Diabetes, Metabolic Syndrome and Obesity

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ORIGINAL RESEARCH

The Active Ingredient Catalpol in *Rehmannia glutinosa* Reduces Blood Glucose in Diabetic Rats via the AMPK Pathway

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Background: Type 2 diabetes mellitus (T2DM) poses a huge threat to population health globally, and more drugs need to be explored for treatment. In this study, we investigated the mechanism of active ingredient catalpol in *Rehmannia glutinosa* on reduces blood glucose in diabetic.

Methods: The T2DM model was constructed by intraperitoneal injection of streptozotocin into Sprague-Dawley (SD) rats, which were randomly grouped into diabetes model group, pioglitazone group, *Rehmannia glutinosa* group, catalpol high-dose group, catalpol low-dose group and normal control group.The intervention was continued for 28 d, and changes in body weight, fasting blood glucose, insulin and lipid levels were observed.

Results: Of all the drugs, pioglitazone had the most pronounced hypoglycemic effect, which began to decline after 2 weeks of treatment in the low-dose catalpol group and had no hypoglycemic effect in the high-dose catalpol group. Among them, *Rehmannia glutinosa* was able to increase serum triglyceride level, and pioglitazone effectively reduced total cholesterol level in rats. The low dose of catalpol decreased the concentration of low-density lipoprotein cholesterol (LDL), while the high dose of catalpol increased the concentration of LDL.

Conclusion: As an active ingredient in *Rehmannia glutinosa*, catalpol has the potential to lower blood glucose and improve blood lipids in diabetes treatment, and its action may be achieved by regulating the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway, which provides a new idea for the development of new diabetes therapeutic approaches.

Keywords: catalpol, *Rehmannia glutinosa*, type 2 diabetes, traditional Chinese medicine

Introduction

Type 2 diabetes mellitus (T2DM), known as a chronic metabolic disease, is showing a rapidly rising epidemic worldwide.[1,2] According to the latest statistics, 537 million people worldwide will have diabetes as of 2021, and this number is expected to increase to 643 million by 2030.[3,4] This trend has attracted widespread attention from the World Health Organization and the international community. Hyperglycemia, insulin resistance and relative insulin deficiency characterize T2DM, while its complications, such as cardiovascular disease and retinopathy, have a serious impact on patients' quality of life and health.[5] And the current major hypoglycemic drugs are mainly divided into insulin analogues, oral hypoglycemic drugs, insulin secretion enhancers, sodium-glucose cotransporter protein-2 inhibitors, and other categories.[6,7] They have various mechanisms of action, including promoting insulin secretion, improving insulin sensitivity, and inhibiting the release of hepatic glycogen.[8] In terms of clinical applications, glucose-lowering drugs are widely used in different types and severities of diabetes, significantly improving glycemic control, reducing symptoms, and lowering the risk of kidney disease and cardiovascular.[9,10] However, they may also be accompanied by side effects and safety concerns, such as hypoglycemia and weight gain. Traditional Chinese medicine (TCM) exhibits potential hypoglycemic effects in diabetes management.

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This mechanism of action is mainly mediated through the active ingredients contained in TCM herbs and their pharmacological properties.[11–14] These drugs may counteract the hyperglycemic state by increasing insulin sensitivity, reducing hepatic glycogen synthesis, and promoting insulin secretion.[14–16]

In addition, molecular pathways have a critical role in the development and complications of T2DM.[17–19] Therapeutic studies for T2DM are increasingly focusing on the regulation of molecular pathways to find new therapeutic targets. Among them, the signaling pathway of adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) has attracted a lot of attention, and AMPK plays an important role in insulin resistance, β-cell function, and complications of T2DM.[20–22] In addition, other molecular pathways, such as miRNA-29, vascular endothelial protein tyrosine phosphatase, heat shock protein-90, Pdia4, and Hedgehog signaling, have also been strongly associated with the development and complications of T2DM.[23–26] These findings provide strong evidence for finding new drug targets to improve the quality of life of T2DM patients and prevent the development of complications.

