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Original Article

Synergistic interactions of catalpol and stachyose in STZ-HFD induced diabetic mice: Synergism in regulation of blood glucose, lipids, and hepatic and renal function

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ABSTRACT

Objective: *Rehmanniae Radix* has been traditionally used to treat diabetes. Catalpol (CAT) and stachyose (STA) are two of the main bioactive compounds in *Rehmannia Radix* and found to have similar therapeutic effects on diabetes and its complications. In this paper, we aimed to investigate whether there were synergistic therapeutic effects of CAT and STA on diabetes.

Methods: Streptozotocin (STZ) with the feeding of high-sugar-high-fat diet (HFD) was applied to induce diabetic C57BL/6 mice. STZ-HFD induced diabetic mice were then divided into model and six medical-treated groups: metformin (MET), STA, CAT, and three combinations of CAT:STA (1:1, 1:2, 2:1). Blood, liver, and kidney samples were isolated after six-week oral administration for biochemical assays of serum lipids, the indicators of kidney and liver functions and HE staining for liver tissues.

Results: It turned out that CAT, STA and their three combinations (1:1, 1:2, 2:1) could effectively control body weight, blood glucose, kidney weight and liver weight index, and well regulate levels of TC, HDL-c, TG, ALT, and TBA. In addition, CAT and its combination with STA at the ratio of 2:1 could significantly improve albumin content, compared to that in model group. STA and CAT and their combinations showed the improvements on kidney function in terms of urinary creatinine (Ucr). However, there were no such consistent observations on serum creatinine (Scr) and creatinine clearance rate (Ccr). The combination of CAT and STA at the ratio of 1:1 exhibited the better adjusting effects on kidney weight and liver weight indexes and the levels of ALT, Ucr, Scr, and Ccr. Our results demonstrated that the combinations of CAT and STA especially 1:1 showed similar or better improvements on diabetes-associated complications, compared to the sole CAT or STA treatment.

Conclusion: Thus, we concluded that there were synergistic therapeutic effects between CAT and STA on STZ/HFD-induced type 2 diabetes. This project provided insights and technical supports for the innovation of discovering bioactive constituents in *Rehmannia Radix* and studying its integrative mechanism in curing diabetes.

1. Introduction

Many historical records and clinical therapies demonstrated that traditional Chinese medicine (TCM) played a vital part in treating diabetes and its complications (Han, 2014; Liu et al., 2014; Tong, Dong, Chen, & Zhen, 2012). The roots of *Rehmannia glutinosa* (Gaetn.) Libosch. ex Fisch. et Mey. (Dihuang in Chinese) are the major medicinal organ, which have the functions of nourishing *yin*, supplementing bloods, and boosting essence and filling the marrow (Pharmacopoeia Committee of P. R. China, 2015). Its dried root is one of the most widely used herbs for the treatment of diabetes among TCMs (Waisundara, Huang, Hsu, Huang, & Tan, 2008; Zhao, Mu, & Liang, 2016). Meanwhile, many studies demonstrated that *Rehmannia Radix* had a wide range of pharmacological effects including ameliorating hyperglycemia, hyperlipemia vascular inflammation (Zhang, Sun, & Wang, 2014; Zhou et al., 2015), diabetic nephropathy in streptozotocin-induced diabetic mice (Dai et al., 2017; Zhao et al., 2015), ameliorating PO279 bone mineral density (Lee, Baek, Jin, Park, & Baek, 2014), and angiogenesis in rats (Zhu et al., 2016). Catalpol (CAT) and stachyose (STA) are two major bioactive compounds in *Rehmannia Radix*, the content of the former in *R. glutinosa* roots ranging from 3% to 4% (Zhao et al., 2015) and the concentration of STA amounting to approximately 26% in fresh roots of *R. glutinosa* (Jia, Zhang, & Liu, 2012). Catalpol has been granted with the clinical approval of the national TCM drugs in dealing with hyperglycemia, and more work on clinical research

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is ongoing now. Additionally, our previous report showed that CAT could effectively control levels of blood glucose and lipids of HFD-induced diabetic KK-*Ay* mice (Wang, Jia, Pang, & Li, 2016). Other work demonstrated that CAT decreased risks of diabetic nephropathy (Dong & Chen, 2013), diabetic encephalopathy (Wang, Li, Xue, & Hu, 2010; Xue et al., 2012), and hepatic mitochondrial dysfunction (Xu et al., 2015). In addition, it was demonstrated that STA together with other RGOs (raffinose family oligosaccharides) of *R. glutisona* could ameliorate CCl_4-induced abnormal hepatic lipid metabolism and liver injury via antioxidant effects (Zhang, Zhao, Sun, Lu, & Yang, 2013). Stachyose which was isolated from *R. glutisona* could lower glucose levels in normal and diabetic mice, indicating its antidiabetic effect (Zhang, Zhou et al., 2004). STA was found to enhance absorption and hepatoprotective effects of tea polyphenols in high fructose-fed mice (Li, Huang, Gao, & Yang, 2016). Given that CAT and STA had the similar therapeutic effects of diabetes and their presentation in a large amount in roots of *R. glutinosa*, we deduced that CAT and STA might have the synergistic effects on treatment of diabetes. That is, STA might improve the antidiabetic effects of CAT, and even widen the therapeutic function of the latter on diabetic complications such as diabetic nephropathy and liver failure. It is possible that multiple compounds inside *Radix Rehmannia* such as CAT and STA coordinatively work to achieve an ideal therapeutic outcome, since TCM has multiple targets. However, little work has been done to evaluate the interaction of CAT and STA on diabetes and its complications.

