Ginsenoside Rg3 has effects comparable to those of ginsenoside re on diabetic kidney disease prevention in db/db mice by regulating inflammation, fibrosis and PPARγ

ZHE SUI[1], DAYUN SUI[2,3], MIN LI[3], QIAN YU[4], HONGJUN LI[1] and YICHUAN JIANG[2,4]

[1]Health Management Medical Center, China-Japan Union Hospital, Jilin University, Changchun, Jilin 130033; [2]Department of Pharmacology; [3]Pharmacological Experiment Center, School of Pharmaceutical Sciences, Jilin University, Changchun, Jilin 130021; [4]Department of Pharmacy, China-Japan Union Hospital, Jilin University, Changchun, Jilin 130033, P.R. China

Abstract. Ginsenoside Rg3 (Rg3) is an adjuvant antitumor drug, while ginsenoside Re (Re) is an adjuvant antidiabetic drug. Our previous studies demonstrated that Rg3 and Re both have hepatoprotective effects in db/db mice. The present study aimed to observe the renoprotective effects of Rg3 on db/db mice, with Re as the control. The db/db mice were randomly assigned to receive daily oral treatment with Rg3, Re or vehicle for 8 weeks. Body weight and blood glucose were examined weekly. Blood lipids, creatinine, and BUN were examined by biochemical assay. Hematoxylin and eosin and Masson staining were used for pathological examination. The expression of peroxisome proliferator-activated receptor gamma (PPARγ) and inflammation and fibrosis biomarkers was examined by immunohistochemical and reverse transcription-quantitative PCR. Although neither had a significant effect on body weight, blood glucose or lipids, Rg3 and Re were both able to decrease the creatinine and blood urea nitrogen levels of db/db mice to levels similar to those of wild type mice and inhibit pathological changes. The expression of PPARγ was upregulated and biomarkers of inflammation and fibrosis were downregulated by Rg3 and Re. The results showed that the potential of Rg3 as a preventive treatment of diabetic kidney disease was similar to that of Re.

Correspondence to: Dr Hongjun Li, Health Management Medical Center, China-Japan Union Hospital, Jilin University, 126 Xiantai Street, Changchun, Jilin 130033, P.R. China
E-mail: hj_li@jlu.edu.cn

Dr Yichuan Jiang, Department of Pharmacy, China-Japan Union Hospital, Jilin University, 126 Xiantai Street, Changchun, Jilin 130033, P.R. China
E-mail: figaro@jlu.edu.cn

Introduction

Chronic kidney disease (CKD) has been recognized as a major public health problem that is associated with substantial morbidity, mortality and financial cost to the healthcare system (1,2). Diabetes is one of the most important causes of CKD and diabetic kidney disease (DKD) is one of the most important complications of diabetes (3,4). DKD prevention is crucial for patients with diabetes.

Although hyperglycemia is an essential requirement for DKD, the pathogenic pathway initiated and maintained in the kidney by elevated glucose levels can be enhanced by a number of different factors. These factors include excess fatty acids, oxidative stress and hemodynamic factors. These factors do not themselves contribute to DKD, but in the presence of diabetes they feed back and reinforce common pathogenic mechanisms, including increased levels of inflammatory cytokines and fibrosis-related growth factors in the kidney (5). In addition, the peroxisome proliferator-activated receptors (PPARs; α, β/δ, γ) are a family of ligand-activated transcription factors of the nuclear receptor superfamily that regulates cellular metabolic homeostasis. Mutations in the PPARγ gene have been demonstrated to be associated with dysfunctional lipid and glucose homeostasis (6). In preclinical and/or clinical studies, activation of PPARγ has been shown to have a positive effect on insulin resistance, diabetic nephrotic syndrome, and renal fibrosis (7). Therefore, PPARγ may serve an important role in DKD.

Ginsenosides are major active ingredients extracted from the roots, stems, leaves or fruits of *Panax ginseng* C.A. Meyer (Ginseng; Fig. 1A), which is widely cultivated in Korea and Northeast China. Ginsenosides are widely used to develop drugs for adjuvant therapy for cancer and chronic cardiovascular and metabolic diseases, especially the complications of these disorders (8-12). These natural products do not have sufficiently strong biological activities to become first-line drugs for those diseases.

