Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Effects of Hydroxysafflor Yellow A on the PI3K/AKT Pathway and Apoptosis of Pancreatic β-Cells in Type 2 Diabetes Mellitus Rats

This article was published in the following Dove Press journal:
Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy

Maosheng Lee[1,2]
Huilin Li[2]
Hengxia Zhao[2]
Miao Suo[1,2]
Deliang Liu[2]

[1]The Fourth Clinical Medical College, Guangzhou University of Chinese Medicine, Guangzhou 510006, People's Republic of China; [2]Department of Endocrinology, Shenzhen Traditional Chinese Medicine Hospital, Shenzhen 518033, People's Republic of China

Background and Aim: Type 2 diabetes mellitus (T2DM), a complex metabolic disease, has become a major public health issue around the world. Hydroxysafflor yellow A (HSYA) is the major active chemical ingredient of *Carthamus tinctorius L.* (safflower), which is widely used in patients with cardiovascular and cerebrovascular diseases in China. The aim of this study was to investigate the anti-diabetic effect and potential mechanism of HSYA on the high-fat diet (HFD) and streptozotocin (STZ-)-induced T2DM rats.

Materials and Methods: T2DM rats were induced by feeding HFD (60% fat) for four weeks followed by intraperitoneal injection of a low dose of streptozocin (35mg/kg). The T2DM rats were treated with HSYA (120mg/kg) or metformin (90mg/kg) for eight weeks. Biochemical analysis, histological analysis and Western blot analysis were conducted after 8 weeks of intervention.

Results: The treatment with HSYA evidently reduced fasting-blood glucose and insulin resistance in T2DM rats, indicated by results from fasting-blood glucose, oral glucose tolerance test, fasting insulin levels and histology of pancreas islets. The Western blot results revealed that HSYA reversed the down-regulation of PI3K and AKT in liver. The TUNEL assay analysis of pancreatic tissue showed that HSYA could inhibit the apoptosis of pancreatic β-cells to a certain extent. Moreover, HSYA-treatment increased the levels of glycogen synthase and hepatic glycogen and improved lipid metabolism by reducing the triglyceride, total and low-density lipoprotein cholesterol levels, even though it did not change the rats' body weights.

Conclusion: The results of this study suggested that HSYA could promote PI3K/Akt activation and inhibit the apoptosis of pancreatic β-cells directly or indirectly, which might be the underlying mechanisms in HSYA to improve insulin resistance and regulate glycolipid metabolism in T2DM rats.

Keywords: hydroxysafflor yellow A, insulin resistance, PI3K/AKT pathway, apoptosis, type 2 diabetes mellitus, traditional Chinese medicine

Introduction

Type 2 diabetes mellitus (T2DM), a complex metabolic disease, is characterized by persistent hyperglycemia, insulin resistance and β-cell dysfunction.[1] T2DM is not terrible; however, the chronic diabetes may cause complications in numerous organs and tissues throughout the body by affecting both small and large blood vessels.[2] These have brought serious influences and burdens to people's life around the world.

Although there are numerous new kinds of anti-diabetic drugs, metformin still is the most widely used clinically as its obvious hypoglycemic effect and broad

Correspondence: Huilin Li
Tel/Fax +86 755-8839368
Email sztcmlhl@163.com

physiological roles. It is all known that metformin' history is linked to Galega officinalis, a traditional herbal medicine in Europe.[3] And nowadays, mining chemical molecules with biological effects on natural plants has become an expanding field, especially for complicatedly non-communicable diseases. Traditional Chinese medicine (TCM) has a history of thousands years and still serves for hundreds of millions people around the world. *Carthamus tinctorius L*. (safflower) (Figure 1A) is one of the commonly used Chinese herbs, which is widely applied in the treatment of cardiovascular, cerebrovascular and gynecological diseases in China.[4] As early as 2012, researchers[5] had confirmed that the extract of Carthamus tinctorius L. could play an anti-diabetic role in alloxan-induced diabetic rats. However, there are more than 104 compounds have been isolated from *Carthamus tinctorius L*.[6] which active ingredient in *Carthamus tinctorius L*. plays an anti-diabetic effect remains unknown.

Hydroxysafflor yellow A (HSYA), a water-soluble monomer, can be extracted from *Carthamus tinctorius L*. (molecular formula, $C_{27}H_{32}O_{16}$; Molecular Weight, 612.5 g/mol),[7] depicted in Figure 1B. HSYA is one of the chemical components with biological effects in *Carthamus tinctorius L*., which is also widely applied in clinical practice in patients with cardiovascular and cerebrovascular diseases in China with its effects of oxygen-free radical scavenging,

