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Hypoglycemic and hypolipidemic effects of total glycosides of *Cistanche tubulosa* in diet/streptozotocin-induced diabetic rats

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ABSTRACT

Ethnopharmacological relevance: Cistanche tubulosa (Schrenk) R. Wight (Orobanchaceae) is a frequently prescribed component in many traditional herbal prescriptions which are used to treat diabetes in China. In recent studies, the antidiabetic activity of *Cistanche tubulosa* extracts have been confirmed. However, no systematic investigation has been reported on the total glycosides of *Cistatnche tubulosa* (TGCT).

Aim of the study: The present study aimed to investigate the hypoglycemic and hypolipidemic effects of TGCT and the potential mechanisms in diet/streptozotocin (STZ)-induced diabetic rats, and to chemically characterize the main constituents of TGCT.

Materials and methods: The major constituents of TGCT were characterized by HPLC/Q-TOF-MS and the analytical quantification was performed with HPLC-DAD. Type 2 diabetic rats were induced by high-fat high-sucrose diet (HFSD) and a single injection of STZ (30 mg/kg). TGCT (50 mg/kg, 100 mg/kg and 200 mg/kg) or metformin (200 mg/kg) were orally administered for 6 weeks. Body weight and calorie intake were monitored throughout the experiment. Fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), area under curve of glucose (AUC-G), glycosylated hemoglobin (HbA1c), fasting insulin, serum C-peptide, glycogen content and insulin sensitivity index were tested. The levels of phosphorylated protein kinase B and phosphorylated glycogen synthase kinase 3β, the activities of hexokinase and pyruvate kinase were assayed. Meanwhile, the changes in serum lipid profiles, superoxide dismutase, glutathione peroxidase, malondialdehyde and inflammatory factors were measured. Histological of pancreas were also evaluated by haematoxylin-eosin stain.

Results: Our investigation revealed the presence of phenylethanoid glycosides (PhGs): echinacoside (500.19 ± 11.52 mg/g), acteoside (19.13 ± 1.44 mg/g) and isoacteoside (141.82 ± 5.78 mg/g) in TGCT. Pharmacological tests indicated that TGCT significantly reversed STZ-induced weight loss (11.1%, 200 mg/kg); decreased FPG (56.4%, 200 mg/kg) and HbA1c (37.4%, 200 mg/kg); ameliorated the OGTT, AUC-G and insulin sensitivity; increased glycogen content (40.8% in liver and 52.6% in muscle, 200 mg/kg) and the activities of carbohydrate metabolizing enzymes; regulated lipid profile changes and the activities of antioxidant enzymes; diminished serum markers of oxidative stress and inflammation in a dose-dependent manner ($p < 0.05$).

Conclusions: This study confirmed that TGCT was an effective nutritional agent for ameliorating hyperglycemia and hyperlipidemia in diet/STZ-induced diabetic rats, which might be largely attributed to the activities of TGCT on inhibitions of oxidative stress and inflammation.

Abbreviations: AUC-G, area under curve of glucose; DC, diabetic control; FINS, fasting insulin; FPG, fasting plasma glucose; GSH-Px, glutathione peroxidase; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; H&E stain, haematoxylin-eosin stain; HFSD, high-fat high-sucrose diet; HK, hexokinase; HPLC, high performance liquid chromatography; IR, insulin resistance; IL-1β, interleukin-1β; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; Met, metformin; MDA, malondialdehyde; NC, normal control; OGTT, oral glucose tolerance test; PhGs, phenylethanoid glycosides; p-GSK3β, phosphorylated glycogen synthase kinase 3β; PK, pyruvate kinase; p-PKB, phosphorylated protein kinase B; SOD, superoxide dismutase; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triacylglycerol; TGCT, total glycosides of *Cistanche tubulosa*; TNF-α, tumor necrosis factor-α.

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1. Introduction

Diabetes mellitus is considered as a metabolic disease, which is characterized by hyperglycaemia resulting from impaired insulin production or/and insulin resistance (IR) (American Diabetes Association, 2019). Chronic hyperglycaemia is associated with multiple severe complications such as nephropathy, retinopathy, neuropathy and cardiac problems (Ekoe, 2019). The number of global diabetic people in 2019 is estimated to be 463 million (9.3%), rising to 578 million (10.2%) by 2030 and 700 million (10.9%) by 2045 (Saeedi et al., 2019). Several drugs such as metformin (Met, augmenting the production of hepatic glycogen), insulin (suppressing glucose production and increasing glucose utilization) and sulfonylureas (stimulating pancreatic islet cell to secrete insulin) are effective in reducing glycaemia. However, lots of undesirable adverse reactions (including weight gain, hypoglycemia, IR and edema) had limited their use (Moller, 2001). Hence, many researchers have been searching for biologically active compounds from traditional plant extracts for the treatment of diabetes over the past years (Kasangana et al., 2019; Liu et al., 2020).

Cistanche tubulosa (Schrenk) R. Wight (Orobanchaceae) has been extensively used in traditional Chinese medicine, which is frequently prescribed in traditional formulas for treating kidney deficiency, female infertility and diabetes mellitus (Li et al., 2016; Han et al., 2017; Su et al., 2017). Recent studies have reported that aqueous extract of *Cistanche tubulosa* showed hypoglycemic and hypolipidemic effects in *db/db* mice with type 2 diabetes mellitus (T2DM) (Xiong et al., 2013), and ameliorated blood glucose levels, IR and lipid peroxidation in streptozotocin (STZ)-nicotinamide-induced diabetic rats (Kong et al., 2018). Phenylethanoid glycosides (PhGs) are the major constituents of *Cistanche tubulosa* (Morikawa et al., 2014), which has exhibited various biological activities such as antioxidation (Xue et al., 2017) and anticancer (Fu et al., 2019). Moreover, PhGs significantly inhibited the increase of postprandial blood glucose levels in starch loaded mice (Morikawa et al., 2014), suppressed the sodium-dependent glucose cotransporter 1-mediated glucose uptake in intestinal epithelial cells (Shimada et al., 2017), and inhibited the activity of aldose reductase in rat lens (Morikawa et al., 2019). However, no previous research has investigated the anti-hyperglycemic activity of total glycosides of *Cistanche tubulosa* (TGCT).

In the present study, the antidiabetic properties of TGCT have been evaluated in high-fat high-sucrose diet (HFSD) and STZ-induced diabetic rats. In addition, the antioxidant and anti-inflammatory activities were also investigated to comprehensively understand the potential mechanism of TGCT.

