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ANTI-DIABETIC EFFECTS OF ZHUODUQING FORMULA, A CHINESE HERBAL DECOCTION, ON A RAT MODEL OF TYPE 2 DIABETES

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Abstract

Background: Zhuoduqing formula (ZDQ) is a Chinese herbal decoction and used to treat type 2 diabetes in clinical practice, but the potential evidence needs to be provided.
Materials and Methods: Type 2 diabetic model rats were induced by feeding high fat diet (HFD) and intraperitoneal injection of streptozotocin (STZ). The model rats were given ZDQ for 4 weeks. Insulin sensitivity was evaluated by homeostasis model assessment of basal insulin resistance (HOMA-IR) and intraperitoneal glucose tolerance test (IPGTT). Blood insulin and tumour necrosis factor-α (TNF-α) levels as well as SOCS-3 levels in skeletal muscles were analyzed by ELISA.
Results: ZDQ significantly decreased fasting blood glucose, ameliorated HOMA-IR and IPGTT, and reduced triglyceride and total cholesterol in type 2 diabetic rats. Moreover, ZDQ remarkably lowered blood TNF-α levels and inhibited SOCS-3 levels in skeletal muscles.
Conclusion: The results display that ZDQ performs anti-diabetic functions in type 2 diabetic rats induced by feeding HFD and intraperitoneal injection of STZ.

Key words: Insulin sensitivity, Chinese medicine, anti-diabetic effect, inflammatory factor, SOCS-3

Abbreviations: ZDQ, zhuoduqing formula; ROS, rosiglitazone; HOMA-IR, homeostasis model assessment of basal insulin resistance; IPGTT, intraperitoneal glucose tolerance test; HFD, high fat diet; SOCS-3, suppressor of cytokine signaling-3; TNF-α, tumour necrosis factor-α.

Introduction

Millions of people suffer from type 2 diabetes and bear a great economic burden. Currently, type 2 diabetes still has a significant increase in prevalence worldwide. It is well known that type 2 diabetes is characterized by insulin resistance. Insulin is in charge of maintaining glucose homeostasis and regulating carbohydrate, lipid metabolism through triggering insulin signaling (Saltiel and Kahn 2001). Insulin resistance means the deficiency of insulin biological function in inducing glucose and lipid metabolism in target tissues, thus leading to metabolic disturbance, indicating chronic hyperglycemia and often accompanying with dyslipidemia including increased triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and decreased high-density lipoprotein cholesterol (HDL-c) in type 2 diabetes (Stamouli et al. 2014; Tian et al. 2015). Additionally, chronic hyperglycemia and dyslipidemia, known as glucose toxicity and lipid toxicity, respectively, in turn deteriorate insulin resistance, thereby

further causing dysfunction of metabolism. It is generally accepted that insulin resistance is the pathophysiological basis for the occurrence and development of type 2 diabetes. Therefore, it is very importance to control hyperglycemia and improve insulin resistance for the treatment of type 2 diabetes.

Chinese herbal medicine is very popular in China and has been used to treat diseases for thousands of years in clinical practice. Zhuoduqing formula (ZDQ), a Chinese herbal decoction extracted from ten Chinese herbs as materials and methods described, is prepared according to the differentiation of signs and symptoms. Recently, this formula has been used to treat type 2 diabetes in clinical practice, and produced beneficial outcomes (Zhao et al. 2013; Zhao et al. 2014). Moreover, the formula brings few side effects (Zhao et al. 2013; Zhao et al. 2014). Nevertheless, the convincing evidence needs to be provided.

In the study, we revealed the anti-diabetic actions of ZDQ in a rat mode of type 2 diabetes induced by feeding high fat diet (HFD) and intraperitoneal injection of streptozotocin (STZ). These implied the potential uses of ZDQ against type 2 diabetes.

Materials and Methods

Reagents

Sigma (St. Louis, MO, USA) provided STZ. Glaxo Company Limited (Tianjin, China) offered rosiglitazone (ROS). Rat insulin ELISA kit was purchased from Mercodia (Uppsala, Sweden). Tumour necrosis factor-α (TNF-α) and suppressor of cytokine signaling-3 (SOCS-3) ELISA kits were from CusaBio (Wuhan, China). TC and TG assay kits were obtained from Changchun Huili Biotech Co., Ltd. (Changchun, China). BCA protein assay kit was from Beyotime Institute of Biotechnology Co., Ltd. (Shanghai, China).

