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Original Research Article

Gancao Xiexin Decoction exerts anti-hyperglycemic and anti-hyperlipidemic effects in type 2 diabetes mellitus rats via IRS2/PI3K/Akt pathway

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Abstract

Purpose: *To evaluate the anti-diabetic effect of the classical traditional Chinese medicine (TCM) formulation, Gancao Xiexin Decoction (GCXXT), as well as unravel its mechanism of action.*
Methods: *A rat model with type 2 diabetes mellitus (T2DM) was induced through ingestion of a high-fat diet, followed by injection of streptozocin. The rats were divided into six groups: control, model, metformin, GCXXT-L (4 g/kg), GCXXT-M (8 g/kg) and GCXXT-H (12 g/kg) groups, each with six rats. The metabolic parameters related to T2DM, expression and phosphorylation levels of IRS2, PI3K, and Akt protein were determined by western blot.*
Results: *GCXXT, especially at the high dose (12 g/kg), not only significantly reduced the levels of fasting blood glucose, hemoglobin A1c, and advanced glycation end products, but also increased the fasting insulin level, HOMA-β index, β-cell mass, and oral glucose tolerance levels in T2DM rats ($p < 0.05$). Furthermore, GCXXT exhibited anti-hyperlipidemic effect by elevating high-density lipoprotein cholesterol level and lowering total cholesterol, triglycerides, and low-density lipoprotein cholesterol levels ($p < 0.05$). Phosphorylated IRS2, PI3K, Akt protein in the pancreas, liver, and adipose tissues of T2DM rats were significantly increased by GCXXT ($p < 0.05$), whereas total IRS2, PI3K, and Akt protein levels did not change significantly.*
Conclusion: *GCXXT exerts anti-hyperglycemic and anti-hyperlipidemic effects, in part, by regulating the IRS2/PI3K/Akt signaling pathway in the pancreas, liver, and adipose tissues of T2DM rats. Thus, GCXXT is a potential agent for the management of T2DM but clinical studies are required to ascertain this.*

Keywords: *Gancao Xiexin Decoction, Type 2 diabetes mellitus, Anti-diabetes, IRS2, PI3K/Akt signaling pathway*

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INTRODUCTION

Diabetes, a universally major metabolic disorder, is distinguished by hyperglycemia subsequent to peripheral insulin resistance (IR), and is becoming a crucial health concern in China [1]. Type 2 diabetes mellitus (T2DM) is the primary type of diabetes, accounting for more than 90 %

of all cases, and is usually accompanied by multiple complications, such as diabetic retinopathy, diabetic nephropathy, and diabetic foot ulcers. Complications in numerous organs and tissues caused by T2DM seriously influence quality of life. Accordingly, the development of effective strategies for T2DM therapy is of great significance. To date, although proper exercise and limiting the consumption of glucose-rich food are the first-line approaches in the early stages of T2DM, pharmacological treatment remains a key strategy to successfully alleviating T2DM. In the last decade, traditional Chinese medicine (TCM) has gained popularity in the treatment of diabetes because of its unique advantages, including fewer side effects and multiple targets of action [2]. In the TCM system, diabetes is known as “Xiaoke” disease, for which knowledge and treatments have been recorded for more than 2000 years. As one of the most widely used Chinese herbs, licorice is often used in multiple TCM formulae to treat metabolic disturbances, especially diabetes [3]. Numerous studies have confirmed the anti-diabetic effect of licorice via its multiple active components, such as liquiritigenin [4] and 18β-glycyrrhetinic acid [5]. For example, a previous study indicated that glabridin, an isoflavonoid isolated from licorice, exerts several anti-diabetic effects including preventing glucose intolerance, decreasing fasting blood glucose (FBG) and blood glucose levels, as well as increasing body weight under hyperglycemic conditions [6]. *Gancao Xiexin* Decoction (GCXXT) is a classical formula of TCM with licorice as a sovereign drug, which was derived from ‘Shang Han Lun’, edited by Zhang Zhongjing. In the past decades, Chinese physicians used GCXXT as a complementary treatment for diabetes to alleviate several diabetic complications, including diabetic gastroparesis, pruritus, and metformin intolerance. However, the effect of GCXXT in the treatment of T2DM has been rarely studied. This study sought to explore the anti-diabetic effect of GCXXT on a T2DM rat model through the incorporation of a high-fat diet (HFD) with streptozotocin injection, and to preliminarily analyze the underlying mechanism.

EXPERIMENTAL

Animals

A total of 36 Sprague-Dawley (SD) rats aged six weeks (SPF grade, male, 180 - 200 g) were purchased from Guangdong Medical Laboratory Center (Guangzhou, China). Prior to the beginning of the experiments, the rats were acclimatized to a new environment for seven days. All experiments involving animals were reviewed and accepted by the Huizhou Central People's Hospital Animal Experimental Ethical Committee.