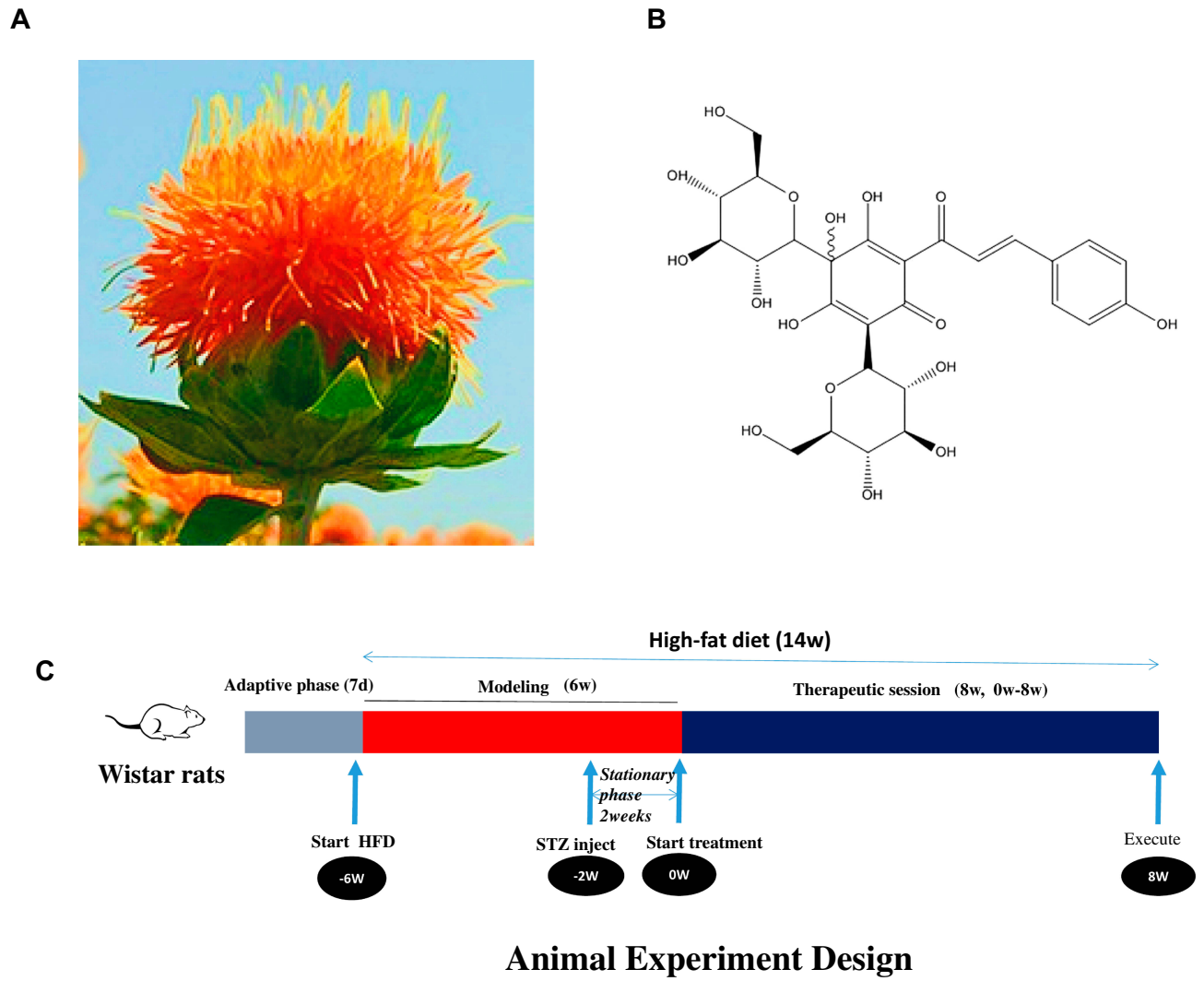

Figure 1 Experimental drug and experimental scheme. (**A**) The picture of *Carthamus tinctorius L*. (*Safflower*). (**B**) The chemical structure (C27H32O16) of *hydroxysafflor yellow A* (HSYA). (**C**) Design of the animal experiment, including modeling method, time points and duration. The Nor group rats fed with standard laboratory diet, the other groups fed with high fat diet (HFD) in the whole course of the experiment. After 7days of adaptive phase, except for the Nor group rats, all rats were fed with HFD for 4 weeks, and followed by Intraperitoneal streptozotocin (STZ, 35 mg/kg). After 6 weeks of T2DM-modeling, the T2DM rats were divided into three groups for different interventions for 8 weeks.

Abbreviations: Nor, Normal group; Mod, Model group; HSYA, hydroxysafflor yellow A; STZ, Streptozocin.

anti-inflammatory, and anti-apoptotic activities.[8–10] Moreover, the HSYA content is used for the standard of controlling quality of *Carthamus tinctorius L*. in authoritative Chinese Pharmacopoeia (The State Pharmacopoeia Commission of China, 2015). The oxidative stress, apoptotic and inflammation are hugely contributed to T2DM. Taken these, HSYA might be a main bioactive substance with hypoglycemic effect in *Carthamus tinctorius L*., which would be a potential anti-diabetic drug.

However, there is still no study of HSYA for the treatment of T2DM. HSYA could protect against cerebral ischemia-reperfusion injury by anti-apoptotic effect through increasing the expression of phosphorylations of Akt and GSK3b.[11] Meanwhile, HSYA could protect neuronal-specific cells by activating the AKT-autophagy pathway in penumbra tissue.[12] Therefore, we hypothesized that HSYA exerted an anti-diabetic effect by regulating the PI3K/AKT pathway and inhibiting apoptosis of pancreatic β-cells to improve insulin resistance and regulate the blood-glucose homeostasis.

The aim of our study was to explore the anti-diabetic effect of HSYA and its potential mechanism in T2DM rats. The T2DM rats were induced by the synthetic approach,[13–16] high-fat diet (HFD) for four weeks and followed by single injections with low-dose streptozotocin (30–40 mg/kg intraperitoneally); which has similar pathological features to human type 2 diabetes as eliciting partial loss of β-cells, and results in hypoinsulinemia and hyperglycaemia. In our study, T2DM rats were induced by feeding HFD (60% fat) for four weeks followed by intraperitoneal injection a low dose of streptozocin (35mg/kg). The T2DM rats were treated with HSYA or metformin for eight weeks.

Materials and Methods

Drugs

STZ was obtained from Sigma (Sigma Chemical Co., St. USA). HSYA was purchased from Nanjing Daosf Biotechnology Co., Ltd. (CAS 78281-02-4, purity≥98%, HPLC, Nanjing city, China). Metformin was provided from Shiguibao (Shiguibao Co., Lt, Shanghai city, China).

Animal Experiment

Male Wistar rats (weighted, 130±20g) were purchased from the Experimental Animal Center of Southern Medical University (Guangzhou city, China). All rats were kept in the Specific Pathogen Free (SPF) animal laboratory with free access to food and water. After seven days of adaptive feeding, they were fed with the HFD diet (60% fat) (the formula of HFD was shown in Supplementary Table 1); and the other eight rats were fed with the standard diet, which set as the normal control group (Nor).

Four weeks after HFD feeding, they were intraperitoneally injected with a low dose of STZ (35mg/kg). Two weeks later, rats with the fasting-blood glucose (FBG) level of ≧11.1 mmol/L were randomly divided into three groups: T2DM model group (Mod, n=8), HSYA treatment group (HSYA, n=8) and metformin treatment group (Met, n=8). The detailed animal experiment design is shown in Figure 1C. After modeling, the T2DM rats were daily oral administrated with HSYA (120mg/kg) or metformin (90mg/kg) or normal saline for eight weeks. The dose of metformin is based on the daily dose conversion in humans (1.0g/d). After eight-week intervention, all animals were anaesthetized by sodium pentobarbital and blood sample was obtained from aorta abdominalis. Liver and pancreatic tissues were dissected, some of them were stored at −80°C immediately and the others were soaked in 10% neutral-buffered formalin for histomorphology experiment.

All animal procedures were conducted with protocol approval from the Bioethics Commission of Tsinghua University, according to the National Institute of Health ethical guidelines, and all efforts were made to minimize animal suffering.

Biochemical Analysis

All rats' body weight, the food consumption (kcal/g) and water consumption (mL) were monitored weekly. The fasting-blood glucose (FBG) level was tested weekly from the tail vein by the glucometer (Roche Diagnostic Products Co. Ltd, Shanghai city, China). After eight-week treatment, the Oral-glucose-tolerance test (OGTT) was performed in 12 hours-fasted rats after oral administrated with glucose solution (2 g/kg, Sigma Aldrich, USA) at the time point of 0, 30, 60 and 120 minutes. The area under the curves (AUC) of OGTT was calculated according to the OGTT results.

The fasting blood insulin (FINS), triglycerides (TG), total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in serum were determined by test assay kits abide by the manufacturer's instructions (Beijing Solarbio Science & Technology Co., Ltd. Beijing city, China; Wuhan Mershack Biotechnology Co. Ltd. Wuhan city, China). The glycogen

synthase (GS) was determined by WST-8 method. The hepatic glycogen in liver was detected by anthrone method according to the manufacturer's directions (Solarbio Science & Technology Co., Ltd. Beijing city, China). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and homeostasis model assessment of β-cell function (HOMA-β) were calculated by the following formulas:[17] HOMA-IR=FINS (mU/L)×FBG (mmol/L)/22.5, HOMA-β=(20×FINS mU/mL)/(FBG mmol/L–3.5).

