

Effects of mulberry leaf polysaccharide on oxidative stress in pancreatic β -cells of type 2 diabetic rats

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Abstract. – OBJECTIVE: To explore and discuss the effects and mechanisms of mulberry leaf polysaccharide (MLP) on oxidative stress in pancreatic β -cells of type 2 diabetic rats.

MATERIALS AND METHODS: The model of diabetic rats was established by inducing the Sprague-Dawley (SD) rats with high-sugar and high-fat diet for 6 weeks and then giving them streptozotocin (STZ) by single intraperitoneal injection. The mulberry leaf polysaccharide was administered via gavage daily for 8 weeks, and the tissue morphology was observed through electron microscopy. The levels of fasting blood glucose (FBG), free fatty acid (FFA), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were detected. The content of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) in the pancreas were tested. The activities of mitochondrial cytochrome C oxidase (CCO) and succinate dehydrogenase (SDH) in the pancreatic cell were examined.

RESULTS: Compared with the normal control group, the level of FBG increased ($p < 0.01$), the levels of FFA, TG, LDL-C, IL-6, and TNF- α were higher ($p < 0.05$), the content of MDA increased ($p < 0.05$) and the activity of SOD, CCO and SDH declined ($p < 0.05$) in the model group. In comparison with the model group, the level of FBG in the group intervened by mulberry leaf polysaccharide decreased [23.9 ± 2.5 vs. 16.8 ± 2.1 mmol·L⁻¹]; the levels of FFA, TG and LDL-C dropped from (1.18 ± 0.24), (2.95 ± 0.65) and (2.18 ± 0.46) mmol·L⁻¹ to (0.65 ± 0.14), (2.20 ± 0.45) and (1.08 ± 0.42) mmol·L⁻¹, respectively; the levels of IL-6 and TNF- α declined from (60.78 ± 5.02) ng·L⁻¹ and (2.34 ± 0.42) μ g·L⁻¹ to (30.94 ± 3.02) ng·L⁻¹ and (1.02 ± 0.12) μ g·L⁻¹, respectively; the content of MDA was reduced from (1.38 ± 0.21) μ mol·g⁻¹ to (0.78 ± 0.12) μ mol·g⁻¹; the activities of SOD, CCO and SDH increased from (25.32 ± 3.58) KU·g⁻¹, (15.00 ± 1.58) mmol·g⁻¹ and (3.23 ± 0.32) KU·g⁻¹ to (32.87 ± 2.62) KU·g⁻¹, (18.30 ± 1.78) mmol·g⁻¹ and (5.62 ± 0.38) KU·g⁻¹, respectively; the electron microscopy results indicated that the intervention of mulberry leaf polysaccharide could improve the morphological structure of the pancreatic β -cells.

CONCLUSIONS: The mulberry leaf polysaccharide can lower down the levels of inflammatory mediators and free fatty acid in the diabetic rats, alleviate oxidative stress injury, improve the mitochondrial functions of islet cells and protect the pancreatic β -cells.

Key Words:

Mulberry leaf polysaccharide, Type 2 diabetes mellitus, Pancreatic β -cells, Oxidative stress.

Introduction

Diabetes Mellitus (DM) is a syndrome of endocrine metabolic disorder caused by genetic and environmental factors together, which has become the third serious disease threatening the health of human after cardiovascular and cerebrovascular diseases and cancers in the world¹. A high percent of the DM patients are diagnosed with type 2 diabetes mellitus². Some studies have shown that oxidative stress is an important reason for the occurrence and development of diabetes mellitus³⁻⁵. Oxidative stress refers to imbalance between production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and elimination of antioxidant defense system in the body, which leads to an excessive generation of ROS and RNS, and damages the protein and nucleic acid in the organism^{6,7}. In addition to disturbing the signal transduction of insulin and inducing insulin resistance, the oxidative stress can injure the pancreatic β -cells, leading to decrease of insulin secretion and apoptosis of the β -cells^{8,9}. Therefore, reducing the level of oxidative stress in the diabetic organism is an important approach to treat diabetes mellitus.