Type 2 diabetes is one of the most prevalent chronic diseases in the 21st century and has been unprecedentedly increasing especially in developing countries in the past decades. Non-independent diabetes mellitus (NIDDM) is the outcome of a pathological process caused by the deficiency of the insulin receptor or a failure in utilization of glucose by muscle, fat, and other tissues resulting in elevated blood glucose. At the same time, due to the defect of islet β cells, the secretion of insulin decreased, hepatic glycogen decomposition and glycogen gluconeogenesis increased, ultimately leading to an increase of glycogen output and a further failure of β-cell function. In addition, numerous studies have shown that type 2 diabetes could lead to complications such as hypercholesterolemia, diabetic nephropathy and diabetic hepatopathy (Pan, 2007). A STZ-induced mice model was applied to identify the anti-diabetic potential of CAT isolated from *Rehmannia Radix* (Huang, Niu, Lin, Cheng, & Hsu, 2010; Shieh et al., 2011). STZ-HFD-induced diabetic model was popular in recent basic research to mimic human diabetes especially in the late stage (Chao et al., 2018; Skovsø, 2014). Therefore, we used a STZ-HFD-induced diabetic mice model to evaluate the synergistic roles of CAT and STA and their superior ratio in treatment of type 2 diabetes through investigations of serum lipid levels and indicators of liver and kidney functions.

2. Materials and methods

2.1. Extraction of CAT and STA from Rehmannia Radix for animal experiment

The fresh roots of *R. glutinosa* were homogenized and refluxed with eight-fold volumes of 50% ethanol at 60 °C for 1 h. The reflux extraction was repeated for three times. The crude extract was then separated into two parts via H103 macroporous resin using water and 10% ethanol as eluents, separately. The water-eluted part contained 90% STA, with the yield 13% of the fresh *R. glutinosa* roots. The ethanol-eluted part contained 45% CAT and was further purified via rotary evaporation and recrystallized by the mixture of acetone and water (3:1). HPLC analysis revealed that the purities of CAT and STA in the final extract were higher than 90% and 94%, respectively, indicating that we could use them for the following animal experiment.

2.2. Induction of diabetic mice and medical treatments

The animal experiment was performed according to the Guidelines of National Health Institutes of China for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Welfare Committee of IMPLAD (Institute of Medicinal Plant Development) with the certificate No. SYXK2013-0023 (Jing). Eighty SPF-grade male C57BL/6 J mice (four weeks old) were purchased from Beijing HFK Bioscience Co., Ltd., China (Approval No. SCXK (Jing) 2016-0004). All the mice were housed at the temperature of 22−23 °C and humidity of 50%−60%, and in a 12/12 h light/dark cycle at the animal center of IMPLAD, Beijing, China. In the beginning, the mice were randomly divided into normal group ($n = 8$) and diabetic group. Normal group was fed with the normal diet during the whole experiment. The mice in the diabetic group were fed with the high-sugar and high-fat diet 1K65 (Beijing HFK Bioscience Co., Ltd., China) throughout the experiment. After two weeks, diabetic group mice fed with HFD were administered with streptozotocin (Sigma, USA) via an intraperitoneal injection at the dose of 70 mg/kg/d for three consecutive days. STZ was prepared in 20% citrate buffer (pH 4.5). Meantime, the normal group was injected only with 20% citrate buffer. One week after STZ injection, blood glucose values of the mice were measured by a blood glucose meter (ACCU-CHEK® Active, Roche Diagnostics GmbH, Mannheim, Germany). STZ-HFD mice with blood glucose ≥ 16.7 mmol/L were diagnosed as diabetic and randomly divided into the model group ($n = 8$) and six medical treated groups ($n = 8$, each group). The medical treated groups were administered by gavage for six weeks, separately with metformin (MET), STA, CAT, and the combinations of CAT and STA (1:1, 1:2, and 2:1). The administration dose for each medical treatment was 200 mg/kg/d. In the meantime, the model and normal control groups were given just with the same volume of normal saline. During the administration experiment, individual body weight and tail fasting blood glucose concentrations of control, model and administered mice were recorded weekly.

2.3. Serum biochemical determination

The next day after the last administration, blood samples were collected through eye vessels and then centrifuged at 4 °C at 3000 rpm for 10 min to isolate serum supernatant for determination of lipid levels, hepatic enzymes, total bile acid, and serum creatinine. Total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), albumin (ALB), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), total bile acid (TBA) and serum creatinine (Scr) in the mouse serum were measured following the manufacturer' protocols of the corresponding kits from Nanjing Jiancheng Bioengineering Institute, China (http://www.njjcbio.com/).

2.4. Kidney weight index and urinary biochemical determination

The 24-h urine was collected at the last administration for the determinations of urea and Ucr. Urea and Ucr concentration were assayed using the kits from Nanjing Jiancheng Bioengineering Institute, China. Ccr was calculated using the formula:

$Ccr = (Ucr \times V)/(Scr \times W \times 1440)$, (V = urinary volume measured during a 24-h period, Ucr = urinary creatinine concentration, Scr = serum creatinine concentration, W = body weight).

The kidney weight index (KWI) was estimated by calculating the ratio of the kidney weight to body weight.

2.5. Liver weight index and histological analysis

The liver weight index (LWI) was estimated by calculating the ratio of the liver weight to body weight. Liver samples were removed, weighed, washed with normal saline, and then fixed in 10% formaldehyde solution for 3 d. After series dehydration in 75%–100% ethanol, liver samples were embedded in paraffin. Tissues were cut into 3 μm slices and mounted on the glass. The slices were stained with hematoxylin and eosin and photographed using an optical microscope (200× magnification). Pathological scores were estimated using the symptoms including liver cell derangement, inflammatory cell infiltration, fiber tissue hyperplasia, Kuffer cell hyperplasia and increment of fatty deposition. The pathological score was obtained from the level of liver damage. A semi quantitative grading method was used to evaluate hepatic steatosis and inflammatory necrosis: zero point for no adipose degeneration and necrosis, 1 point for mild steatosis and necrosis, 2 points for moderate steatosis and necrosis, 3 points for severe steatosis and necrosis.