Ginsenoside Rg3 (Rg3; chemical structure shown in Fig. 1B) is one of the major active ingredients extracted from Korean red ginseng. It can also be chemically or biologically converted from other ginsenosides (13-15). Rg3 is well known
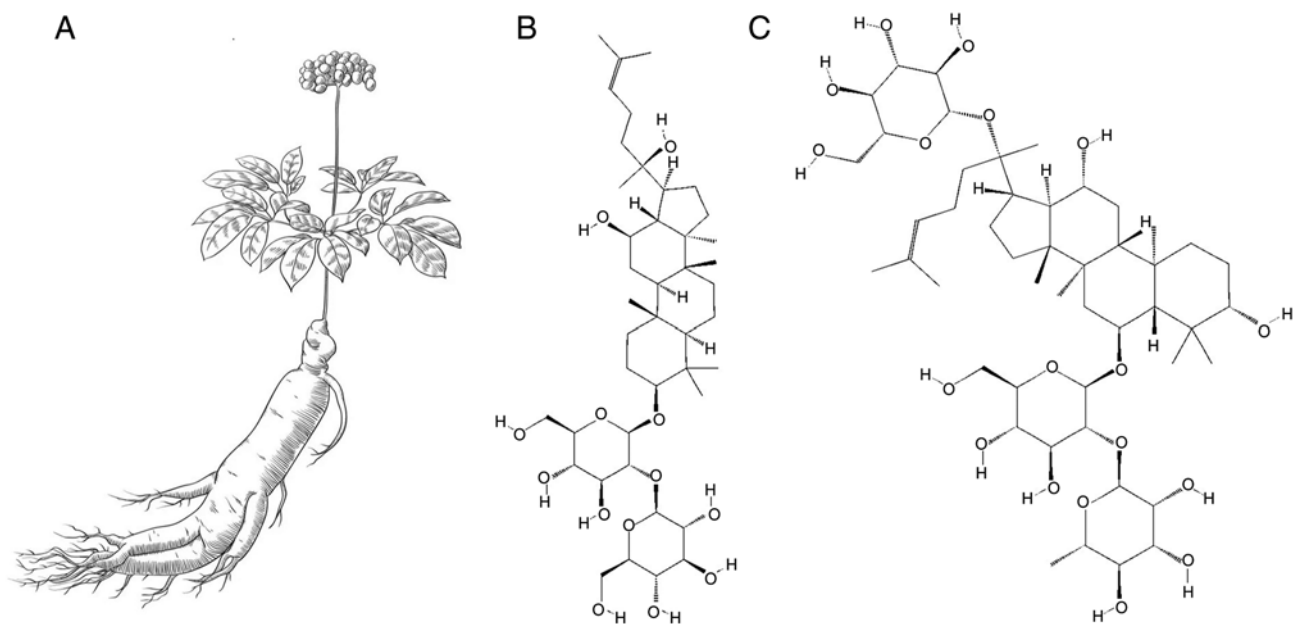
Figure 1. Ginseng and ginsenosides. Pictures of (A) Ginseng (whole plant) and chemical structures of (B) Rg3 and (C) Re. Rg3, ginsenoside Rg3; Re, ginsenoside Re.

for its antitumor effects (16-20) and has been developed into an adjuvant antitumor drug called Shenyi capsule (SYC) in China (11,21-23).

Ginsenoside Re (Re; chemical structure shown in Fig. 1C) is the essential component of total saponins from ginseng fruits and was developed into a drug called Zhenyuan capsule (ZYC) in China (10). According to the quality control standard documents for ZYC, the content of Re is the only technical standard in its quality control. Re alleviates the complications of cardiovascular and metabolic diseases (24-26) and ZYC is widely used for adjunctive treatment of type II diabetes (T2DM) and coronary heart disease in the clinic in China (10,27,28).

Our group is currently undertaking a long-term study assessing the effects of various ginsenosides (including Rg3 and Re) in various animal models of chronic diseases, such as hypertension, hyperlipidemia and T2DM (21-24,29,30). Notably, our previous studies demonstrated that Rg3, the essential component of SYC, has various beneficial effects on the target organs of those chronic diseases (21-23,29), although SYC is used for adjunctive cancer treatment in the clinic (11,18). In particular, Rg3 attenuated atherosclerosis in hyperlipidemic mice (29) and nonalcoholic steatohepatitis (NASH) in T2DM mice (22), both of which are chronic metabolic diseases. In these two studies, the mechanisms of the protective effects included regulation of inflammation, fibrosis and PPARγ.

In another study, the authors demonstrated that Re attenuated NASH in T2DM mice, also via the regulation of inflammation, fibrosis and PPARγ (24). In contrast to Rg3/SYC, Re/ZYC has already been widely used for T2DM treatment in the clinic. The present study observed the renoprotective effects of Rg3 on db/db mice (BKS-Lepr[em2Cd479]/Gpt), and Re was used as the control. The leptin receptors are defective in this type of mice, leading to obesity and disorders of glucose-lipids metabolism. Hyperleptinemia, hyperglycemia and hyperlipidemia also lead to DKD and non-alcoholic

fatty liver disease (NAFLD) in db/db mice >20 weeks old, with abnormal serum renal and liver function indicators. The symptoms of T2DM in db/db mice are similar to those in humans, and it is one of the best-known gene-deficient animal models of T2DM with DKD (31,32). The main features of DKD in humans are renal hypertrophy, enlarged glomeruli, albuminuria and dilated thylakoid stroma and the db/db mice simulates these pathological features successfully.

Materials and methods

Reagents. Rg3 (95% purity) was obtained from Dr Li Fu at the Institute of Dalian Fusheng Natural Medicine, Dalian Fusheng Pharmaceutical Co., Ltd. (Dalian, China). Re (95% purity) was obtained from Dr Yanping Chen at the Department of Natural Medicinal Chemistry, School of Chemistry, Jilin University (Changchun, China). Rg3 and Re were dissolved in 0.5% sodium carboxymethyl cellulose solution (0.5% CMC-Na) for use. All other chemicals were analytical reagents.

Animals. A total of 24 db/db mice (25-27 g) and 8 wild-type (WT) mice (45-47 g) were purchased from GemPharmatech Co., Ltd. All the mice were male and 12-13 weeks old. The experimental animal house was of specific pathogen-free (SPF) grade, and the mice were kept in individually ventilated cages (four mice per cage). The house was maintained at a constant temperature of 22-24°C relative humidity of 45-55%. All mice had free access to water and food and were maintained in a 12-h light/dark cycle. All feeding conditions were the same as described in previous studies (22,24). The mouse chow diet (cat. no. SZS9126-1010082) was purchased from Jiangsu Xietong Pharmaceutical Bioengineering Co., Ltd.

