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Ginsenoside Re, Produces Multi-targeted Potent Antidiabetic Effects Via Estrogen Receptor Signaling Pathway in Rat, an Alternative Multi-Targeted Therapeutic for Type 2 Diabetes

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Authors' contributions

This work was carried out in collaboration among all authors. Author JG designed the experiments, investigation, methodology, biochemical and molecular biology data analysis and visualization of the experiment in Protocol 2, and writing the original draft. Author XM advised experimental design, conceptualization, manuscript review, and project administration. Author BZ biochemical data acquisition, molecular biology data analysis and visualization of the experiment in Protocol 1. Author YZ methodology, data curation, manuscript review, funding acquisition. Author SW carried out the animal experiment in Protocol 1 and data curation Author HM carried out the animal experiment in Protocol 2 and data curation. NH preparation of blood and tissues samples, and data statistical analysis. Author QS histological analysis and visualization, and manuscript review; author TJ data curation and data statistical analysis. Author CB advised experimental design, conceptualization, funding acquisition, project administration, manuscript review, writing and editing. All authors have read and agreed to the published version of the manuscript.

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ABSTRACT

Aims: To identify more effective ginsenoside for type 2 diabetes (T2D) and clarify whether the ginsenoside characterizing estrogenic multi-targeted antidiabetic effects.
Study Design: Identifying more effective ginsenoside through preclinical evaluation of antidiabetic effects of representative ginsenosides with T2D rat model, and further test pharmacological mechanism underlying the potent antidiabetic effects of the ginsenoside in the same model.
Place and Duration of Study: Key laboratory for Pharmacy, Inner Mongolia Medical University, March 2018 to November 2020.
Methodology: Used a total of 240 female adult rats. Rat model of T2D induced by high-fat diet fed and streptozotocin. Five tapes of representative ginsenosides (Rb1, Rd, Rg3, Re, Rg1) administrated at low (20 mg/kg daily) and high (40 mg/ kg daily) doses to T2D rats with orally for 4 weeks. Detect testing indexes with biochemical, histological, Quantitative Real-Time PCR, and western blots analysis.
Results: Ginsenoside Re (Re), very significantly lowered blood glucose (P<0.01), lipids (P<0.001), free fatty acid (P<0.001), and glucagon (P<0.01) levels, markedly improved impaired insulin sensitivity (P<0.01), ameliorated oxidative stress (P<0.01) and inflammation (P<0.01) in T2D rats, exhibited potent antidiabetic effects. Moreover, Re, phosphorylate serine/threonine kinase (Akt) (P<0.01) and endothelial nitric oxide synthase (eNOS) (P<0.01), up regulates B-cell lymphoma-2 (P<0.01) and insulin gene expression (P<0.01), down regulates glucagon gene expression(P<0.01), reverse impaired glucagon-like peptide 1 (P<0.01); exhibits multi-targeted effects; these effects of Re were inhibited by estrogen receptor (ER) inhibitor (ICI-182,780) (P<0.01). Functionally, the antidiabetic effects of Re were sequentially inhibited by inhibitor of ER, Akt, and eNOS, respectively (P<0.01).
Conclusion: These findings, revealed a novel pharmacological property of Re that characterized in multi-targeted potent antidiabetic effects mediated by ER/Akt/eNOS/NO signaling pathway, provide the first evidence for the potential use of Re, as a multi-targeted therapeutic for T2D, particularly, a novel candidate for replacement of estrogen therapy and NO therapy in diabetes.

Keywords: Ginsenoside Re; Type 2 diabetes; Multiple targeted antidiabetic effects; Estrogen receptor signaling pathway; Nitric oxide; An alternative multi-targeted therapy.

1. INTRODUCTION

Type 2 diabetes (T2D) is one of the leading causes of morbidity and mortality in world-wide. Pathogenesis of T2D is involved multifactor such as dysfunction of pancreatic beta cell (β-cell) as well as alpha cell (α-cell), dysregulated insulin as well as glucagon (Gcg), and insulin resistance (IR) [1,2,3], which induced by multiple pathological processes including oxidative stress, inflammation, glucose and lipids toxicity, and apoptosis [4,5]. The multifactorial pathogenesis of T2D means that treatment needs to involve multiple targeted therapies characterized in more effective and less adverse effects [6,7]. However, developing multi-targeted more effective therapeutic measures for the multifactorial T2D has been challenging [8,9,10].

Recently, numerous clinical and experimental studies have demonstrated that estrogen and 17β-estradiol (E2) characterize insulinotropic and multi-targeted antidiabetic effects that including anti-oxidative stress, anti-inflammation, anti-apoptosis, enhances β-cell survival and generation , increases pancreatic insulin content, gene expression and insulin release , reducing IR, glycemic and lipids control in various animal models and diabetes [11,12] by signaling through estrogen receptor (ER) alpha (ERα) /or ERβ [13,14]. It strongly suggests that estrogen and ER signaling pathway are potential multi-targeting therapeutic candidate for diabetes therapy [15,16], but it has serious adverse events [17]. Moreover, currently, available pharmacological agents for T2D, however, have many limitations including lack of multi-targeted

synergistic beneficial effects, and adverse effects [18,19].

Therefore, considering complementary and alternative approaches, including use of medicinal herbs that proposed synergistic multiple actions on diabetes [20,21]. In fact, ginseng is the best choice of herbal medicines that show promising result in the treatment of diabetes [22,23]. Interestingly, ginseng also characterize estrogenic and insulinotropic properties [21, 23]. The active components of ginseng are considered to ginsenosides, sixteen types of ginsenosides were found to have antidiabetic effects, such as ginsenoside Re, Rg1, Rg3, Rg5, Rb1, Rb2, Rb3, and compound K [24]. The molecular mechanisms underlying the antidiabetic effects and medical applications of ginsenosides have attracted much attention [24]. Multiple molecular targets including glucose transporters (GLUTs), glucagon-like peptide 1 (GLP-1), peroxisome proliferator-activated receptor gamma (PPAR-γ), phosphatidylinositol 3-kinase (PI3K)/threonine kinase (Akt) signaling, and AMP-activated protein kinase (AMPK) are involved in the antidiabetic effects of ginsenosides [24]. However, the potential systemic pharmacological mechanisms of ginsenosides are still unclear, which was major hindrance for use of ginseng or ginsenoside in diabetes therapy.

Estrogenic activities [25] and nitric oxide (NO) actions [26] have suggested as mechanisms of ginseng's actions, whether these actions involve in the antidiabetic effects of ginseng and ginsenosides have not been tested. Several studies indicated that antidiabetic effects of ginseng extract could be attributed to ginsenoside Re (Re) which the most representative and a major bioactive component in the ginseng [27, 28]. Our previous study reveals that Re, a main phytosterol of panax ginseng, activates cardiac potassium channels via a nongenomic pathway of sex hormones [29], and involve NO actions [30].