Pancreatic Histology Analysis

The pancreatic tissues which dissect from the animals were immersed in 10% neutral-buffered formalin for 48 hours. After fixation, they were prepared into paraffin sections of 4 μm, and stained with hematoxylin-eosin, and then observed on a fluorescence microscope. The fresh paraffin sections of pancreatic tissues were used for Immunohistochemical staining with the specific antibodies directed against insulin (Cat YT2357, ImmunoWay Biotechnology Company, North American). The sections were incubated with the HRP-conjugated secondary antibodies (Cat RS0002, Biotechnology Company, North American) and imaged with a fluorescence microscope. Briefly, the fresh paraffin sections would be gone through dewaxing, antigen repair, blocking, first and second antibody incubation, coloration and dewatering.

The pancreatic islets' cells apoptosis were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay with the flesh tissue paraffin sections. Pancreas slides, made into 3.5μm paraffin sections after dehydration, were incubated with the TUNEL reaction mixture in a humidified chamber for 60 minutes at 37°C in light avoidance condition. Then, the sections were stained with DAPI at a concentration of 460 nm. The percentage of TUNEL-positive nuclei was used to access the apoptosis of the pancreatic islets' cells.

Western Blot Analysis

The expression of liver tissue proteins, including PI3K, AKT and p-AKT were analyzed by Western blotting. Briefly, the total proteins of liver tissue were extracted with the RIPA buffer, and the concentrations were detected by using a Bradford Protein Assay Kit (Beyotime Biotechnology, Shanghai city, China). The protein was separated on 10% SDS-PAGE gels and then transferred onto nitrocellulose membranes. Then, the membranes were incubated in blocking solution for 2hours, and immersed with the primary antibodies for overnight at 4° C. Antibodies against PI3K (#4292,100kDa, CST, USA), AKT (#9272, 60kDa, CST, USA) and p-AKT (Ser473) (#4060, 60kDa, CST, USA) were obtained from Cell Signaling Technology and used at a dilution of 1:1000; antibodies against GAPDH were used at a dilution of 1:1000. After that, the membranes were washed in TBST and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies for 2hours. Finally, the protein bands were exposed to the chemiluminescent reagent (ECL) for about 3–10 minutes. All the proteins' expressions were obtained with fluorescence captured on X-ray photographic film in a dark room. The Image J software (NIH, Bethesda, MD, United States) was used for quantitative analysis the band densities of the above proteins.

Statistical Analysis

The data of this study were expressed as mean±SD in each group. Statistical Product and Service Solutions (SPSS) statistics 22.0 and GraphPad Prism 8.0 software (San Diego, CA, United States) were applied for the data statistical analysis and graphics. Unpaired *t*-test was used to analyze statistical comparisons between two groups when necessary. Multiple comparisons were compared by one-way (or two-way) analysis of variance (ANOVA) followed by Bonferroni hoc tests. Statistically significant changes were classified as significant (*) when $P<0.05$.

Results

Effects of HSYA on Body Weight, Fasting-Blood Glucose, and Glucose Tolerance in T2DM Rats

The body weight (Supplementary Figure 1A), the daily food and water consumption were monitored weekly. The rats' body weight in the Nor group was significantly higher than the Mod groups after STZ-modeling although there were no significant statistical differences between the other three groups ($P>0.05$). The daily food and water consumption of the normal group were lower than the T2DM rats' groups after modeling. Expectantly, the daily food and water consumption of the HSYA group (Supplementary Figure 1B and C) were observably lower than Mod group and Met group after 4-week treatment, although no difference in the body weight.

We monitored the FBG weekly to evaluate the efficacy of HSYA in T2DM rats. The figure of FBG levels for modeling is shown in Figure 2A, the FBG of all the T2DM groups rats

were ≧11.1 mmol/L after 6-week's modeling. The FBG of the Mod group was remarkably higher than the Nor group in the whole course of the experiment ($P<0.0001$). The FBG significantly reduced in the Met and HSYA group after 2-week's treatment when compared to the Mod group ($P<0.001$), and the best curative effect emerged at 8th week although the process fluctuates (Figure 2B). The glucose tolerance test, OGTT value and AUC-OGTT in the Met group (46.78) and HSYA group (58.73) were evidently lower than the Mod group (74.88)($P<0.0001$).

Effects of HSYA on Insulin Resistance and Pancreatic Islet Function

As depicted in Figure 3, the levels of HOMA-IR and HOMA-βinT2DM rats were apparently higher than those in the Mod group. Although there was no statistical difference in the fasting insulin levels, the Mod group (14.06) still showed relatively high insulin resistance than the Met group (11.51) and HSYA group (13.47).

Effects of HSYA on the Histopathological Change and the Insulin Expression in Pancreatic Tissues

The hematoxylin-eosin (HE) stained sections of the pancreatic tissue are shown Figure 4. The T2DM rats indicated a decrease in acinar staining intensity that reflecting the defects in digestive function of the pancreas. Expectantly, remarkably differences in the number and pattern of the islets were appeared between the Mod group and the other three groups, as the islets with lots of vacuous areas in the Mod group. The immunohistochemical (IHC) staining results of insulin in pancreas tissues indicated that the Mod group rats' islet structure changed in both of the endocrine portion and exocrine (Acinus) portion when compared to the other three groups. The borders of the

