Effect of sophora japonica total flavonoids on pancreas, kidney tissue morphology of streptozotocin-induced diabetic mice model

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Abstract To observe effect of sophora japonica total flavonoids on pancreas, kidney tissue morphology of streptozotocin-induced diabetic mice model. Mice received tail vein injection of streptozotocin (60 mg/kg) for diabetes modeling. The model mice were divided into five groups, to be respectively fed with high, middle and small doses of sophora japonica total flavonoids solution, metformin solution and saline of the same volume. Another blank control group was set to be fed with saline of the same volume. The mice were administered once a day for 30 consecutive days, to be euthanatized after fasting blood glucose level testing on 30th day with pancreas, kidney taken out for pathological section and microscopic examination. The mice chain streptozotocin diabetes modeling was successful, with significant pathological changes (P < 0.01) in pancreas, kidney. Compared with model group, high, middle and small doses of sophora japonica total flavonoids could significantly alleviate streptozotocin-induced pancreas, kidney damage (P < 0.01). Conclusion: Sophora japonica total flavonoids can effectively alleviate pancreas, kidney injury of streptozotocin-induced diabetic mice model.

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1. Introduction

Sophora japonica, as flower and bud of Sophora japonica L., is bitter, slightly cold, which can cool blood and stop bleeding, clear liver fire, so many doctors choose it as one of the traditional Chinese medicines for treatment of diabetes. Streptozotocin can establish a good diabetes model for related research. It has been previously reported that sophora japonica has a good hypoglycemic effect on a variety of diabetic animal models. This paper focuses on effect of sophora japonica total
flavonoids on pancreas, kidney tissue morphology of streptozotocin-induced diabetic mice model.

2. Materials and methods

2.1. Experimental materials

2.1.1. Pharmaceutical agents

Sophora japonica total flavonoids (provided by Chinese Medicine Development Engineering Research Center in Henan Province); streptozotocin, produced by Sigma Company; metformin hydrochloride, produced by Shanghai Pharmaceutical (Group) Co., Ltd. Xinyi Pharmaceutical Factory; saline, produced by Zhengzhou Yonghe Pharmaceutical Co., Ltd; concentrated sulfuric acid, produced by Luoyang Chemical Reagent Factory; citric acid (AR), Hubei Pharmaceutical Company Chemical Glass Station; sodium citrate (AR), Tianjin Chemical Reagent Wholesale Unit; glucose kit, produced by Zhejiang Dongou Biological Engineering Co., Ltd.

2.1.2. Experimental animals

Mice: Wistar, clean grade, male, weighing 180–200 g; provided by Experimental Animal Center of Hebei Province, Certificate No. 701022.

2.1.3. Experimental equipment

UV-2000 UV–Vis spectrophotometer, produced by UNICO (Shanghai) Instrument Co., Ltd; AL204 series electronic balance, Beijing Rayleigh Analytical Instrument Co., Ltd; adjustable pipette (OLYMPUS BX61), Shanghai Labsystems Analytical Instrument Co., Ltd.

2.1.4. Solution preparation

Preparation of citric acid buffer solution: Weigh 1292.4 mg citric acid and 1132.3 mg sodium citrate to prepare 100 ml solution. The solution is citric acid buffer solution with pH 4.2 pH instrument calibration preparation can be employed to ensure its accuracy. (Streptozotocin is not very stable, with greater stimulation to blood vessels, while buffer preparation of streptozotocin can reduce these negative impacts); prepare before use. Preparation of 10% formalin fixation: 36% formaldehyde and sliced to observe ultrastructural changes via TEM.

Preparation of 4% glacial aldehyde fixation: Dilute disodium hydrogen phosphate 35.61 g/100 ml with double distilled water; Dilute sodium dihydrogen phosphate 27.60 g/100 ml with double distilled water. Blend disodium hydrogen phosphate solution 40.5 ml and sodium dihydrogen phosphate solution 9.5 ml, to be added with 25% glutaraldehyde 16.0 ml and double distilled water to 100 ml.

2.1.5. Statistical treatment

Data were analyzed by SPSS 17.0 for windows statistical software, one-way ANOVA was employed for measurement data comparison between groups and Ridit analysis was employed for ranked data.