In the present study, therefore, we examined and compared the antidiabetic efficacies of Re to those of ginsenosides in ginseng, to test whether Re is more effective component of ginseng regarding its antidiabetic effects; more importantly, to test the possibility that whether molecular mechanism underlying the antidiabetic effects of Re mediated by ER signaling pathway. Re could mimics the multi-targeted antidiabetic effects of estrogen that can greatly facilitate developing multi-targeted new treatments for T2D from natural products.

2. MATERIALS AND METHODS

2.1 Reagents

Ginsenoside Re (CAS#PS000790), Rg1(CAS#PS010153), Rg3(CAS#PS010045), Rb1(CAS#PS010160), Rd (CAS#PS010161) were purchased from Chengdu Pusi Biotechnology Co., Ltd (Sichuan, China) and the Re, Rg1, Rg3, Rb1, Rd had a purity of 98%~99%. Streptozotocin (STZ) (CAS#S0130), L-N5 -(l-iminoethyl) ornithine (L-NIO) (CAS#150403-88-6), 17β-estradiol (E2) (CAS#50-28-2) and S-nitroso-N-acetylpenicillamine (SNAP) (CAS#67776-06-1) were purchased from Sigma-Aldrich (St Louis, MO, USA). Rosiglitazone (CAS#H20030569) bought from Chengdu Hengrui Pharmaceutical Co., Ltd (Sichuan, China). ICI182,780 (CAS#129453-61-8) was purchased from Tocris (Ellisville, MO, USA). MK-2206 (CAS#1032350-13-2) was purchased from Selleck Chemicals LLC (Houston, TX, USA). All other chemicals purchased from Sigma-Aldrich (St Louis, MO, USA) or Selleck Chemicals LLC (Houston, TX, USA) unless otherwise mentioned.

2.2 Experimental Animals

Sprague Dawley (SD) adult (12 weeks old) female rats（260-280g）were purchased from Beijing Viton Lihua Animal Center (Beijing, China; SCXK (jing) 2016-0006). Rats were individually housed in a temperature-controlled vivarium on a 12/12-h light/dark cycle and free access to water and either a normal chow diet or a high-fat diet (HFD). HFD contained 20% (w/w) sucrose, 10% (w/w) lard oil, 2.5% (w/w) cholesterol and 1.5% (w/w) bile salt were added to the normal diet (SCXK-Beijing, 2014-0010, Research Diets, Beijing, China).

2.3 Experimental Design

Rat experiments carried out using two different protocols (Protocols 1 and 2).

2.3.1 Protocol 1

Preparation of type 2 diabetes (T2D) model: The rats of the T2D model group were feded a HFD diet for 8 weeks and then received an intraperitoneal (IP) injection of Streptozotocin

(STZ) (40 mg/kg, dissolved in citrate buffer, pH 4.5; Sigma-Aldrich, USA), twice at an interval of 24 h. After 72 h, collection of blood from tail vein of rats after 12 h of fasting, fasting blood glucose (FBG) was measured with spectrophotometry-based assays using commercially available kits (Invitrogen, USA) at once a day for three consecutive days, each measurement of FBG level in all of rats is ≥16.7 mmol/L and insulin sensitivity index decreased (compared with normal control group rats) significantly (*P* value <0.05), considered as model established. One hundred and twenty (120) rats in the HFD group fulfilled this T2D criterion. Ten rats of the normal control group (NC) were fed standard rat chow and received an IP injection of the same volume of citrate buffer. The T2D rats that induced by HFD and STZ were subsequently apportioned randomly and equally to a T2D model control group (MC, administered saline, 4 ml/d by gavage) and eleven different treatment groups: administered ginsenoside Re, Rg1, Rg3, Rb1and Rd at low (20 mg/kg/day), high (40 mg/ kg/day) doses by gavage (qd) for 4 weeks, respectively, hereafter referred to as the Re, Rg1,Rg3,Rb1and Rd low (Re-l, Rg1-l,Rg3-l,Rb1-l, Rd-l) and high (Re-h, Rg1-h,Rg3-h,Rb1-h, Rd-h) groups; administered Rosiglitazone (30m/kg/day) to rats of positive control group (PC); each group has 10 rats, respectively.

2.3.2 Protocol 2

Preparation of T2D model is the same as described in Protocol 1. One hundred (100) T2D rats were subsequently apportioned randomly and equally to a MC (administered saline, 4 ml/d by gavage) and eight different treatment groups: administered ginsenoside Re 40 mg /kg/day (gavage, Re-h the same below) , Re-h + MK-2206 (240 mg/ kg/day, gavage), Re-h + ICI 182 , 780 (5mg/ kg/day , gavage), Re-h + SNAP (13.5 mg/kg/day, it), Re-h+ L-NIO (100 mg/kg, iv), E2 (100 ug/kg/day, sc) and Rosiglitazone (30mg/ kg/day) to rats of PC, respectively; rats in NC and PC were treated the same as in Protocol 1. Each group has 10 rats, respectively.

2.4 Preparation of Samples and Biochemical Analysis

Preparation of blood samples and biochemical analysis: At the end of the 4 weeks treatment, blood was collected after 12 h fast and serum samples are prepared. FBG, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and free fatty acids (FFAs) were measured with automatic analyzer (Hitachi 7080 Automatic Biochemical Analyzer, Tokyo, Japan); malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) using commercial kits (Beijing bioscience Biomedical Technology Co., Ltd., Beijing, China) and a spectrophotometer (Beijing PLANON New Technology Co., Ltd., China), in accordance with the manufacturer's protocols. Plasma glucagon, insulin, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α) and nuclear factor κB (NF-κB) were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Millipore, St. Charles, MO, USA). Insulin sensitivity index (ISI) was calculated according to the formula: insulin sensitivity index = 1/ [blood glucose (mmol/l) × blood insulin (pmol/l)].

Preparation of tissues samples and biochemical analysis: At the end of the 4 weeks treatment, rats were sacrificed after a 12 h fasting. The pancreas and intestine (ileum and colon) were dissected immediately and frozen at -20℃ until analysis. Tissue was homogenized on ice and centrifuged at 12000 rpm for 20 min, then the supernatants were collected and stored at -20℃ until analysis. Pancreatic Insulin and ileocolonic glucagon-like peptide 1 (GLP-1) concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (Millipore, St. Charles, MO, USA).