Experimental protocols. A total of eight WT mice were designated as Group WT. The 24 db/db mice were randomly divided into three groups with eight mice in each: Group

db/db, 8 db/db mice; Group Rg3, 8 db/db mice; and Group Re, eight db/db mice. Mice in Rg3 and Re were orally administered Rg3 and Re, respectively, at a dose of 30 mg·kg^{-1}day^{-1}, the same dose used in previous studies (22,24). Mice in Group WT and Group db/db were orally administered 0.5% CMC-Na as a placebo in the same volume given to those in Group Rg3 and Group Re. The administration of Rg3, Re and placebo was carried out for eight weeks. During these eight weeks, the blood glucose and body weight of the mice were measured weekly while general health and behavior states of mice were monitored daily during oral administration.

After the eight week treatment, the mice in the four groups were sacrificed (carbon dioxide euthanasia, the carbon dioxide concentration in the container rose from 0.03-99% in 2 min) for renal tissue sample collection immediately after collecting blood samples from the ophthalmic venous. All the samples were processed as described in previous studies (22,24). Serum was isolated from blood samples for biochemical assays. Renal tissue samples were fixed in 4% formaldehyde (20°C overnight) for histopathological and immunohistochemistry (IHC) assessment, snap-frozen and kept at -80°C for biochemical assays and reverse transcription-quantitative (RT-q) PCR.

Fasting blood glucose measurement. Throughout the eight weeks of treatment, blood from the lateral tail vein of the WT and db/db mice was collected, and the fasting blood glucose level was monitored weekly using a blood glucose test meter and strips (GlucoLab, Infopia Co., Ltd.) according to the manufacturer's protocol as previously described (22,24).

Serum biochemical assays. Biochemical assay kits were purchased from Nanjing Jiancheng Bioengineering Institute: Creatinine (CRE; cat. no. C011-2-1), blood urea nitrogen (BUN; cat. no. C013-2-1), triglyceride (TG; cat. no. A110-1-1), total cholesterol (TC; cat. no. A111-1-1), high-density lipoprotein cholesterol (HDL; cat. no. A112-1-1), and low-density lipoprotein cholesterol (LDL; cat. no. A113-1-1). Levels of CRE, BUN, TG, TC, HDL and LDL in serum were assayed in accordance with the manufacturer's protocols as previously described (22-24).

Histopathological assessment. Renal tissue specimens fixed in 4% formalin were embedded in paraffin, cut into 4-μm-thick sections and then stained with hematoxylin and eosin (H&E) at 5 and 1 min respectively at room temperature or Masson's trichrome stain (Masson) as previously described (22-24). A Masson kit (cat. no. BSBA-4079B) was purchased from OriGene Technologies, Inc. Sections stained with H&E and Masson were examined, and images were captured using a Nikon E100 light microscope (Nikon Corporation).

IHC. Primary antibodies against PPARγ (cat. no. bs-4590R), tumor necrosis factor-α (TNF-α; cat. no. bs-10802R), transforming growth factor β1 (TGF-β1; cat. no. bs-0103R) and connective tissue growth factor (CTGF; cat. no. bs-0743R) were purchased from BIOSS. A DAB kit (cat. no. ZLI-9017) and a two-step rabbit IHC kit (cat. no. PV-9001) were purchased from OriGene Technologies, Inc. IHC was performed in accordance with the manufacturer's protocols for the IHC kit and DAB kit as previously described (22-24). Photomicrograph images

Table I. Primer sequence for reverse transcription-quantitative PCR.

Gene	Sequence (5'-3')
β-actin	
Forward	GGCTGTATTCCCCTCCATCG
Reverse	CCAGTTGGTAACAATGCCATGT
CTGF	
Forward	GTAACCGGGGAGGGAAATTA
Reverse	GCTTTATCACCTGCACAGCA
IL-6	
Forward	GTCCTTCAGAGAGATACAGAAACT
Reverse	AGCTTATCTGTTAGGAGAGCATTG
Col-I	
Forward	CTTCACCTACAGCACCCTTGTG
Reverse	TGACTGTCTTGCCCCAAGTTC
Col-III	
Forward	TGTCCTTTGCGATGACATAATCTG
Reverse	AATGGGATCTCTGGGTTGGG

CTGF, connective tissue growth factor; Col, collagen type.

were then captured with a light microscope as aforementioned and were further analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Inc.).

RNA purification and RT-qPCR. Isolation of total RNA was carried out using TRIzol® reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol as previously described (22-24). RT and qPCR were performed were performed according to the manufacturer's protocol with TransScript Green TwoStep RT-qPCR SuperMix (Beijing Transgen Biotech Co., Ltd.) on a Stratagene Mx3000P Real-Time PCR System (Agilent Technologies, Inc.). The thermocycling conditions were denaturation 94°C for 5 sec, annealing 60°C for 15 sec and extension 72°C for 10 sec, 40 cycles. The 2$^{-\Delta\Delta Cq}$ method (33) was employed for analysis of the expression of genes of interest, and β-actin was used as a housekeeping gene. All primers are listed in Table I.