2.6. Statistical analysis

All the data were expressed as mean ± SD. The significance analysis between the groups was performed with nonparametric one-way ANOVA in Prism 6.0, followed by *Student–Newman–Keuls* test for multiple comparisons. $P < 0.05$ indicated the significant difference between the two compared groups.

3. Results

3.1. Determination of CAT and STA in extract of R. glutinosa

The purity of CAT and STA were determined through HPLC analysis with results of 95% and 95.75% by the normalization method, respectively. The HPLC analysis diagrams of CAT and STA in extracts were presented in Fig. 1.

3.2. Effects of CAT, STA, and their combinations on body weight and blood glucose of STZ-HFD induced diabetic mice

One week after STZ injection, physiological changes such as somnolence, polydipsia and polyuria had been observed in STZ-HFD treated mice. Although the body weight of mice in control group was increased steadily during the experiment, it was lower than that of HFD-fed mice till the beginning to the end of experiment, which indicated that high-sugar and high-fat diet (HFD) could quickly increase the body weight of mice (Fig. 2A). During the experiment, the body weights of mice in model and MET groups were increased in the first four weeks and reached the relatively steady platform. However, body weights of CAT, STA, and CAT-STA groups were changed in a different way. Compared with their initial body weight on week 1, CAT, STA and CAT-STA groups had the decreased body weight on week 2. However, it remained relatively steady throughout the rest experimental time, indicating administration of CAT, STA, and their combinations stopped the continuous reduction or increased of body weight in diabetic mice. Weight loss of CAT, STA, and CAT-STA groups on week 2 was possibly due to their adaption on medical treatment. On week 4 and 5, there was the significant difference in body weights of CAT, STA, and CAT-STA groups compared to the model group ($P < 0.05$).

The blood glucose of STZ-HFD mice was increased to over 20 mmol/L one week after the STZ injection, indicating the successful establishment of diabetic model (Fig. 2B). The blood glucose of model mice was increased on the last two weeks of the experiment, which meant the continuous intake of HFD feed aggravated the diabetes. This provided the scientific evidence of the clinical suggestion that less high-fat food would contribute to the effective control of blood glucose in diabetic patients. All the medical treatments could effectively decrease blood glucose of STZ-HFD-induced diabetic mice, even when high-fat diet was still given to medical groups. The blood glucose levels in all the medical treated groups were decreased to around 15 mmol/L on last week of the experiment. However, the positive medical treatment MET showed the earlier and better effect on the reduction of blood glucose in diabetic mice than other medical treatments especially STA and its combination with CAT (1:1, 2:1). STA and the combination of CAT and STA (1:1) decreased the blood glucose until week 3 compared to the model group. Among three combinations of CAT and STA, the ratio of CAT /STA 1:2 (66.7 mg CAT/133.3 mg STA) seemed to regulate blood glucose better than other two ratios of CAT and STA. CAT/STA 1:2 decreased the blood glucose level in the diabetic mice earlier than other two combinations, although there was no difference in glucose levels in all the three combination groups at the end of our experiment.

3.3. Effects of CAT, STA, and their combinations on serum lipid levels of STZ-HFD induced diabetic mice

Diabetes is a metabolic disease and causes the abnormal lipid metabolism in diabetic patients and mice. As shown in Fig. 3, serum TC, TG, and LDL-c were markedly elevated in model mice at the end of six weeks' experiment ($P < 0.001$) and HDL-c was decreased significantly ($P < 0.001$), compared to the normal control group. STA, CAT and all three combinations of STA and CAT except MET treatment could significantly improve TC levels of dia-

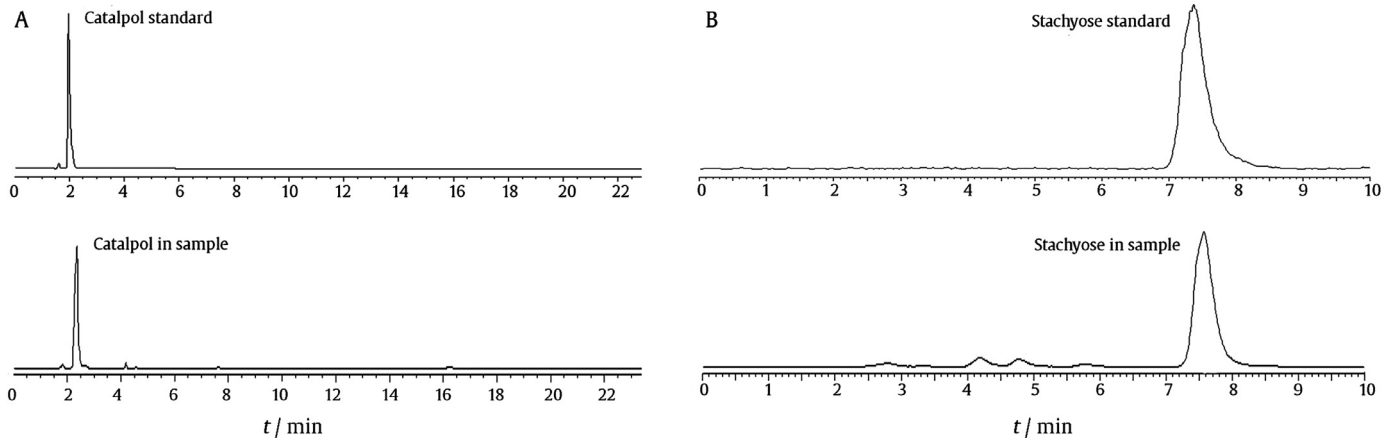

Fig. 1. HPLC chromatograms of catalpol (A) and starchyose (B) in *R. glutinosa* extracts.