Catalpol, an iridoid glucoside obtained from the roots of *Rehmannia glutinosa*. This plant is traditionally used in China and Korea for the treatment of aging-related diseases and is extensively referred to as Di-Huang in TCM for treating diabetic disorders.[27] Catalpol exerts a wide variety of biological activities including analgesic, sedative, liver protective, purgative, anti-inflammatory, anti-microbial, anti-tumour, and anti-apoptosis activity.[28,29] In the last few years, catalpol has been extensively investigated and several studies have reported its multiple biological activities. The antioxidant and free radical scavenging activity of catalpol are the key mechanisms for exhibiting neuroprotection, anti-atherosclerosis, cardioprotective, and antidiabetic activity. Researchers reported that catalpol activated AMPK/PGC-1α/TFAM signaling, which augments mitochondrial biogenesis in skeletal muscle, thereby increasing glucose uptake and adenosine triphosphate production.[30]

However, the mechanism of how catalpol exerts its hypoglycemic effect is unclear. In this study, we explored the potential role of catalpol in diabetes treatment and its mechanism of action by exploring the AMPK signaling pathway. This study provides new ideas for the development of new diabetes therapeutics to improve the quality of life and prevent the development of complications in T2DM patients.

Materials and Methods

Experimental Animals

Animals 60 male Sprague-Dawley (SD) rats, body mass 180–200 g, were housed in the animal laboratory at a room temperature of 20 ± 2°C, relative humidity of 40–60%, and a light-dark cycle (12 h/12 h). During the experiment, the animals were fed freely. According to the grouping of animals, normal feed and high fat feed (HFD, 20% sucrose, 2.5% cholesterol, 10% lard, 1% sodium cholate, 66.5% basal feed) were given.

Grouping, Modeling and Observation

Male SD rats were acclimatized for 1 week, and 10 rats were randomly selected as the normal group and fed with normal chow. The remaining 50 rats were fed a high-fat diet for 4 weeks, then fasted for 12 h. Streptozotocin (STZ), a chemical widely used to induce diabetes in laboratory animals, and its cytotoxicity to the β-cells leads to a decrease in insulin release and a subsequent increase in blood glucose levels. STZ was injected intraperitoneally at 30 mg/kg. The control was injected with the same volume of citric acid-sodium citrate buffer at the same time. Fasting blood glucose in each rat was measured in triplicate at 72 h post-STZ injection; a sustained fasting blood glucose level ≥ 16.7 mmol/L was considered indicative of successful induction of diabetes mellitus modelling. After the modelling was completed, the animals were randomly grouped according to blood glucose and body weight, with diabetes model group, pioglitazone group (5 mg/kg-d), *Rehmannia glutinosa* group (6 g/kg-d,10 times the daily dose for human use), catalpol high-dose group (50 mg/kg-d), and catalpol low-dose group (25 mg/kg-d), 10 animals in each group.[31] Juice the fresh *Rehmannia glutinosa* in a juicer and then add purified water until the ratio of the weight of the juice to the weight of the herbs reaches 1:1. Once the drug solution was ready, the drug was administered to the rats by gavage. The volume of gavage was the same in all groups. The original feeding method was continued during the experimental period, and the drug was administered for 4 weeks in total. The body weights of the rats were recorded before the start of the intervention, 2 and 4

weeks after the intervention, and fasting blood glucose levels were determined by blood collection from the tail. The protocol was approved by the Animal Ethics Committee (No. NKYY-DWLL-2023-194).

Animal Harvesting

At the end of 4 weeks of drug intervention, all rats were anesthetized with 1% sodium pentobarbital after fasted overnight 20 h fasting and 2 h water fasting, and blood was collected from abdominal aorta, 8–10 mL of blood was collected. The blood was allowed to stand at room temperature for 30 min, then centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant was taken and stored in a refrigerator at −20°C. The liver was stored in liquid nitrogen and then transferred to −80°C for storage.

Measurement of Blood Lipid Levels

Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) levels were measured in rats using a blood biochemistry analyzer.

Western Blotting

Total proteins were extracted from trigeminal ganglion and nasal mucosa tissues using RIPA lysate containing protein phosphatase inhibitor. Western blotting was performed as previously described.[32] After 3 washes in Tris-Buffered Saline Tween-20 (TBST), the PVDF membranes were incubated for 2h at room temperature using horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (Cell Signal Technology). After washing 3 times with TBST, ECL reagent was used for visualization and imaging was conducted by chemiluminescence imaging system.

Statistical Analysis

Statistical analysis of the data was done by SPSS Statistics 22.0 software. Differences between groups were determined by one way analysis of variance (One Way ANOVA), and the pairwise comparison between groups were performed using the Least Significance Difference method.