Study design

Based on random principles, the rats were shared into six groups (n = 6) numbered as follows: (1) control, (2) model, (3) metformin, (4) GCXXT-L, (5) GCXXT-M, and (6) GCXXT-H. The T2DM rat model was established as described previously [7]. Except for the rats from the control group which were fed a chow diet (CD), other rats were fed a HFD throughout the experimental period. Streptozotocin (STZ; Sigma-Aldrich, cat. No S0130, MO, USA) was dissolved in fresh 0.1 M citrate buffer to a concentration of 50 mg/mL before use. After two weeks of dietary manipulation, the fed rats were intraperitoneally injected with STZ (40 mg/kg) to create the T2DM rat model. The rats fed with CD were then administered with 0.1 M citrate buffer. T2DM was confirmed by examining the blood glucose level in the rats (≥ 16.7 mM) [8].

After successfully establishing the T2DM rat model, rats in all the groups received different treatments by oral gavage every day at a specific time point for 6 weeks. The treatment groups were as follows: control group (distilled water); model group (distilled water); metformin group was considered as positive control (300 mg/kg standard metformin (Sigma-Aldrich, cat. no 1396309, MO, USA); GCXXT-L group (low dose of GCXXT (4 g/kg)); GCXXT-M group (middle dose of GCXXT (8 g/kg)); GCXXT-H group (high dose of GCXXT (12 g/kg)). The standard recipe of GCXXT was as follows: Radix glycyrrhizae preparata (12 g), Radix scutellariae (9 g), Rhizoma zingiberis (9 g), Pinellia ternate (9 g), Fructus jujubae (24 g), and Rhizoma coptidis (3 g). During the treatment, the body weight of the rats was recorded once every two weeks. Biochemical index and oral glucose tolerance test (OGTT) were performed after concluding the treatment. After the last treatment for one week, the body weight of the rats was recorded for the last time before euthanization by cervical dislocation. Then, the pancreas, liver, and adipose tissues were collected from each rat, and then they were anesthetized in order to collect their pancreas after recording their body weight for the last time. Finally, the rats were euthanized by cervical dislocation.

Determination of biochemical parameters/indices

Approximately 1 mL of blood was taken from the ocular vein within 24 h of the last administration,

followed by centrifugation at 1,500 × g to separate and obtain serum for subsequent determinations. The serum levels of hemoglobin A1c (HbA1c), advanced glycation end products (AGEs), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assessed using the corresponding assay kits.

We computed the rats FBG and fasting insulin (FINS) after 6 h fasting [9], in addition to the HOMA-β index through the following formula [10]:

$$HOMA - \beta = 20 \times \frac{\text{FINS}}{(\text{FBG} - 3.5)}$$

In order to identify β-cell mass quantitatively, half of the pancreas isolated from the rats was embedded in paraffin, followed by longitudinal serial sectioning, then staining with insulin as previously described [11].

Oral glucose tolerance test (OGTT)

An OGTT was performed after measurement of Fasting blood glucose (FBG) levels. Glucose dissolved in distilled water (2.0 g/kg) was orally administered to each group of rats. Blood was then collected from the tail vein at 0 (prior to glucose intake), 30, 60, and 120 min to measure both the blood glucose and insulin levels of rats using an automated blood glucose analyzer (Bayer, Germany) and commercial quantitative enzyme-linked immunosorbent assay [(ELISA) (Thermo Scientific, Austin, Texas. USA), respectively.

Western blot assay

To study the molecular mechanism underlying the effect of GCXXT on T2DM rats, the total and phosphorylated protein expression levels of IRS2, PI3K, and Akt in the pancreas, liver, and adipose tissue from rats were investigated in each group. The pancreatic tissues were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer on ice to isolate total proteins. Bicinchoninic acid (BCA) protein assay kit was used to quantify the proteins, followed by purification with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, the membrane was blocked with 8% skimmed milk for 1 h prior to overnight incubation with specific primary antibodies at 4°C. The membranes were rinsed thrice with Tris-buffered saline-Tween (TBST), then, to determine proteins through ECL™ western blotting reagents, we incubated the corresponding secondary antibody (polyclonal goat anti-rabbit horseradish peroxidase-conjugated secondary antibody; Abcam, Cambridge, MA, USA, cat. No ab7090, MA, USA; 1/30000 dilution) with the membranes at room temperature for 1 h. In conclusion, we generated the gray values of each protein band by the ImageJ software (version 1.46). We computed the protein expression levels by comparing with the gray value of the β-actin band.

The dilutions of the primary antibodies used in this study were as follows: IRS2 (Abcam, Cambridge, MA, USA, cat. no ab134101, MA, USA; 1/1000 dilution), phosphorylated-IRS2 (p-IRS2) Ser731 (Abcam, Cambridge, MA, USA, cat. no ab3690, 1/1500 dilution); PI3K (Abcam, Cambridge, MA, USA, cat. no ab154598, MA, USA; 1/1000 dilution); p-PI3K P85α (Abcam, Cambridge, MA, USA, cat. no ab191606, 1/1000 dilution); Akt (Abcam, Cambridge, MA, USA, cat. no ab18805, 1/1000 dilution); p-Akt Ser473 (Abcam, Cambridge, MA, USA, cat. no ab38449 ab81283, MA, USA; 1/1000 dilution), and β-actin (Abcam, Cambridge, MA, USA, cat. No ab8226, MA, USA; 1/1500 dilution).