Statistical analysis. SPSS 16.0 (SPSS, Inc.) was employed for all statistical analyses. Data are presented the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's post-hoc test was employed for group comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Rg3 and Re had no significant effects on body weight, blood glucose or lipids. Prior to and following eight weeks of treatment, the mice in the db/db group had significantly higher body weight (Fig. 2A) and blood glucose levels (Fig. 2B) compared with the mice in the WT group, which is the basic feature of db/db mice (31,32). The body weights of the mice in the WT and db/db groups after eight weeks of treatment were
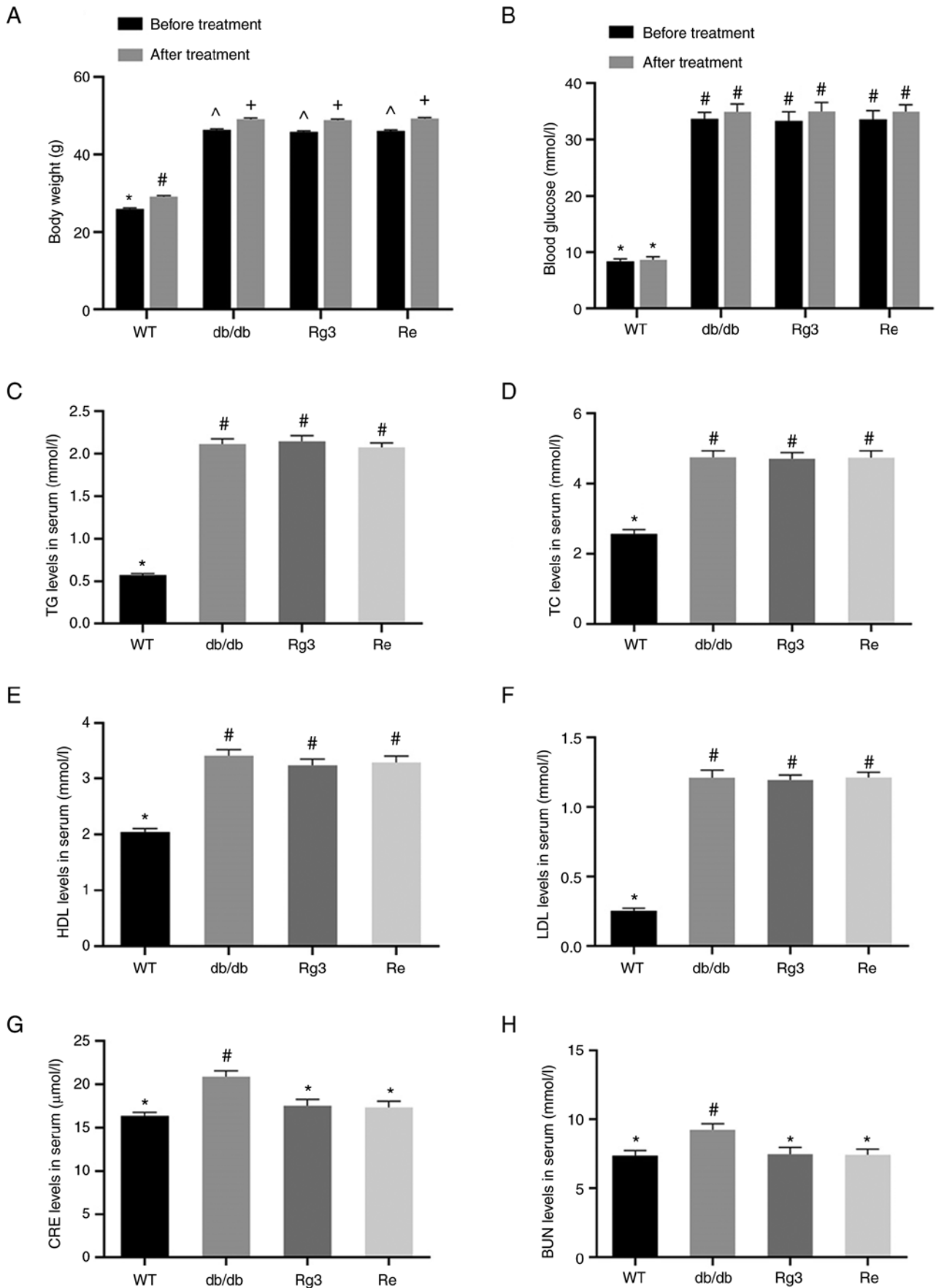
Figure 2. Body weight, blood glucose, lipids and renal function index in serum. (A) Body weight and (B) blood glucose of mice prior to and following 8 weeks of treatment; serum (C) TG, (D) TC, (E) HDL, (F) LDL, (G) CRE and (H) BUN levels in mice after treatment. Data are presented as the mean ± standard deviation, n=8. The same superscript symbols indicate no significant difference between groups (P>0.05); a significant difference existed between groups that do not have the same superscript symbol (P<0.05). TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; CRE, creatinine; BUN, blood urea nitrogen; WT, wild type; Rg3, ginsenoside Rg3; Re, ginsenoside Re.
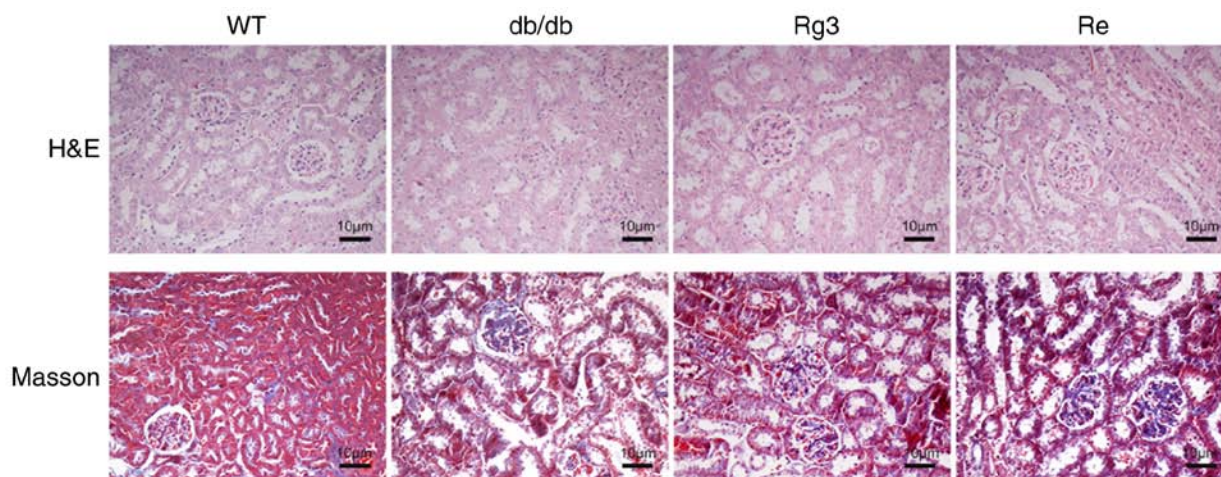
Figure 3. Representative H&E and Masson staining images of the renal tissue in mice. Scale bars, 10 μm. H&E, hematoxylin and eosin; WT, wild type; Rg3, ginsenoside Rg3; Re, ginsenoside Re.