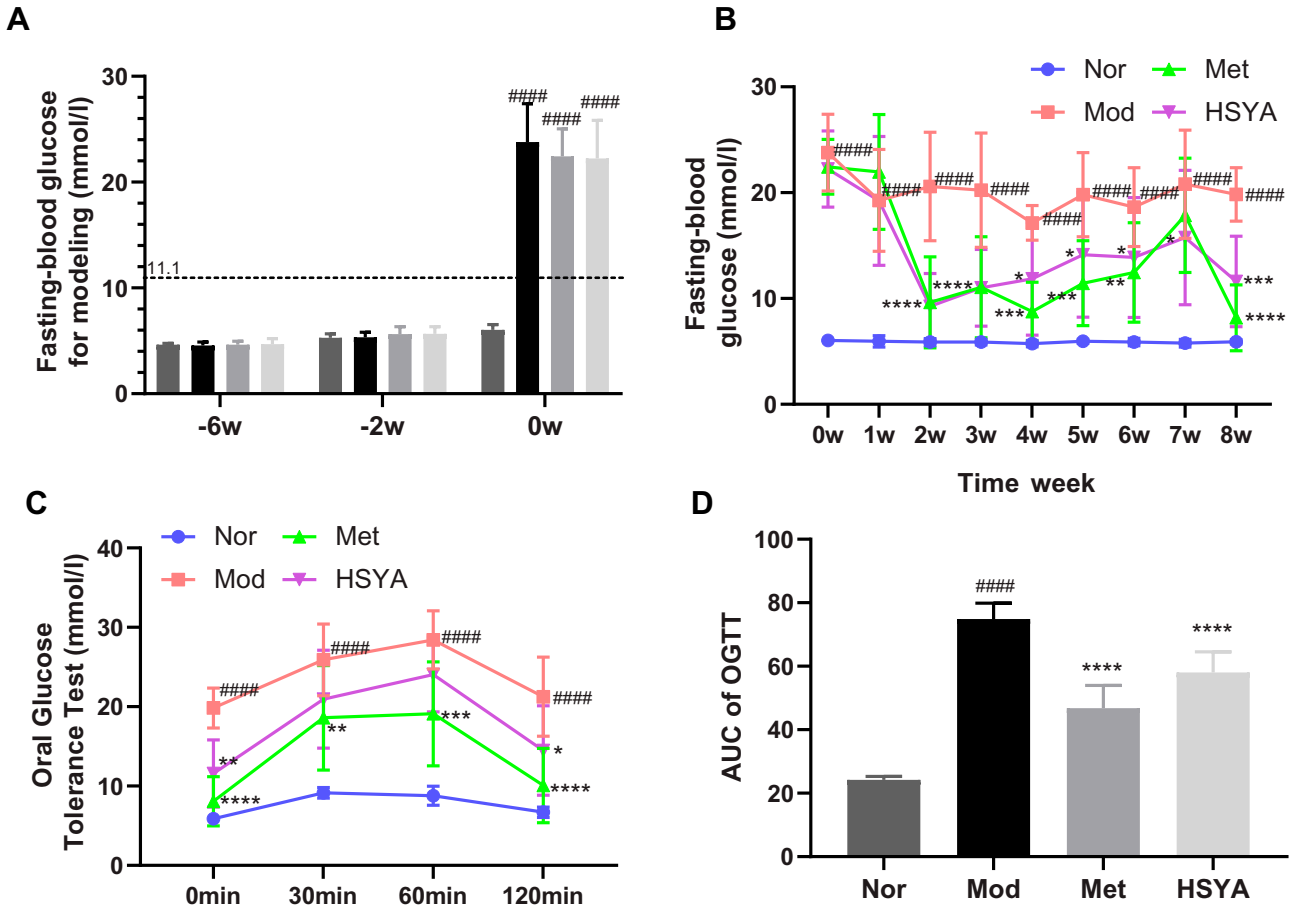

Figure 2 Effects of HSYA on fasting blood-glucose and glucose tolerance. (**A**) Fasting blood-glucose for modeling. (**B**) Fasting blood-glucose level. $^{\#\#\#\#}P<0.0001$, vs Nor; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$, vs Mod. (n=8 rats/group). (**C**) Oral glucose tolerance test (OGTT). (**D**) Area under curve (AUC) of OGTT. $^{\#\#\#\#}P<0.0001$, vs Nor; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$, vs Mod. Results are presented as means ± SD and n=8 in each group.

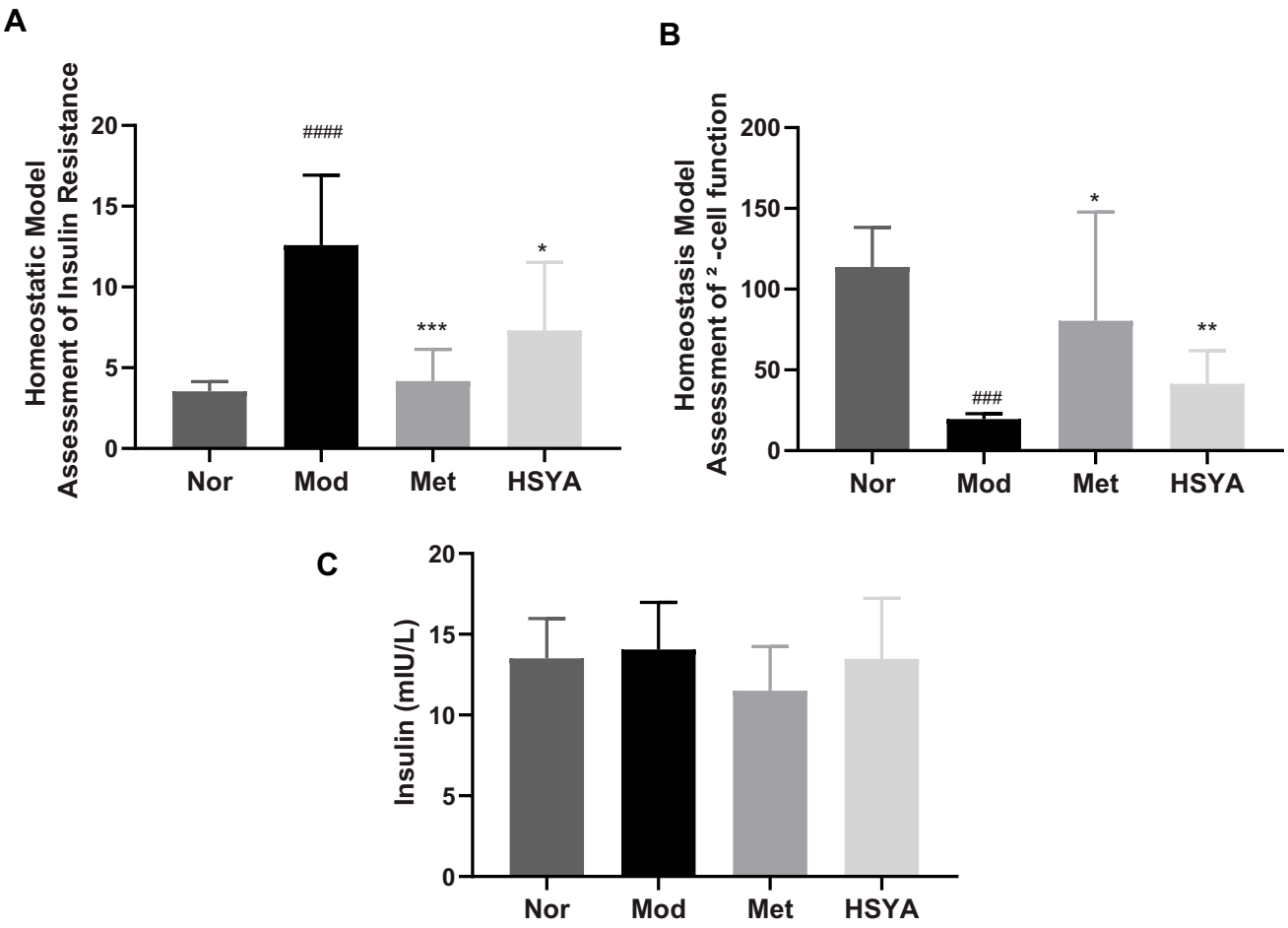

Figure 3 Effects of HSYA on the homeostasis model assessment of insulin resistance (HOMA-IR) and Homeostasis model assessment-β (HOMA-β). (**A**) Homeostasis model assessment of insulin resistance (HOMA-IR). (**B**) Homeostasis model assessment-β (HOMA-β). (**C**) Fasting insulin level. ####*P*<0.001, #####*P*<0.0001, vs Nor; **P*<0.05, ***P*<0.01, ****P*<0.001, vs Mod. Results are presented as means ± SD and n=8 in each group.

structures of the pancreas islets were distinctive from the exocrine glands due to the adequate zymogen granules; and the brown stained was shown the insulin expression in pancreas islets with immunohistochemical analysis. It suggested that HSYA and metformin might restore the impaired islets cells caused by the HFD and STZ in T2DM rats.

Effect of HSYA on Apoptosis of Pancreatic β-Cells in T2DM Rats

In order to access the apoptosis of pancreatic β-cells in T2DM rats, TUNEL stain analysis was performed in this study. The TUNEL assay showed an observable increase in TUNEL-positive cells in the pancreatic islets in T2DM rats when compared to the Nor group (Figure 5A and B). In the Mod group, the TUNEL-positive (%) was markedly higher than the HSYA and Met groups; the HSYA-treatment group exhibited fewer numbers of TUNEL-positive cells.