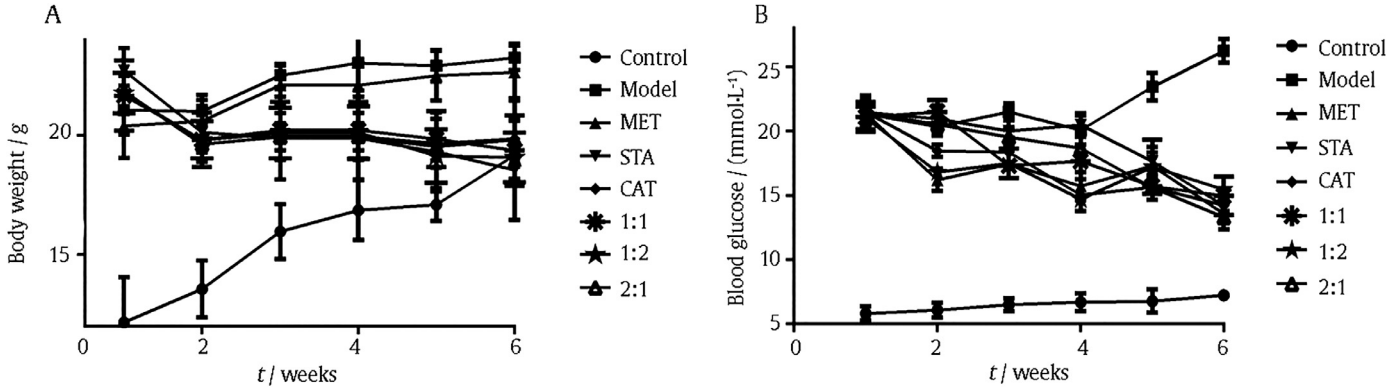

Fig. 2. Effects of catalpol, starchyose, and their combinations on body weight (A) and blood glucose (B) of STZ-HFD induced diabetic mice.

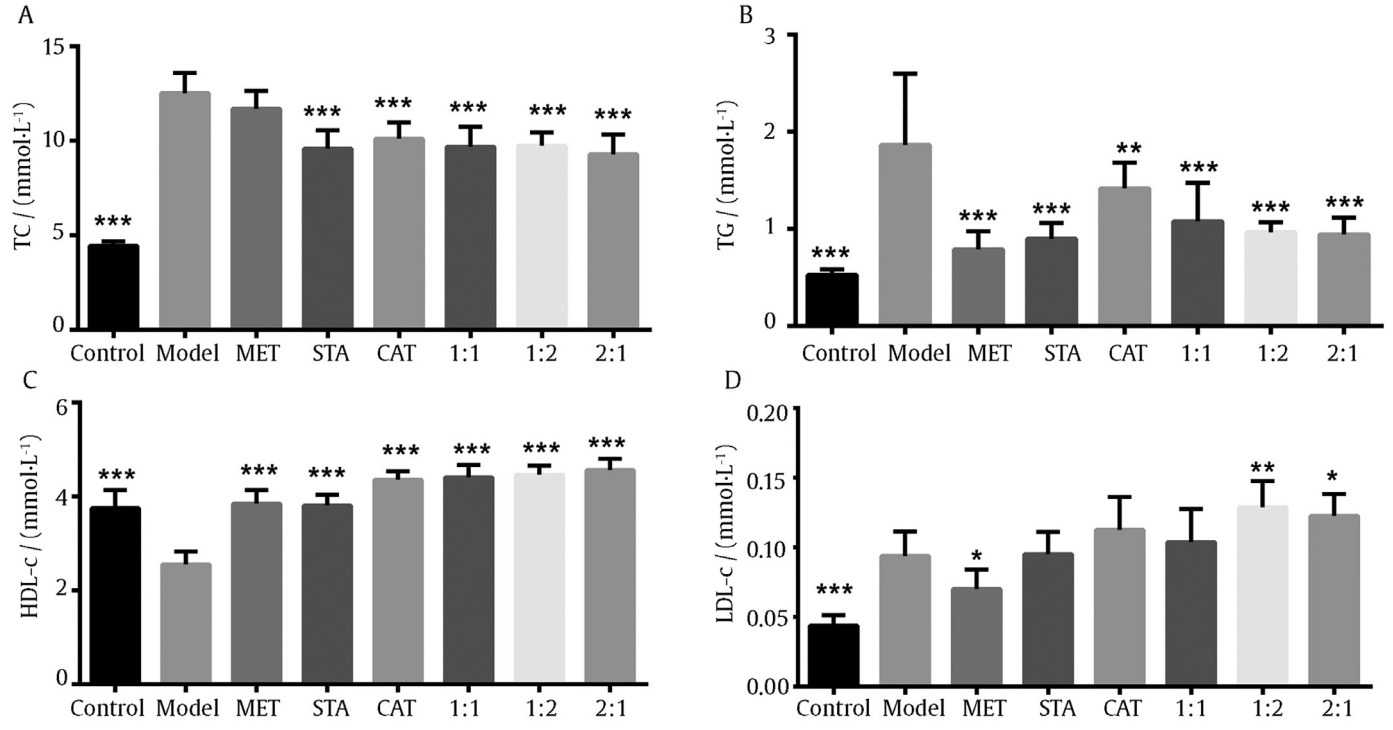

Fig. 3. Effects of catalpol, starchyose, and their combinations on serum lipid levels (A: TC, B: TG, C: HDL-c, D: LDLc) in STZ-HFD induced diabetic mice (***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$ *vs* model group).