Statistical analysis

All the tests were completed and repeated three times. GraphPad Prism software (Version 8.0.1, La Jolla, CA, USA) was used to conduct statistical analyses of the data. We compared the groups and determined significant differences by applying one-way ANOVA, followed by Bonferroni's post hoc test. Significance was set at $P < 0.05$.

RESULTS

Effect of GCXXT on body weight of T2DM model rats

Before the beginning of treatment, the body weight of T2DM model rats was not significantly different from that of the normal rats (Table 1). Compared to the control group, the rats weight in the model group was significantly less after seven weeks ($p < 0.05$; Table 1). Treatment with metformin decreased the discrepancy in body weight between control rats and T2DM model rats ($p < 0.05$; Table 1). Similarly, the treatment with GCXXT slightly improved the body weight of T2DM model rats ($p < 0.05$), suggesting that GCXXT might have partially alleviated the deleterious effect of T2DM on body weight in rats.

Table 1: Body weight (g) of rats in different groups within 7 weeks

Group (n=6)	week 0	week 2	week 4	week 6	week 7
Control	195.38±7.82	228.40±6.39	255.28±5.21	291.27±5.46	312.29±6.41
Model	189.43±2.60	198.30±4.47	212.35±4.50	238.00±4.26	252.02±7.19
Metformin	193.48±6.69	230.56±6.01	255.12±6.48	285.28±5.54	305.80±8.63
GCXXT-L	196.28±3.57	215.32±2.42	235.23±6.63	253.97±6.13	270.83±8.94
GCXXT-M	196.52±3.95	224.03±4.10	241.45±4.64	262.90±3.33	285.62±5.54
GCXXT-H	199.80±3.26	225.18±5.42	246.78±2.74	278.03±2.48	301.28±5.40

Effect of GCXXT on glucose metabolic parameters in T2DM model rats

Glucose metabolic parameters (FBG. FINS, HOMA-β, HbA1c, and AGEs) of rats in different groups were evaluated to explore the anti-diabetic effect of GCXXT after six weeks of treatment. As shown in Figures 1 and 2, rats in the model group exhibited significant hyperglycemia, when compared with those in the control group, which was characterized by a significant increase in FBG, HbA1c and AGE levels, and a reduction in FINS level, HOMA-β index, and β-cell mass. As expected, metformin alleviated the deleterious effects induced by T2DM in such glucose metabolic parameters (Figure 1 and Figure 2). Similar to the treatment with metformin, treatment with GCXXT decreased FBG levels in T2DM model rats, and this effect was exerted in a concentration-dependent manner (Figure 1 A). As depicted in Figures 1 B and C, the FINS and HOMA-β of rats in the GCXXT group were significantly elevated in the model group, suggesting that GCXXT significantly improved the function of β-cells in T2DM model rats. After treatment with the medium- or high-dose GCXXT, there was a significant increase in total β-cell mass in T2DM model rats (Figure 1 D). In addition, treatment with GCXXT reduced the elevation of HbA1c and AGE levels in T2DM model rats (Figures 2 A and B). Collectively, these data revealed that GCXXT exerts an anti-hyperglycemic effect in a concentration-dependent manner in T2DM model rats.

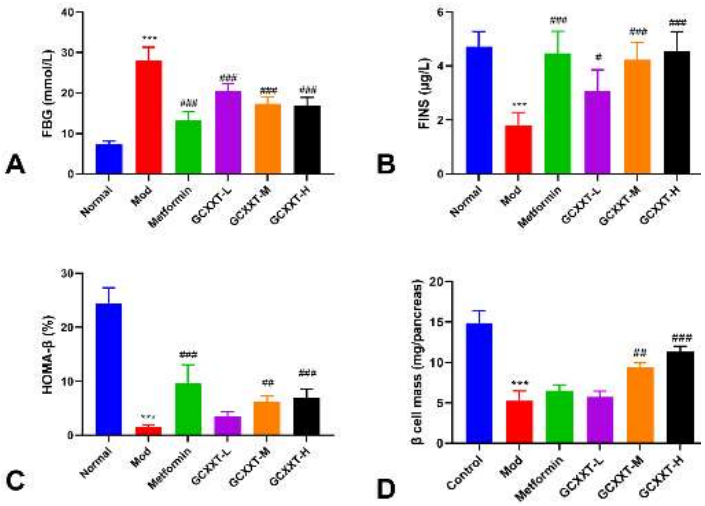

Figure 1: Fasting blood glucose (FBG), fasting insulin (FINS), HOMA-β index, and β-cell mass in rats from different groups. (A) FBG. (B) FINS. (C) HOMA-β; (D) β-cell mass. Note: $^{***}p < 0.001$ compared with the control group; $^{\#}P < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with the model group; n = 6