Preparation of ZDQ

ZDQ was extracted from ten Chinese herbs as follows: the rhizome of *Coptis chinensis* Franch. (Ranunculaceae), the herba of *Eupatorium fortunei* Turcz. (Compositae), the rhizome of *Dioscorea hypoglauca* Palibin (Dioscoreaceae), the root of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Leguminosae), the rhizome of *Pinellia ternate* (Thunb.) Breit. (Araceae), the herba of *Pogostemon cablin* (Blanco) Benth (Lamiaceae), the rhizome of *Panax notoginseng* (Burk.) F. H. Chen (Araliaceae), the seed of *Coix lacryma-jobi* L. var. *mayuen* (Roman.) Stapf (Gramineae), the seed of *Prunus armeniacae* L. (Rosaceae), and the fructus of *Amomum kravanh* Pierre ex Gagnep. (Zingiberaceae). The First Affiliated Hospital of Guangxi University of Chinese Medicine (Nanning, China) provided the herbs, and the specimens were kept in the hospital. The ratio of the ten herbs as above order was 13.16, 13.16, 10.53, 13.16, 7.89, 7.89, 10.53, 10.53, 7.89 and 5.26, respectively. The herbs were prepared and processed according to the Good Manufacturing Practice as Leung et al. previously described (Leung et al. 2006). The decoction was stored in 4°C fridge, and warmed in 37˚C water bath for 10 min before administration to the rats.

Animal and Experiment Design

The Ethics Committee of Guangxi University of Chinese Medicine approved the research. Male Sprague-Dawley rats weighing 160-200 g were used in the study. Shanghai Slaccas Laboratory Animal Company Limited (Shanghai, China) offered chow diet including normal pellet diet (NPD) and high fat diet (HFD). NPD contained 4.6% fat which produced about 10% of calories, and the fat in HFD provided about 40% of calories. The animals were maintained in an ambient humidity- and temperature-controlled room with a 12/12 h light/dark cycle, and free access to water and chow diet. On the tenth day of adaption, type 2 diabetic model rats were induced by feeding HFD followed by an intraperitoneal injection of low-dose STZ according to the past report (Feng et al. 2015). In brief, the rats were

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randomly assigned to two groups based on the chow diet: the NPD group and the HFD group. The rats in the former group were fed NPD, whereas the latter given HFD. After 6 weeks, the rats in the HFD group were given a single intraperitoneal injection of STZ (35 mg/kg, dissolved in freshly prepared citrate buffer), while the rats in the NPD group were injected intraperitoneally with citrate buffer. After 72 h of STZ injection, the rats whose blood glucose concentration was not less than 16.7 mmol/L were considered to have diabetes. The diabetic rats were randomly divided into the model group (MOD), the Zhuoduqing formula group (ZDQ), and the rosiglitazone group (ROS). And the rats in the NPD group were served as a control group (CON). Then, the rats were treated according to the following regimens: the Zhuoduqing formula group, receiving ZDQ (26.6 g/kg·d) by intragastric administration; the rosiglitazone group, receiving rosiglitazone (4 mg/kg·d) by oral gavage; the control and model groups, receiving an equal volume of water by oral gavage. The treatment course lasted for 4 weeks. All experimental procedures were carried out according to the guidelines of Guangxi University of Chinese Medicine for laboratory animal use and care.

Blood Glucose Assay

Blood samples of rats were collected from caudal vein by acupuncture blood. Blood glucose concentration was measured by glucose oxidase method using a portable Glucometer (Accu-Check Active, Roche Diagnostics Limited, Germany).

Insulin Sensitivity Evaluation

The homeostasis model assessment of basal insulin resistance (HOMA-IR) was used to evaluate insulin sensitivity. The value of HOMA-IR was calculated as the following: HOMA-IR = fasting glucose × fasting insulin/22.5. This value was inversely proportional to insulin sensitivity. Additionally, intraperitoneal glucose tolerance test (IPGTT) was also used to assess insulin sensitivity.