betic mice compared to the model group ($P < 0.001$). In addition, all the medical treatments greatly modulated HDL-c and TG levels of diabetic mice ($P < 0.001$ or 0.01). LDL-c content of diabetic mice was relatively poorly regulated by medical treatments, which was only decreased slightly in MET group and even increased in 1:2 and 2:1 groups. In terms of the first three assayed lipids and their administration amount, the combinations of CAT and STA had the same or even better regulating effects on lipid levels than STA or CAT treatment alone, which indicated that there was a synergistic role of CAT and STA in regulation of serum lipid metabolism in diabetes.

3.4. Effects of CAT, STA, and their combinations on kidney functions of STZ-HFD induced diabetic mice

Diabetic nephropathy is one of the most serious complications in diabetes. Compared to the normal control group, the kidney weight index (KWI) of the model group was not significantly but slightly increased, which was decreased by MET, CAT and the combinations of CAT/ STA (1:2, 2:1) ($P < 0.001$ or 0.01) (Fig. 4A). Scr, Ucr, Ccr and 24 h urea amount are important indicators on the kidney condition. The levels of Scr, Ucr, Ccr and 24 h urea amount in the control group were significantly different from those in the model group ($P < 0.001$), indicating kidney damage worsened as diabetes progressed (Fig. 5). Metformin showed more alterations on Scr, Ucr, and Ccr of STZ-HFD induced diabetes than CAT, STA, and their combinations. Ucr was increased closely to the normal level by treatments of CAT, STA, and their combinations. Metformin, stachyose and 1:1 of CAT and STA decreased serum creatinine of diabetic mice, although its contents in these three medical treatments were still higher than the normal control. In addition, STA, the combinations of CAT and STA effectively enhanced Ccr of diabetes. The level of 24 h urea in diabetic mice was not changed by almost all the medical treatments except the administration of STA,

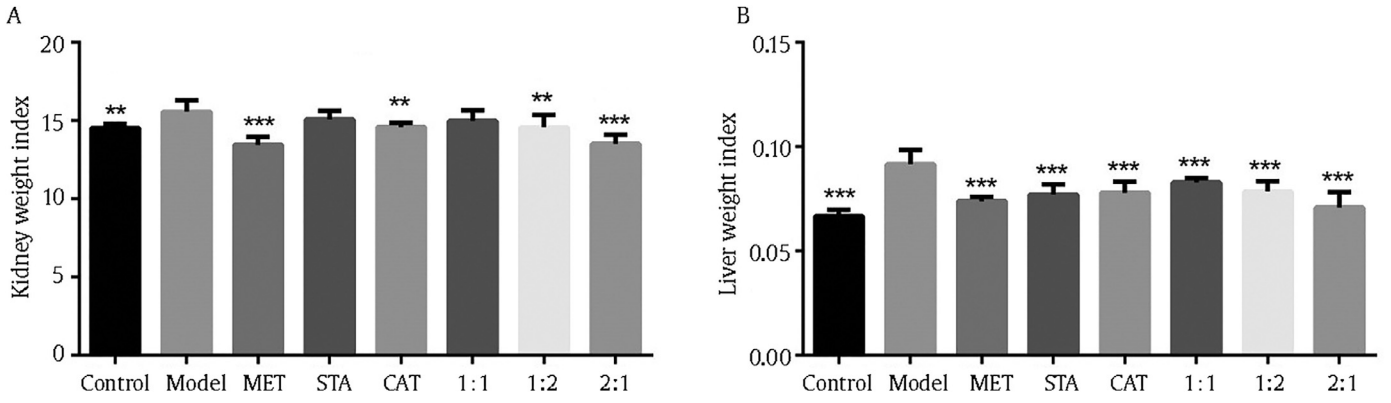

Fig. 4. Effects of catalpol, starchyose, and their combinations on weight indexes of kidney (A) and liver (B) in STZ-HFD induced diabetic mice ($^{***}P < 0.001$, $^{**}P < 0.01$, *vs* model group).