2.5 Oral Glucose Tolerance Test (OGTT) and Insulin tolerance Test (ITT)

In the Protocol 1 experiment, at the end of the three weeks, the rats (in the normal control group-NC, T2D model control group-MC, positive control group-PC treated with Rosiglitazone, treated with high dose of panaxadiols and panaxatriols groups, each group has five rats) were performed OGTT and ITT, respectively. In the OGTT experiment, the rats were fasted for 12 h and then orally administrated glucose 2g/kg. Blood glucose levels were measured from tail blood samples at 0 min (before glucose administration), 30 min, 60 min, and 120 min after glucose administration. Analyze the area under blood glucose curve (AUC). AUC was calculated according to the formula: AUC= [(0 min blood glucose + 2×30 min blood glucose + 3×60 min blood glucose +2 ×120 min blood glucose)]/4. In the ITT experiment, the rats were fasted for 10 h and then received intraperitoneal (IP) administration of insulin (0.75U/kg). Blood

glucose levels were measured from tail blood samples at 0 min (before glucose administration), 30 min, 60 min, and 120 min after insulin administration. Analyze the area under blood glucose curve (AUC). AUC was calculated according to the formula: AUC= [(0.25×0 min blood glucose + 0.5×30 min blood glucose + 0.5×60 min blood glucose +0.25×120 min blood glucose)]/4.

2.6 Histological Analysis and Morphometry

Pancreas tissue was fixed in 10% formalin for 1 month and then embedded with paraffin. Tissue sections (10 um) were cut with a microtome (Leica, Germany) and mounted on super frost /plus microscope slides. After being air-dried, they were stained with hematoxylin and eosin (HE), and photographed at 100 × magnification. At least two fields per slice and six slices per islet mass were analyzed for the purpose of quantifying tissue size.

2.7 Quantitative Real-Time PCR (qRT-PCR) for measurement of BcL-2, Bax, caspase3, insulin and glucagon Mrna

Total RNA (100 ng), extracted from the pancreas of rats (in NC, MC, Re-h, Re-h+ ICI 182, 780, and PC group, respectively) with different treatments using Trizol Reagent (Invitrogen, USA), was reverse transcribed to first-strand complementary DNA (GE Healthcare Bio-Sciences, Piscataway, NJ, USA). qRT-PCR was performed in a 25-μl final reaction volume with an iCycler iQ Detection System using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA). Sequences of the primers for measuring mRNA levels of Bcl-2 , Bax, caspase3, insulin, and glucagon were as follows: 5'-TTGCTACAGGGTTTCATCCAGG-3' [forward primer] and 5'-CACTCGCTCAGCTTCTTGGT-3' [reverse primer] for Bcl-2;

5'- TTGCTACAGGGTTTCATCCAGG -3' [forward primer] and
5'- CACTCGCTCAGCTTCTTGGT-3' [reverse primer] for Bax;
5'- GGAGCTTGGAACGCGAAGAA -3' [forward primer] and
5'- GTCCATCGACTTGCTTCCAT -3' [reverse primer] for Caspase3;
5'-GACCATCAGCAAGCAGGTCA-3'[forward primer] and
5'- CACCAGGTGAGGACCACAAA-3' [reverse primer] or
5'-TTCTCACTTGGTGGAAGCTCT-3' [forward primer] and
5'-GTTGTGCCACTTGTGGGTCC-3'[reverse primer] for insulin;
5'- GGAACCGGAACAACATTGCC-3'[forward primer] and
5'- CTTCCTCGGCCTTTCACCAG-3'[reverse primer] for glucagon;
5'-AATGTGTCCGTCGTCGTGATCT-3' [forward primer] and
5'-CATCGAAGGGGTGGAAGAGTGG-3' [reverse primer] for GAPDH; respectively.

2.8 Western Blots Analysis for Akt and eNOS Protein

Extraction of protein from pancreas of rats (in NC, MC, Re-l, Re-h, Re-h+ ICI 182780, Re-h+ MK-2206, and E2 treated group, respectively), and immunoblotting followed standard procedures. Briefly, those of 30 ug total protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred to nitrocellulose membranes. Then, the membranes were blocked and subjected to immunoblot analysis by incubation with the following antibodies. Using a 1:1000-diluted anti-phospho Akt (^{473}Ser) antibody (CAS#9271) (Cell Signaling, Danvers, MA, USA) or a 1:1000-diluted anti-Akt antibody (CAS#9272) (Cell Signaling) for detection of Akt in pancreas. Using a 1:1000-diluted anti-phospho eNOS antibody (CAS#S1177) (Cell Signaling, Danvers, MA) or a 1:1000-diluted anti-eNOS antibody (eNOS/NOS Type III, CAS#610296; BD Biosciences) for detection of eNOS in pancreas. Followed incubation with a 1:20,000-diluted horseradish peroxidase-conjugated anti-rabbit IgG (Dako Japan Co. Ltd., Kyoto, Japan). Beta-actin (anti-beta actin antibody, CAS# sc1616; Santa Cruz, CA, USA) was detected for verification of protein loading. Proteins were detected using an advanced enhanced chemiluminescence system (GE Healthcare, Buckinghamshire, UK) and quantified by densitometry using IPP, version 6.0, software (Media Cybernetics, Silver Spring, MD, USA). The reagents for electrophoresis were obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA).

2.9 Statistical Analysis

Origin Pro 8.5 analysis software was used for Statistical analysis. All data are expressed as

mean ± SD. The differences between two groups were analyzed by Student's t test. More than two conditions were analyzed by repeated-measures analysis of variance (rANOVA). Differences were considered statistically significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of Panaxadiols and Panaxatriols Ginsenosides on Glucose Metabolism in Type 2 Diabetes Rats

High-fat diet (HFD) feeding and injection of Streptozotocin (STZ) very significantly increased fasting blood glucose (FBG), fasting blood insulin (FINS), glycosylated hemoglobin (HbA1c) and glucagon (Gcg) levels, and very significantly decreased insulin sensitivity index (ISI), in the type 2 diabetes (T2D) model control group rats (MC) that compared with normal control (NC) (Fig. 1B-E). This indicated that glucose metabolic disorder established in the T2D rats.

After treated T2D rats with low and high dose of panaxadiols (Rb1, Rd, Rg3) and panaxatriols (Re, Rg1) in ginseng (Fig. 1A), for 4 weeks, respectively. Compared panaxadiols and panaxatriols treated 2TD rats with those of the non-treated MC , the differences in FBG, FINS, ISI, HbA1c, and Gcg level are no significant change in Rd-l and Rd-h treated 2TD group rats (P>0.05); significant decrease of FBG and FINS observed in Rb1-l, Rb1-h, Rg3-l, Rg3-h, and Rg1-l treated (*P*<0.05); significant increase of ISI observed in Rb1-l, Rb1-h, Rg3-l, Rg3-h ,Rg1-l and Rg1-h treated (*P*<0.05); significant decrease of HbA1c observed in Rb1-l, Rg3-l, and Rg1-l treated (*P*<0.05); significant decrease of Gcg observed in the Re-l treated only (*P*<0.05); more significant decrease of FBG and FINS observed in the Rg1-h, Re-l and Re-h treated (*P*<0.01); more significant increase of ISI observed only in the Re-h treated (*P*<0.01); more significant decrease of HbA1c observed in the Rb1-h, Rg3-h, Rg1-h and Re-l treated (*P*<0.01); more significant decrease of Gcg observed in the Re-h treated only (*P*<0.01); The most significant decrease of FBG and HbA1c observed in the Re-h treated only (*P*<0.001); compared Re-l treated rats with those of rats treated by other ginsenosides at low dose, respectively; the differences in FBG, ISI, HbA1c, and Gcg level are significant (*P*<0.05) or very significant (*P*<0.01); similar significant (*P*<0.05) or very significant (*P*<0.01) differences are observed in compared Re-h treated rats with those of rats treated by other ginsenosides at high dose, respectively; compared between Re-l and Re-h treated group rats , the differences in FBG, ISI, HbA1c, and Gcg level are significant (*P*<0.05) ; as shown in Fig1B-F. In sum of above, the intensity of the regulative actions induced by panaxadiols and panaxatriols ginsenosides on the glucose metabolic disorder are: Re-h>Re-l>Rg1-h>Rg1-l>Rg3-h>Rg3-l>Rb1-h. This suggesting that the panaxatriols (Re and Rg1), especially Re elicit potent regulative effect on glucose metabolic disorder. Rosiglitazone showed similar regulation to that of Re (Fig. 1B-E).