2.2. Experimental methods

Take 100 mice, after fasting 12 h, 90 of which received tail vein injection of streptozotocin 60 mg/kg (prepared by pH 4.2 citric acid buffer solution). The remaining 10 served as completely blank control group to be received with tail vein injection of citric acid buffer solution of an equal volume. On 10th day, take blood from tail to test blood glucose level. Select 50 mice with blood glucose level > 11.1 mmol/L and significant polydipsia, polyphagia, diuresis symptoms, to be randomly divided into five groups according to blood glucose level and fed with high, middle, small doses of sophora japonica total flavonoids (600 mg/kg, 300 mg/kg, 150 mg/kg, with concentration at 30 mg/ml, 15 mg/ml, 7.5 mg/ml, gavage volume at 2 ml/100 g) solution, metformin tablet 208 mg/kg (20.8 mg/ml, 2 ml/100 g). Model group and control group received the same volume of saline, to be administered once a day for 30 consecutive days with blood glucose measured on 10, 20, 30th days. The results are shown in the literature [2]. The mice were euthanatized then with pancreas, kidney taken and fixed with formalin. After it was sliced, observe effect on tissue morphology via microscope; pancreas was fixed with glutaraldehyde and sliced to observe ultrastructural changes via TEM.

3. Results

3.1. Effect on pancreas, kidney tissue morphology of streptozotocin-induced diabetic mice model

Take pancreas and kidneys of mice to be fixed with formalin, sliced and stained for microscopic observation. Results of pathological change in pancreas of mice are shown in Table 1, the mice pancreas pathology photographs are shown. As can be seen from Table 1, by Ridit test, compared with the control group, the model group pancreas showed significant pathological changes ($P < 0.01$), indicating that model mice suffered...
Table 2 Effect of sophora japonica total flavonoids on mitochondria volume density ($V_v$), membrane density ($\delta_m$), endoplasmic reticulum membrane density ($\delta_{em}$) of islet cell of diabetic mice model ($\mu m^3$, $\mu m^2$).

| Group                              | n  | $V_v$          | $\delta_m$      | Endoplasmic reticulum $\delta_{em}$ |
|------------------------------------|----|----------------|-----------------|-------------------------------------|
| Blank group                        | 30 | $17.28 \pm 4.36$ ** | $26.42 \pm 3.12$ ** | $47.62 \pm 3.51$ **             |
| Model group                        | 30 | $10.62 \pm 3.28$   | $12.63 \pm 2.43$ | $32.18 \pm 4.22$                  |
| Metformin group                    | 30 | $18.72 \pm 4.16$ ** | $25.18 \pm 3.50$ ** | $38.27 \pm 2.36$ **             |
| High dose sophora japonica total flavonoids group | 30 | $17.19 \pm 2.60$ ** | $22.68 \pm 3.04$ ** | $45.08 \pm 4.36$ **             |
| Middle dose sophora japonica total flavonoids group | 30 | $18.26 \pm 4.27$ ** | $23.12 \pm 2.36$ ** | $36.17 \pm 2.18$ **             |
| Small dose sophora japonica total flavonoids group | 30 | $17.03 \pm 2.21$ ** | $16.58 \pm 2.62$ ** | $34.32 \pm 2.76$ **             |

Note: Compared with model group.

* $P < 0.05$

** $P < 0.01$

Table 3 Effect of sophora japonica total flavonoids on kidney tissue morphology of streptozotocin-induced diabetic mice model.

| Group                              | Number of animals | Dose (g/kg) | – | + | ++ | +++ |
|------------------------------------|------------------|-------------|---|---|----|-----|
| Blank control group                | 10               | –           | 10| 0 | 0  | 0   |
| Model group                        | 10               | 0.25        | 0 | 0 | 2  | 8   |
| Metformin group                    | 10               | 0.6         | 0 | 0 | 9  | 1   |
| High dose sophora japonica total flavonoids group | 10 | 0.3         | 0 | 0 | 7  | 3   |
| Middle dose sophora japonica total flavonoids group | 10 | 0.15   | 0 | 0 | 4  | 6   |

Note: “–” means that glomerulus, renal capsule, tubular epithelial cells were normal; “+” means that glomerular cell had slight proliferation, a small part of renal capsule was expanded, parietal cells were flat, a small part of renal tubular epithelium had edema; “++” means that 25% glomerular cell had proliferation, a small part of renal capsule was expanded, parietal cells were flat, part of renal tubular epithelium showed edema and vacuolation; “+++” means that 50% glomerular cell had proliferation, 75% renal capsule was expanded, parietal cells were flat, most renal tubular epithelium showed vacuolation.

Figure 1 The rat pancreas pathological morphology.
from significant damage to the pancreas. Based on comparison with model group, high, and middle doses of sophora japonica total flavonoids group can significantly reduce pathological damage to the pancreas ($P < 0.01$). The results showed that: High and middle doses of sophora japonica total flavonoids and metformin have better protection for pancreas of streptozotocin-induced diabetic mice model.