As fodder for *bombyx mori*, the mulberry leaves can also be used as a kind of drug which has been applied in clinical treatment of diabetes since ancient times. Polysaccharide is a vital

active component of the mulberry leaf and it is proved by modern pharmacological studies that it has significant hypoglycemic effects. During prophase studies, it was found that mulberry leaf polysaccharide could improve glucose metabolism as well as insulin resistance and promote insulin secretion¹⁰⁻¹². It is also discovered that mulberry leaf polysaccharide (MLP) has a distinct antioxidant activity while its mechanism still remains unknown^{13,14}. This research attempted to observe whether MLP possessed protective effects on oxidative stress of the islet cells of type 2 diabetic rats and to further explore and discuss its possible mechanisms by establishing the type 2 diabetic rat model, giving MLP intervention and detecting oxidative stress and other indexes.

Materials and Methods

Model Establishment, Animal Grouping and Specimen Collection

This study was approved by the Animal Ethics Committee of Ankang Center Hospital Animal Center. 32 SD male rats aged 6-8 weeks and weighed 160-180 g were randomly divided into normal control group (n=8), diabetic model group (n=12) and MLP treatment group (n=12) after they were fed adaptively for 1 week. The rats in the diabetic model group and MLP treatment group were deprived of food but not water for 12 h after they had been fed with high-sugar and high-fat diet for 6 weeks. Then, the rats were administered with STZ (35 mg·kg⁻¹), which was prepared by fresh citrate buffer solution (pH 4.5), by intraperitoneal injection. The fasting blood glucose was measured 3-7 d later; if the blood glucose level was >13 mmol·L⁻¹, it indicated that the type 2 diabetic rat model was established successfully; the success rate was about 70%. The rats in the diabetic model group and MLP treatment group were continuously fed with high-sugar and high-fat diet every day. The irrigation dosage in the MLP treatment group was calculated at 100 mg/kg which was dissolved as 6 mg/mL solution and administered by gavage. The irrigation dosages in the normal control group and the diabetic model group were calculated in the same way, but the normal saline was utilized instead; the time of administration was 8 weeks. At the end of the experiment, the rats were abstained from food except water for over 12 h, and then they were anesthetized

by intraperitoneal injection of 2% pentobarbital 35 mg·kg⁻¹. The FBG were measured through the tail veins; the blood samples were obtained at the hepatic portal veins to detect the levels of FFA, TG, LDL-C and inflammatory factors (IL-6 and TNF- α) after the rats were sacrificed by disarticulating the cervical spine.

Observation of Pancreatic β -cells Through Transmission Electron Microscope

The fresh pancreatic tissues were fixed in 2.5% glutaraldehyde, dehydrated with conventional PSB (Statistical Package for Social Science) and acetone of gradient concentrations, embedded in Epon 812, sliced with LKB-V type microtome and stained with lead and uranium. The photographs were observed via the Hitachi H-7500 transmission electron microscope (Hitachi Ltd, Tokyo, Japan).

Measurement of Relevant Serum Biochemical Indexes

The glucose oxidase-peroxidase antiperoxidase (GOD-PAP) method was used to test the glucose. The levels of free fatty acids (FFA), TG, LDL-C, IL-6, and TNF- α were measured according to the standards in the instructions of the kits.

Extraction of Pancreatic Mitochondria and Preparation of Pancreatic Tissue Homogenate

The pancreatic tissues were weighed and then put into a 10 mL beaker. The tissue was halved by ophthalmic scissors, one half of which was cut into pieces as soon as possible. The ice-cold homogenate transmitter was measured and taken with pipettes (Tris-HCl 0.1 mol·L⁻¹, pH 7.4, KCl 1 mmol·L⁻¹ and sucrose 0.25 mol·L⁻¹), of which the volume was 9 times of the weight of the obtained tissue blocks. And then it was made into 10% tissue homogenate and centrifuged by low speed centrifuge 448 \times g for 10 min at low temperature (4°C). The supernatant was taken and centrifuged by high speed centrifuge 11200 \times g for 15 min (4°C). The precipitate which was mitochondrion was obtained and made into standby mitochondrial suspension by adding 0.5 mL mixture (TrisHCl 0.1 mol·L⁻¹, pH 7.4, KCl 1 mmol·L⁻¹, prepared at a ratio of 1:1) to it. The other half of the pancreas was put into the ice-cold (4°C) normal saline of which the volume was 9 times of the weight of the tissue block and it was homogenized in ice water for 10 min. The obtained 10% tissue homogenate was

centrifuged by high speed centrifuge 11200×g for 15 min and then the supernatant was extracted to test the superoxide dismutase (SOD) and malondialdehyde (MDA).