2. Materials and methods

2.1. Chemicals and reagents

STZ was purchased from Sigma-Aldrich Corp. (Saint Louis, USA). Met was obtained from Sino-American Shanghai Squibb Pharma. The ELISA kits of insulin, C-peptide were supplied by Elabscience Biotechnology Co., Ltd (Wuhan, China). The ELISA kits of glucose, glycosylated hemoglobin (HbA1c), total cholesterol (TC), triacylglycerol (TG), low-density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), interleukin 6 (IL-6) were purchased from Nanjing Jiancheng Bioengineering Institute. The ELISA kits of phosphorylated protein kinase B (p-PKB) and phosphorylated glycogen synthase kinase 3β (p-GSK3β) were acquired from Shanghai Enzyme-linked Biotechnology Co., Ltd. Echinacoside (purity ≥ 98%) and acteoside (purity ≥ 97%) were purchased from National Institutes for Food and Drug Control. Isoacteoside (purity ≥ 98%) was provided by Chengdu Must Biotechnology Co., Ltd.

2.2. Plant resources and preparation of TGCT

Dried succulent stem of *Cistanche tubulosa* was purchased from Bozhou Yihongtang Pharmaceutical Co., Ltd., (Anhui, China) and identified by Dr. Puyang Gong of Pharmaceutical Botany Department, Southwest Minzu University. A voucher specimen (No. 20161103) was crushed into a powder and deposited in the herbarium of Jiangsu Kanion Parmaceutical Co., Ltd. According to the SFDA national drug standard "Congrong Zonggan Jiaonang" (YBZ07482005-2011Z), the crude drug (1 kg) was extracted with water for three times after soaking for 1 h. After filtration, the filtrate was concentrated under reduced pressure, added alcohol to the concentrated solution until the ethanol concentration reached to 60%. Liquid supernatant was concentrated to no alcohol taste and then be purified by macroporous resin. Firstly, water eluent and 40% ethanol eluent were collected successively for later use. Secondly, water eluent was reinjected into the macroporous resin and eluted with water. The water eluent was discarded. Thirdly, elution with 40% ethanol and the eluent was collected for later use. Finally, 40% ethanol eluents were combined and concentrated by rotary evaporator, then the solution was dried by spray drying. About 60 g brown power was obtained (i.e. TGCT). The purity of TGCT was detected according to the standard (YBZ07482005-2011Z), which is reached up to 853 mg/g. TGCT (0.21 mg) and three mixed standards (echinacoside: 131.3 μg, acteoside: 4.2 μg, isoacteoside: 39.4 μg) were respectively dissolved in 1 mL methanol: water (50/50, v/v), and then filtered through a 0.45 μm membrane prior to injection.

2.3. Qualitative analysis of TGCT by HPLC/Q-TOF-MS

The HPLC system was interfaced with an Agilent 6538 Q-TOF-MS (Agilent Corp, USA) equipped with electro-spray ionization. The analysis was carried out on Zorbax SB-C18 column (150 mm × 4.6 mm, 5 μm). Mobile phase was composed of methanol-water (containing 0.1% H_3PO_4). The flow rate was 1.0 mL/min. Gradient elution programs were summarized as follows: 0–20 min, 18%–28% A; 20–50 min, 28%–32% A; 50–60 min, 32% A; 60–70 min, 32%–50%. TOF-MS was performed in both positive and negative ion mode over m/z 100–3000 under the following operation parameters: capillary voltage 3500 V (ESI-) or 4000 V (ESI+); drying gas, 10.0 L/min; gas temperature, 350 °C; nebulizer pressure, 35 psi; skimmer voltage, 65 V; fragmentor voltage, 135 V; OCTRFV, 750 V. All data were controlled by Data Acquisition for TOF/Q-TOF Ver. B.03.01 and Qualitative Analysis Ver. B.03.01 (Agilent Technologies, USA) respectively.

2.4. Quantitative analysis of TGCT by HPLC-DAD

Separation was performed on Zorbax SB-C18 column (150 mm × 4.6 mm, 5 μm) within 50 min (1.0 mL/min). Chromatographic conditions for HPLC-DAD were same as the qualitative analysis. The injection volume, column temperature, and UV wavelength were set at 5 μL, 30 °C and 330 nm, respectively.

2.5. Experimental animals

Male SD rats, weighing 180 ± 20 g, were purchased from the Laboratory Animal Center of Nanjing Qinglongshan [Certificate No. SCXK (SU) 2017-0001] and housed at the Animal Care Facility in Jiangsu Kanion Pharmaceutical Co., Ltd. (Jiangsu, China). The animal care and experimental procedures were approved by Institutional Animal Care and Use Committee, Huzhou Institute for Food and Drug Control (Approval No. 19018), and were performed according to the Regulations for Animal Experiments of China. Rats were maintained under controlled temperature (24 ± 2 °C) and humidity (50 ± 10%) with a 12 h light and dark cycle, acclimatized to living conditions for 7 days by normal laboratory chow and water ad libitum.

2.6. Induction of diabetes

Rats in normal control group (NC, n = 10) were fed with a normal diet, whereas the experimental rats were fed with HFSD (a normal diet supplemented with 20% sucrose, 10% lard, 2.5% cholesterol and 1% cholate, 3.95 kcal/g) for 4 weeks. After a 12 h fasting, rats were intraperitoneally injected with a single dose of STZ (30 mg/kg), which dissolved in cold citrate buffer (0.1 M, pH 4.5) immediately before use. On the 8th day of STZ-injected, blood sample was taken from the tail-end by disposable blood taking needle, and fasting plasma glucose (FPG) was determined by the portable glucometer (LifeScan, Inc. UK). Rats with the symptoms of polyuria, polydipsia and FPG ≥11.1 mmol/L were considered as diabetic rats and randomly divided into five groups (n = 10).

Group I: NC, fed with 0.5% sodium carboxyl methylcellulose (CMC-Na, 10 mL/kg).

Group II: diabetic control (DC), fed with 0.5% CMC-Na (10 mL/kg).

Group III: TGCT-50, treated with TGCT (50 mg/kg).

Group IV: TGCT-100, treated with TGCT (100 mg/kg).

Group V: TGCT-200, treated with TGCT (200 mg/kg).

Group VI: Met-200, treated with Met (200 mg/kg).