significantly elevated compared to those before eight weeks of treatment, while their blood glucose levels were slightly elevated but not significantly different. The body weight and blood glucose levels in Groups Rg3 and Re were similar to those in Group db/db, and there was no significant difference between them either before or after the eight week treatment.

The levels of blood lipids (TG, TC, HDL and LDL) in the mice in Group db/db were all significantly higher than those in Group WT after eight weeks of treatment (Fig. 2C-F). Similar to body weight and blood glucose, lipid levels in Groups Rg3 and Re were similar to those in Group db/db.

In summary, all the db/db mice presented significant abnormalities and glucose and lipid metabolism disorders, which are all typical symptoms of T2DM. Treatment with Rg3 and Re were not able to significantly attenuate these symptoms, which is similar to our previous studies (22-24).

Rg3 and Re improved early renal injury in T2DM. According to the histology images of kidney H&E-stained sections from the mice in Group WT, the outer cortical glomerulus was of normal size and configuration (Fig. 3). The capillary tuft was fully expanded with patent capillary loops and the glomerular basement membrane appeared thin without matrix expansion, inflammation, or sclerosis. The histology images from mice in Group db/db showed striking differences in some glomeruli. The visceral epithelial cells were swollen and appeared prominent. The glomerular capillary basement membranes appeared thickened, and the peripheral capillary loop appeared to be collapsed; 30-40% of glomeruli had a similar appearance and the remaining had a lesser degree of mesangial matrix expansion. The histology images from mice in Groups Rg3 and Re showed that treatments with these two ginsenosides significantly attenuated the pathological changes; only 10-20% of glomeruli had an appearance similar to that aforementioned and most of the glomeruli had a lesser degree of mesangial matrix expansion. According to the histology images of Masson-stained kidney sections, mice from Group db/db presented slight tubulointerstitial fibrosis, absent in the mice from the other three groups.

The levels of CRE and BUN supported the results of the pathological examination (Fig. 2G and H). The two were

significantly higher in the mice from Group db/db compared with the mice from Group WT. The levels of the other three groups did not present significant differences.

These results showed that mice from Group db/db were in an early stage of DKD. There were slight pathological changes according to the pathological examination and slight renal function decompensation according to the blood biochemical examination. Although Rg3 and Re had no significant effects on body weight, blood glucose or lipids, their treatment prevented early-stage DKD in db/db mice from Groups Rg3 and Re. Their protective effects were comparable, and both were able to reduce the levels of CRE and BUN to those in WT mice. These findings were also consistent with our previous studies (22-24), in which ginsenoside treatment improved renal or hepatic early-stage injury independent of reducing the levels of blood pressure or blood glucose.

Rg3 and Re upregulated PPARγ expression and inhibited inflammation and fibrosis in renal tissue. In our two previous studies, Rg3 (22) and Re (24) were demonstrated to ameliorate hepatic injury in db/db mice by upregulating PPARγ expression and inhibiting inflammation and fibrosis in hepatic tissue. The present study also performed IHC and RT-qPCR to assess the levels of PPARγ expression and the inhibition of inflammation and fibrosis in renal tissue.

According to the integrated optical density (IOD) analysis of the IHC images, PPARγ expression in renal tissue from mice in Group db/db was significantly higher than that in Group WT (Fig. 4A and B). This increase was a compensatory upregulation related to the high blood glucose levels in db/db mice (34). The PPARγ expression in the renal tissue was further upregulated by Rg3 and Re treatment, which was similar to the findings in hepatic tissue in the previous aforementioned studies (22,24).