3.2 Effects of Panaxadiols and Panaxatriols Ginsenosides on Glucose Tolerance and Insulin Tolerance in 2TD Rats

In the OGTT, compared with NC rats, the rats in MC have very significant higher level of FBG (*P*<0.01) at the all point of test tiame and very significant increase of AUC (*P*<0.01); compared panaxadiols and panaxatriols treated 2TD rats with those of the non-treated MC , the difference in FBG level and AUC are no significant change in Rd-h treated 2TD group rats (P>0.05); significant decrease of FBG observed in Rb1-h, Rg3-h, and Rg1-h treated (*P*<0.05); more significant decrease of FBG and AUC observed in the Re-h treated (*P*<0.01), more significant decrease of AUC also observed in the Rg1-h treated (*P*<0.01); rosiglitazone showed similar effect of Re; at the all point of test time, respectively; compared Re-h treated rats with those of rats treated by other ginsenosides at high dose, respectively; the differences in FBG level and AUC are significant (*P*<0.05) ; as shown in Fig. 2A-B. In the ITT, after administration of insulin for 30min, FBG levels were decreased in the all groups of rats. Compared with NC rats, the rats in the MC still have very significant higher level of FBG (*P*<0.01) at the all point of test time and very significant increase of AUC (*P*<0.001); compared with MC rats, the FBG level and AUC, are not significantly changed in the Rd-h treated 2TD group rats (P>0.05); significantly decreased in the Rb1-h, Rg3-h, and Rg1-h treated (*P*<0.05), respectively; more significantly decreased in the Rd-h and rosiglitazone treated (P>0.05); compared Re-h treated rats with those of rats treated by other ginsenosides at high dose, respectively; the differences in FBG level and

AUC are significant (P<0.05) ; as shown in Fig.e 2C-D.

3.3 Effects of Panaxadiols and Panaxatriols Ginsenosides on Lipid Metabolism in 2TD Rats

In the above experiments, compared with the NC group rats, the rats in the MC group had very significantly higher mean serum levels of total cholesterol (TC) (P<0.01), triglyceride (TG) (P<0.001), low-density lipoprotein cholesterol (LDL-C) (P<0.001), and free fatty acid (FFA) (P<0.001), as shown in Figure 3A-D; and had very significantly lower mean serum levels of high-density lipoprotein cholesterol (HDL-C) (data not shown). This indicated that lipid metabolic disorder established in the 2TD model rats. The model rats treated by the panaxadiols and panaxatriols for 4 weeks, respectively; compared panaxadiols and panaxatriols ginsenosides treated rats with those of non-treated MC group rats, respectively; the mean serum levels of TC were no significant change in the Rd-l treated group rats, significant decrease in Rd-h, Rb1-l, Rb1-h, Rg1-l, and Rg1-h treated group rats (P<0.05); more significant decrease in the Rg3-h, Re-l and Re-h treated (P<0.01); the