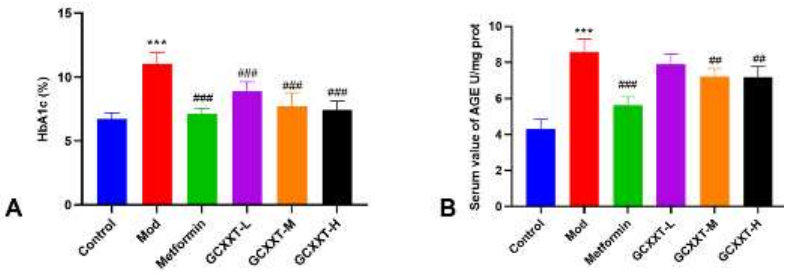

Figure 2: Levels of hemoglobin A1c (HbA1c) and advanced glycosylation end-products (AGEs) in rats from different groups. (A) HbA1c. (B) AGEs. Note: $^{***}p < 0.001$, compared with the control group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the model group; n = 6

Effect of GCXXT on lipid profiles in T2DM model rats

As depicted in Figure 3 A, B, and D, significant increases in TC, TG, and LDL-C levels were detected in the model group when compared with the control group. Simultaneously, the HDL-C level in the model group was significantly decreased compared to the control group (Figure 3 C). Metformin effectively corrected the lipid metabolism disorder induced by T2DM (Figure 3). As can be seen, among the different doses of GCXXT treatment, only the high-dose GCXXT (12 g/kg) significantly decreased the serum TC level of the T2DM rats (Figure 3 A). As shown in Figure 3 B, there was no obvious discrepancy in serum TG levels between the GCXXT-L group (4 g/kg) and the model group, while the increased serum level of TG in T2DM model rats was significantly countered by medium-dose (8 g/kg) and high-dose (12 g/kg) GCXXT. In addition, treatment with GCXXT significantly increased serum HDL-C levels in T2DM model rats in a concentration-dependent manner (Figure 3 C). Moreover, treatment with GCXXT also reduced serum LDL-C level in T2DM model rats in a concentration-dependent manner (Figure 3 D). These data suggest that GCXXT played a certain role in improved blood lipid profiles of T2DM model rats.

Effect of GCXXT on oral glucose tolerance in T2DM model rats

In the OGTT, the model group showed significantly higher levels of blood glucose than the control group, whereas, the blood insulin levels at 0 and 30 min were significantly lower. These results further confirmed that the T2DM rat model was successfully established (Figures 4A and 4B; Figure 5A). In addition, both metformin and GCXXT significantly reduced blood glucose levels in T2DM model rats. The OGTT-AUC of the different groups (Figure 5 C) showed that the values of OGTT-AUC in metformin, GCXXT-L, GCXXT-M, and GCXXT-H groups were 42.1, 49.2, 6.8, and 44.4, respectively, which were statistically lower than those of the model group (61.4). Compared with that in the control group, the AUC of OGTT-insulin was significantly decreased in the model group, and this decrease was partly reversed by metformin and GCXXT at middle and high doses (Figure 5B). The results revealed that the oral glucose tolerance of T2MD model rats was significantly impaired compared with the normal rats, and GCXXT significantly improved the oral glucose tolerance of T2MD model rats.