Intraperitoneal Glucose Tolerance Tests

After 4 weeks of treatment, the rats in each group were done IPGTT. Briefly, after deprivation of food for 12-16 h, the rats were administered by intraperitoneal injection with glucose (2 g/kg). Blood samples of the rats were collected to analyze glucose concentration from caudal vein immediately (0 min), 30, 60, and 120 min after glucose injection. The areas under the glucose curves (AUC) were evaluated according to the trapezoidal rule. Smaller AUC stands for a higher insulin sensitivity, and vice versa.

Measurement of Plasma Insulin, Fatty Profile, and TNF-α

After 4 weeks of treatment, the rats were deprived of food for 12-16 h, and then anaesthetized with pentobarbital sodium (40 mg/kg, i.p.), blood was collected from aortaventralis. Plasma was separated by centrifuge at 2000 × g for 10 minutes at 4 °C, and then used to analyze TC and TG using commercial assay kits according to manufacturer's instructions. And the levels of insulin and TNF-α were analyzed by ELISA per the manufacturer's instructions.

Measurement of SOCS-3 Levels in Skeletal Muscle Tissues

The rats were treated for 4 weeks, skeletal muscles were collected after anaesthesia with pentobarbital sodium (40 mg/kg, i.p.). The tissue was rinsed with PBS, and then homogenized in PBS. The homogenates were kept in -20°C fridge overnight. After two freeze-thaw cycles, the homogenates were centrifuged (5000 x g, 5 min, 4 °C) to remove

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the tissue debris. The supernatant was collected immediately and used to analyze SOCS-3 levels by ELISA per the manufacturer's instructions. The protein concentration of supernatant was analyzed using a BCA protein assay kit.

Statistical Analysis

The data were analyzed with SPSS 16.0 for Windows and are presented as means ± standard error (SE). Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by LSD test. A level of $P < 0.05$ was regarded as statistically significant.

Results and Discussion

ZDQ Improved Insulin Resistance in Type 2 Diabetic Rats

Type 2 diabetes is characterized by chronic hyperglycemia due to worsening insulin resistance, a hallmark of type 2 diabetes. In fact, insulin resistance exists in prediabetes, thus leading to metabolic dysfunction and promoting the development of diabetes. Currently, enhancing insulin sensitivity is a key strategy for clinical treatment of type 2 diabetes. In the present study, we fed the rats HFD following intraperitoneal injection of STZ to induce type 2 diabetes, and the model rats finally showed rised fasting blood glucose (Figure 1A) and HOMA-IR (Figure 1C); moreover, the model rats still suffered from impaired glucose tolerance, exhibiting greater AUC (Figure 1E) for IGPTT (Figure 1D) compared with the control group ($P < 0.01$). These results indicate that the model rats had hyperglycemia and great insulin resistance which were consistent with the previous report (Cao et al. 2016), implying the successful induction of type 2 diabetes. Therefore, we observed the effects of ZDQ on insulin resistance. Just as our clinical report (Zhao et al. 2014), ZDQ treatment not only significantly decreased fasting blood glucose (Figure 1A) in type 2 diabetic rats, but also markedly reduced HOMA-IR (Figure 1C), ameliorated IPGTT (Figure 1D), and decreased AUC (Figure 1E), suggesting the beneficial effects of ZDQ on type 2 diabetes. Additionally, hyperinsulinemia is indeed another characteristic of type 2 diabetes at early stage due to the compensatory elevation of insulin secretion in response to hyperglycemia. But the blood insulin level is gradually declined with the development of the disease because of the deterioration of pancreatic β cells. In this study, fasting blood insulin was decreased in type 2 diabetic rats (Figure 1B), which was also in according with the report (Cao et al. 2016). Administration of ZDQ reduced fasting blood insulin to a certain extent, implying the improvement of insulin resistance in type 2 diabetic rats in consideration of decreased fasting blood glucose and HOMA-IR by ZDQ. In clinical practice, agonists of peroxisome proliferator-activated receptors-γ (PPAR-γ) such as rosiglitazone, an insulin sensitization agent, are often utilized to treat type 2 diabetes. In this study, rosiglitazone was used as a positive control for ZDQ. As was expected, rosiglitazone lowered fasting blood glucose and improved insulin resistance, which were similar to ZDQ and strongly suggested the anti-diabetic activity of ZDQ. These results indicate that ZDQ improved insulin resistance in type 2 diabetic rats.