The doses used in this study were selected based on Chinese Pharmacopoeia (2015 edition). All groups were administered orally once daily and continued to receive their respective diets for another 6 weeks.

2.7. Observe the general condition of rats

Fur condition, urine output and survival of rats were observed every day. Body weight (BW) and calorie intakes were monitored throughout the experiment. FPG was evaluated at 0, 2nd, 4th and 6th weeks after treatment with TGCT.

2.8. Oral glucose tolerance test (OGTT)

The OGTT was performed on overnight-fasted rats at the terminal stage of the whole study. Only 60 μL blood samples were collected with capillary pipet from the orbital sinus (0 h), then administered with TGCT (50 mg/kg, 100 mg/kg and 200 mg/kg) or Met (200 mg/kg) respectively. Blood samples were collected at 0.5 h, 1 h, 2 h after glucose load (2 g/kg). The rats were anaesthetized with isoflurane for a few minutes before taking blood, and then immediately press to stop the bleeding by hemostatic cotton. All the experiments were done with good care to ensure the welfare of the animals. Plasma glucose concentrations were determined by glucose kit based on glucose oxidase peroxidase method. Area under curve of glucose (AUC-G) was calculated refer to the literature (Shao et al., 2013).

2.9. Determination of fasting insulin (FINS) and insulin sensitivity index (ISI)

Interval of 1 day after OGTT, all rats were in good conditions without any symptoms such as blindness and inflammation. Then, they were anaesthetized with pentobarbital sodium (40 mg/kg, i.p.) after fasting for 12 h and blood samples were collected from the abdominal aorta with and without heparin for biochemical estimations. Serum was collected from blood samples (without heparin) by centrifugation. FINS was assayed by the ELISA kit. FPG was determined using commercial kit based on the glucose peroxidase method. ISI was calculated in accordance with the formula: ISI = 1/[FINS (pmol/L) × FPG (mmol/L)] (Wang et al., 2013).

2.10. Estimation of glycogen synthesis in liver and muscle

Liver and gastrocnemius muscle were excised, rinsed, weighed and stored at −70 °C. Glycogen in liver and muscle were measured by the anthrone method as described previously (Ren et al., 2015). The content of glycogen was expressed as mg/g wet weight of tissue. The activities of hexokinase (HK) and pyruvate kinase (PK) in liver were determined by commercially available kits according to the manufacturer's instructions.

2.11. Biochemical analysis

HbA1c in whole blood (with heparin) was measured by diagnostic kit. Serum C-peptide, p-PKB, p-GSK3β, TC, TG, LDL-C, HDL-C, SOD, GSH-Px, MDA, TNF-α, IL-6 and IL-1β were tested using commercial kits in accordance with the manufacturer's directions.

2.12. Histological evaluation of pancreas

Pancreas tissues were also excised, rinsed and fixed in 10% neutral formalin, then dehydrated in gradient ethanol (75%, 85%, 95% and 100%) and xylene (100%). After permeation, they were embedded in paraffin and cut into 3 μm thick sections with a rotary microtome. Tissue sections were stained with haematoxylin-eosin (H&E) for light microscopic examinations (Chen et al., 2014).

2.13. Statistical analysis

Statistical analysis was performed by SPSS version 16.0 software. The data were presented by the mean ± SD. Statistical comparisons between the groups were performed using one-way ANOVA followed by Tukey's test and a value of $p < 0.05$ was taken as statistically significant.

3. Results

3.1. Phytochemical analysis of TGCT

The qualitative analysis was carried out and presented in the supplementary materials. Total ion chromatogram in negative ion mode were demonstrated in Fig. S1. MS data were tentatively assigned by comparison with data in a previous report (Li et al., 2015) and summarized in Table S1. The analytical methods for quantifying the markers also have been validated and briefly described in the supplementary materials. The HPLC chromatograms were presented in Fig. 1. Three major constituents (echinacoside, acteoside and isoacteoside) of TGCT were identified compared with the reference substances. The identification of the compounds and their concentrations in the TGCT were shown in Table 1.

3.2. Effects of TGCT on BW and calorie intake

As shown in Fig. 2A, rats fed HFSD showed the weight increased by an average of 41 g compared with NC group after 4 weeks. However, STZ evidently reduced the BW of rats when compared with NC group. In contrast, BW in TGCT groups (100 and 200 mg/kg) were progressively and significantly increased ($p < 0.05$) by 8.1% and 11.1% respectively compared with DC group by the end of the experiment period, which indicated that TGCT could prevent excessive weight loss under pathological conditions. The calorie intake of the NC group was observably lower than the other groups, and there was no any significant difference of calorie intakes between the other five groups (Fig. 2B).

3.3. Effects of TGCT on FPG, OGTT and HbA1c

STZ-induced diabetic rats showed remarkable increase in FPG compared with NC group ($p < 0.01$) as depicted in Fig. 3A. Oral administration of TGCT demonstrated hypoglycemic effect in a time and dose-dependent manner. TGCT (100 and 200 mg/kg) significantly decreased the FPG levels at 4th (22.1% and 24.8%) and 6th (23.2% and 56.4%) weeks compared with DC group. As depicted in Fig. 3B and C, TGCT (100 mg/kg and 200 mg/kg) obviously decreased the blood