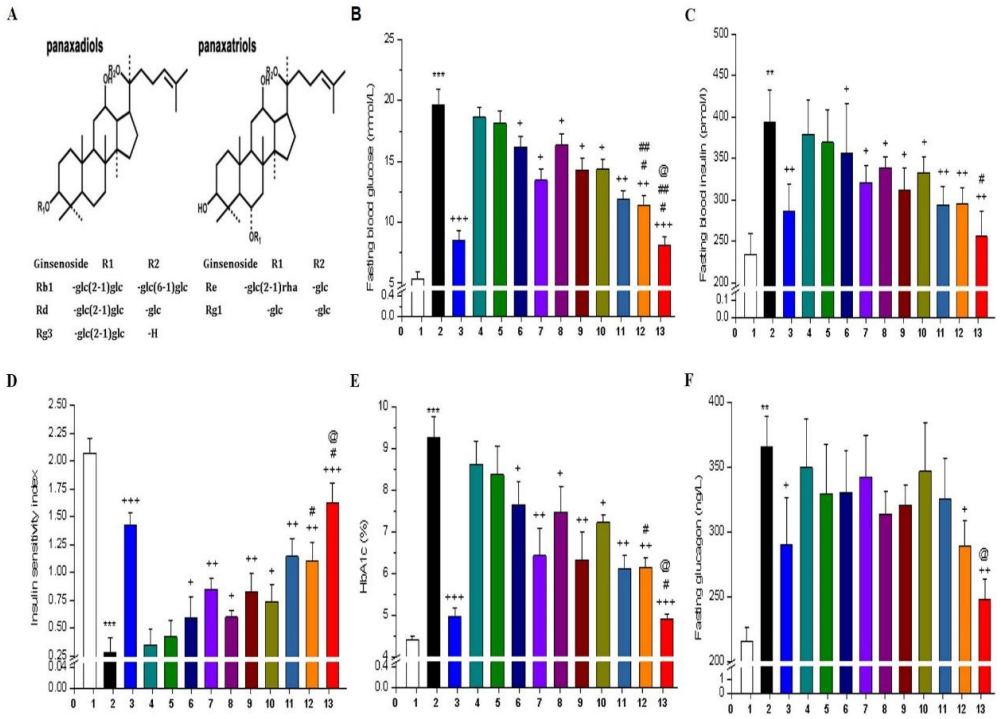

Fig. 1. Effects of panaxadiols and panaxatriols on FBG, FINS, ISI, serum HbA1c and glucagon in type 2 diabetes rats. A. Chemical structures of panaxadiols and panaxatriols derivatives of ginseng used. B. Fasting blood glucose (FBG) levels, C. Fasting blood insulin (FINS) levels, D. Insulin sensitivity index (ISI), E. Serum glycosylated hemoglobin (HbA1c), and F. Fasting blood glucagon levels, in the rats of normal control group (NC), type 2 diabetes (T2D) model control group (MC), 2TD treated by Rosiglitazone (positive control group, PC), and treated by low and high dose of panaxadiols and panaxatriols, respectively. In the B, C, D, F, and E, the 1is NC group, 2 is MC, 3 is PC, 4 is low dose of ginsenoside Rd (Rd-l) treated, 5 is high dose of Rd (Rd-h) treated, 6 is low dose of Rb1(Rb1-l) treated ,7 is high dose of Rb1(Rb1-h) treated, 8 is low dose of Rg3 (Rg3-l) treated, 9 is high dose of Rg3 (Rg3-h) treated,10 is low dose of Rg1 (Rg1-l) treated,11is high dose of Rg1(Rg1-h) treated, 12 is low dose of Re (Re-l) treated and 13 is high dose of Re-h (Re-h) treated group, respectively. Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001; compared with MC, ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001; Re-l compared with Rd-l, Rb1-l, Rg3-l and Rg1-l, Re-h compared with Rd-h, Rb1-h, Rg3-h and Rg1-h, respectively, $^{\#}P$ <0.05, $^{\#\#}P$ <0.01; compared with Re-l, $^{@}P$ <0.05. Each group has 10 rats in the 13 groups (N=10).

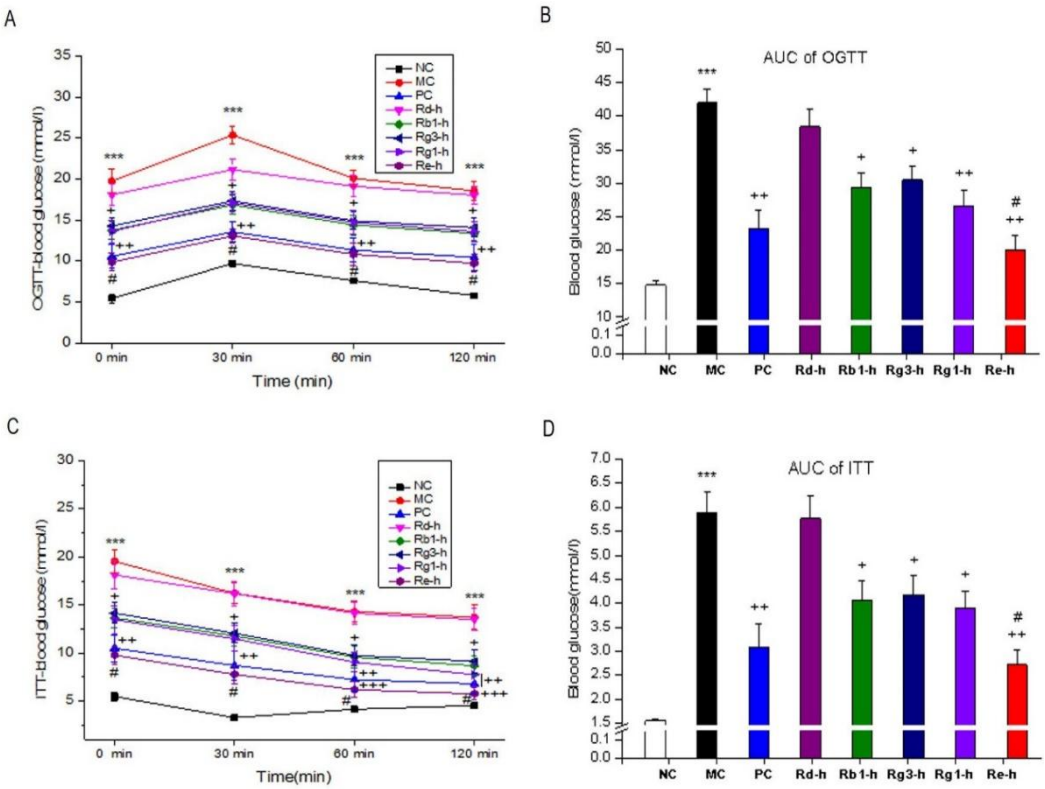

Fig. 2. Effects of panaxadiols and panaxatriols on glucose tolerance and insulin tolerance in type 2 diabetes rats. A. Fasting blood glucose (FBG) levels in the rats of normal control group (NC), type 2 diabetes (T2D) model control group (MC), 2TD treated by Rosiglitazone (positive control group, PC), and treated by high dose of panaxadiols (Rd-h, Rb1-h, Rg3-h) and panaxatriols (Rg1-h, Re-h) at 0 min (before glucose administration), 30 min, 60 min, and 120 min after glucose administration, respectively. B. Calculated area under blood glucose curve (AUC) in the NC, MC, and PC, Rd-h, Rb1-h, Rg3-h, Rg1-h, Re-h treated group rats, respectively. C. FBG levels in the NC, MC, and PC, Rd-h, Rb1-h, Rg3-h, Rg1-h, Re-h treated group rats, at 0 min (before insulin administration), 30 min, 60 min, and 120 min after insulin administration, respectively. D. Calculated AUC in the NC, MC, and PC, Rd-h, Rb1-h, Rg3-h, Rg1-h, Re-h treated group rats, respectively. Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001; compared with MC, ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001; Re-h compared with Rd-h, Rb1-h, Rg3-h and Rg1-h, respectively, $^{\#}P$ <0.05. Each group has 5 rats (N=5).

most significant decrease in the Rg3-l treated group rats (*P*<0.001); as shown Fig. 3A. The intensity of the lowering blood TC by various ginsenosides is: Rg3-l > Re-l> Rg3-h > Re-h> Rb1-l > Rb1-h > Rg1-h> Rd-h>Rg1-l>Rd-l. Rosiglitazone showed similar regulation to that of Rb1-l (Fig. 3A). The mean serum levels of TG were no significant change in the Rd-l and Rd-h treated group rats, significant decrease in Rg1-l and Rg1-h treated (*P*<0.05), more significant decrease in the Rb1-l and Rb1-h treated (*P*<0.01), the most significant decrease in the Rg3-l, Rg3-h, Re-l, and Re-h treated group rats (*P*<0.001); as shown in Fig. 3B. The intensity of the lowering blood TG by various ginsenosides is: Rg3-l > Re-h >Rg3-h > Re-l> Rb1-h > Rb1-l > Rg1-h> Rg1-l. Rosiglitazone showed similar regulation efficacy to that of Rb1-h (Fig. 3B). The mean serum levels of LDL-C that were no significant change in the Rd-l and Rd-h treated group rats (*P*>0.05), significant decrease in Rb1-l, Rb1-h, Rg3-l, and Rg3-h treated(*P*<0.05), more significant decrease in the Rg1-l, Re-l and Re-h treated(*P*<0.01), the most significant decrease in the Rg1-h treated group rats (*P*<0.001); as shown in Fig. 3C. The intensity of the lowering blood LDL-C by various ginsenosides is: Rg1-h > Re-l > Rg1-l > Re-h > Rg3-h >Rg3-l >Rb1-h >Rb1-l. Rosiglitazone showed similar regulation efficacy to that of Re-l (Fig. 3C).The mean serum