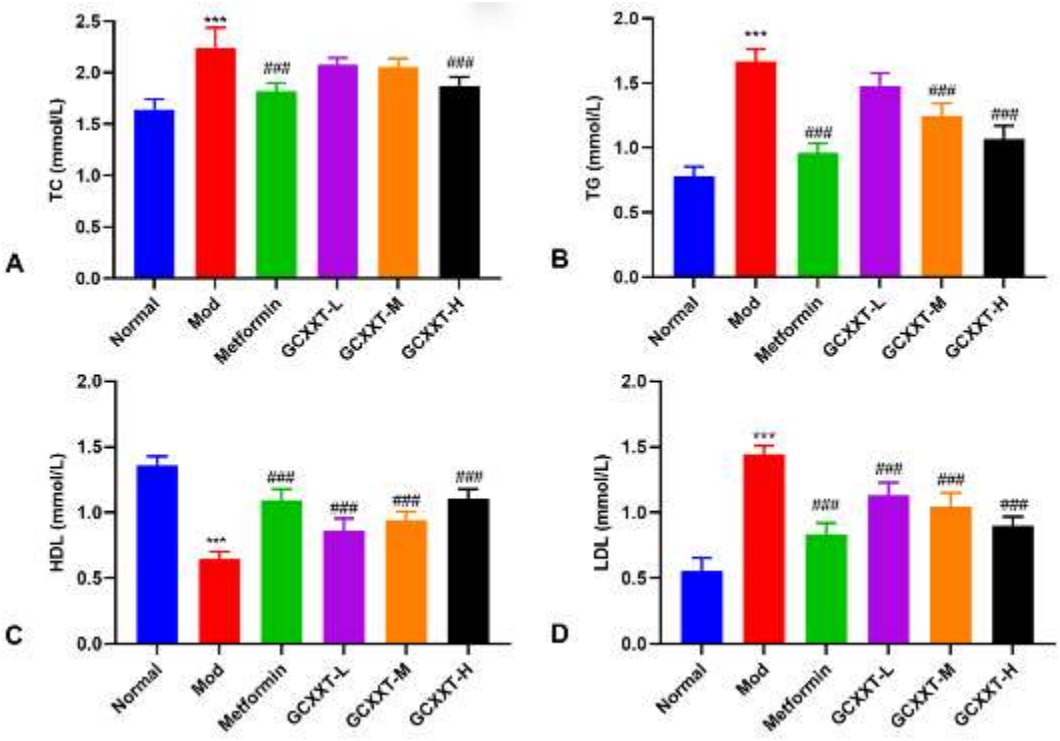

Figure 3: Lipid levels of rats from different groups. (A) Total cholesterol (TC). (B) Triglyceride (TG). (C) High-density lipoprotein cholesterol (HDL-C). (D) Low-density lipoprotein cholesterol (LDL-C). $^{***}P < 0.001$ compared with the control group; $^{###}p < 0.001$ compared with model group; n = 6

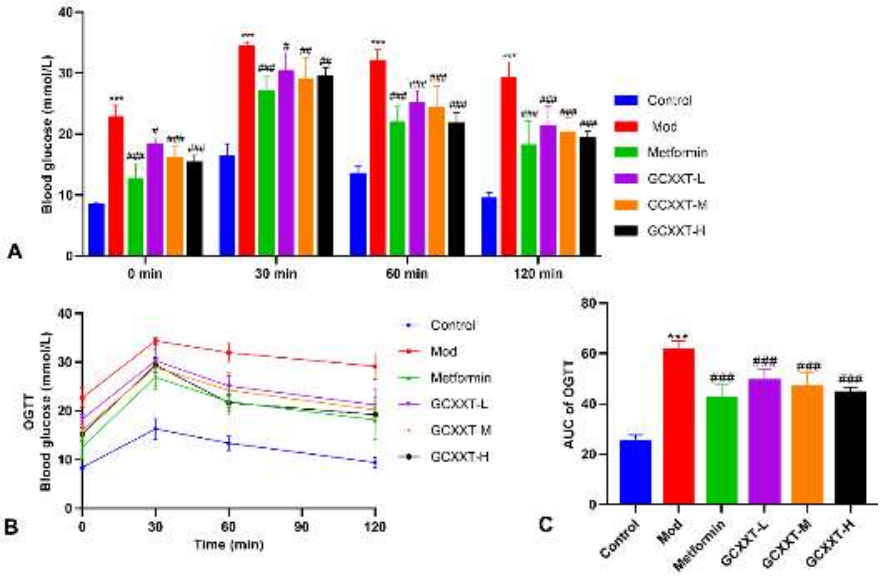

Figure 4: Oral glucose tolerance test (OGTT) results for rats in different groups. (A) Bar plot showing the blood glucose level of rats at different time points (0, 30, 60, and 120 min) after glucose intake. (B) OGTT curve of rats from each group. (C) Area under the OGTT curve of rats from each group. $^{***}p < 0.001$ compared with the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.00,1$ compared with model group; n = 6

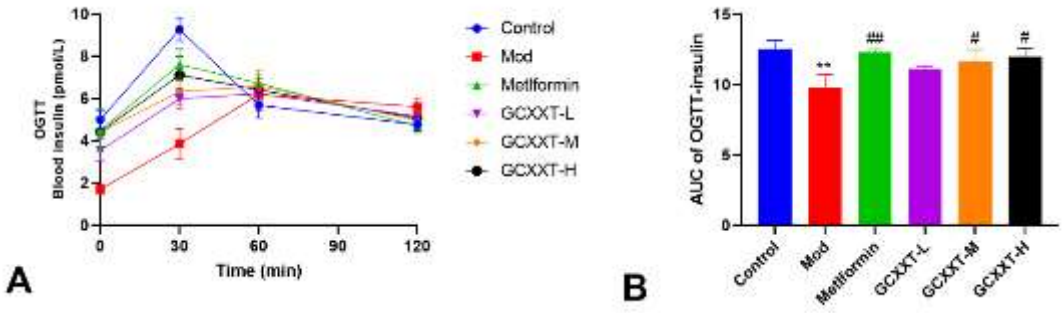

Figure 5: Insulin levels in rats in different groups. (A) OGTT-insulin curve of rats from each group. (C) Area under the OGTT-insulin curve of rats from each group. $^{**}p < 0.01$, compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the model group; n = 6