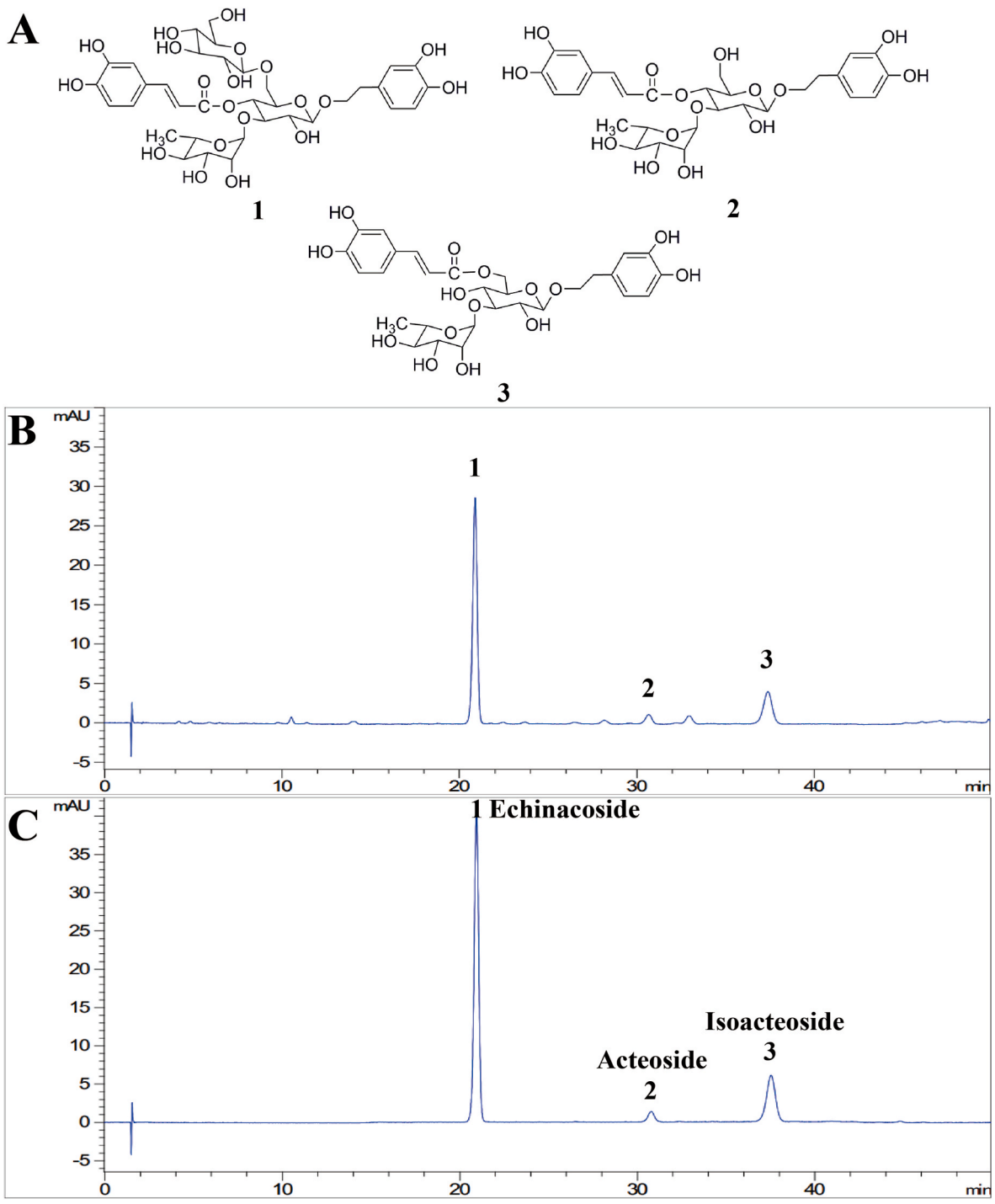

Fig. 1. The HPLC spectrum of TGCT and the chemical structures of the major compounds in TGCT. (A) Chemical structures of echinacoside, acteoside and isoacteoside. (B) HPLC fingerprint spectrum of TGCT. (C) HPLC profiles of standard mixtures.

glucose by 16.1% and 22.2% at 0.5 h and decreased by 17.2% and 26.5% at 1 h, and the AUC-G of TGCT groups were also reduced by 8.1%, 18.5% and 25.4% respectively. As illustrated in Fig. 3D, there was a significant increase in HbA1c (93.3%) compared with NC group, while oral administration of TGCT (100 mg/kg and 200 mg/kg) to diabetic rats remarkably ($p < 0.05$) decreased HbA1c (26.7% and 37.4%, respectively) compared with DC group.

3.4. Glycogen contents in liver and muscle

As demonstrated in Fig. 4A and B, glycogen levels were significantly decreased in diabetic rats. When different concentrations of TGCT were administered to diabetic rats for 6 weeks, liver glycogen in TGCT groups (100 mg/kg and 200 mg/kg) were higher (25.2% and 40.8%) than those in DC group ($p < 0.05$, Fig. 4A). Similar effect was demonstrated in muscle that glycogen in TGCT groups (100 mg/kg and 200 mg/kg) were higher (40.7% and 52.6%) than those in DC group ($p < 0.05$, Fig. 4B).

3.5. Effects of TGCT on serum insulin, C-Peptide and ISI

As shown in Table 2, insulin and C-peptide were obviously ($p < 0.01$) decreased in diabetic rats compared with NC group. However, TGCT at

Table 1
The contents of phytochemical markers of TGCT. Results for HPLC-DAD method validation.

Peak No.	PhGs	Regression equations	Linear ranges (μg/mL)	Correlation coefficient/r	LOD (ng/mL)	LOQ (ng/mL)	Retention time (min)		Content (mg/g)
							Mixed TGCT	Standards	
1	Echinacoside	Y = 0.123 6X-0.2193	27.792–277.92	0.9996	2.86	8.58	20.89 ± 0.18	20.82 ± 0.16	500.19 ± 11.52
2	Acteoside	Y = 0.3782X-0.0522	2.4184–24.184	0.9996	3.40	10.2	30.76 ± 0.25	30.74 ± 0.22	19.13 ± 1.44
3	Isoacteoside	Y = 0.0501X-0.0255	5.106–51.06	0.9998	3.11	9.65	37.72 ± 0.21	37.68 ± 0.26	141.82 ± 5.78

Results are expressed as mean ± SD of three replicates.