levels of FFA that were no significant change in the Rd-l and Rd-h treated group rats (P>0.05), significant decrease in Rb1-l, Rb1-h, Rg3-l, and Rg3-h treated (P<0.05), more significant decrease in the Rg1-l, Rg-h and Re-l treated (P<0.01), the most significant decrease in the Re-h treated group rats (P<0.001); as shown in Fig. 3D. The intensity of the decreasing serum FFA level by various ginsenosides is: Re-h > Re-l > Rg1-h > Rg1-l > Rg3-h >Rg3-l >Rb1-h >Rb1-l. Rosiglitazone showed similar regulation efficacy to that of Rg1-l (Fig. 3D). The various ginsenosides had significant increasing effects on the serum HDL-C, high dose of them showed more obvious effects except the Re-1also has obvious effects (data not shown). In sum of above, data suggesting the Rg3 and Re has potent regulative effects on lipid metabolic disorder.

3.4 Effects of Panaxadiols and Panaxatriols Ginsenosides on Oxidative Stress and Inflammation in 2TD Rats

The same the above experiments, the effects of various ginsenosides on serum superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) levels showed as in Fig. 4A-C; The intensity of anti-oxidative stress by various ginsenosides is: Re-h> Rg1-h >Re-l> Rb1-h > Rg3-h> Rg1-l >Rd-h> Rb1-l >Rg3-l> Rd-l. As showed in the Fig.e 4D-F, the Re more significantly decreased serum tumor necrosis factor alpha (TNF-α), nuclear factor κB (NF-κB) and *interleukin 6 (*IL-6) levels (*P*<0.01) compared with those of other ginsenosides tested (*P*<0.05); especially, very significant decrease in serum IL-6 observed only in the Re-l and Re-h treated rats (*P*<0.01). These suggesting that the Re has potent anti-oxidative stress and anti-inflammation in 2TD rats.

3.5 Effects of Ginsenoside Re on Bcl-2, Bax and Caspase3 mRNA Expression and Histological Change in pancreas of 2td Rats

As showed in the Fig. 5A-D, compared with the NC rats, the rats in MC had very significant lower level of B-cell lymphoma-2 (Bcl-2) mRNA expression (P<0.01), very significant higher level of B-cell lymphoma-2 associated X protein (Bax) (P<0.01) and caspase3 mRNA (P<0.01) expression in pancreas. Compared with the MC, Re very significantly up regulates Bcl-2 mRNA (P<0.01), down regulates Bax (P<0.01) and Caspase3 mRNA (P<0.05 for Re-l, P<0.01 for Re-h) expression in the pancreas of 2TD rats, exhibit potent anti-apoptosis effects. The histological analysis and morphometry data were shown in Fig. 5E-I, the pancreas of NC rats are mostly oval or round cell clusters, with different sizes, regular structures, and a large number. The islet boundaries are clear, the cells are abundant in the islets, the size is regular, full, and the arrangement is regular and compact. Compared with NC, the pancreas of MC rats is atrophy, the number of islets is significantly reduced, the distribution of islets is loose, sparse, and irregular, the structure of the islets is disordered, the boundary is unclear, the cells in the islets are deformed, swollen, and the cytoplasm is vacuolated or shallow, which can be seen Apoptotic or dead cells with missing or pyknotic nuclei. Compared with MC rats, the islet damage of the rats treated with Re and Rosiglitazone were significantly repaired, the islets and the cells in the islets increased significantly, the structure of the islets was more regular and the atrophy was significantly improved, the islet cells were arranged more uniformly, and the morphology was more regular. The arrangement is relatively neat, swelling, vacuolating cells, and apoptotic and dead cells are reduced; the effect of large doses of Re is better than that of small doses.

Altogether, these data suggesting that Re produces potent antidiabetic effects in T2D rats. To understand the molecular mechanisms by which Re produce potent antidiabetic effects, we employed following experiments.

3.6 Ginsenoside Re Activates Akt via ER Signaling Pathway in T2D rats

Estrogenic activities have suggested as a mechanism of ginseng's actions, and Re was one of the major component that possess estrogenic activities in ginseng [25,31]. Moreover, our previous report demonstrated that Re activates cardiac potassium channels via a nongenomic pathway of sex hormones (including estrogen) [29]. Importantly, sex hormones and sex hormone receptor, particularly, estrogen and estrogen receptor (ER), play an important role in the pathogenesis and remission of T2D [16]. These findings encouraged us to hypothesis that whether Re produce potent antidiabetic effects via ER signaling pathway. To test this hypothesis, we, first, proceeded western blot analysis to determine whether Re phosphorylate

threonine kinase (Akt) that was a critical node cascade in the classical sex hormone receptors signaling pathway, and test the effects of inhibitors of ER and Akt. Results of western blot analysis were shown as in Fig. 6A-B, compared with MC, Re-l treated significantly phosphorylate Akt (P<0.05), Re-h treatment more significantly phosphorylated Akt (P<0.01) in T2D rats; ICI-182,780, an inhibitor of ER, and MK-2206, a Akt inhibitor, inhibited Re-h induced Akt phosphorylation (P<0.01). These data indicate that Re activates Akt via ER signaling pathway. Second, we tested whether potent antidiabetic effects of Re mediated by ER/Akt signaling pathway. We analyzed the effects of ICI-182,780 and MK-2206 on the hypoglycemic and improving ISI actions of Re. As shown in the Fig.e 6C-D, ICI-182,780 and MK-2206 abolished Re-induced hypoglycemic and improving ISI effects (P<0.01). This functional analysis suggesting that the antidiabetic effects of Re mediated by ER/Akt signaling pathway.