TNF-α is one of the most important markers of inflammation. The IHC results of TNF-α showed significantly higher levels of inflammation in renal tissue from mice in Group db/db compared with those in Group WT (Fig. 4A and C). The levels of TNF-α were significantly decreased in Groups Rg3 and Re and were not significantly different from those in Group WT.
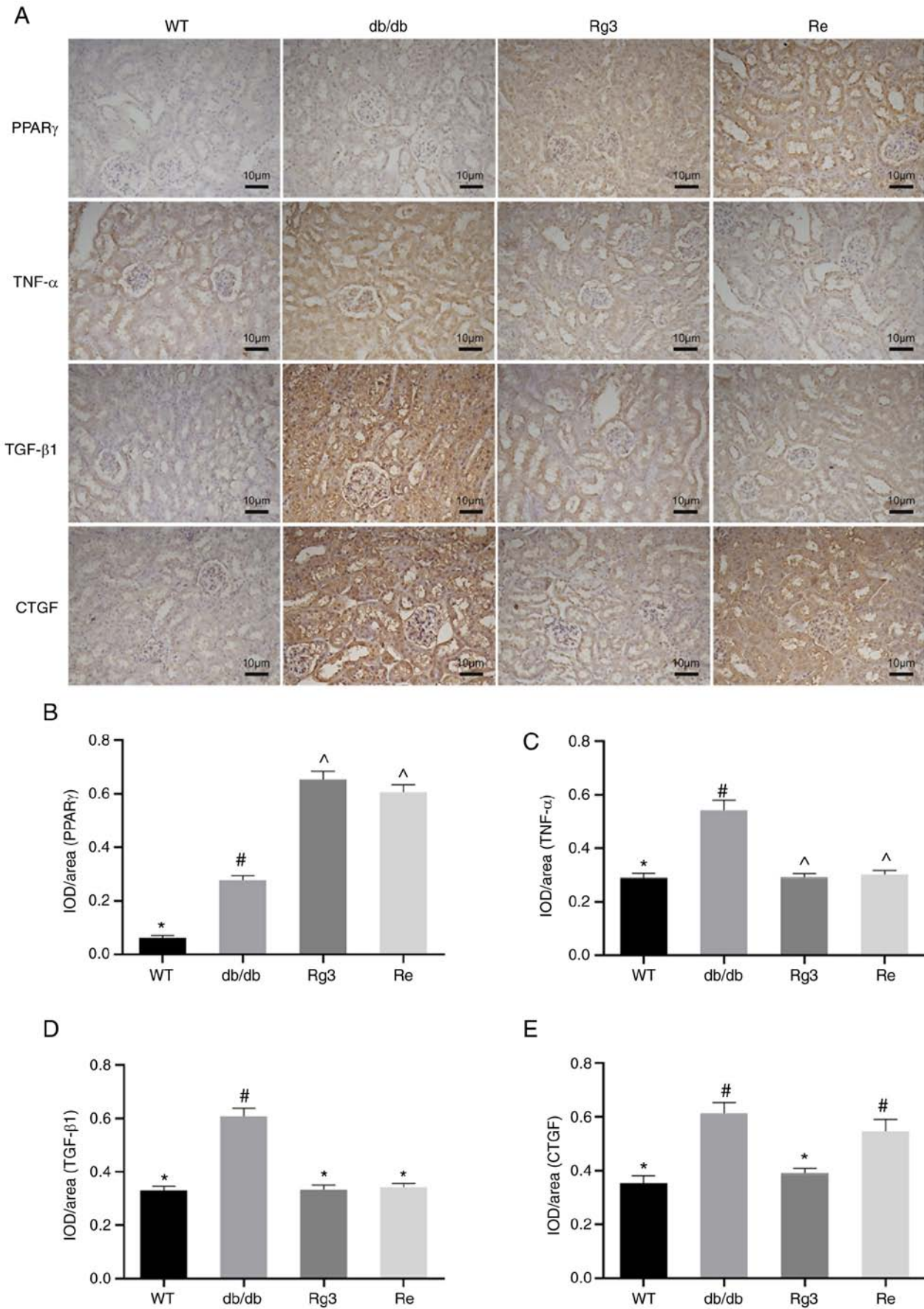
Figure 4. IHC staining images of the renal tissue in mice and analyses. (A) Representative IHC staining images of the renal tissue in mice and quantitative results of IHC staining, which are presented as IOD/area and are proportional to the levels of (B) PPARγ, (C) TNF-α, (D) TGF-β1 and (E) CTGF. Scale bars, 10 μm. Data are presented as the mean ± standard deviation, n=4. The same superscript symbols indicate no significant difference between groups (P>0.05); a significant difference existed between groups that do not have the same superscript symbol (P<0.05). IHC, immunohistochemistry; PPARγ, peroxisome proliferator-activated receptor γ; CTGF, connective tissue growth factor; WT, wild type; Rg3, ginsenoside Rg3; Re, ginsenoside Re.
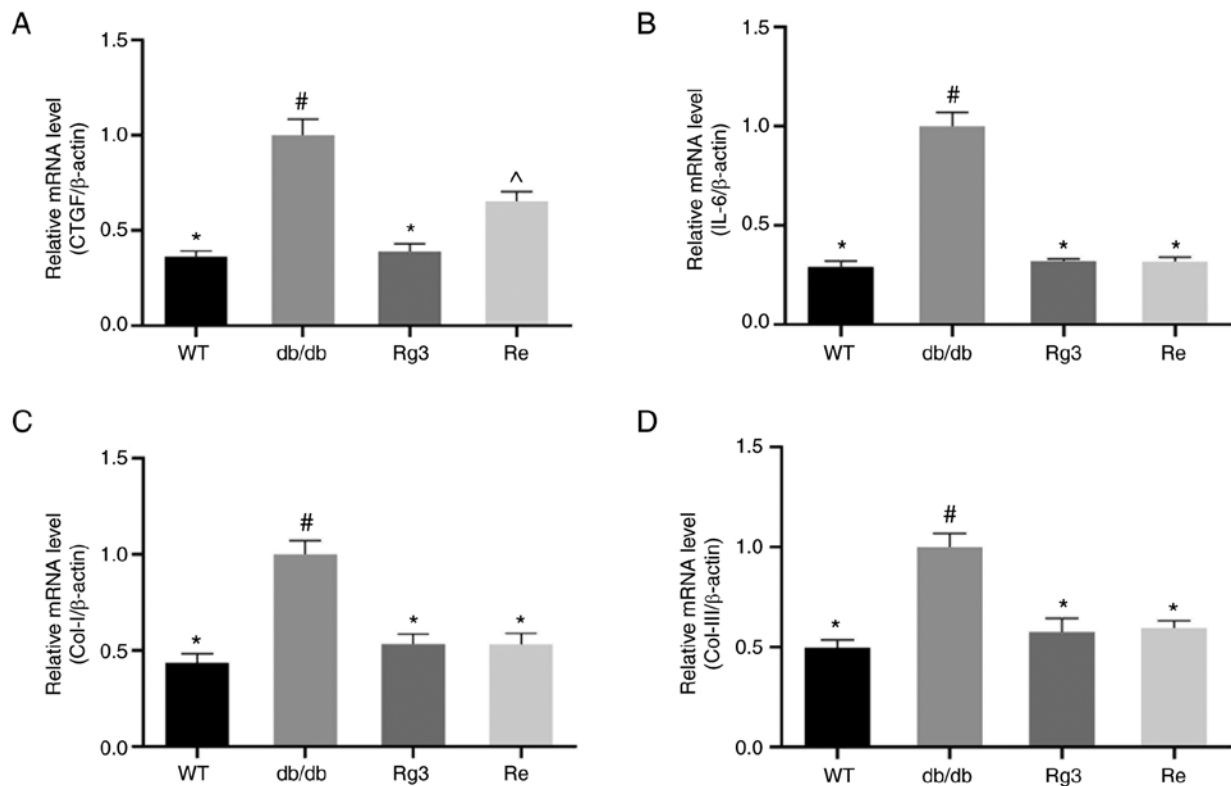
Figure 5. Levels of mRNA associated with inflammation and fibrosis in renal tissue in mice. Relative mRNA levels of (A) CTGF, (B) IL-6, (C) Col-I and (D) Col-III. β-Actin was used as a housekeeping gene. Data are presented as the mean ± standard deviation, n=4. The same superscript symbols indicate no significant difference between groups (P>0.05); a significant difference existed between groups that do not have the same superscript symbol (P<0.05). CTGF, connective tissue growth factor; Col, collagen type.