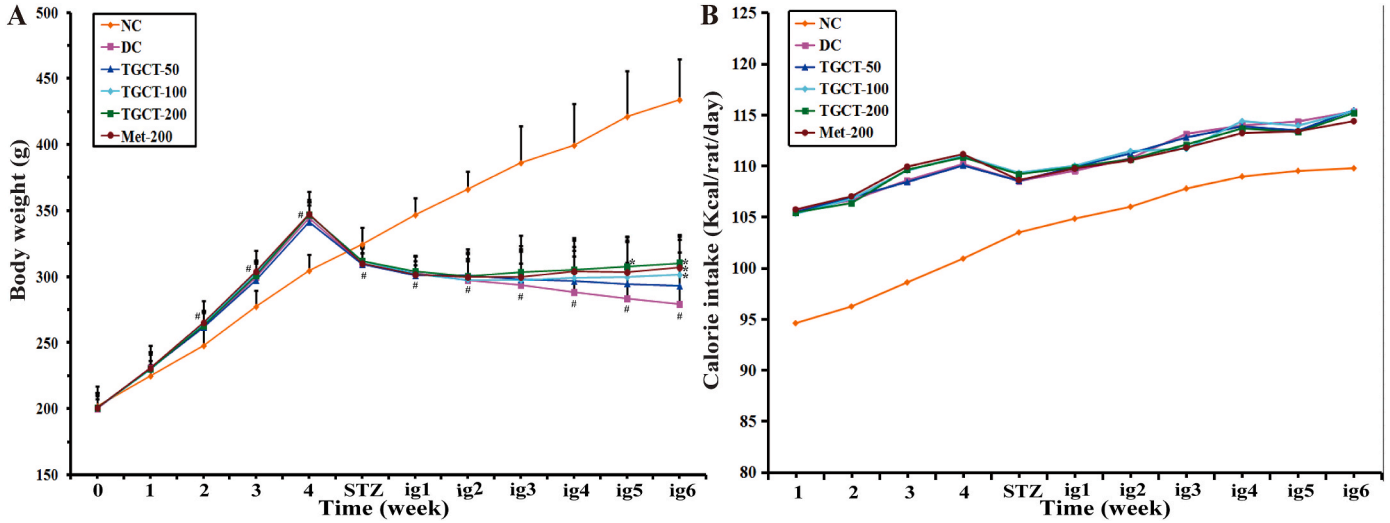

Fig. 2. Effects of TGCT on body weight (A) and calorie intake (B) in STZ-induced diabetic rats. NC: normal control; DC: diabetic control; TGCT (50 mg/kg, 100 mg/kg and 200 mg/kg); Met (200 mg/kg); "ig" means intragastric administration. Results were represented by mean ± SD (n = 10). #p < 0.05 versus NC group; *p < 0.05 versus DC group.

all doses caused no significant increase in insulin and C-peptide levels ($p > 0.05$), even if insulin and C-peptide in TGCT-treated groups were slightly higher than that in DC group. Meanwhile, we calculated the ISI in Fig. 4C. In contrast to the insulin secretion, ISI were respectively elevated 32.9% and 37.8% by TGCT (100 mg/kg and 200 mg/kg) compared with DC group ($p < 0.01$).

3.6. Histopathological examination of the pancreas

In order to verify the effect of TGCT on regeneration in pancreatic islets, the histological analysis of pancreas were performed. In Fig. 5A, normal histological structure and sized islets were observed in NC group. In contrast, STZ-injected resulted in decreasing the number and diameter of islets with marked microvesicular changes (Fig. 5B). Both TGCT and Met without significantly increased the number and size of islets by scoring analysis (Fig. 5C–F).

3.7. Effects of TGCT on p-PKB, p-GSK3β, HK and PK

In Table 2, the phosphorylation levels of PKB and GSK3β were found to be significantly ($p < 0.05$) decreased in diabetic rats. TGCT (100 mg/kg and 200 mg/kg) remarkably increased the concentrations of p-PKB (TGCT-100: 13.5%, $p < 0.05$ and TGCT-200: 16.7%, $p < 0.05$) and p-GSK3β (TGCT-200: 18.3%, $p < 0.01$). Similar effects of TGCT on HK and PK were also observed. TGCT groups increased the activities of HK (TGCT-100: 30.2%, $p < 0.05$ and TGCT-200: 59.1%, $p < 0.01$) and PK (TGCT-200: 32.7%, $p < 0.05$) than those in DC group.

3.8. Effects of TGCT on dyslipidemia

As shown in Table 3, Rats treated with TGCT (100 mg/kg and 200 mg/kg) were notably ameliorated the lipid abnormalities. TG were reduced by 19.8% ($p < 0.05$) and 25.9% ($p < 0.01$); TC were decreased by 28.5% and 31.4% ($p < 0.05$); LDL-C were reduced by 20.0% ($p < 0.01$) compared with DC group. However, the suppressed HDL-C level in DC group was significantly elevated by 26.8% ($p < 0.05$) in TGCT-200 group.

3.9. Effects of TGCT on oxidative stress and inflammation

In the light of the important roles of oxidative stress and inflammation in the pathophysiology of diabetes, we evaluated the capability of TGCT on oxidative stress and inflammation in diabetic rats. TGCT (100 and 200 mg/kg) effectively increased (14.7%, $p < 0.05$ and 20.5%, $p < 0.01$) the activity of SOD, remarkably elevated (16.3%, $p < 0.01$ and 22.3%, $p < 0.01$) the GSH-Px activity and significantly decreased (15.0%, $p < 0.05$ and 19.7%, $p < 0.05$) the MDA formation compared with DC group in Table 3. Similarly, TGCT (200 mg/kg) treatment also blocked the STZ-induced overproduction of pro-inflammatory cytokines TNF-α (21.8%, $p < 0.01$), IL-6 (14.0%, $p < 0.05$) and IL-1β (15.2%, $p < 0.05$).

4. Discussion

Diabetes is a progressive and chronic metabolic disorder which is mainly characterized by hyperglycemia. At present, FPG is a specific