Results and Discussion

As an important part of TCM, herbal medicine has played an important role in the field of medicine since ancient times.[33–36] They are widely used in medical practice and cover the treatment and regulation of many diseases, reflecting their unique therapeutic characteristics and theoretical system.[33,37–39]

In recent years, an increasing number of studies have focused on the potential of herbs in the treatment of diabetes. Clinical studies have shown that some herbs can significantly improve glycemic control and insulin sensitivity in diabetic patients. For example, a clinical trial of bitter melon found that bitter melon extract significantly reduced fasting blood glucose and glycated hemoglobin levels in diabetic patients.[40,41] In addition, a study on yam found that yam polysaccharides significantly reduced blood glucose levels and improved insulin resistance in diabetic rats.[42,43] These clinical findings provide preliminary evidence for the use of herbs in the treatment of diabetes.

Catalpol in Rehmannia glutinosa Reduces Blood Glucose

After 4 weeks of treatment, changes in body weight, blood glucose and serum insulin were determined in rats. All the rats in diabetic model group showed a significant decrease in body weight compared to the initial body weight when compared to the healthy control. There was no significant difference between each drug intervention group and the model group alone (Figure 1A). The pioglitazone group showed a significant decrease in fasting blood glucose after 2 weeks of treatment compared to the start of treatment, and the diabetic intervention group started to show a decrease in blood glucose at 4 weeks after treatment. The blood glucose of catalpol low dose group started decreasing at 2 weeks, whereas the catalpol high dose group showed no glucose lowering effect (Figure 1B). After 4 weeks of treatment, the insulin content of rats in the pioglitazone treatment group and *Rehmannia glutinosa* group increased, whereas the insulin content of the catalpol low-dose and high-dose groups decreased. Among all the drugs, pioglitazone treatment group had the highest serum insulin concentration compared to the HFD group after 4 weeks of treatment (18.50 ± 4.485 mU/L). However, there was no difference in serum insulin levels between the two groups (Figure 1C).

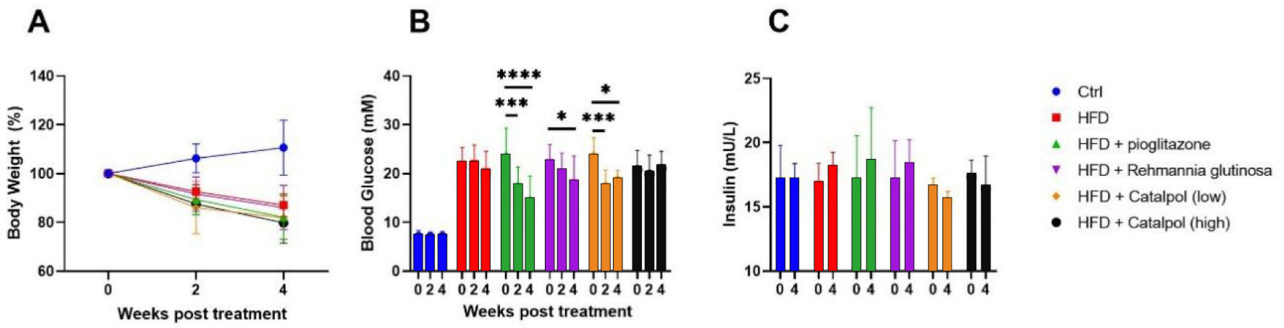

Figure 1 Changes in diabetes indications (n = 10). (**A**) Weight; (**B**)Blood Glucose; (**C**) Blood insulin. [blue: Ctrl group, red: HFD group, green: HFD + pioglitazone group purple: HFD + *Rehmannia glutinosa* group, Orange: HFD + catalpol (low) group, black: HFD + catalpol (high) group] Ctrl, control. HFD, high-fat diet. **P* < 0.05, ****P* < 0.001, *****P* < 0.0001.

Effect of Rehmannia glutinosa and Catalpol on Blood Lipids in Diabetes

After 4 weeks of treatment, changes in serum TG, TC, HDL and LDL were determined. TG, relevant to the accumulation of lipid repository in the liver, is concerned with metabolic syndrome and T2DM. It was found that *Rehmannia glutinosa* was able to increase the serum TG level and pioglitazone was effective in lowering the TC level in the rats. There was no significant change in blood HDL levels among the groups. For LDL concentration, low dose of catalpol decreased significantly after 4 weeks of administration, and high dose of catalpol significantly increased lipoprotein LDL concentration. (Figure 2).