Detection of Activities of Cytochrome C Oxidase and Succinate Dehydrogenase, Content of Malondialdehyde and Activity of Superoxide Dismutase By Spectrophotometric Method

The mitochondrial suspension was taken to measure the activities of cytochrome C oxidase and succinate dehydrogenase via the spectrophotometric method. The supernatant of the centrifuged pancreatic tissue homogenate was extracted to test the content of Malondialdehyde (MDA) and the activity of Superoxide Dismutase (SOD) by the spectrophotometric method.

Statistical Analysis

All the data were presented as mean ± standard deviation (SD) and the Statistical Package for Social Science 11.0 (SPSS Inc., Chicago, IL, USA) statistical software was used for data processing. The one-way analysis of variance was adopted to indicate the significance of differences among groups. The Student-Newman-Keuls (SNK) test was utilized for statistical processing of comparisons among groups. $p < 0.05$ meant that the differences were statistically significant.

Results

Effects of Mulberry Leaf Polysaccharide on The Ultrastructure of Pancreatic β -Cells In Diabetic Rats

The transmission electron microscopy results indicated that there were plentiful endocrine granules in the β -cells of the normal control group, of which the nuclei were in large oval shape and the nuclear membranes were smooth (Figure 1A). The number of endocrine granules declined significantly in the diabetic model group, the density of dense core decreased, the mitochondria swelled, the nuclei were deformed, the nuclear membranes were not smooth and the dilatation of endoplasmic reticulum was visible (Figure 1B). Those phenomena were greatly improved after MLP treatment, of which the quantity of endocrine granules increased, and the density of dense core recovered gradually (Figure 1C).

Improvement of Glucose Metabolism State By Mulberry Leaf Polysaccharide

The level of fasting blood glucose in the diabetic model group increased distinctly ($p < 0.05$) and the level of blood glucose in the rats were declined after MLP treatment ($p < 0.05$). It indicated that the MLP could ameliorate the glucose metabolism state of the diabetic rats. The FFA level in the diabetic model group rose significant-