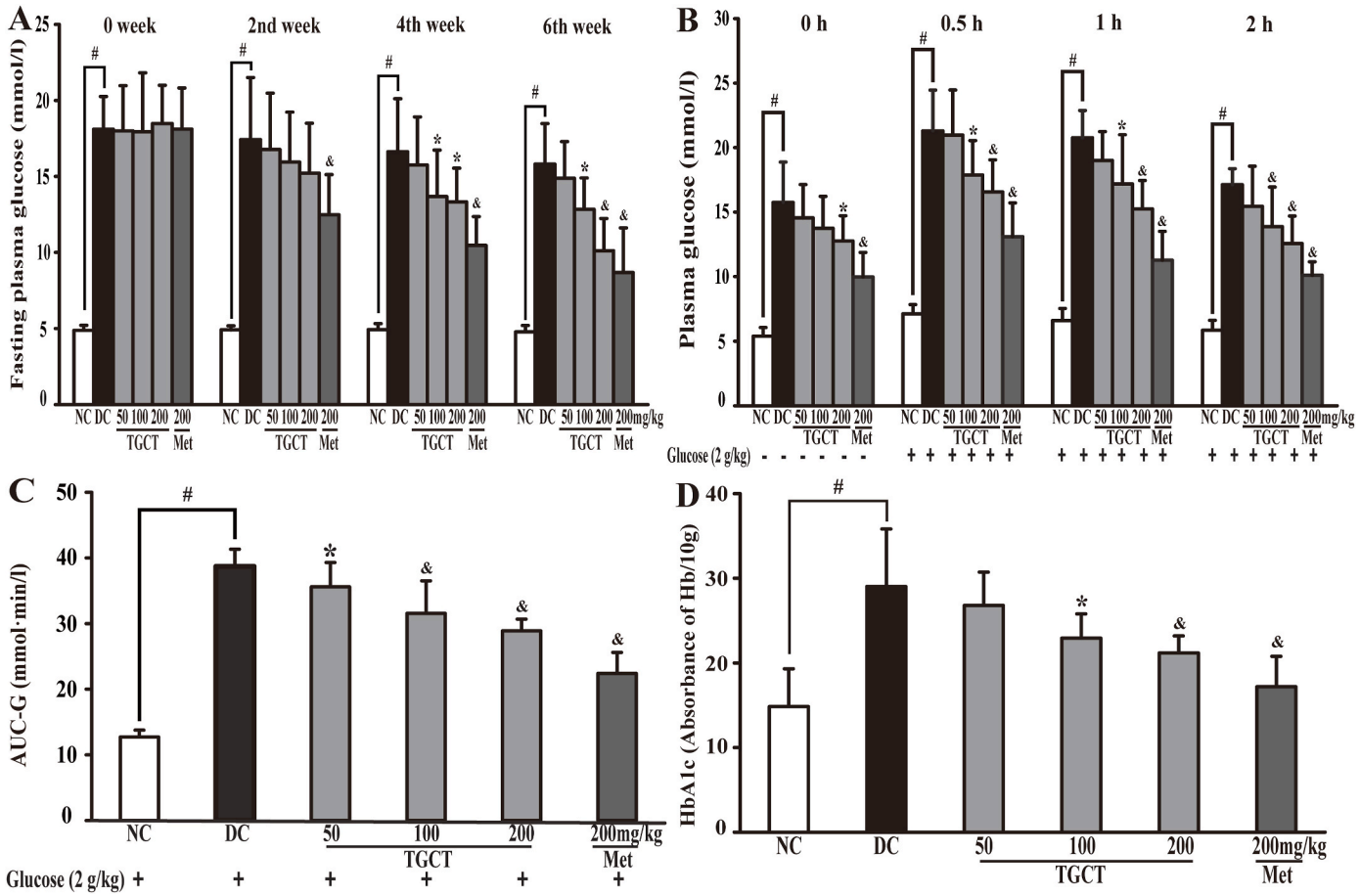

Fig. 3. The change of FPG in diabetic rats during being administrated with TGCT for 6 weeks (A). Effects of TGCT on OGTT (B), AUC-G (C) and HbA1c level (D) in diabetic rats after 6 weeks of treatment. NC: normal control; DC: diabetic control; TGCT (50 mg/kg, 100 mg/kg and 200 mg/kg); Met (200 mg/kg). Data were expressed as mean ± SD (n = 10). $^{\#}p < 0.05$ versus NC group; *p < 0.05 and &p < 0.01 versus DC group.

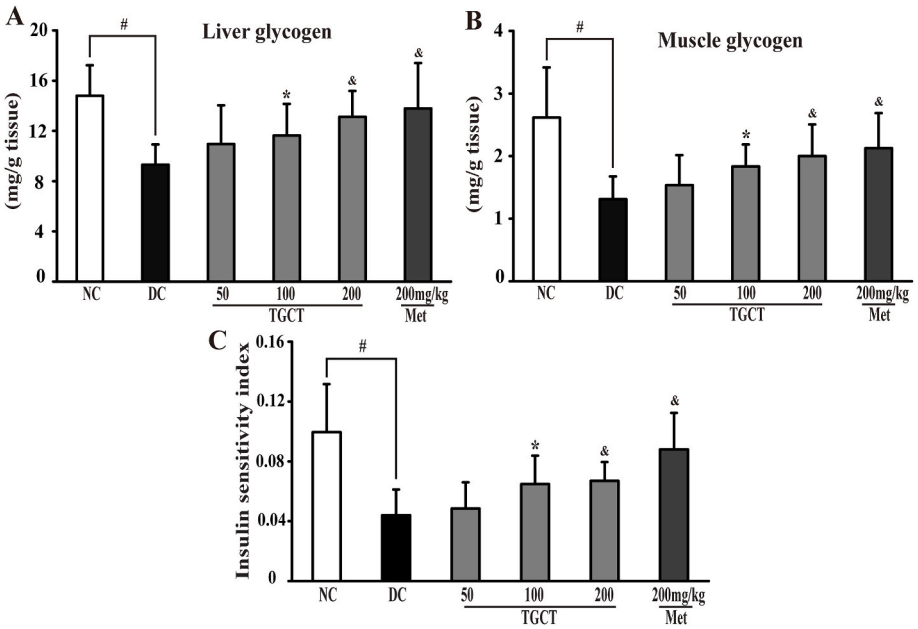

Fig. 4. Effects of TGCT on liver glycogen (A), muscle glycogen (B) and insulin sensitivity index (C) after 6 weeks of treatment in diabetic rats. NC: normal control; DC: diabetic control; TGCT (50 mg/kg, 100 mg/kg and 200 mg/kg); Met (200 mg/kg). Values are expressed as the mean ± SD (n = 10). #p < 0.05 versus NC group; *p < 0.05 and &p < 0.01 versus DC group.

Table 2
Effects of TGCT on insulin secretion and carbohydrate metabolizing enzymes in diabetic rats.

Groups	Insulin (μg/L)	C-peptide (μg/L)	p-PKB (ng/L)	p-GSK3β (ng/L)	HK (U/g protein)	PK (U/g protein)
NC	2.51 ± 0.51	2.39 ± 0.34	48.08 ± 6.46	34.57 ± 2.76	8.73 ± 1.08	154.10 ± 30.82
DC	1.32 ± 0.11$^{\#}$	1.22 ± 0.14$^{\#}$	41.48 ± 4.48$^{\#}$	28.19 ± 3.33$^{\#}$	5.14 ± 0.95$^{\#}$	103.51 ± 33.03$^{\#}$
TGCT-50	1.38 ± 0.33	1.26 ± 0.21	44.68 ± 7.55	30.74 ± 7.49	6.08 ± 1.40	123.47 ± 23.45
TGCT-100	1.42 ± 0.17	1.32 ± 0.30	47.06 ± 6.02*	32.02 ± 5.01	6.69 ± 1.67*	125.53 ± 25.49
TGCT-200	1.45 ± 0.18	1.34 ± 0.29	48.41 ± 5.93*	33.36 ± 2.60$^{\&}$	8.18 ± 0.50$^{\&}$	137.38 ± 11.14*
Met-200	1.41 ± 0.15	1.31 ± 0.14	49.29 ± 6.61$^{\&}$	34.78 ± 1.76$^{\&}$	9.32 ± 1.33$^{\&}$	147.18 ± 10.94$^{\&}$