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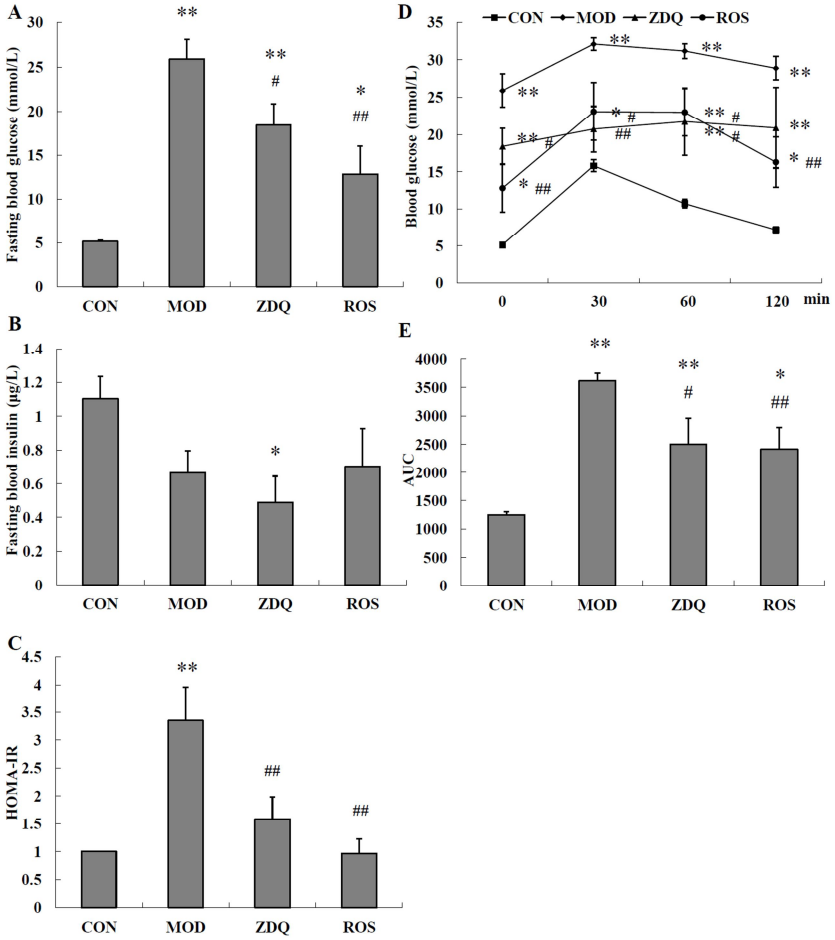

Figure 1: ZDQ ameliorated insulin resistance in type 2 diabetic rats. The diabetic rats were treated for 4 weeks, and then were analyzed fasting blood glucose (A) and insulin (B). Both of HOMA-IR (C) and IPGTT (D) were used to assess insulin sensitivity of rats as materials and methods described. AUC (E) stands for areas under the glucose curves. * P < 0.05, ** P < 0.01 *vs*. CON; $^{\#}$ P < 0.05, $^{\#\#}$ P < 0.01 *vs*. MOD. CON, control group; MOD, model group; ZDQ, Zhuoduqing formula group; ROS, rosiglitazone group. n = 5-6.

ZDQ Reduced Plasma TC and TG in Type 2 Diabetic Rats

Type 2 diabetes often associates with dyslipidemia (Tian et al. 2015) which not only increases the risk of cardiovascular disorder and initiates atherosclerosis, but also aggravates insulin resistance through inhibiting insulin signaling in insulin-targeted tissues including skeletal muscles (Park et al. 2016). In addition, chronic dyslipidemia causes lipid deposition and induces pancreatic lipotoxicity, thus impairing insulin secretion and promoting the progression of type 2 diabetes (Litwak et al. 2015). In this study, both of plasma TC and TG levels were significantly increased in type 2 diabetic rats (Figure 2), which were agreed with the previous report (Irudayaraj et al. 2016). And ZDQ treatment remarkably lowered TG, and to a certain extent decreased TC. Interestingly, rosiglitazone was also confirmed to significantly reduced TC and TG levels. The past study ever showed that rosiglitazone ameliorated dyslipidemia (Cai et al. 2016), which was in line with the results of this study. The data suggest that ZDQ improved dyslipidemia in type 2 diabetic rats in addition to anti-insulin resistant actions.