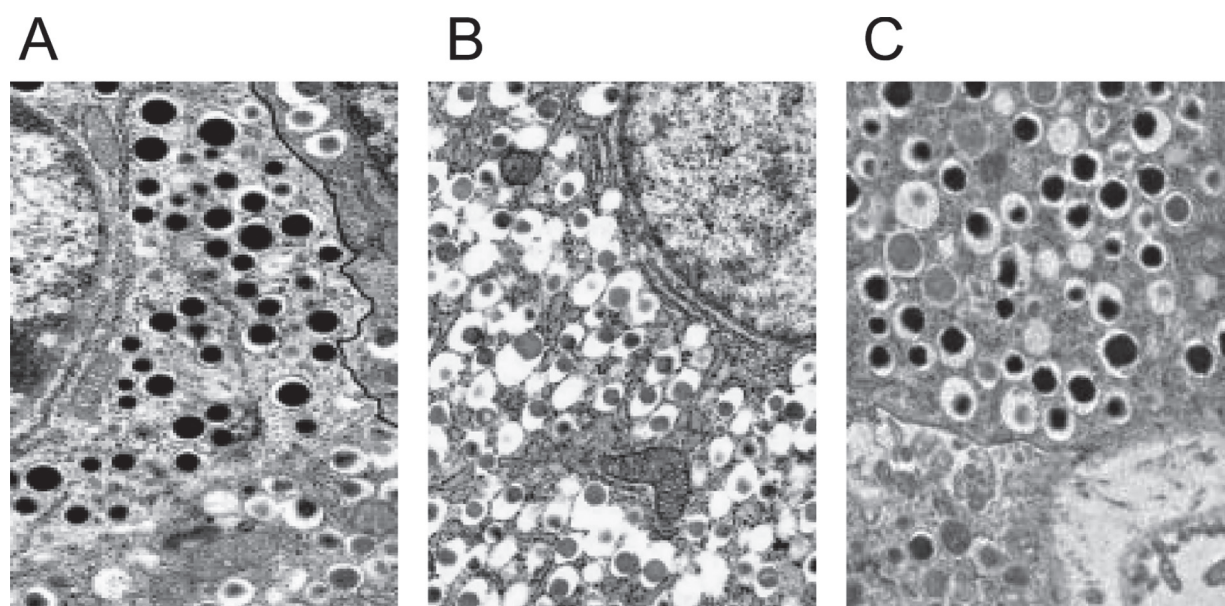


Figure 1. Effect of MLP on pancreatic beta cells by transmission electron microscope (magnification *400). **A**, Normal group. **B**, Diabetic group. **C**, MLP group.

ly ($p < 0.05$) while it dropped greatly in the MLP treatment group ($p < 0.05$), but it was still higher than that in the normal control group ($p < 0.05$) (Figure 2).

Improvement of Lipid Metabolism Disorder Through Mulberry Leaf Polysaccharide

The levels of TG and LDL-C in the diabetic model group increased obviously ($p < 0.05$). Compared with the diabetic model group, the levels of TG and LDL-C were declined in the MLP treatment group ($p < 0.05$), but they were still higher than those in the normal control group ($p < 0.05$). It suggested that the lipid metabolism disorder of diabetic rats can be improved to some extent by mulberry leaf polysaccharide (Figure 3).

Effects of Mulberry Leaf Polysaccharide on Reducing Inflammatory Mediators

In terms of inflammatory mediators, the levels of IL-6 and TNF- α in the diabetic rats increased significantly ($p < 0.05$), showing that the diabetic rats were in an inflammatory state. Those levels dropped after the rats were treated with mulberry leaf polysaccharide ($p < 0.05$), but they were still higher than those in the normal control group ($p < 0.05$). It manifested that the inflammatory state could be ameliorated by the mulberry leaf polysaccharide (Figure 4).

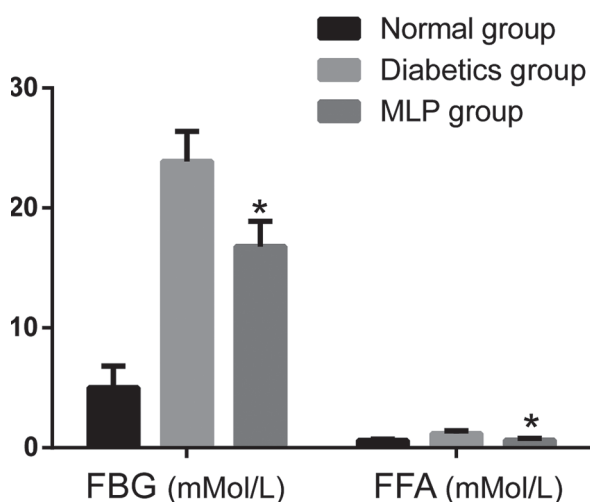


Figure 2. Effect of MLP on fasting blood glucose (FBG) and free fatty acid (FFA) in serum of diabetic rats. * $p < 0.05$, compared with Diabetics group.

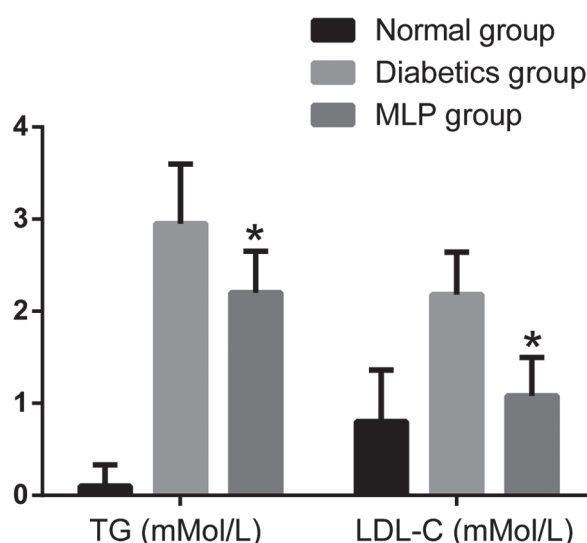


Figure 3. Effect of MLP on triglyceride (TG), low density lipoprotein cholesterol (LDL-C) in serum of diabetic rats. * $p < 0.05$, compared with Diabetics group.