All results are expressed as mean ± SD. #$p < 0.05$, significant differences versus NC group; *$p < 0.05$ and &$p < 0.01$, significant differences versus DC group.

measure of blood glucose concentration, OGTT is a sensitive detection criterion of early abnormalities in glucose disposal, while HbA1c is widely used as a gold standard index for glycemic control reflects average glucose level over 120 days preceding the test (Nagy et al., 2018; Gan et al., 2018). FPG, OGTT and HbA1c are used clinically for the diagnosis and management of prediabetes and diabetes (Chai et al., 2017). In our study, it is clear that FPG of diabetic rats treated with TGCT (100 mg/kg and 200 mg/kg) were significantly decreased compared with DC group. As the data shown in OGTT and AUC-G, the impaired glucose tolerance and glucose uptake rate were reversed by TGCT in diabetic rats. Meanwhile, the result was also supported by the measurement of HbA1c content. These data indicated that TGCT ameliorated the physiological indexes of diabetic rats by regulating blood glucose homeostasis.

Impaired insulin secretion and IR play a crucial role in the development of hyperglycemia. Targeting either of them is appropriate to improve glycemic control and prevent T2DM (Szoke and Gerich, 2005; Punthakee et al., 2018). Several researches have reported that a combination of high-fat diet and low-dose STZ is an effective means of inducing T2DM in experiments animals. Low-dose STZ causes mild impairment of insulin secretion that more closely resembles the later stages of T2DM (Gheibi et al., 2017). In this study, TGCT neither significantly increase the insulin secretion nor restore the pancreas islet of diabetic rats, even if insulin and number of islets in TGCT-treated groups were higher than that in DC group. These findings are in line with the previous study in *db/db* mice (Xiong et al., 2013). Our results showed significant weight increase in diabetic rats treated with TGCT, which can be explained by a slight increase of insulin that can inhibit protein catabolism in muscle tissue (Adams et al., 2019). Furthermore, the result of ISI displayed that TGCT obviously ameliorated the IR of diabetic rats that is consistent with previous report (Kong et al., 2018), which provided a novel evidence in regard to the potential mechanism on the antidiabetic effect of TGCT.

As we all known, PKB/GSK3β pathway is one of the most critical insulin signaling pathways, has been suggested to mediate insulin-induced glycogen synthesis (Zheng et al., 2015). HK and PK act as the potential drug-targets in the pharmacological treatment of diabetes. Lowered HK and PK activities have been confirmed in IR, while activation of HK and PK cause more glycogen reserves or glycolysis producing fuller energy by utilizing blood glucose (Hu et al., 2014). In the present study, TGCT treatment simultaneously increased the phosphorylated proteins expression of PKB and GSK3β, led to a significant reversal in the activities of HK and PK, and markedly restored the glycogen contents in liver and muscle as the blood glucose reduced. These results indicated that TGCT activated the key enzymes of insulin signaling pathway, and further provided the evidence that insulin sensitivity was really improved in diabetic rats.

Long-term diabetes also contributes to boost LDL-C and decrease HDL-C levels that cause lipid dysregulation (Jayashankar et al., 2016), and dyslipidemia is an established marker for endothelial dysfunction and cardiovascular risk in diabetes (Shahwan et al., 2019). In our study, TGCT (200 mg/kg) remarkably lowered the levels of TC, TG and LDL-C, and enhanced the level of HDL-C in diabetic rats, which is consistent with the previous reports that *Cistanche tubulosa* effectively regulated the lipid metabolism in mice (Shimoda et al., 2009; Xiong et al., 2013).

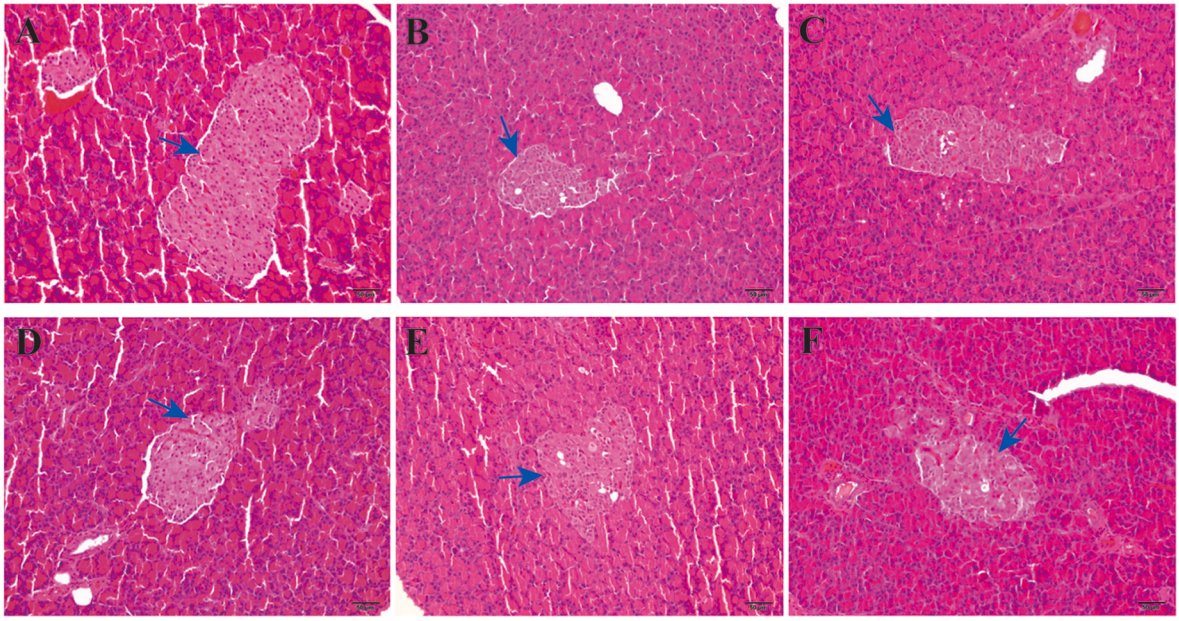

Fig. 5. Histopathological observation of diabetic rats in pancreas after 6 weeks treatment. Microscopic images of × 200 magnification were acquired and observed. (A) Normal control group-presence of normal islet cells; (B) Diabetic control group-microvesicular changes and decrease of islet cells; (C–F) TGCT (50 mg/kg, 100 mg/kg and 200 mg/kg) and Met (200 mg/kg)-microvesicular changes and decrease of islet cells.