TGF-β1 and CTGF are both important profibrotic factors and markers of fibrosis. According to the IHC results, the levels of TGF-β1 and CTGF in renal tissue were significantly higher in the db/db group compared with the WT group. The levels of both TGF-β1 and CTGF in Group Rg3 were significantly reduced and were similar to those in Group WT (Fig. 4A, D and E). Notably, only TGF-β1 levels were significantly decreased in Group Re compared to those in Group db/db, while CTGF levels were not significantly different from those in Group db/db.

Thus, the present study measured the levels of CTGF repeatedly using RT-qPCR. The relative mRNA levels of CTGF supported the IHC results (Fig. 5A). The levels of CTGF in Group Re were significantly lower than those in Group db/db but significantly higher than those in Group WT. The levels of CTGF in Group Rg3 were not significantly different from those in Group WT.

Levels of collagen type (Col)-I, Col-III, markers of collagen deposition, and IL-6, another marker of inflammation, were also measured using RT-qPCR (Fig. 5B-D). The relative mRNA levels of all three markers were higher in the renal tissue of mice from the db/db group than in that of mice from the WT group. Rg3 and Re treatment were both able to significantly downregulate these mRNA levels in db/db mice. Moreover, the degree of downregulation was similar.

In summary, Rg3 and Re both upregulated the expression of PPARγ in the renal tissue of db/db mice and downregulated inflammatory and fibrotic factors, such as TNF-α, TGF-β1 and CTGF. These regulatory effects all contributed to the

renoprotective effects of Rg3 and Re. Notably, Rg3 had a stronger effect of downregulating CTGF than Re. However, the overall effects of Rg3 on inhibiting inflammation and fibrosis in renal tissue were comparable to those of Re in this research.

Discussion

DKD has become the leading cause of end-stage renal disease worldwide, which increases the risk of premature death and represents a serious financial burden. Although DKD is controllable with different drugs, such as angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs) and hypoglycemic agents, such as thiazolidinediones (TZDs), the preventive and therapeutic merits of natural medicines, such as ginsenosides, have been widely recognized (4).

ACEIs and ARBs, blockers of the renin-angiotensin-aldosterone system, are widely used to control the progression of DKD, especially in patients with both diabetes and hypertension. However, as ACEIs and ARBs also have a strong biological ability to reduce blood pressure, they are not recommended for diabetic patients without hypertension or any DKD symptoms (35,36).