Improvement of Functions of Pancreatic Mitochondrion Via Mulberry Leaf Polysaccharide

The activities of CCO and SDH in the diabetic rats decreased greatly, which indicated that there were mitochondrial dysfunctions in the pancreatic cells of the diabetic rats. The abovementioned indexes recovered remarkably after the rats had been treated with mulberry leaf polysaccharide for 8 weeks; it meant that the functions of pancreatic mitochondrion had been improved (Figure 5).

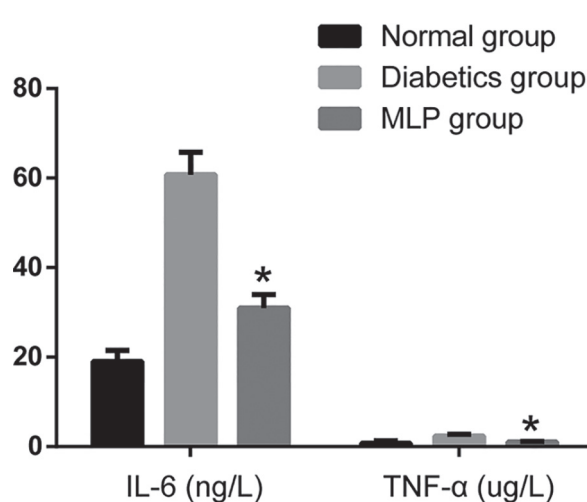


Figure 4. Effect of MLP on interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) in diabetic rats. * $p < 0.05$, compared with Diabetics group.

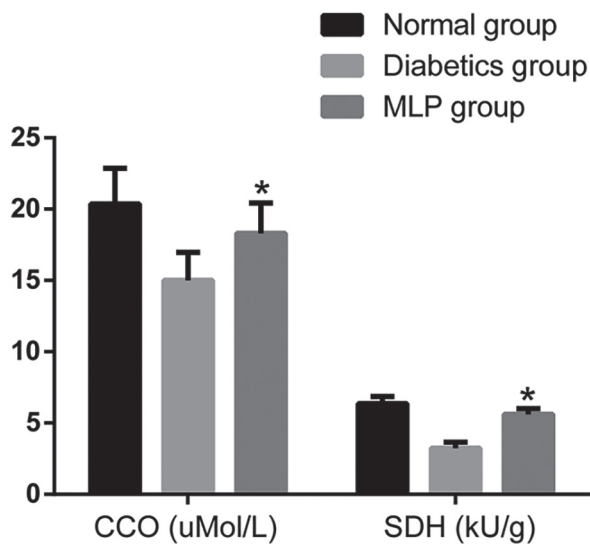


Figure 5. Effects of MLP on activity of cytochrome C oxidase and succinate dehydrogenase in diabetic rats. * $p < 0.05$, compared with Diabetics group.

Improvement of Oxidative Stress State of Pancreatic Cells Through Mulberry Leaf Polysaccharide

The content of MDA in the diabetic rats was elevated remarkably ($p < 0.05$) while the activity of SOD decreased obviously ($p < 0.05$). After 8 weeks of MLP treatment, the content of MDA declined while the activity of SOD increased significantly. It suggested that the oxidative stress state of the pancreatic cells had been improved (Figure 6).