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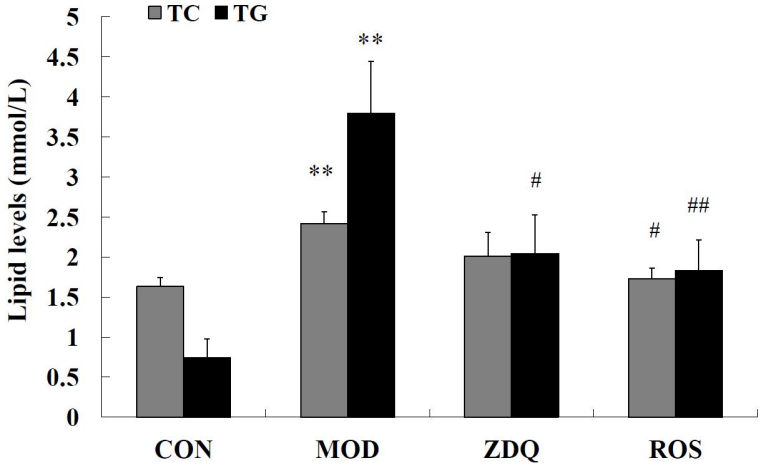

Figure 2: Effects of ZDQ on plasma TC and TG levels in type 2 diabetic rats. After 4 weeks of treatment, plasma TC and TG levels of the rats were determined. $^{**}P < 0.01$ *vs.* CON; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* MOD. CON, control group; MOD, model group; ZDQ, Zhuoduqing formula group; ROS, rosiglitazone group.

ZDQ Decreased Plasma TNF-α Levels and SOCS-3 Levels in Skeletal Muscles of Type 2 Diabetic Rats

It is generally accepted that diabetes is an inflammatory disease. Type 2 diabetes often coexists with increased inflammatory factors such as TNF-α and interleukin-6 (IL-6) (Mirza et al. 2012). Inflammation contributes to insulin resistance and promotes the development of type 2 diabetes (Daniele et al. 2014). In this study, plasma TNF-α was increased in type 2 diabetic rats (Figure 3A). Sharma et al. reported that HFD and STZ-induced type 2 diabetic rats showed elevated serum TNF-α levels (Sharma et al. 2012), which was in line with our study. Interestingly, ZDQ significantly decreased TNF-α. Meanwhile, rosiglitazone also reduced plasma TNF-α.

It is well known that SOCS-3 is a negative regulator of cytokine signaling. SOCS-3 is sharp increased in type 2 diabetes (Broholm et al. 2012; Feng et al. 2014). In addition to an increase in body weight, constitutive elevated SOCS-3 by transgenic technology aggravates whole-body insulin resistance in HFD-fed mice (Lebrun et al. 2009). But SOCS-3 deletion impedes the development of hyperinsulinemia in HFD-fed mice, and ameliorates systemic insulin sensitivity by promoting glucose uptake in skeletal muscles, which involve in increased activity of insulin signal pathway (Jorgensen et al. 2013). Further studies indicated that SOCS-3 contributes to insulin resistance through inhibiting phosphatidylinositol-3-OH kinase (PI3K) insulin signal pathway (Yang et al. 2010), a key signaling involving the regulation of most of the metabolic actions of insulin. And SOCS-3 knockout relates to enhanced activity of PI3K insulin signaling and improves insulin resistance (Jorgensen et al. 2013). In addition to leading to insulin resistance, SOCS-3 associates with inflammation. Elevated inflammatory factors including TNF-α promote SOCS-3 expression through triggering cytokine signal pathway, thereby resulting in dysfunction of PI3K signaling via SOCS-3 inhibition (Weigert et al. 2006; Waller et al. 2012). Therefore, SOCS-3 has become an important node between inflammation and insulin resistance. In this study, SOCS-3 protein levels was obviously increased in skeletal muscles of type 2 diabetic rats, and ZDQ treatment markedly reduced SOCS-3 protein levels (Figure 3B). Interestingly, rosiglitazone also decreased SOCS-3, which was similar to ZDQ. Study reported that rosiglitazone inhibited mRNA expression of SOCS-3 in IL-6-induced adipocytes with improved insulin resistance (Lagathu et al. 2003). Pioglitazone, another PPAR-γ agonist, reduced hepatic inflammatory responses and SOCS-3 expression, and ameliorated insulin responsiveness in rats fed a high-cholesterol fructose diet (Collino et al. 2010), suggesting that decreased SOCS-3 links reduced inflammation and improved insulin sensitivity. Here, ZDQ decreased plasma TNF-α and SOCS-3 in skeletal muscles, further confirming the actions of ZDQ in improving insulin resistance.