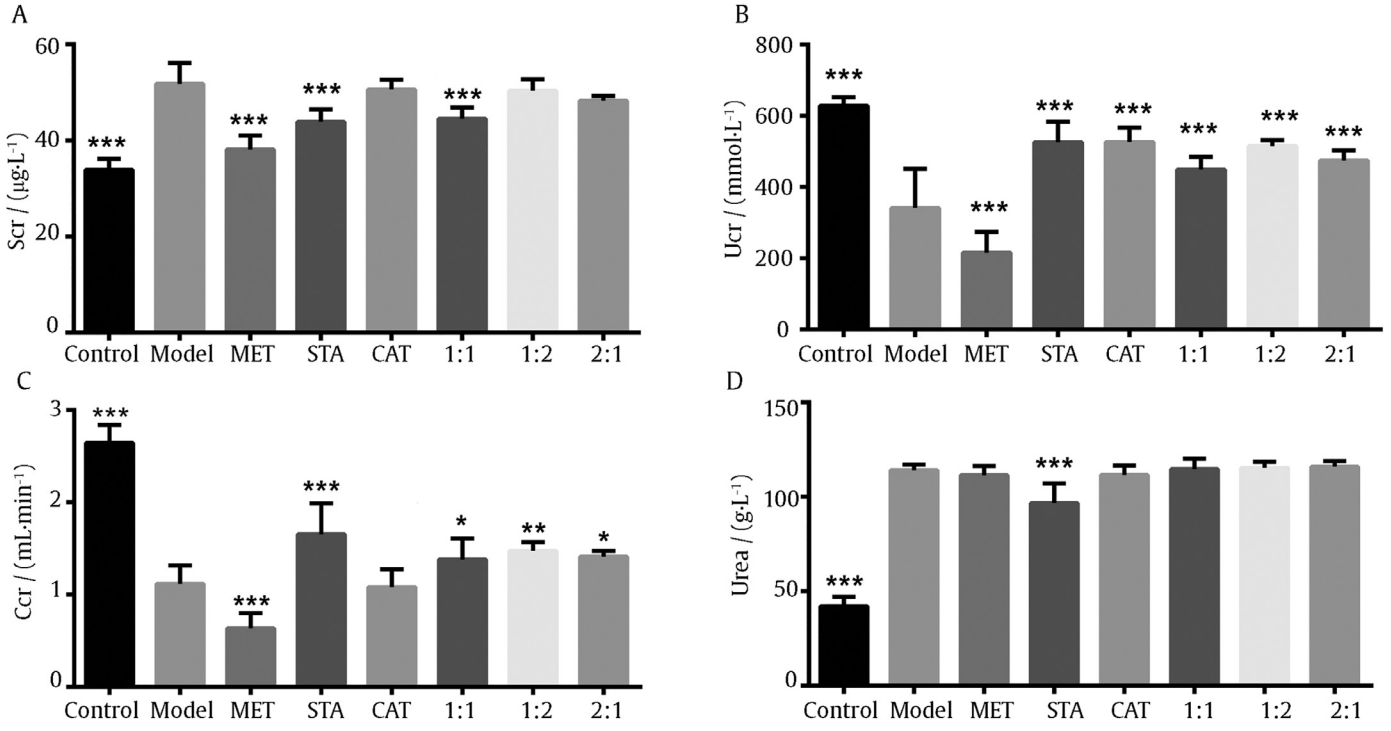

Fig. 5. Effects of catalpol, starchyose, and their combinations on kidney function (levels of Scr (A), Ucr (B), Ccr (C), and Urea (D)),in STZ-HFD induced diabetic mice ($^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ *vs* model group).

which indicated that it was difficult to reduce serum urea by CAT at least in our experiment.

3.5. Effects of CAT, SAT, and their combinations on liver functions of STZ-HFD induced diabetic mice

Diabetes can lead to liver dysfunction. Compared to the normal control group, the liver weight index (LWI) of the model group was significantly increased, which was remarkably decreased by all the medical treatments ($P < 0.001$) (Fig. 4B). However, there was no difference in therapeutic effects among all the six medical administrations. The activities of ALB, ALT and AST, and the level of TBA in the serum are clinically used as active biochemical indicators of liver damage. The activities of ALT, AST, and TBA level were increased significantly in the model group than control group, while ALB content was decreased ($P < 0.001$) (Fig. 6). Serum AST level was not improved by all the medical treatments, compared to model control ($P > 0.05$). Only ALT was improved by all the medical treatments. Moreover, 1:1 of STA and CAT showed better improvement on ALT activity than STA, CAT or other two combinations of CAT and STA. Administrations of STA, CAT, and their combinations could significantly attenuate TBA content of diabetic mice compared to that in model group ($P < 0.05$ and $P < 0.001$), while there was no change in MET-treated group. CAT and its combinations with STA all showed stronger reduction on TBA content in STZ-HFD induced diabetic mice than STA alone. ALB concentration of diabetic mice can only be improved by treatments of MET, CAT, and the ratio of CAT/ STA 2:1, indicating that higher dose of CAT would possibly be required.

We further investigated the damage of STZ-HFD on diabetic liver and compared the medical improving effects of CAT, STA, and their combinations by HE staining method. It was observed

that the histopathological changes of diabetic liver were consistent with our above results of the biochemical parameters (Fig. 7). Compared with the normal control, model mice had irregular and swollen liver cells. In addition, other liver damage symptoms including the disappeared hepatic lobules, the damaged hepatic cord structure, bullous fatty degeneration and ballooning, and irregular nuclei were observed in untreated model mice. The size of the hepatic sinusoid disappeared, and a large number of inflammatory cells were found in the portal area of model mice, compared with control group. HE staining revealed that all the medical treatments could relieve liver damage of diabetic mice. The histopathological scores on the liver symptoms further revealed that all medical treatments could ameliorate liver damage of diabetic mice (Fig. 8). However, there was no difference in ameliorating effects on liver damage among different medical treatments.