Effects of HSYA on the Blood Lipids in T2DM Rats

As shown in Figure 6A–D, the levels of the blood lipids including TC, TG and LDL-C in T2DM group rats were obviously higher than that in the Nor group. HSYA remarkably decreased the levels of TC, TG and LDL-C although there were no statistical differences between the Mod group and the other three groups.

Effects off HSYA on PI3K, AKT and p-AKT Expression and the Contents of Hepatic Glycogen and Glycogen Synthase in Liver

To evaluate whether HSYA reduced the blood glucose and improves insulin resistance through PI3K/AKT pathway, the expressions of PI3K, AKT and p-AKT in liver were analysed by Western blot (Figure 7A–D). The expressions of PI3K, AKT and p-AKT levels were significantly

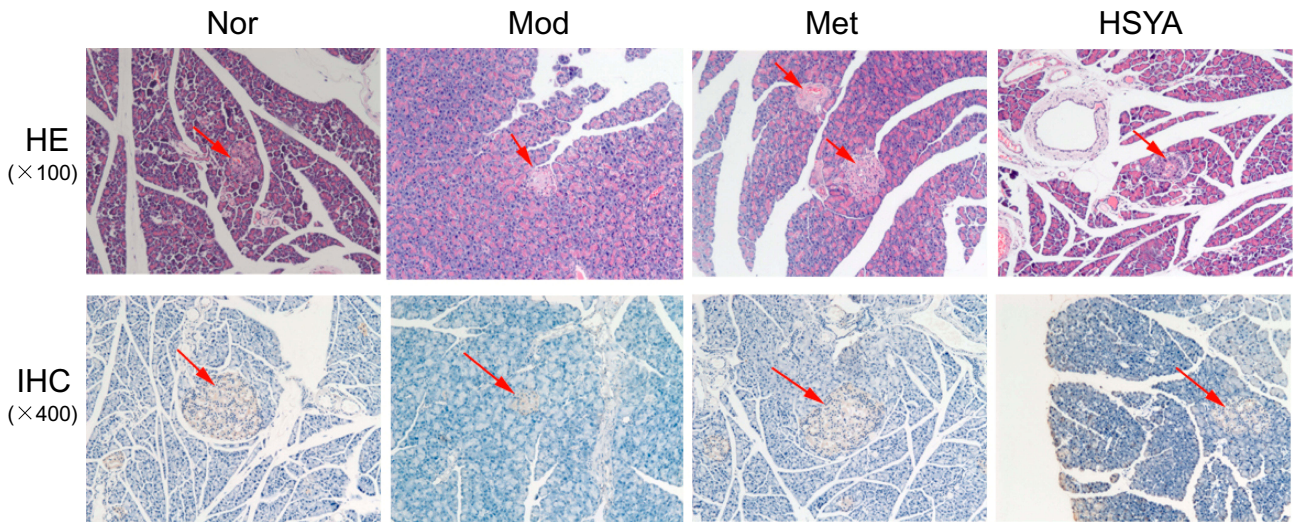

Figure 4 Effect of HSYA on the pathomorphism of pancreas tissues. Representative pictures of hematoxylin and Eosin (HE) staining (first row) (×100, n=6 each group) and insulin expression in pancreatic islets by immunohistochemistry (IHC) (second row) (×400, n=6 each group) of the tails of pancreas from each group rats after treatment. The red arrows indicate the pancreatic islets in the microscopic view (second row). The borders of the structures of the pancreas islets were distinctive from the exocrine glands due to the adequate zymogen granules; and the brown stained was shown the insulin expression in pancreas islets with immunohistochemical analysis.

depressed in T2DM rats when compared to the Nor group. The HSYA and metformin treatment groups evidently reversed the down-regulation of the above proteins' expression when compared to the Mod group. Meanwhile, the contents of hepatic glycogen and glycogen synthase in liver in the HSYA were distinctly higher than that of the Mod group (Figure 7E and F).

Discussion

The insulin resistance and the impaired insulin secretion were the major characteristic of T2DM, which associate with the high-fat diet, inflammation and other environmental factors. The T2DM rats' model, simulating the pathology of human T2DM, was critical to access the curative effect of the preclinical drugs for T2DM. The compositive method for T2DM modeling, conducting HFD for some weeks (4–8 w) followed by treating with a low dose of streptozotocin, which was evidenced to be a highly simulated human T2DM modeling method.[13,18,19] In our study, given 4 weeks' HFD and a low dose of STZ (35 mg/kg) for T2DM modeling, as shown in the Figures 2 and 3, the levels of FBG, OGTT, HOAM-IR, HOMA-β and the pathological results of T2DM rats suggested that the T2DM rats were induced successfully. After 8-week treatment of HSYA, the results indicated that HSYA could lower FBG and increase the glucose tolerance level, improve insulin resistance and activated insulin signaling pathway.

The improving of insulin resistance and inhibit the apoptosis of pancreatic β-cells would be the major methods prevent and treat T2DM. In our study, HSYA-treatment reduced the HOMA-IR level at a rate of 42.06% and increased the HOMA-βlevel at a rate of 112% when compared to the Mod group. Feeding with HFD could induce hyperlipemia and insulin resistance,[20,21] HSYA-treatment not only decreased FBG but had the anti-hyperlipidemic effect which would improve insulin resistance indirectly. Although HDL-C had the antioxidant and anti-inflammatory effects[22] and some studies[23,24] showed that the HDL-C level in T2DM rats would be higher than that of the normal rats, our founding suggested that there was no statistical difference between them as the previous study.[25]

In this study, the T2DM rats indicated a decrease in acinar staining intensity that reflecting the defects in digestive function of the pancreas. The insulin granules and their intensity in β-cells were reduced in T2DM rats, and HSYA could reverse the impaired islets β-cells as shown in the Immunohistochemical results. As the previous studies described, HSYA could alleviate apoptosis and autophagy of neural stem cells[26] and reduces apoptosis after I/R injury in kidney.[27] There were numerous TUNEL-positive cells appeared in the islets of the Mod group, while HSYA-treatment exhibited fewer numbers of TUNEL-positive cells.