Hypoglycemic Effect of AMPK Pathway in Rehmannia glutinosa and Catalpol

AMPK, the AMP-activated protein kinase, is an important regulatory molecule for intracellular energy metabolism. It has been reported in the literature that the expression of both AMPK and its activated form, p-AMPK is significantly decreased in the liver of diabetes rats, is a key enzyme in the fatty acid synthesis process.[44] AMPK knockdown resulted

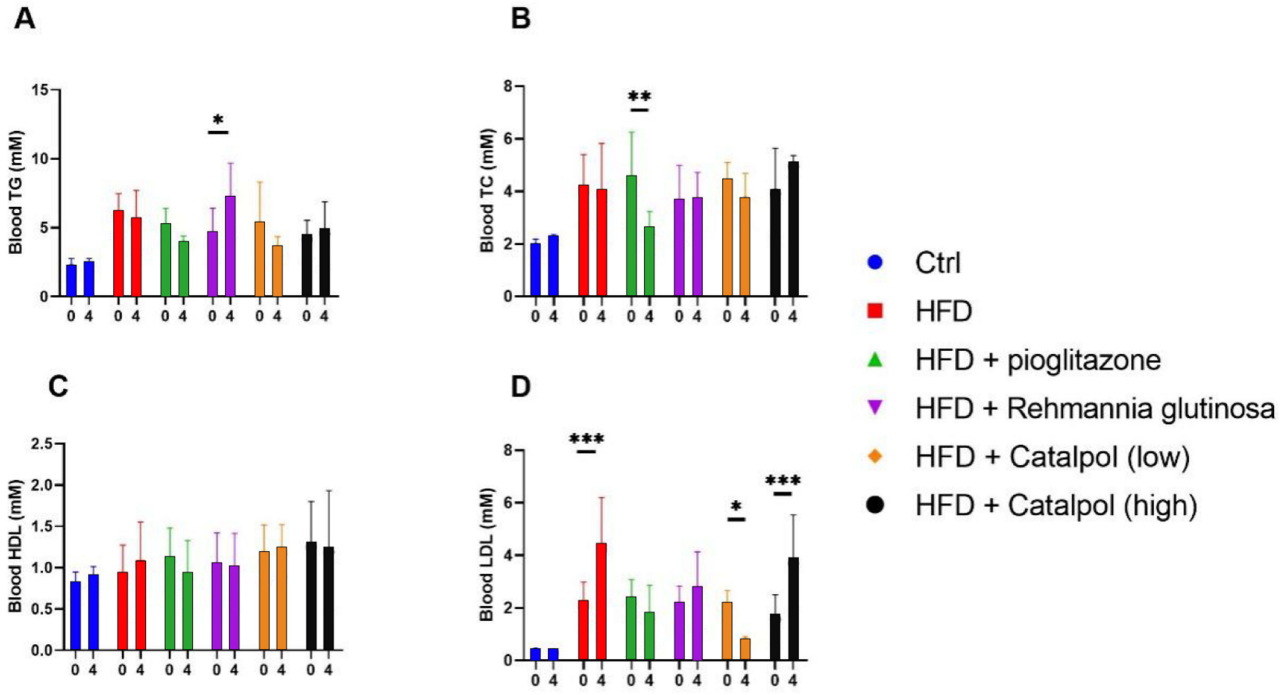

Figure 2 Blood lipid level (n = 10). (**A**) Triglyceride; (**B**) total cholesterol; (**C**) high density lipoprotein; (**D**) Low-density lipoprotein. [blue: Ctrl group, red: HFD group, green: HFD + pioglitazone group purple: HFD + *Rehmannia glutinosa* group, Orange: HFD + catalpol (low) group, black: HFD + catalpol (high) group]. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.
Abbreviations: Ctrl, control; HFD, high-fat diet.