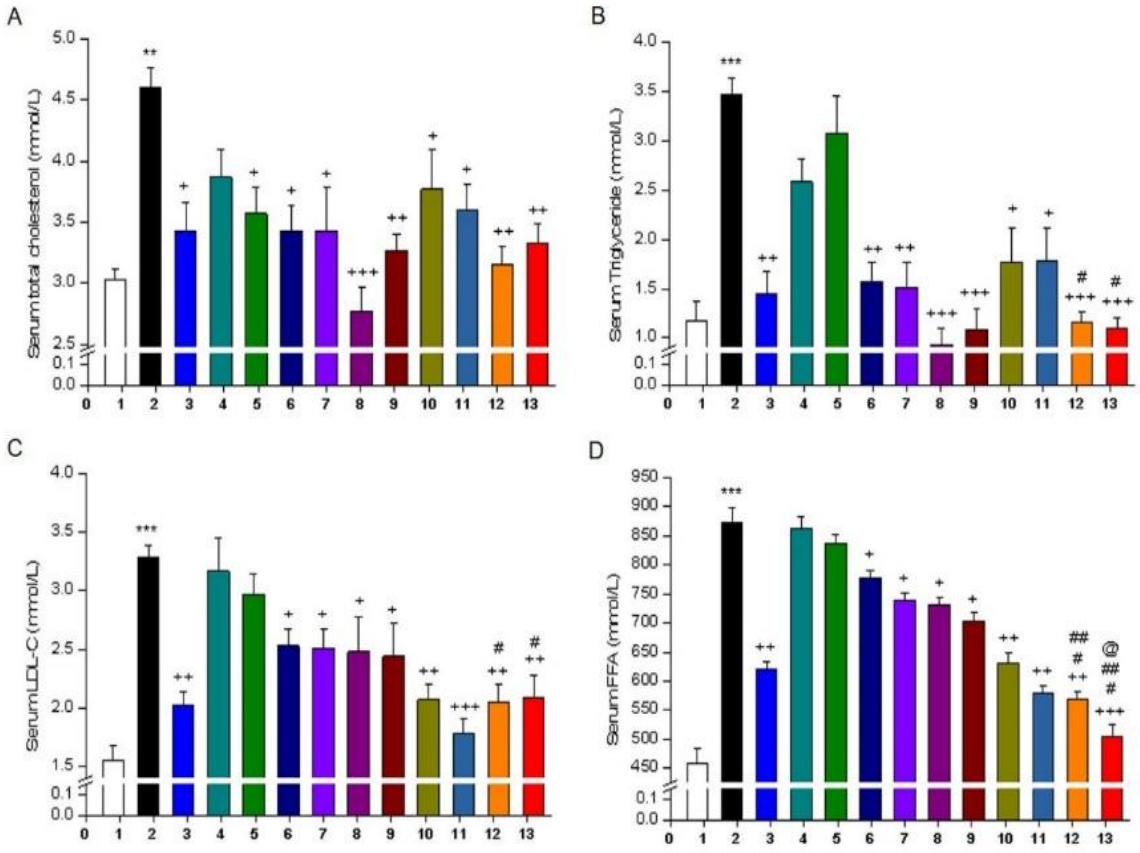

Fig. 3. Effects of panaxadiols and panaxatriols on serum TC, TG, LDL-c and FFA in type 2 diabetes rats. A. Serum levels of TC, B. Serum levels of TG, C. Serum levels of LDL-C, and D. Serum concentrations of FFA, in the rats of normal control group (NC), type 2 diabetes (T2D) model control group (MC), 2TD treated by Rosiglitazone (positive control group, PC), and treated by low and high dose of panaxadiols and panaxatriols, respectively. In the A, B, C, and D, the 1 is NC, 2 is MC, 3 is PC, 4 is Rd-l treated, 5 is Rd-h treated, 6 is Rb1-l treated, 7 is Rb1-h treated, 8 is Rg3-l treated, 9 is Rg3-h treated,10 is Rg1-l treated,11is Rg1-h treated, 12 is Re-l treated and 13 is Re-h treated group, respectively. Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001; compared with MC, ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001; Re-l compared with Rd-l, Rb1-l, Rg3-l and Rg1-l, Re-h compared with Rd-h, Rb1-h, Rg3-h and Rg1-h, respectively, $^{\#}P$ <0.05. Each group has 10 rats in the 13 groups (N=10).

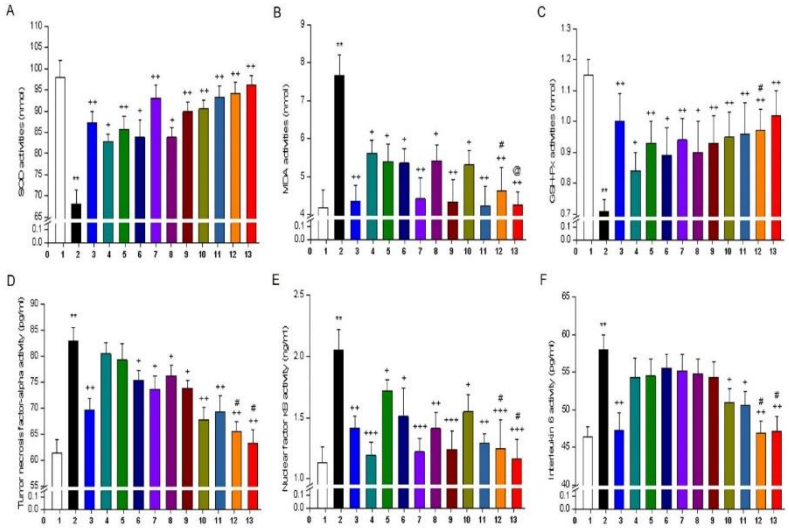

Fig. 4. Effects of panaxadiols and panaxatriols on serum SOD, MDA, GSH-Px, TNF-α, NF-κB and IL-6 levels in type 2 diabetes rats. A. Serum SOD activities, B. Serum MDA levels, C. Serum GSH-Px activities, D. Serum TNF-α levels, E. Serum NF-κB levels, and F. Serum IL-6 levels, in the rats of normal control group (NC), type 2 diabetes (T2D) model control group (MC), 2TD treated by Rosiglitazone (positive control group, PC), and treated by low and high dose of panaxadiols and panaxatriols, respectively. In the A, B, C, D, E, and F, the 1 is NC, 2 is MC, 3 is PC, 4 is Rd-l treated, 5 is Rd-h treated, 6 is Rb1-l treated ,7 is Rb1-h treated,8 is Rg3-l treated, 9 is Rg3-h treated,10 is Rg1-l treated,11is Rg1-h treated, 12 is Re-l treated and 13 is Re-h treated group, respectively. Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01; compared with MC, ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001; Re-l compared with Rd-l, Rb1-l, Rg3-l and Rg1-l, Re-h compared with Rd-h, Rb1-h, Rg3-h and Rg1-h, respectively, $^{\#}P$ <0.05; compared with Re-l, $^{@}P$ <0.05. SOD: superoxide dismutase; MDA: Malondialdehyde; GSH-Px: glutathione peroxidase; TNF-α: tumor necrosis factor alpha; IL-6: Interleukin 6. Each group has 10 rats in the 13 groups (N=10).

3.7 Ginsenoside Re Activates Endothelial Nitric Oxide Synthase via ER/Akt Signaling and the Antidiabetic Effects of Ginsenoside Re Involve Nitric Oxide Actions

It has been suggested that nitric oxide (NO) action as a mechanism of ginseng's actions [26]. Moreover, our previous report demonstrated that Re activates endothelial nitric oxide synthase (eNOS) through sex hormone receptor including estrogen receptor alpha (ERα) and androgen receptor (AR) signaling, Re-induced delayed rectifier K^{+} current enhancement and L-type Ca^{2+} current suppression involved NO (release from eNOS) actions in guinea-pig cardiomyocytes [29,30]. Importantly, results from present study strongly suggested that eNOS might be a main downstream signal cascade in the ER/Akt signaling pathway. We, therefore, reasoned that NO derived from eNOS should be an important effector in the ER/Akt signaling pathway and the antidiabetic effects of Re involve the NO actions.

