, a traditional Chinese medicine, has a history of thousands of years. The *Schisandra chinensis* used in this study is considered the best one because it was collected from Changbai Mountain, Jilin province, China. Polysaccharide is the major active component of *Schisandra chinensis*, and has shown some obvious effects and less toxicity, with a great development value.\(^{11-13}\) *Schisandra chinensis* polysaccharide has been reported to have a hypoglycemic effect on diabetic rat induced by STZ through antioxidant action.\(^{14}\) The results in this study also showed that SCAP could significantly reduce FBG, the serum TC, TG, LDL-C and MDA content, elevate the insulin level, HDL-C content and the SOD activity, and further improve the pathological changes of islets in T2D rats. SCAP treatment also prevented the body weight from excessive decrease in T2D rats. These data indicated that SCAP has a good therapeutic effect on the diabetes in rats.

The rat T2D model in this study was established by giving high-fat diet combined with a single intraperitoneal injection of low-dose STZ. STZ, an alkylating agent with nitroso-group, can produce a specific toxicity to islet β-cells by selectively destroying them by oxidative stress and alkylation reaction, thereby inducing the apoptosis of islet β-cells,\(^{15, 16}\) so it is widely used to establish a model of diabetes.\(^{17}\) Both in vitro and in vivo studies have shown that STZ can promote the expression of Caspase-8, Caspase-9 and Cleaved Caspase-3 proteins, and increase the ratio of BAX/Bcl-2 in islet cells to induce the apoptosis of β-cells.\(^{18-20}\) In order to investigate the protective effect of SCAP on islet cells, we detected the pro-apoptotic protein BAX and anti-apoptotic protein Bcl-2 in Bcl-2 family members, as well as Cleaved Caspase-3. The results showed that SCAP could decrease the ratio of BAX/Bcl-2 and the expression of Cleaved Caspase-3, suggesting that SCAP has an anti-apoptotic effect.

In order to further explore the mechanism through which SCAP could inhibit the apoptosis of islet cells, we also examined the expression of JNK protein. JNK, one of the members of mitogen-activated protein kinase (MAPK), is involved in the regulation of cell proliferation, differentiation and apoptosis.\(^{21, 22}\) One of the important mechanisms to induce the apoptosis of β-cells in diabetes is that ROS causes the apoptosis of cells by activating
JNK, and then inhibiting the expression of anti-apoptotic protein and promoting the expression of the pro-apoptotic protein in Bcl-2 family. This results in the change of cell membrane permeability to induce the release of Cytochrome C, further leads to the Caspase cascade reaction. Therefore, to regulate the activity of the JNK signal and inhibit the excessive apoptosis and necrosis of the islet cells contribute to the protection against the damage of islet $\beta$-cells in diabetes. Our results in this study showed that with the increase in SCAP concentrations, the expression level of P-JNK protein decreased, suggesting that SCAP might inhibit the apoptosis of pancreatic islet cells by regulating the activity of JNK signaling pathway.

In conclusion, SCAP has a significant preventive and therapeutic effect on rat T2DM induced by high-fat diet combined with STZ, and inhibit the excessive apoptosis of islet cells by regulating the activity of JNK signal, decreasing the ratio of BAX/Bcl-2 and down-regulating the expression of Cleaved Caspase-3, thereby increasing insulin secretion to lower the blood glucose, which may provide a theoretical basis for the further application of *Schisandra chinensis* in the prevention and treatment of diabetes.

**Acknowledgements**

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**Conflict of Interest**

The authors declare no conflict of interest.

**Supplementary Materials**

The online version of this article contains supplementary materials.
REFERENCES


Fig. 1 Effects of SCAP on Serum Fasting Blood Glucose (FBG) and Fasting Insulin (FINS) Levels. A. serum FBG level; B. serum FINS level. Rats in SCAP groups were administered SCAP at 25, 50 mg/kg/day for 8 weeks. Data are expressed as means ± SD (n=10). Between-group differences were assessed using one-way ANOVA with post hoc test. *P < 0.05, **P < 0.01 vs. CON group, #P < 0.05, ##P < 0.01 vs. MOD group.
**Fig. 2 Effects of SCAP on Serum MDA Level and SOD Activity.** A. MDA level in serum; B. SOD activity in serum. Data are expressed as means ± SD (n=10). *P < 0.05, **P < 0.01 vs. CON group, #P < 0.05, ##P < 0.01 vs. MOD group.
Fig. 3 Effects of SCAP on Blood Lipid Profile. A. TG level in serum; B. TC level in serum; C. LDL-C level in serum; D. HDL-C level in serum. Data are expressed as means ± SD ($n=10$). *$P < 0.05$, **$P < 0.01$ vs. CON group, #$P < 0.05$, ##$P < 0.01$ vs. MOD group.
Fig. 4 Effects of SCAP on Oral Glucose Tolerance Test (OGTT). A. Oral glucose tolerance test curve of each group; B. Area under the curve of OGTT in each group. Data are expressed as means ± SD (n=10). *$P < 0.05$, **$P < 0.01$ vs. CON group, #$P < 0.05$, ##$P < 0.01$ vs. MOD group.
Fig. 5 Effects of SCAP on the Pathomorphology of Pancreatic Tissue in Rrat.
H&E stained sections of pancreatic tissue (×200)
(Color figure can be accessed in the online version.)
Fig. 6 Effects of SCAP on Protein Expression of P-JNK, JNK, BAX, Bcl-2, and Cleaved Caspase-3. A & C. The protein expression of BAX, Bcl-2, Cleaved Caspase-3 and β-actin. B & D. The protein expression of P-JNK, JNK. All data are expressed as mean (±SD) of three independent experiments. *P < 0.05, **P < 0.01 vs. CON group, ##P < 0.01 vs. MOD group.
### Table 1

Chemical composition of SCAP.

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<th>Total sugar (%)</th>
<th>Uronic acid (%)</th>
<th>Monosaccharide components (mol %)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>GalA</td>
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<td>SCAP</td>
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<td>52.5</td>
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Table 2

Composition of experimental diet ND and HFD (AIN-93 modified), maintenance (M)

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<tr>
<td>Cholesterol(%)</td>
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