GCXXT exerts anti-diabetic effects in T2DM model rats via IRS2/PI3K/Akt pathway

To understand the molecular mechanism of the anti-diabetic effects of GCXXT in T2DM model rats, we assessed the effects of GCXXT on IRS2, PI3K, and Akt protein levels using western blot assay in the pancreas of rats. The results of the protein bands are shown in Figure 6 A. As shown in Figure 7C and D, there was no significant difference in total protein expressions of PI3K and Akt between the groups. Furthermore, the total protein expressions of IRS2 did not differ between control and model groups (Figure 6 B). In addition, the phosphorylation levels of IRS2 in the model group were significantly reduced compared to control group, but metformin, medium-dose and high-dose GCXXT reversed this reduction to a certain degree (Figure 7 B). Moreover, treatment with metformin simultaneously increased the total protein levels of IRS2 in T2DM model rats. As shown in Figure 6 C, no statistical difference in the phosphorylation levels of PI3K was observed between control and model groups. Notably, both metformin and GCXXT treatment significantly elevated the levels of p-PI3K. It was also observed that the elevation of p-PI3K in GCXXT groups was concentration-dependent and was more pronounced than that in the metformin group. Moreover, the phosphorylation levels of Akt exhibited a similar tendency to those of IRS2, indicating that both metformin and GCXXT treatments significantly reversed the reduction of p-Akt induced by T2DM (Figure 6 D).

Furthermore, insulin signaling in other insulin target tissues, including the liver and adipose tissue, was also observed (Figure 7). The results showed that insulin signaling was suppressed in

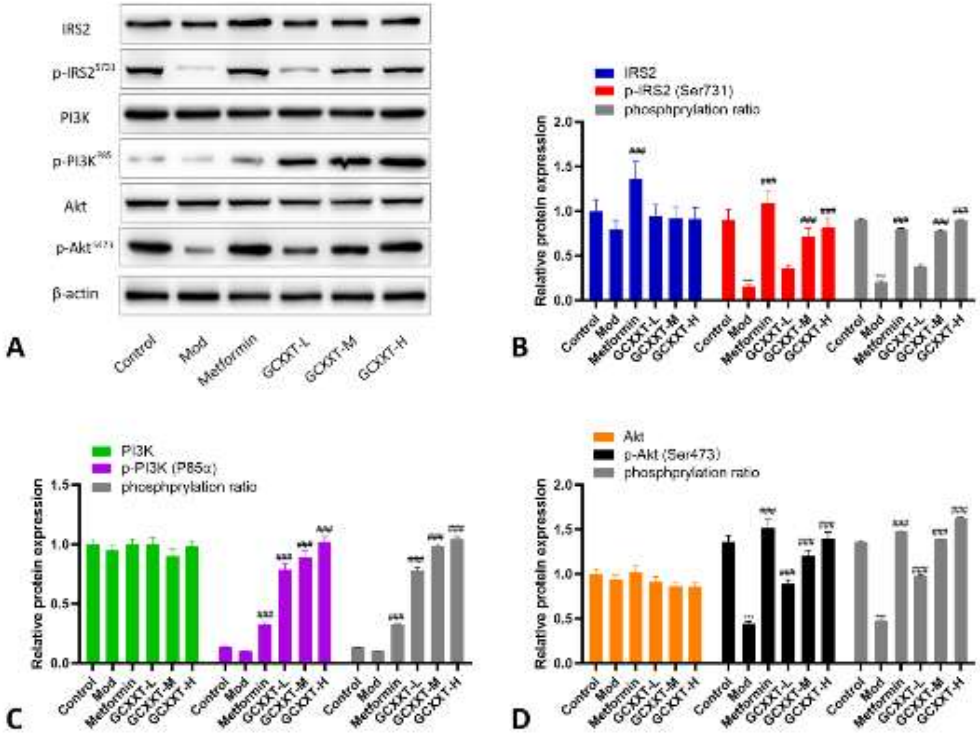

Figure 6: Total and phosphorylated protein levels of IRS2, PI3K, and Akt in rat pancreas f. (A) Relative expression levels of total protein and phosphorylated protein of (B) IRS2, (C) PI3K, and (D) Akt. $^{***}P < 0.001$ compared with the control group; $^{###}p < 0.001$ compared with the model group; n = 6

both the liver and adipose tissues of T2MD model rats. Treatment with GCXXT partly reversed the suppression. Taken together, these results suggest that GCXXT exerts anti-diabetic effects in T2DM model rats by upregulating the phosphorylation levels of IRS2, PI3K, and Akt.