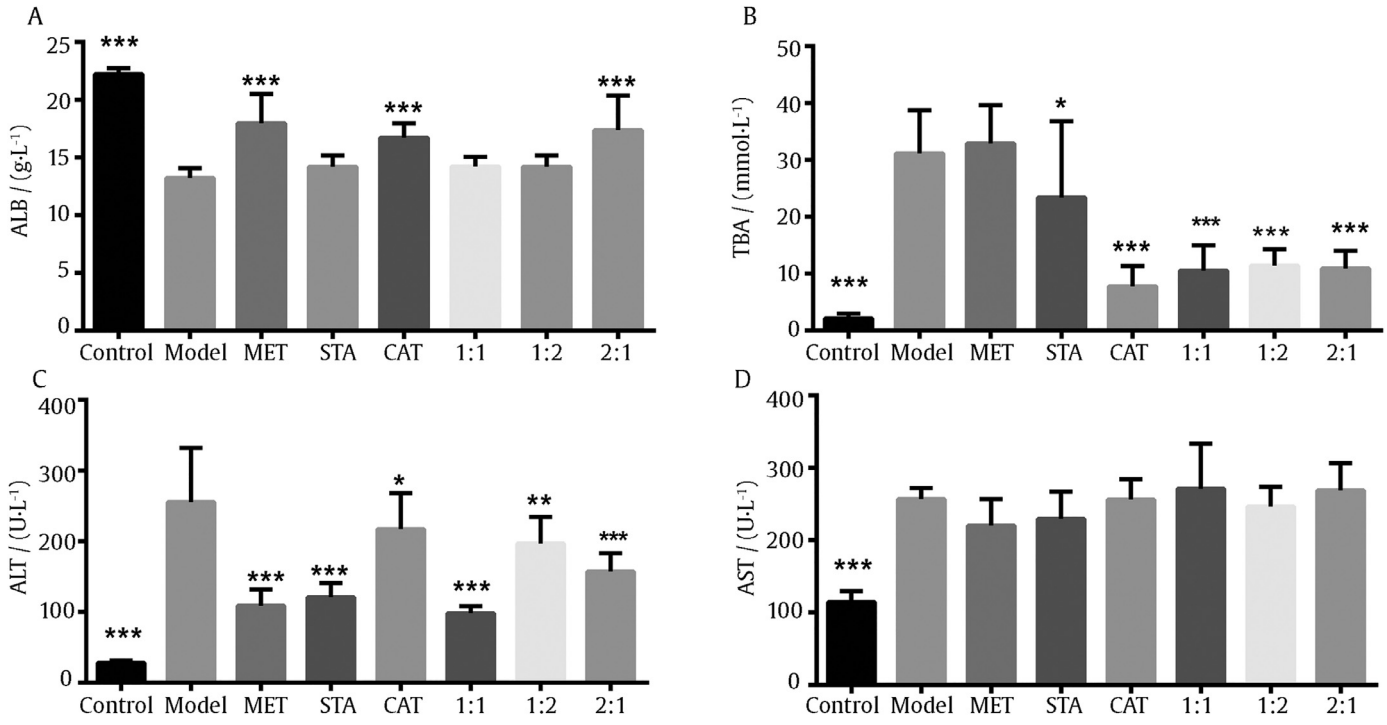

Fig. 6. Effects of catalpol, starchyose, and their combinations on liver function (levels of ALB (A), TBA (B), ALT (C), and ATS (D)),in STZ-HFD induced diabetic mice ($^{***}P < 0.001$, $^{**}P < 0.01$, and $^{*}P < 0.05$ *vs* model group).

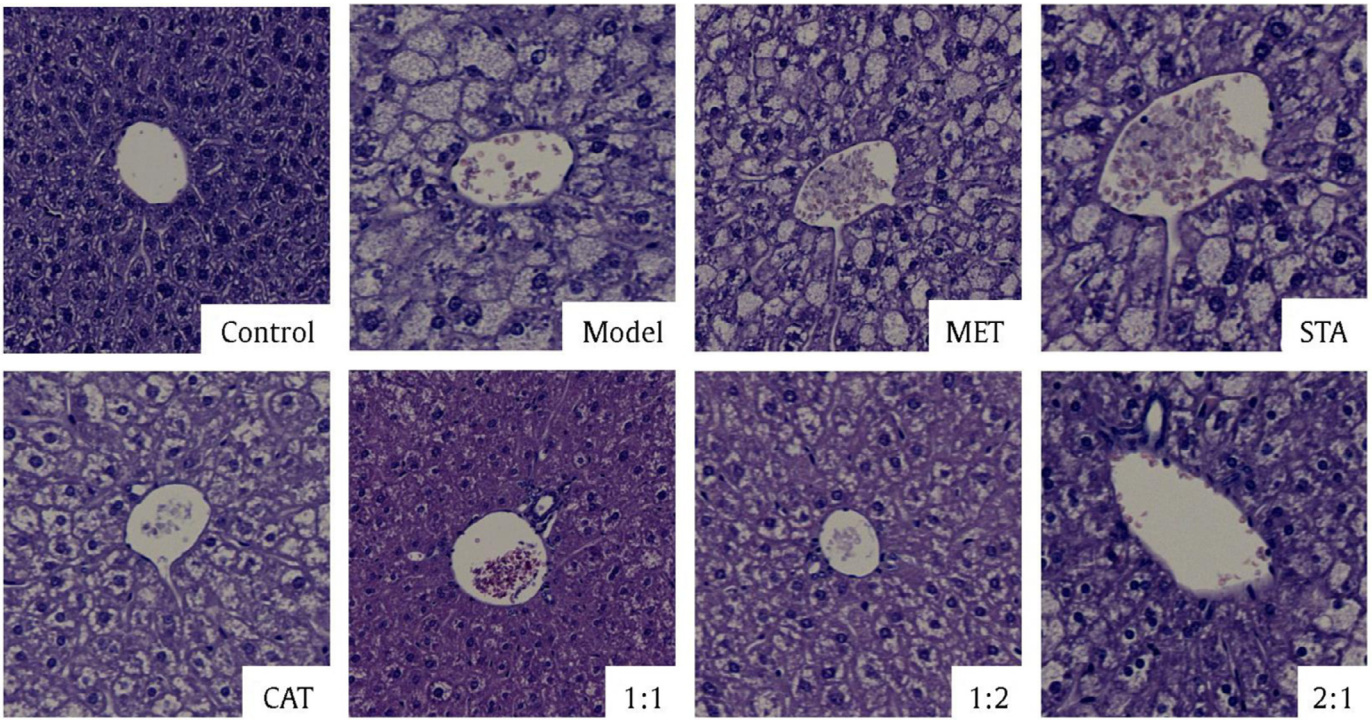

Fig. 7. Histopathological analysis of effects of catalpol, stachyose, and their combinations on liver in STZ-HFD induced diabetic mice.