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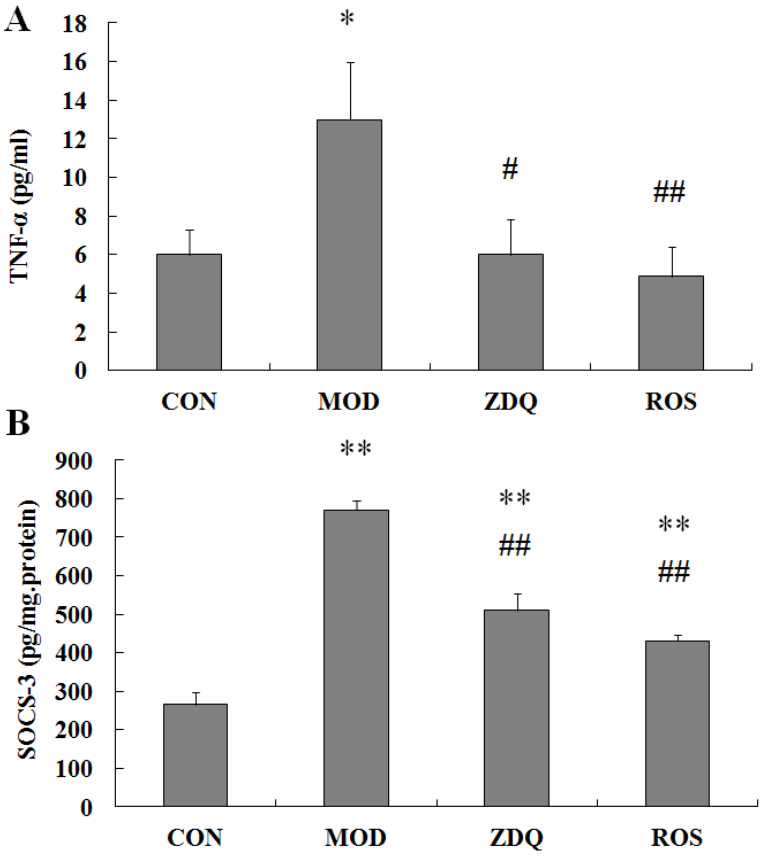

Figure 3: Effects of ZDQ on plasma TNF-α levels and SOCS-3 levels in skeletal muscles of type 2 diabetic rats. After 4 weeks of treatment, plasma TNF-α levels (A) and SOCS-3 levels (B) in skeletal muscles were analyzed by ELISA. * P < 0.05, ** P < 0.01 *vs*. CON; $^{\#}$ P < 0.05, $^{\#\#}$ P < 0.01 *vs*. MOD. CON, control group; MOD, model group; ZDQ, Zhuoduqing formula group; ROS, rosiglitazone group.

ZDQ contains ten Chinese herbal medicines including Coptis chinensis Franch, Astragalus membranaceus, and Panax notoginseng. The extracts of the above medicines have anti-diabetic activity according to the recent reports: coptis chinensis polysaccharide exerts an antioxidant action in HFD and STZ-induced type 2 diabetic rats (Jiang et al. 2015); both of astragalus polysaccharide extracted from Astragalus membranaceus and dammarane-type triterpene extracted from Panax notoginseng ameliorated hyperglycemia and insulin resistance in diabetic KKAy mice (Liu et al. 2010; Kitamura et al. 2016).

Conclusion

We concluded that ZDQ performs anti-diabetic functions in type 2 diabetic rats induced by feeding HFD and intraperitoneal injection of STZ.

Acknowledgments

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Conflict of Interest: The authors declared that there was no potential conflict of interest relevant to this article.

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