Table 3
Effects of TGCT on antioxidant activity and lipid profiles in diabetic rats.

Groups	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	SOD (U/mL)	GSH-Px (μmol/L)	MDA (nmol/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1β (pg/mL)
NC	0.73 ± 0.12	1.19 ± 0.25	0.72 ± 0.23	1.73 ± 0.34	339.47 ± 20.60	1005.33 ± 87.58	13.88 ± 2.45	57.25 ± 6.14	46.84 ± 5.60	37.68 ± 5.83
DC	1.16 ± 0.22$^{\#}$	1.72 ± 0.59$^{\#}$	1.50 ± 0.21$^{\#}$	1.27 ± 0.26$^{\#}$	263.75 ± 22.76$^{\#}$	737.27 ± 50.90$^{\#}$	18.94 ± 2.89$^{\#}$	94.39 ± 8.21$^{\#}$	85.43 ± 7.28$^{\#}$	65.29 ± 6.97$^{\#}$
TGCT-50	1.04 ± 0.15	1.51 ± 0.46	1.43 ± 0.34	1.38 ± 0.32	287.96 ± 24.11	832.91 ± 74.58*	17.67 ± 1.89	86.87 ± 9.36	82.48 ± 6.89	63.36 ± 7.58
TGCT-100	0.93 ± 0.20*	1.23 ± 0.29*	1.29 ± 0.29	1.51 ± 0.45	302.62 ± 30.66*	857.76 ± 71.13$^{\&}$	16.09 ± 1.57*	80.46 ± 6.28*	79.73 ± 8.32	59.75 ± 8.27
TGCT-200	0.86 ± 0.20$^{\&}$	1.18 ± 0.30*	1.20 ± 0.21$^{\&}$	1.61 ± 0.29*	317.91 ± 21.09$^{\&}$	901.95 ± 61.70$^{\&}$	15.21 ± 2.08*	73.83 ± 6.83$^{\&}$	73.49 ± 5.53*	55.38 ± 4.82*
Met-200	0.77 ± 0.24$^{\&}$	1.21 ± 0.17*	0.98 ± 0.25$^{\&}$	1.58 ± 0.16$^{\&}$	292.73 ± 23.85*	830.89 ± 69.88*	15.61 ± 1.70*	63.45 ± 4.74$^{\&}$	59.78 ± 7.14$^{\&}$	48.49 ± 5.62*

Data are expressed as mean ± SD. #$p < 0.05$, significant differences versus NC group; *$p < 0.05$ and &$p < 0.01$, significant differences versus DC group.

These findings indicated that TGCT may be more beneficial to diabetic individual with blood lipid abnormalities.

STZ is a broad-spectrum antibiotic, which has a high selective toxicity effect on pancreatic islet β-cells resulting from the increasing of superoxide radical, and further to a poor glycemic control in turn (Ghosh et al., 2015; Swain et al., 2020). Meanwhile, oxidative stress is also an important cause for IR in numerous environments (Taniguchi et al., 2006). IR and diabetes are associated with the reduced activities of antioxidant enzymes, such as SOD and GSH-Px (Styskal et al., 2012). Previous studies had implied that suppressing oxidative stress was able to lower blood glucose in diabetic rats (Lim et al., 2012; Gao et al., 2016). In this study, treatment with TGCT significantly restored the cellular defensive functions of SOD and GSH-Px, and reduced MDA level in diabetic rats, which manifested that TGCT has the properties in antioxidation.

The major constituents in TGCT are PhGs and the total content of echinacoside, acteoside and isoacteoside is more than 661 mg/g. Plants which possess high antioxidant components, such as PhGs, generally were proved to have hypoglycemic effect (Morikawa et al., 2014; Shimada et al., 2017; Spínola et al., 2019). Therefore, we speculated that the anti-diabetic effect of TGCT might be partly attributed to the antioxidant activity of PhGs. In consideration of the content of echinacoside in TGCT, further studies are required to investigate the effects of echinacoside on IR and diabetes in vivo and in vitro models.

It is widely recognized that diabetes mellitus is an inflammatory disease. Inflammatory cytokines such as TNF-α, IL-6 and IL-1β can interfere with the insulin receptor signaling pathway, and further leads to IR (Bastard et al., 2006). Inflammation appears to be a viable drug target in the treatment of IR, and by extension diabetes (Chen et al., 2015). In the current study, TGCT could decrease the level of TNF-α, IL-6 and IL-1β, which demonstrated the anti-inflammatory effect. This may be another antidiabetic mechanism of TGCT in diabetic rats.

Accumulating evidence shows that persistent high blood glucose gives rise to the abnormalities in the structure and function of circulating proteins and lipids, which leads to glycoxidation and peroxidation, and then promotes the production of inflammatory cytokines. Similarly, increased inflammatory cytokines result in the production of reactive oxygen species and other reactive moieties, which promotes oxidative stress and oxidative damage. This result in a vicious cycle (Aghadavod et al., 2016; Domingueti et al., 2016). Therefore, reducing blood glucose and lipids may be contributed to the antioxidant and anti-inflammatory effects of TGCT. To some extent, the exact antidiabetic mechanism is not fully clear and needs more investigations.

5. Conclusion

In summary, this study indicated that TGCT was an effective agent for treating hyperglycemia and hyperlipidemia in diet/STZ-induced diabetic rats. Furthermore, the antidiabetic effect might be largely related to the antioxidant and anti-inflammatory properties of TGCT. This study would put forward the possibility of introducing TGCT in diabetes treatment. However, the detailed antidiabetic mechanism of TGCT remains uncertain, and further studies in vivo and in vitro are necessary.

Author contributions

Conception and design: ZQM, KNZ and WX. Preparation of TGCT, chemical constituents analysis and methodology validation: RG, ZKX and WJL. Diabetes induction and daily treatment: KNZ and HF. Preparation of samples for biochemical analysis: KNZ, HF and ZQM. Data analysis and manuscript drafting: KNZ, ZQM and YST. Data validation and manuscript revision: WZH, GD and YST.

Declaration of competing interest

The authors have declared no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.113991.

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