DISCUSSION

Cumulative evidence has proven that TCM is a promising method for T2DM treatment [12]. In the present study, a classical TCM formula, GCXXT, was applied to treat T2DM rat models to discover the possible role of GCXXT in reducing T2DM and its underlying mechanism. STZ is the most common chemical inducer in the establishment of diabetes models because it can specifically destroy β cells, resulting in diabetic renal damage and hyperglycemia, thereby mimicking the pathological condition of diabetes [13]. Our study combined an HFD that induces insulin resistance (IR) and a low dose of STZ administration that causes initial β-cell dysfunction to establish a T2DM rat model. The model incorporating an HFD and STD imitates not only the phenotype, but also the initiation and development of human T2DM [14]. Metformin was chosen as a positive control in this study as it is currently the 'gold standard' drug for the treatment of T2DM. In this study, rats exhibited symptoms of hyperglycemia and hyperlipidemia, including significant increases in FBG, FINS, HbA1c, AGE, TG, TC, and LDL-C, and an obvious decrease in LDL-H and oral glucose tolerance, which confirmed the successful establishment of the T2DM model. GCXXT is a classical TCM decoction for treating epigastric fullness, and consists of Radix Glycyrrhizae Preparata (licorice), *Radix scutellariae*, Rhizoma zingiberis, *Pinellia ternata*, Fructus jujubae, and Rhizoma coptidis. Most of the above herbal drugs have been proven capable of improving diabetes conditions.

The most direct and crucial way to control T2DM is the management of FBG. Our results demonstrated that GCXXT significantly reduced FBG levels in a T2DM rat model. A previous study demonstrated that licorice extracts ameliorated IR in HFD-induced obese rats [15], which might explain the FBG reducing effect of GCXXT. This study also showed that GCXXT has the capacity to improve the function of β-cells and reduce the serum levels of HbA1c and AGEs, which further confirmed the anti-hyperglycemic effect of GCXXT in T2DM rats. Hyperlipidemia is another common symptom of T2DM.