Mice pancreatic tissue morphology in each group: Control group of mice had islet cells with rich cytoplasm, larger cell body and dispersedly distributed cell nucleus; model group mice had significantly shrunk islet cells, with decreased cytoplasm, shrunk cell body and dense cell nucleus. Some cells had edema and vacuolar degeneration; a small fraction of islet cells of metformin group mice were significantly restored, with rich cytoplasm and increased cell body. Most cells shrank, with reduced cytoplasm, shrunk cell body and dense cell; most cells of mice pancreatic tissue in high dose sophora japonica total flavonoids group were restored, with rich cytoplasm and increased cell body. A small number of cells shrank, with disperse cell nucleus; some cells of mice pancreatic tissue in middle dose sophora japonica total flavonoids group shrank, partially restored with rich cytoplasm, increased cell body; islet cells of mice pancreatic tissue in small dose sophora japonica total flavonoids group had apparent atrophy, with shrunk cell volume, significantly reduced cytoplasm and dense cell nucleus. Individual cells had rich cytoplasm. The results showed that: high dose of sophora japonica total flavonoids can protect streptozotocin-induced islet with effect greater than the metformin group.

Submicroscopic structure of mice islet cells in each group: For mice islet cells of blank control group, mitochondria and mitochondrial cristae were in neat dense arrangement, without expansion in intercristal space; endoplasmic reticulum was in neat and dense arrangement, without expansion, vesicle. For mice islet cells of model group, mitochondria was significantly decreased, mitochondrial cristae significantly shortened or disappeared with intercristal space in vacuolization; endoplasmic reticulum was disorganized in expansion or vesicle shape. For metformin group, mitochondria in islet cells were increased, with most mitochondrial cristae in neat arrangement and partial disordered. Cristae were shorter or in vacuolization; endoplasmic reticulum was disorganized with endoplasmic reticulum expansion or in vesicle shape. For mice islet cells of high dose sophora japonica total flavonoids group, mitochondria was increased, with part mitochondrial cristae neatly arranged and part cristae shortened or in vacuolization; endoplasmic reticulum was neatly and densely arranged, with a small portion of endoplasmic reticulum expanded.

For mice islet cells of middle dose sophora japonica total flavonoids group, mitochondria was increased, a small part of mitochondrial cristae was disordered, with part cristae shortened or in vacuolization; endoplasmic reticulum was disorganized, in expansion or vesicular shape. For mice islet cells of small dose sophora japonica total flavonoids group, mito-
chondria was increased, a small part of mitochondrial cristae was disordered, with part cristae shortened or in vacuolization; endoplasmic reticulum was disorganized, mostly in expansion or vesicular shape. The results showed that: each sophora japonica total flavonoids group can make mice islet cell cytoplasm gradually rich, increase the cell body with cell nucleus slowly into disperse distribution.

With three-dimensional metrology point analysis, determine mitochondrial volume density ($V_v$) membrane density ($\delta_m$) and endoplasmic reticulum membrane density ($\delta_m$), with the measurement results shown in Table 2.

Table 2 shows that, compared with the blank group, mitochondria volume density $V_v$, membrane density $\delta_m$ and endoplasmic reticulum membrane density $\delta_m$ of islet cell in model group were significantly reduced ($P < 0.01$). Compared with model group, mitochondria volume density, endoplasmic reticulum membrane density, membrane density of streptozotocin-induced islet cell of high, middle dose sophora japonica total flavonoids group and metformin group were significantly or obviously increased ($P < 0.01$), and mitochondria volume density, endoplasmic reticulum membrane density of streptozotocin-induced islet cell of small dose sophora japonica total flavonoids group were increased ($P < 0.01$), membrane density was increased ($P < 0.05$), with endoplasmic reticulum and mitochondria richer compared with the model group, indicating from microscopic point of view that sophora japonica total flavonoids has a protective effect on islet cells (Table 3).

3.2. Effect on kidney tissue morphology of streptozotocin-induced diabetic mice model

Take kidneys of mice to be fixed with formalin, sliced and stained for microscopic observation. Results of pathological change in kidney of mice are shown in Table 2, the mice kidney pathology photographs are shown in Fig. 1 and pancreatic tissue ultramicro photos are shown in Fig. 2.

As can be seen from the table, by Ridit test, compared with the blank control group, the model group kidney showed significant pathological changes ($P < 0.01$), indicating that model group mice showed significant kidney damage. Based on comparison with model group, high and middle doses of sophora japonica total flavonoids group and metformin group can significantly reduce pathological damage to the kidney ($P < 0.01$). The results showed that: High and middle doses of sophora japonica total flavonoids and metformin have better protection for kidney of streptozotocin-induced diabetic mice model (see Fig. 3).