As the previous studies,[28–30] The PI3K/AKT signal pathway related to the glucose metabolism, which was

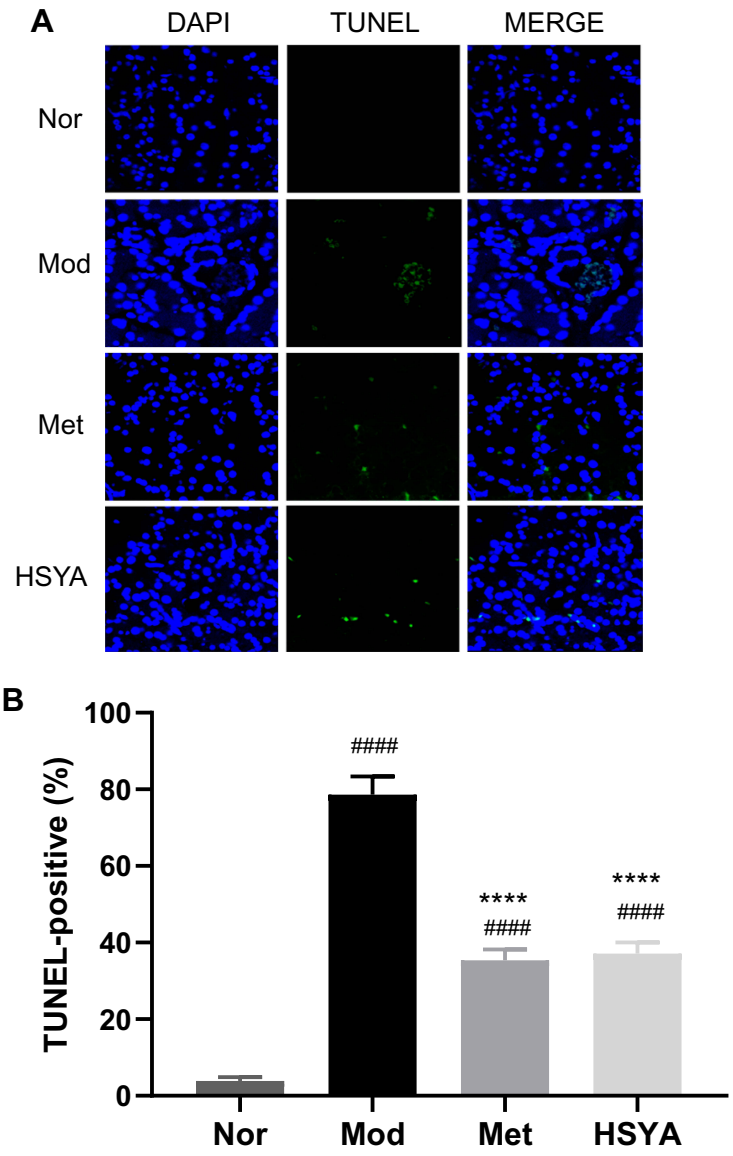

Figure 5 Effect of HSYA on apoptosis of pancreatic β-cells in T2DM rats. Representative images of the TUNEL staining in the four groups. (**A**) (First column) DAPI staining (blue) indicates total nuclei (middle column) apoptotic nuclei detected by TUNEL staining (green), and (Last column) overlay of both types of staining. (**B**) The number of TUNEL-positive myocytes was performed as a percentage of total nuclei detected by DAPI staining (fluorescence microscopy, magnification, x60). ####P<0.0001, vs Nor; ****P<0.0001, vs Mod. Results are presented as means ± SD and n=6 in each group.

Abbreviations: Nor, Normal group; Mod, Model group; HSYA, hydroxysafflor yellow A.

critical for insulin aroused glucose intake in liver. The glycogen synthase kinase 3b (GSK3b) was one of the numerous downstream targets of Akt phosphorylates.[31,32] Akt activation promoted the cells' survival by phosphorylation, and GSK3b inactivation of apoptosis-inducing factors.[33] GSK3b is the key enzyme involved in hepatic glucose metabolism, which can decrease the synthesis of hepatic glycogen by phosphorylation of glycogen synthase (GS).[34] In our study, the glycogen synthase level in liver was lower in the Mod group than that of Nor group, while HSYA-treatment could reverse the down-regulated GS. GS promoted the conversion of excess glucose into glycogen in the blood. HSYA could protect against cerebral ischemia-reperfusion injury by anti-apoptotic effect through increasing the expression of phosphorylations of Akt and GSK3b.[11] HSYA reversed the down-regulated expression of PI3K, AKT and p-AKT levels in T2DM rats in this study.

Additionally, the sub-chronic toxicity of HSYA (180 mg/kg) with 90 days of intraperitoneal injections in rats showed that there is not an obvious pathological change in the organs in rats.[35] Although HSYA has a poor oral bioavailability (1.2%),[36] it was with high uptake and eliminated slowly in the rats with blood

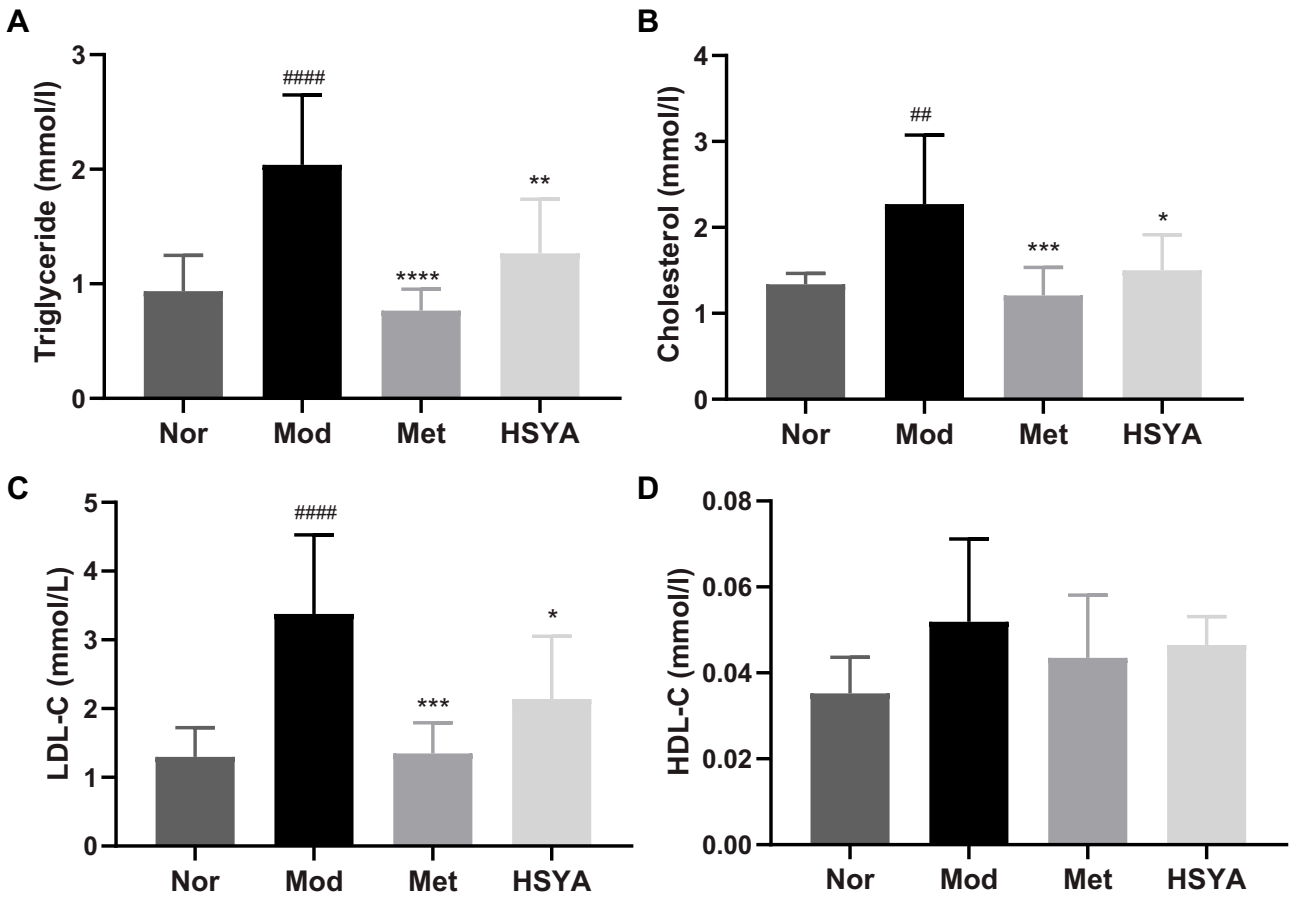

Figure 6 Effects of HSYA on the blood lipid profiles in T2DM rats. (**A**) Triglycerides (TG) level; (**B**) Total cholesterol (TC) level; (**C**) Low-density lipoprotein cholesterol (LDL-C) level. (**D**) High-density lipoprotein cholesterol (HDL-C) level. The blood lipid profile was measured in each group after 8 weeks of treatment. Results are presented as means ± SD and n=8 in each group. $^{\#\#}P<0.01$, $^{\#\#\#\#}P<0.0001$, vs Nor; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$, vs Mod.