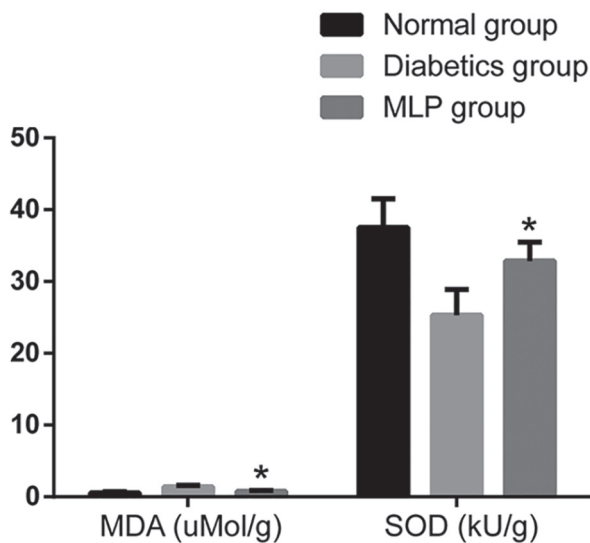


Figure 6. Effects of MLP on content of malondialdehyde and activity of superoxide dismutase. * $p < 0.05$, compared with Diabetics group.

Discussion

There are multiple pathways to generate ROS in the type 2 diabetic rats, of which mitochondrial pathway is the main source^{15,16}. Under the high-sugar and high-fat conditions, the activity of the glycolytic pathway is strengthened. And there are more electron donors entering into the mitochondria, which lead to constant accumulation of ROS and reactive nitrogen species (RNS)¹⁷, and the oxidative stress state is reached at the end. The pancreatic β -cells, however, are extremely vulnerable to injury due to their weak resistance to oxidative stress as well as the synergistic effects of inflammatory factors, Free Fatty Acids (FFA) and other factors. Those factors contribute to the occurrence and development of diabetes mellitus.

The results of this research indicate that the significant increase in the levels of inflammatory factors (IL-6 and TNF- α) in the blood of type 2 diabetic rats means the existence of the inflammatory state. It is discovered through the research that, in the case of metabolic syndrome, there is a close correlation between inflammation and oxidative stress, of which the inflammation could trigger the oxidative stress¹⁸. In this research, the content of MDA increased obviously along with the elevated levels of inflammatory factors while the activity of SOD decreases significantly. It suggests that the level of oxidative stress in the rats is much higher than that in the normal ones. In addition to the elevated level of blood glucose in the model rats with type 2 diabetes mellitus which were fed with high-sugar and high-fat diet, the lipid metabolism disorder was significant, which manifested typical metabolic syndromes. The lipid metabolism disorder could lead to increase of FFA in the blood which exceeded the storage capability of the adipose tissue and its ability to oxidize FFA. Previous studies had shown that high FFA could cause apoptosis of β -cells through oxidative stress, endoplasmic reticulum stress and other pathways. In the research results, the changes of MDA content and SOD activity which reflect the level of oxidative stress also mean that the increased level of FFA has aggravated the oxidative stress of the pancreatic cells. On the other hand, the inflammatory factors IL-6 and TNF- α can promote the decomposition of adipocyte fat, stimulating the liver to synthesize and release more FFA. Excessive FFA will be accumulated in the pancreatic β -cells, which could damage

the mitochondria and glucokinase and cause disorders of synthesis and secretion of insulin. This proves that the inflammatory factors and FFA were closely related to oxidative stress. During the intervention process to type 2 diabetic rats with mulberry leaf polysaccharide, the blood glucose and lipid level was improved, the levels of IL-6, TNF- α and FFA were declined obviously, the MDA content dropped and the SOD level was elevated, which indicated that the oxidative stress is ameliorated. The mulberry leaf polysaccharide might have remitted the oxidative stress by restraining inflammatory responses and high FFA. Therefore, the damage to the β -cells was alleviated. It could be seen from the electron microscopy results that the ultrastructure of the β -cells was also restored to some extent. It manifested that such anti-inflammatory effect and regulating effect on lipid metabolism disorder are fairly conducive to the restoration of the pancreatic cells.

Conclusions

The mulberry leaf polysaccharide may mitigate the oxidative stress of β -cells by restraining inflammatory responses and regulating lipid metabolism disorder. The mitochondrial functions of the β -cells can be recovered, the level of insulin secretion is increased, and the metabolic status of type 2 diabetes mellitus is improved.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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