Mice kidney tissue morphology in each group: For blank control group, glomerulus, renal capsule and tubular epithelial cells in mice kidney were normal; for model group, glomerular endothelial cells and mesentery in mice kidney

![Figure 3](image-url)  Endoplasmic reticulum.
had slight proliferation, capsular space was expanded, pari-
etal cells were flat, and renal tubular epithelium showed vac-
ruolation; for metformin group, glomerular endothelial cell
and mesangial cell in mice kidney were significantly reduced,
most capsular space was not expanded, only a small portion
of parietal cells was flat, tubular epithelial cells were also
recovered to some extent, and a few cells showed vacuolation;
for high dose sophora japonica total flavonoids group, part
of glomerular endothelial cells and mesangial cells in mice
kidney was suppressed, most capsular space was not
expanded, a small part was expanded, parietal cells were flat,
and tubular epithelial cell had slight edema with a small part
showing vacuolation; for middle dose sophora japonica total
flavonoids group, some glomerular cells in mice kidney had
proliferation, part capsular space was expanded, parietal cells
were flat, part tubular epithelial cells had edema, and a few
cells showed vacuolation; for small dose sophora japonica
total flavonoids group, glomerular cells in mice kidney had
obvious proliferation, capsular space was expanded, parietal
cells were flat, tubular epithelial cells had edema, and a few
cells showed vacuolation. The results showed that: for
sophora japonica total flavonoids group in different doses,
glomerular endothelial cells and mesangial cells in mice kid-
ney were partially suppressed, capsular space gradually
turned to non-expansion, parietal cells were flat and tubular
epithelial cell edema became smaller (see Fig. 4).

4. Discussion

In the Chinese medicine view, diabetes (DM) belongs to con-
sumptive thirst as a disorder characterized by polydipsia,
polyphagia, polyuria, body weight loss, or turbid urine, sweet
urine. But according to modern medicine, it is regarded as a
chronic metabolic disease (Zhang et al., 2011) caused by a vari-
ety of reasons of the pancreas as the major lesion. And dia-
betes can easily cause kidney disease. Common
complications of diabetes is diabetic nephropathy and diabetic
secondary infection (Miao et al., 2014a,b,c), which further
demonstrates that diabetes can easily cause kidney disease,
and may also lead to abnormal immune function (Li and
Liu, 2010).

Studies have shown that (Arya et al., 2015), the volume size
of pancreas islet, intensity of cell nucleus as well as cytoplasm
richness can reflect function of islet cells, as the stronger insulin
generation function, the bigger islet volume, the more and
richer cytoplasm; when islet cell function decreases, cytoplasm
significantly reduces and cell nucleus becomes very dense. Part
pancreatic islet cells of diabetic mice model showed significant
atrophy, with cytoplasm decreased obviously, cell body shrank
and cell nucleus dense, while some cells showed edema and
vacuolation; in high, middle dose sophora japonica total flavo-
noids group, some damaged islet cells were restored with rich
cytoplasm and increased cell body, and some cells were in
shrinking state with sparse cell nucleus, suggesting that pancreatic function was partially restored. Mitochondrion in islet cells of diabetic mice model was significantly reduced, mitochondrial cristae significantly shortened or disappeared, with intercristal space showing vacuolization, and endoplasmic reticulum was disorganized in expansion or vesicle shape; high, middle dose of sophora japonica total flavonoids can make mitochondria in mice islet cells increased. Part mitochondrial cristae was neatly arranged, while part cristae shortened or showed vacuolization. Endoplasmic reticulum was neatly and densely arranged, while a small portion of endoplasmic reticulum was expanded, suggesting that pancreas energy metabolism was partially restored with pancreatic secretion partially restored.

5. Results

Kidney of streptozotocin-induced diabetic mice model showed significant pathological changes, with obvious damage in tubular epithelial cells (Dai, 2015). Apparent kidney damage also appeared in this experiment. In kidney of mice model, glomerular endothelial cells and mesangial cells had slight proliferation, capsular space was expanded, parietal cells were flat, tubular epithelial cells showed vacuolation; part of glomerular endothelial cells and mesangial cells in mice kidney of high, middle dose sophora japonica total flavonoids group were suppressed, part capsular space was expanded, while some were not, parietal cells were flat, tubular epithelial cell had slight edema, and part showed vacuolation, which significantly reduced kidney damage.

Sophora japonica total flavonoids can effectively alleviate streptozotocin-induced diabetic model, which can significantly improve a variety of life indicators, prevent pancreas, kidney pathological changes of streptozotocin-induced diabetic mice model and significantly improve ultrastructure of the pancreas. This study further confirmed hypoglycemic characteristic of sophora japonica total flavonoids from morphology, which lays the foundation for further development of sophora japonica.

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