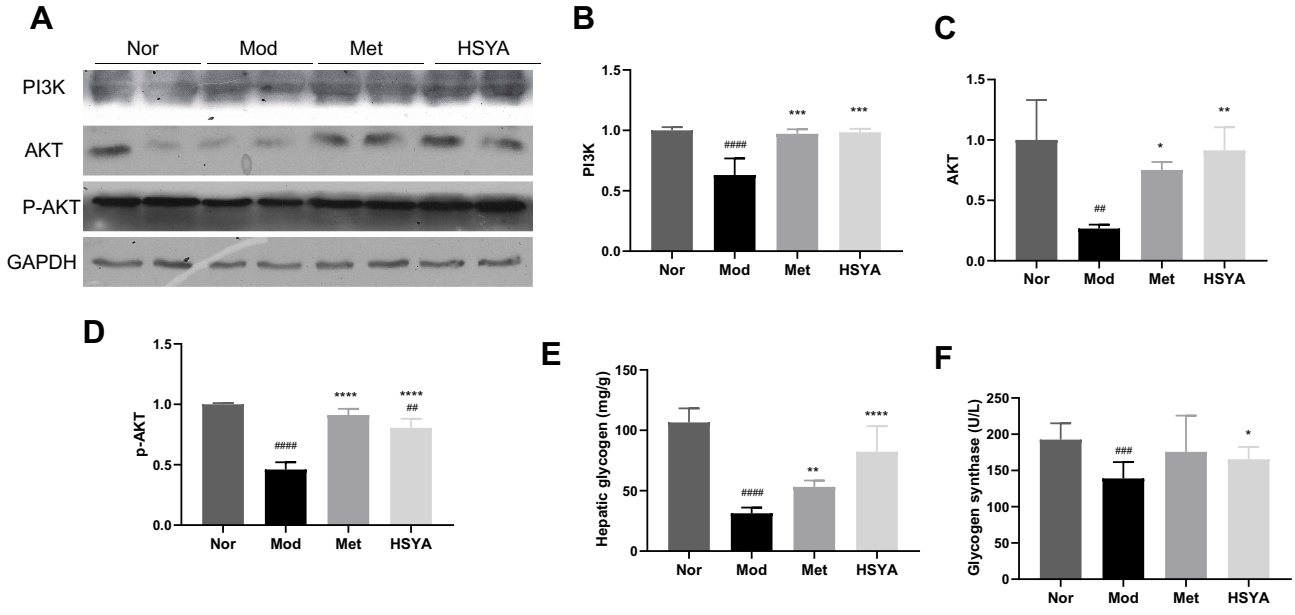

Figure 7 Effects of HSYA on PI3K, AKT and p-AKT expression and the content of hepatic glycogen and glycogen synthase in liver tissue. (**A, B**) The expression of PI3K in liver tissue. (**A, C**) The expression of AKT in liver tissue. (**A, D**) The expression of p-AKT in liver tissue. Results are presented as means ± SD and n=4 in each group. $^{\#\#}P<0.01$, $^{\#\#\#\#}P<0.0001$, vs Nor; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$, vs Mod. (**E**) The level of hepatic glycogen in liver tissue. (**F**) The level of glycogen synthase in liver tissue. Results are presented as means ± SD and n=8 in each group. $^{\#\#\#}P<0.001$, $^{\#\#\#\#}P<0.0001$, vs Nor; $^{*}P<0.05$, $^{**}P<0.01$, $^{****}P<0.0001$, vs Mod.

stasis syndrome.[37] Moreover, HSYA might be a potential therapeutic drug for obesity by regulating the gut microbiota.[38] Therefore, a higher dose of HSYA (120 mg/kg) was used for intervention in our study.

Conclusion

In conclusion, our results suggested that HSYA could promote PI3K/Akt activation and inhibit the apoptosis of pancreatic β-cells directly or indirectly, which might be the underlying mechanisms in HSYA to improve insulin resistance and regulate glycolipid metabolism on HFD and STZ-induced T2DM rats.

Abbreviations

TCM, Traditional Chines medicine; HSYA, Hydroxysafflor yellow A; T2DM, Type 2 diabetes mellitus; HFD, High-fat diet; STZ, streptozotocin; OGTT, Oral glucose tolerance test; FBG, Fasting blood glucose; FINS, Fasting insulin; AUC, Area under the curves; TC, Total serum cholesterol; TG, Triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol; HOMA-IR, Homeostatic model index-insulin resistant; HOMA-β, Homeostasis model assessment of β-cell function.

Acknowledgments

We appreciate all the participants in this study.