TZDs are agonists of the PPARγ pathway and have anti-inflammatory and antifibrotic biological activities, increase insulin sensitivity and reduce blood glucose; they are also used to control DKD. However, TZDs have recently fallen into disuse for glycemic control due to concerns over side effects and adverse events. These drugs are recommended

only for diabetic patients with complications such as DKD and NASH to control their progression (37,38).

In traditional Chinese medicine (TCM) theory, the main causes of diabetic kidney disease are congenital deficiencies, dietary disorders and emotional disorders. Insufficient innate endowment (a type of birth defect) and weakness of the internal organs by birth make these individuals more susceptible to DKD. The best way to manage the complications of diabetes is 'Zhi Wei Bing' (39,40). Zhi Wei Bing means taking appropriate measures to prevent the development of the disease. The main idea in TCM theory is to prevent disease before it occurs and to prevent the development of disease after it has occurred. Correctly anticipating the development of the disease can stop its exacerbation or transformation in time, which is why TCM, such as Re/ZYC, is widely used in China for patients with T2DM. According to our previous studies, ginsenosides such as Rg3 and Re exerted anti-inflammatory and anti-fibrotic effects on vital organs such as the heart, liver and kidneys, although they do not significantly lower blood pressure, blood glucose and lipids in animals (21-24). As aforementioned, natural products such as Rg3 and Re do not have strong enough biological activity to be first-line antihypertensive or hypoglycemic agents. However, it is also the mild biological activity of Rg3 and Re that makes it possible for them to be developed as safe drugs with few side effects for use in early-stage diabetic patients without hypertension or any symptoms of DKD.

As aforementioned, natural products such as Rg3 and Re do not have sufficiently strong biological activities to become first-line hypotensive or hypoglycemic agents. On the other hand, Rg3 and Re have the potential to be used for early-stage diabetes patients without hypertension or any DKD symptoms because of their mild biological activities. Ginseng has been widely consumed as a food/diet supplement from natural sources, and the safety of using ginseng and ginsenosides has been widely recognized (4,41,42).

The situation of Rg3/SYC is different. Rg3 was widely used for cancer patients in China at first (11,18). Indeed, 10 years ago most studies focused on the role of Rg3 in inducing apoptosis of tumor cells and inhibiting their growth (43-45). More recently, an increasing number of antitumor effects of Rg3 have been discovered (18-20,46-48) and include inhibition of drug resistance, angiogenesis and epithelial-mesenchymal transformation. The mechanisms include regulation of the Wnt/β-catenin pathway, Hippo pathway, VEGF pathway and TGF-β signaling pathway (18-20,46-48), all of which have a close connection to inflammation, fibrosis and the PPARγ pathway (49-51).

Although the use of TZDs in T2DM has been controversial in recent years (37,38), increasing evidence shows that activation of the PPARγ pathway is beneficial to tumor treatments in most fields (52). Activation of the PPARγ pathway has a positive correlation with the Hippo pathway (51) and a negative correlation with the Wnt/β-catenin pathway (50). These interactions occur not only in diabetes but also in tumors. The Hippo pathway and Wnt/β-catenin pathway are also popular tumor therapeutic targets. Moreover, activation of PPARγ usually has a negative correlation with insulin resistance, inflammation and fibrosis (37,38,50-52), which are all important for the treatment of diabetes and its complications.

Although Rg3 and Re belong to the ginsenosides family, their aglycone structures are different, with Rg3 being a protopanaxadiol saponin and Re being a protopanaxatriol saponin. Nevertheless, both Rg3 and Re are protective against DKD or other complications of T2DM, as well as several kinds of other protopanaxadiol or protopanaxatriol saponins, all of which have anti-inflammatory and anti-fibrosis effects. In addition, comparing the effects of Rg3 and Re in the present study, the major difference was that Rg3 had a stronger effect of regulating CTGF in renal tissue. These results were consistent with our previous studies of Rg3 and Re in db/db mice with NASH (22,24). As CTGF has a close connection to the Hippo pathway (49), these results were also consistent with the report of Rg3 activating the Hippo pathway in antitumor research (48). These studies might explain why Rg3 has more antitumor effects than Re while the renoprotective effects are comparable. More experiments are needed to confirm this hypothesis in the future.

Although neither has significant effects on body weight, blood glucose and lipids, Rg3 and Re both have effects in the prevention of DKD in db/db mice. The mechanisms of their renoprotective effects include alleviation of inflammation and fibrosis and upregulation of PPARγ expression in renal tissue. The effects of Rg3 and Re were comparable, as they were both able to reduce the CRE and BUN levels of db/db mice to levels similar to those of WT mice, as well as the inhibition of pathological changes in the renal tissue of those mice. The only difference was that Rg3 had a stronger effect of regulating CTGF in renal tissue. The present study showed that Rg3, an adjuvant antitumor drug, has a potential similar to that of Re, which has already been widely used in the clinic for the preventive treatment of T2DM complications, such as DKD. The timing of using Rg3 or Re could be much earlier than that of using chemical drugs, such as ACEIs, ARBs or TZDs.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

ZS completed the biochemistry and molecular biology experiments. DS, the recipient of the funded projects, lead the design of the experiment. ML completed the animal experiments. QY processed the experimental data. HL and YJ designed the

experiment and wrote the manuscript. ZS and ML confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the experimental procedures involving animals were conducted in accordance with the Institutional Animal Care Guidelines of Jilin University and approved by the Laboratory Animal Ethics Committee of School of Pharmaceutical Sciences, Jilin University (approval no. 20210010).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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