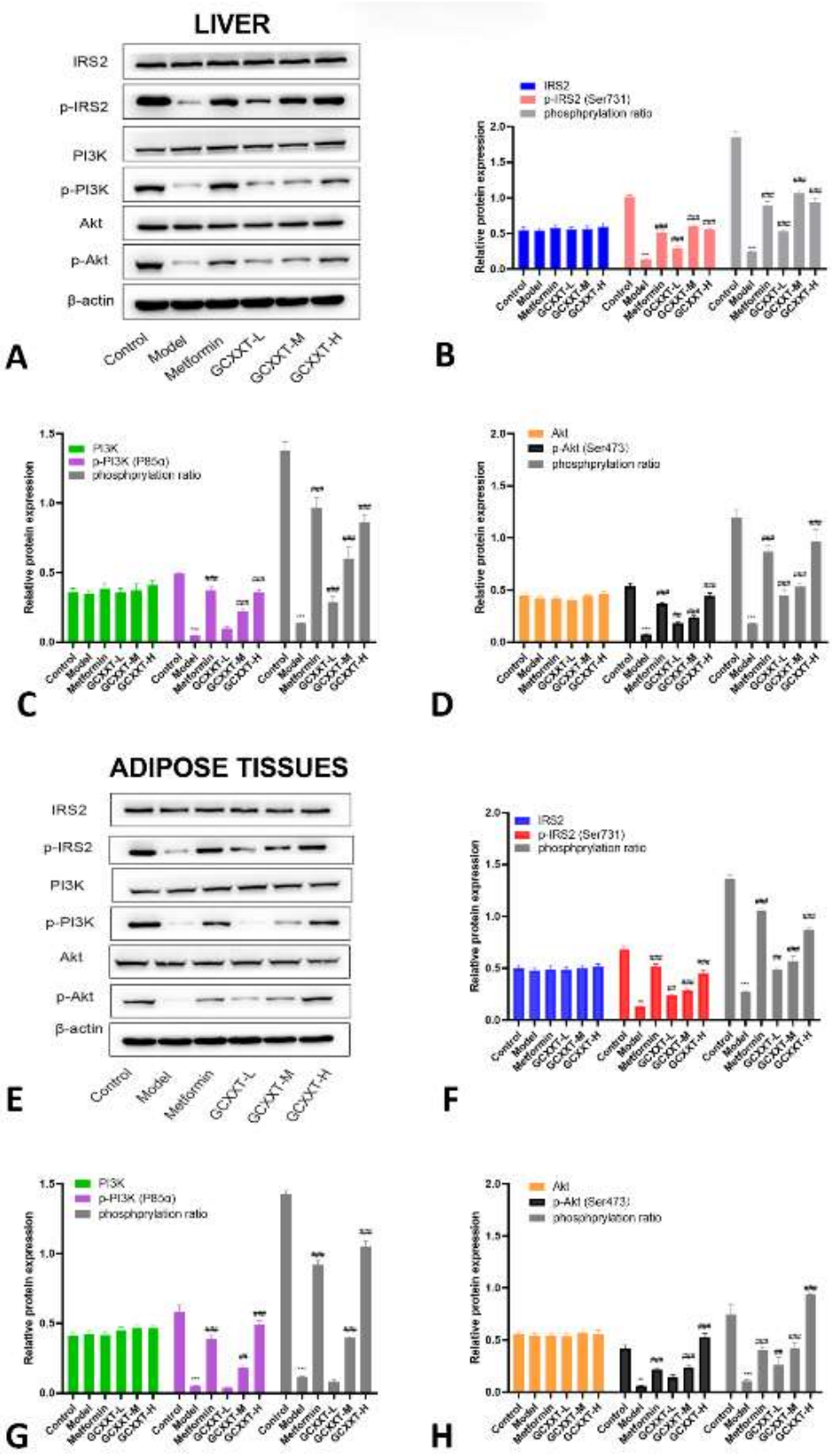

Figure 7: Total and phosphorylated protein levels of IRS2, PI3K, and Akt in the livers and adipose tissue of rats (A) total protein and phosphorylated protein of IRS2 (B), PI3K(C), and (D) Akt. (E) The result of western blot from rat adipose tissue; and the quantification of total protein and phosphorylated protein of (F) IRS2, (G) PI3K, and (H) Akt. $^{**}P < 0.01$, $^{***}p < 0.001$ compared with control group; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the model group; n = 6

Therefore, improving diabetic dyslipidemia is an important approach for T2DM treatment. In this study, T2DM rats were characterized by dyslipidemia, such as increased serum levels of TC, TG, and LDL-C, and decreased serum HDL-C levels. GCXXT treatment effectively reversed the changes induced by T2DM.

IR and impaired pancreatic β-cell function are key elements in the T2DM pathogenesis. The deterioration of β-cell function is important for the occurrence and development of T2DM. Our study showed that GCXXT has the capacity to ameliorate the β-cell function. Multiple studies have showed that insulin signaling (IRS/PI3K/Akt) is important in pancreatic β-cell multiplication, apoptosis, and function, as well as the glucose metabolism in insulin-targeted tissues during T2DM pathology [16,17]. Thus, we further explored the mechanism behind the improving effect of GCXXT on β-cells by detecting the expression of insulin signaling proteins in the pancreas, liver, and adipose tissues. In insulin signaling, activated insulin receptor makes the tyrosine site of IRS2 phosphorylation, subsequently activates PI3K; activated PI3K can catalyze PIP2 and generate PIP3 to activate Akt, thereby exert diverse physiological and biochemical effects by regulating downstream molecules (GSK-3, GLUT-4, and ERK). Consistent with previous studies [18], the present study showed that the insulin signaling in β-cells was significantly suppressed after IR, whereas this suppression was partly reversed by the treatment of GCXXT. The activation of insulin signaling has been implicated in the normal proliferation and anti-apoptosis of β-cells [19,20]. It has been reported that several bioactive compounds of licorice increased the induction of IRS2 [21]. Besides, *Radix scutellariae* also has been proven to promote β-cell proliferation through IRS2 induction [22]. Hence, GCXXT improves β-cell mass and insulin secretion by regulating insulin signaling in the pancreas via licorice and *Radix scutellariae*. Notably, this study showed that GCXXT increases β-cell mass more than metformin, but insulin levels during OGTT and Akt phosphorylation levels in β-cell are not different between them. This may be because of the different hypoglycemic mechanisms between metformin and GCXXT in T2DM. Insulin exerts a hypoglycemic effect by stimulating the liver and adipose tissue to uptake glucose and fatty acids via insulin signaling activation in the liver and adipose tissue [23]. IRS2 has a critical function in lowering gluconeogenesis and controlling the PI3K/Akt cascade. Interestingly, the examination of IRS/PI3K/Akt in the liver and adipose tissues revealed that GCXXT also regulated insulin signaling in these insulin-targeted tissues. Given the critical role of IR in the development of dyslipidemia [24], HDL-raising and hypocholesterolemic effects of GCXXT treatment might be associated with its regulatory role in insulin signaling. These data revealed that GCXXT probably improved β-cell function and alleviated IR to achieve anti-hyperglycemic and anti-hyperlipidemic effects in T2DM rats by affecting the IRS2/PI3K/Akt signaling pathway in both pancreatic β-cell and insulin-targeted tissues (liver and adipose tissues). However, this study is preliminary in nature; the mechanisms by which GCXXT modulates downstream molecules of IRS-2/PI3K/Akt remain far from clear. Hence, further investigations at the molecular level are required to further clarify the underlying mechanism of the effect of GCXXT in the future. This research is the first to study the anti-diabetic effects of GCXXT and its corresponding molecular mechanism. The present study indicated that GCXXT has anti-hyperglycemic and anti-hyperlipidemic effects in T2DM rats by regulating IRS2/PI3K/Akt signaling pathway, which provides new evidence and scientific information for the clinical application of GCXXT in T2DM.

DECLARATIONS

Acknowledgements

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Funding

None provided.

Ethical approval

Approval for this study was obtained from Huizhou Central People's Hospital Animal Experimental Ethical Committee, China.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the correspon-

ding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. **Bo Yang and Junxian Chen contributed equally.** Bo Yang, Junxian Chen and Hongtao Tan designed the study and performed the experiments, Yiqun Li and Lingling Liu collected the data, Yiqun Li, Lingling Liu and Yunchang Zhong analyzed the data, Bo Yang, Junxian Chen and Hongtao Tan prepared the manuscript. All authors read and approved the final manuscript.

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