Funding

This study was supported by the National Natural Science Foundation of China (No. 81774225) and Sanming Project of Medicine in Shenzhen (SZSM201512043).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Kahn SE, Cooper ME, Prato SD. Pathophysiol ogy and treatment of type 2 diabetes: perspectives on the past, present, and future. *Lancet*. 2014;383(9922):1068–1083. doi:10.1016/S0140-6736(13)62154-6
2. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev*. 2013;93(1):137–188. doi:10.1152/physrev.00045.2011
3. Bailey CJ. Metformin: historical overview. *Diabetologia*. 2017;60(9):1566–1576. doi:10.1007/s00125-017-4318-z
4. Zhang L, Tian K, Tang Z, et al. Phytochemistry and pharmacology of Carthamus tinctorius L. *Am J Chin Med*. 2016;44(2):197–226. doi:10.1142/S0192415X16500130
5. Asgary S, Rahimi P, Mahzouni P, Madani H. Antidiabetic effect of hydroalcoholic extract of Carthamus tinctorius L. in alloxan-induced diabetic rats. *J Res Med Sci*. 2012;17(4):386–392.
6. Zhou X, Tang L, Xu Y, Zhou G, Wang Z. Towards a better understanding of medicinal uses of Carthamus tinctorius L. in traditional Chinese medicine: a phytochemical and pharmacological review. *J Ethnopharmacol*. 2014;151(1):27–43. doi:10.1016/j.jep.2013.10.050
7. Chu D, Liu W, Huang Z, Liu S, Fu X, Liu K. Pharmacokinetics and excretion of hydroxysafflor yellow A, a potent neuroprotective agent from safflower, in rats and dogs. *Planta Med*. 2006;72(5):418–423. doi:10.1055/s-2005-916249
8. Tian J, Li G, Liu Z, Fu F. Hydroxysafflor yellow A inhibits rat brain mitochondrial permeability transition pores by a free radical scavenging action. *Pharmacology*. 2008;82(2):121–126. doi:10.1159/000141653
9. Wu Y, Wang L, Jin M, Zang B. Hydroxysafflor yellow A alleviates early inflammatory response of bleomycin-induced mice lung injury. *Biol Pharm Bull*. 2012;35(4):515–522. doi:10.1248/bpb.35.515
10. D B J, Zhang LY, C L L, Ye J, Zhu H. Effect of hydroxysafflor yellow A on human umbilical vein endothelial cells under hypoxia. *Vascul Pharmacol*. 2009;50(3–4):137–145. doi:10.1016/j.vph.2008.11.009
11. Chen L, Xiang Y, Kong L, et al. Hydroxysafflor yellow A protects against cerebral ischemia–reperfusion injury by anti-apoptotic effect through PI3K/Akt/GSK3β pathway in rat. *Neurochem Res*. 2013;38(11):2268–2275. doi:10.1007/s11064-013-1135-8
12. Qi Z, Yan F, Shi W, et al. AKT-related autophagy contributes to the neuroprotective efficacy of hydroxysafflor yellow A against ischemic stroke in rats. *Transl Stroke Res*. 2014;5(4):501–509. doi:10.1007/s12975-014-0346-x
13. Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacol Res*. 2005;52(4):313–320. doi:10.1016/j.phrs.2005.05.004
14. Sahin K, Tuzcu M, Orhan C, et al. Anti-diabetic activity of chromium picolinate and biotin in rats with type 2 diabetes induced by high-fat diet and streptozotocin. *Br J Nutr*. 2013;110(2):197–205. doi:10.1017/S0007114512004850
15. Reed MJ, Meszaros K, Entes LJ, et al. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metab Clin Exp*. 2000;49(11):1390–1394. doi:10.1053/meta.2000.17721
16. Skovso S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *J Diabetes Investig*. 2014;5(4):349–358. doi:10.1111/jdi.12235
17. Hanley AJ, Williams KC, Stern MP, Haffner S. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease the San Antonio heart study. *Diabetes Care*. 2002;25(7):1177–1184. doi:10.2337/diacare.25.7.1177
18. Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. *Exp Biol Med*. 2012;237(5):481–490. doi:10.1258/ebm.2012.011372
19. Jiang B, Qu Z, Gu Y, et al. Renoprotective effect of JinQi-JiangTang tablet on high-fat diet and low-dose streptozotocin-induced type 2 diabetic rats. *RSC Adv*. 2018;8(73):41858–41871. doi:10.1039/C8RA07858K
20. Flanagan AM, Brown JL, Santiago CA, Aad PY, Spicer J, Spicer MT. High-fat diets promote insulin resistance through cytokine gene expression in growing female rats. *J Nutr Biochem*. 2008;19(8):505–513. doi:10.1016/j.jnutbio.2007.06.005
21. Ji G, Zhao X, Leng L, Liu P, Jiang Z. Comparison of dietary control and atorvastatin on high fat diet induced hepatic steatosis and hyperlipidemia in rats. *Lipids Health Dis*. 2011;10(1):23. doi:10.1186/1476-511X-10-23
22. Barter PJ, Nicholls SJ, Rye K, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. *Circ Res*. 2004;95(8):764–772. doi:10.1161/01.RES.0000146094.59640.13
23. Mi J, He W, Lv J, Zhuang K, Huang H, Quan S. Effect of berberine on the HPA-axis pathway and skeletal muscle GLUT4 in type 2 diabetes mellitus rats. *Diabetes Metab Syndr Obes Targets Ther*. 2019;12:1717–1725. doi:10.2147/DMSO.S211188
24. Atta MS, Elfar AH, Farrag F, Abdeldaim MM, Jaouni SK, Mousa SA. Thymoquinone attenuates cardiomyopathy in streptozotocin-treated diabetic rats. *Oxid Med Cell Longev*.2018;2018:7845681. doi:10.1155/2018/7845681

25. Chen Y, Wu Y, Yang Y, et al. Transcriptomic and proteomic analysis of potential therapeutic target genes in the liver of metformin-treated sprague-dawley rats with type 2 diabetes mellitus. *Int J Mol Med*. 2018;41(6):3327–3341. doi:10.3892/ijmm.2018.3535
26. Li H, Liu Y, Wen M, et al. Hydroxysafflor yellow A (HSYA) alleviates apoptosis and autophagy of neural stem cells induced by heat stress via p38 MAPK/MK2/Hsp27-78 signaling pathway. *Biomed Pharmacother*. 2019;114:108815.biopha.2019.108815. doi:10.1016/j.biopha.2019.108815.
27. Juan B, Jinyi Z, Dongxiao C, et al. Protective effect of hydroxysafflor yellow A against acute kidney injury via the TLR4/NF-κB signaling pathway. *Sci Rep*. 2018;8(1):9173. doi:10.1038/s41598-018-27217-3
28. Nandipati KC, Subramanian S, Agrawal DK. Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. *Mol Cell Biochem*. 2017;426(1–2):27–45. doi:10.1007/s11010-016-2878-8
29. Nana W, Tiegang L, Ping H. The effect of tianmai xiaoke pian on insulin resistance through PI3-K/AKT signal pathway. *J Diabetes Res*. 2016;2016:1–8.
30. Yu N, Fang X, Zhao D, et al. Anti-diabetic effects of Jiang Tang Xiao Ke granule via PI3K/Akt signalling pathway in type 2 diabetes KKAy mice. *PLoS One*. 2017;12(1):e0168980. doi:10.1371/journal.pone.0168980
31. Martin M, Rehani K, Jope RS, Michalek SM. Toll-like receptor–mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat Immunol*. 2005;6(8):777–784. doi:10.1038/ni1221
32. Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci*. 2004;29(2):95–102. doi:10.1016/j.tibs.2003.12.004
33. Woodgett JR. Recent advances in the protein kinase B signaling pathway. *Curr Opin Cell Biol*. 2005;17(2):150–157. doi:10.1016/j.ceb.2005.02.010
34. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther*. 2015;148:114–131. doi:10.1016/j.pharmthera.2014.11.016
35. Liu Z, Li C, Li M, et al. The subchronic toxicity of hydroxysafflor yellow A of 90 days repeatedly intraperitoneal injections in rats. *Toxicol*. 2004;203(1–3):139–143. doi:10.1016/j.tox.2004.06.007
36. Zhang HF, Guo JX, Huang LS, et al. Pharmacokinetics of hydroxysafflor yellow A in rats. *J China Pharm Univ*. 2006;37(5):456–460.
37. Tian Y, Yang ZF, Li Y, et al. Pharmacokinetic comparisons of hydroxysafflower yellow A in normal and blood stasis syndrome rats. *J Ethnopharmacol*. 2010;129(1):0–4. doi:10.1016/j.jep.2010.02.023
38. Liu J, Yue S, Yang Z, et al. Oral hydroxysafflor yellow A reduces obesity in mice by modulating the gut microbiota and serum metabolism. *Pharmacol Res*. 2018;134:40–50. doi:10.1016/j.phrs.2018.05.012