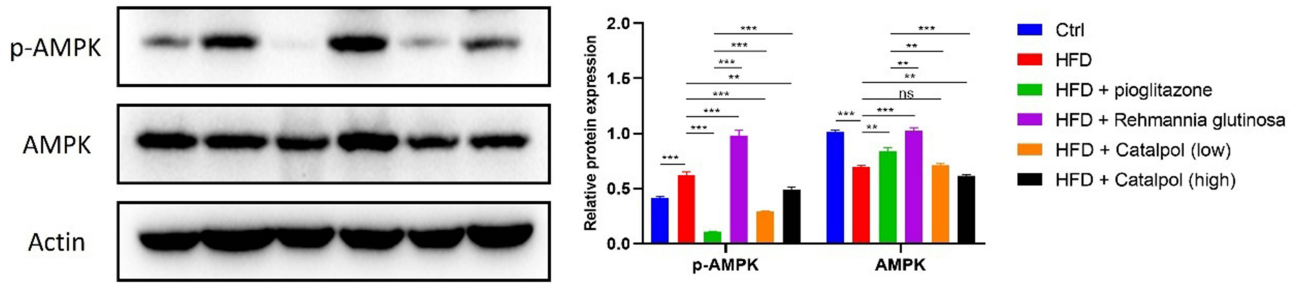

Figure 3 The AMPK pathway in diabetes (n = 3). [blue: Ctrl group, red: HFD group, green: HFD + pioglitazone group purple: HFD + *Rehmannia glutinosa* group, Orange: HFD + catalpol (low) group, black: HFD + catalpol (high) group]. $^{**}P < 0.01$, $^{***}P < 0.001$.
Abbreviations: Ctrl, control; HFD, high-fat diet.

in a significant decrease in phosphorylation levels.[45,46] Overexpression of recombinantly activated AMPK negatively reduces malonyl coenzyme A content in hepatocytes, thereby inhibiting fatty acid synthesis, and enhances mitochondrial utilization and oxidation of fatty acids, reduces lipid deposition in peripheral tissues, and thereby increases insulin sensitivity.[47–49] The results of Western blotting showed that diabetes was able to increase the level of the p-AMPK pathway in the liver and decrease the concentration of AMPK. Pioglitazone reversed this trend, decreasing the level of the p-AMPK pathway and increasing the concentration of AMPK in the liver. Unexpectedly, *Rehmannia glutinosa* appeared to exacerbate the elevation of p-AMPK andAMPK pathway associated with diabetes. This suggests that the mechanism by which *Rehmannia glutinosa* ameliorates T2DM may be to reduce the activation of nuclear factor kappa-B by increasing the expression level of p-AMPK/AMPK. And then control the inflammatory response mediated by the activation of PYD domains-containing protein 3 inflammasome.[50] For low concentration of catalpol treatment, it had no effect on AMPK concentrations, but was able to reduce p-AMPK concentrations. High concentration of catalpol inhibited both p-AMPK and AMPK concentrations in the liver (Figure 3).

Molecular mechanisms and pharmacological studies have revealed the role of herbs in regulating glucose metabolism and insulin signaling pathways.[47,51] For example, it was found that bitter melanin was able to increase insulin sensitivity by activating the AMPK signaling pathway, thereby promoting glucose uptake and utilization.[52,53] Panax ginseng saponin, on the other hand, can improve insulin resistance by inhibiting inflammatory responses and oxidative stress.[48,54] These findings provide a theoretical basis for the mechanism of herbal medicine in diabetes treatment. In summary, this study found that catalpol in *Rehmannia glutinosa* can affect blood glucose by affecting the AMPK pathway, thus lowering the blood glucose of T2DM rats. Its effect is comparable to that of pioglitazone, indicating that *Rehmannia glutinosa* is able to activate AMPK and play the role of protecting liver function, improving liver lipid metabolism in rats with T2DM, and then treating T2DM through the AMPK signal transduction pathway. However, there are some limitations to this study. Due to significant biological differences between species, rat-based animal studies may be affected by species when extrapolated to populations. For a long-term chronic disease as diabetes, further studies are still needed to investigate the safety and efficacy of long-term medication.

Conclusion

T2DM, known as a chronic metabolic disease, poses a huge threat to population health globally, and more drugs need to be explored for treatment. Our findings suggest that catalpol, the active ingredient in *Rehmannia glutinosa*, has a certain therapeutic effect in the STZ-induced diabetic rats. It was found that a low dose of 25 mg/kg-d was found to be able to lower blood glucose and effectively improve lipid levels, while high doses above 50 mg/kg-d were ineffective. Exploration of the AMPK pathway also suggests that catalpol may produce hypoglycemic effects by modulating the AMPK pathway.

Declarations

The study protocol was approved by the Animal Ethical and Welfare Committee of NanKai Hospital (No. NKYY-DWLL -2023-194). All experiments were performed following the Animal Ethics and Welfare Committee of Nankai Hospital, as well as the Animal Protection Law and the Animal management regulations.

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Disclosure

The authors report no conflicts of interest in this work.

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