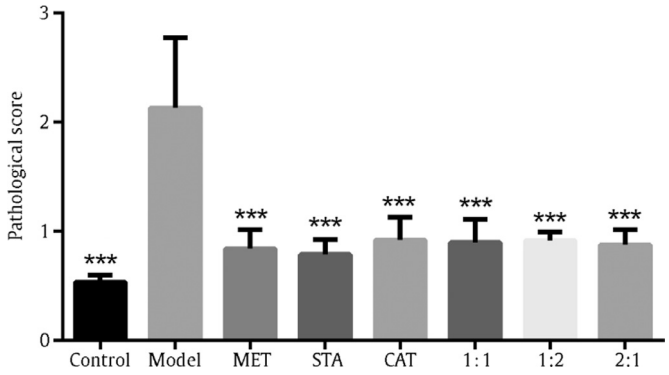

Fig. 8. Histopathological scores of effects of catalpol, starchyose, and their combinations on liver in STZ-HFD induced diabetic mice (***$P < 0.001$ *vs* model group).

4. Discussion and conclusion

TCM has played a vital part in preventing the occurrence of healthy problems or the treatment of these problems thousands of years ago. Some researches had been done to reveal the synergistic actions of drugs in the formula containing *Rehmanniae Radix* (Wen et al., 2011). There was a synergistic interaction between *Rehmanniae Radix* and *Cornus officinalis* on renal injury in db/db diabetic mice (Lv et al., 2016; Tao, Zhao, Ling, Jiang, & Qiu, 2017). Besides, the synergistic action of *Rehmanniae Radix* and *Astragali Radix* promoted diabetic wound healing (Lau et al., 2012). To further develop the therapeutic efficacy of TCM in the formulae, pharmacological study on combinations of bioactive compounds in multiple drugs were recommended (Zhang et al., 2014). In this paper, STZ/HFD-induced diabetic mice were established to evaluate the effects of CAT, STA, and their combinations on regulation of blood glucose, lipids, damage of liver and kidney and further compared their therapeutic effects. Our experiment showed that CAT, STA, and their combinations could effectively control the increase of body weight, blood glucose, liver, and kidney index of diabetic mice. Meanwhile, the levels of TG, TC, LDL-c, and HDL-c were important parameters for lipid metabolism in the living organism. It was found that CAT, STA, and their combinations showed good adjustment of blood lipid levels in this study. It was reported that CAT reduced blood glucose of diabetic mice via regulation of glucose uptake, utilization and lipid metabolism via upregulating the expression of phosphorylation-AMPKα1/2 in liver, skeletal muscle, and adipose tissue (Bao et al., 2016). The improved glucose and lipid metabolisms were also found in insulin-induced insulin-resistant HepG2 cells which were treated with Zengye decoction containing three main compounds: catalpol, aucubin, and harpagide (Liu et al., 2018). Catalpol (200 mg/kg) could rescue mitochondrial ultrastructure in skeletal muscle and improve its mitochondrial function contributing to the reduction of blood glucose level in HFD/STZ-induced diabetic mice (Li et al., 2014). However, we did not make deeper investigation of the effects of CAT or its combinations with STA on mitochondrial biogenesis in this experiment.

ALT, AST, ALB, and TBA together with histopathological changes of liver structure were indicators of abnormal alteration of liver function. Our observation revealed that CAT and its combination with STA reduced liver damage of diabetes. The parameters Scr, and Ucr, Ccr, and urea were commonly used to judge the situation of kidney failure. It turned out that STA and its combinations could protect the kidney of diabetic mice from diabetic-caused injuries. The combinations of CAT and STA especially at the ratio of 1:1 were superior to CAT alone in ameliorating kidney and liver injury. Therefore, our work provided some information about the relationship between CAT and SAT in treatment of diabetes for the first time, which will benefit for the development of *Rehmannia Radix* and the utilization of CAT on diabetes and its complications. Bile acid is a regulator of lipid solubilization and bile acid homeostasis. It is also a complex metabolic integrator and signal factor. The signal function of bile acids plays a vital role in the treatment of obesity, type 2 diabetes, hypertriglyceridemia, and atherosclerosis, as well as other related chronic diseases such as non-alcoholic steatohepatitis (Thomas, Pellicciari, Pruzanski, Auwerx, & Schoonjans, 2008). CAT and SAT could relief diabetes through ways of adjusting levels of bile acids in serum. It is reported that the extract of *Rehmannia Radix* can inhibit the excessive proliferation of human glomerular mesangial cells (HRMC) and cytokine release in glomerular mesangial cells. Besides, it could control the synthesis of extracellular matrix (ECM) components, reduce the oxidative stress of glomeruli and the risk of diabetic nephropathy (Lv, Xu, & Lv, 2015). The project provides insights and technical supports for the innovation of discovering bioactive constituents and studying integrative mechanism for *Rehmannia Radix* in curing diabetes.

In summary, we concluded that administration of CAT, STA, and their combinations could improve diabetic complications. Based on all the assayed parameters, the combination of CAT/STA 1:1 showed similar or better improvements on diabetes-associated diseases compared with the sole CAT or STA treatment. Therefore, we concluded that there were synergistic effects between CAT and STA. However, the underlying mechanisms on how CAT/STA combination synergistically reduced blood glucose and risks of kidney and liver failure in diabetes are needed to be explored.

Conflict of interest

The authors declare no conflicts of interest.

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