To test the propose, first, we employed western blot analysis to determine whether Re activates eNOS via Akt signaling that a critical node in the downstream of the ERα signaling pathway and upstream of the eNOS activation. The results of western blot analysis shown as in the Fig. 7A, compared with NC rats, the rats in MC had very significant lower phosphorylated eNOS proteins (P<0.01) in the pancreas of T2D rats, Re-treated very significantly increase the impaired phosphorylated eNOS protein levels (P<0.01), MK-2206 (a Akt inhibitor) treated completely inhibited Re-induced eNOS phosphorylation (P>0.05). Consistently, these data led us to conclude that Re activate eNOS through Akt signaling. Second, we examined whether the antidiabetic effects of Re involve the NO actions. We use eNOS inhibitor (L-N5 -(l-iminoethyl) ornithine, L-NIO) and NO donor (S-nitroso-N-acetylpenicillamine, SNAP) to determine NO involved in the antidiabetic effects of Re. As shown in Fig. 7B-D, L-NIO treated very significantly inhibited Re-induced actions of lowering FBG, decreasing serum HbA1c and

improving insulin sensitivity (P<0.01); Re plus SNAP treated that compared with Re-treated alone, did not significant changed in Re-induced lowering FBG, serum HbA1c and increasing insulin sensitivity (P>0.05); whereas, SNAP treated alone that compared with MC very significantly decreased FBG, serum HbA1c and increased insulin sensitivity (P<0.01). These data suggested that NO (release from eNOS) actions involved in the antidiabetic effects of Re and NO acts as a main effector in the ER/Akt/eNOS signaling pathway that mediated the antidiabetic effects of Re. However, it should be noted that L-NIO treatment did not completely revers Re-induced lowering FBG, serum HbA1c and increasing insulin sensitivity; there are still significant difference between MC and Re plus L-NIO treated in the FBG, serum HbA1c and insulin sensitivity subjects (P<0.05); this data suggesting that might be alternative effectors or signals molecules are also participated in the antidiabetic effects of Re.

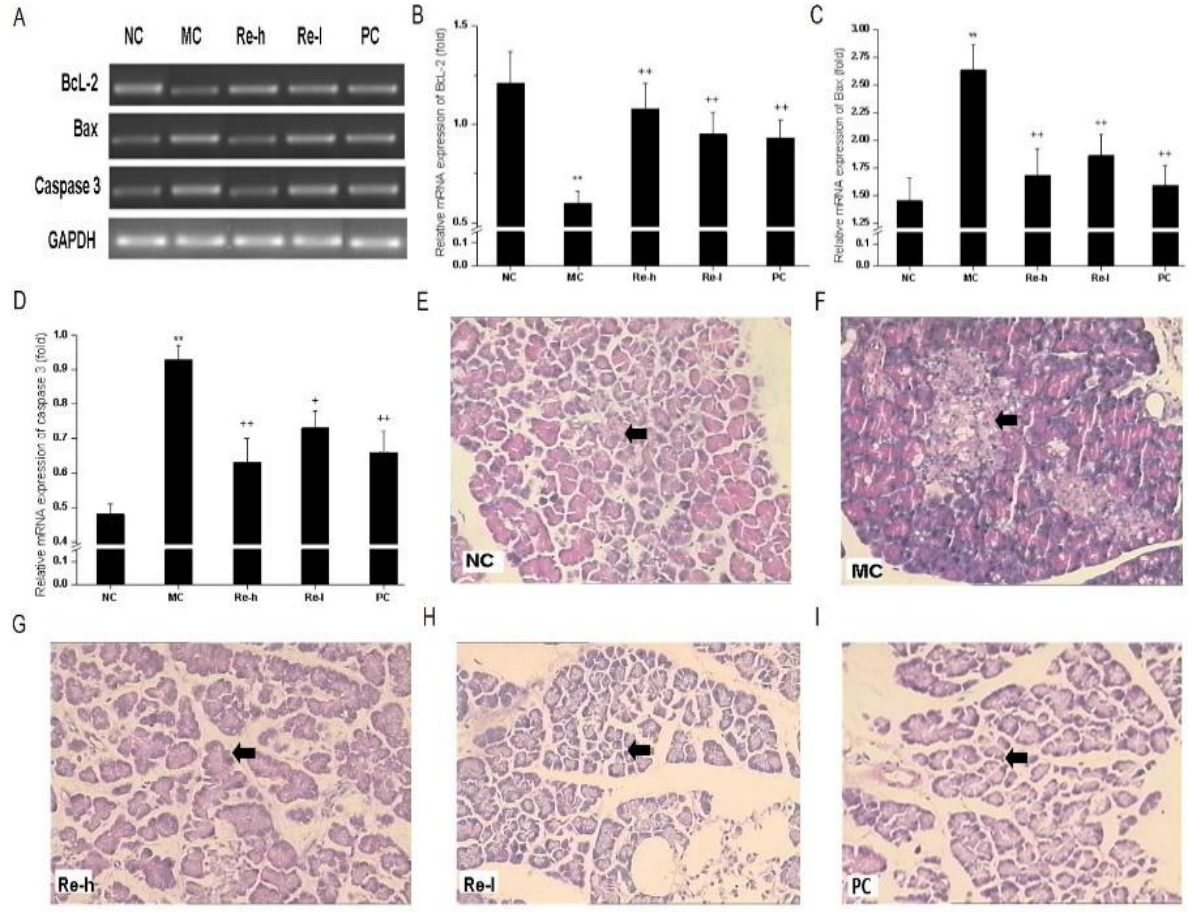

Fig. 5. Effects of Re on Bcl-2, Bax and Caspase3 mRNA expression and histological change in pancreas of type 2 diabetes rats. A. Representative northern blot of Bcl-2, Bax and Caspase3 mRNA expression of pancreas in the rats of normal control group (NC), type 2 diabetes (2TD) model control group (MC), type 2 diabetes rats treated by Rosiglitazone (positive control group, PC), and Re low (Re-l) and Re high dose (Re-h) treated group, respectively. B. Calculated Bcl-2 mRNA expression levels, C. Calculated Bax mRNA expression levels, and D. Caspase3 mRNA expression levels, in the NC, MC, PC, and Re-l, Re-h treated group, respectively (N=3). The E, F, G, H, and I, presents hematoxylin and eosin (HE) staining of pancreas of rats in the NC (E), MC (F), Re-l (G), Re-h(H), and PC(I) group, respectively. Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01; compared with MC, ^{+}P <0.05, ^{++}P <0.01; Compared with Re-l, $^{@}P$ <0.05.

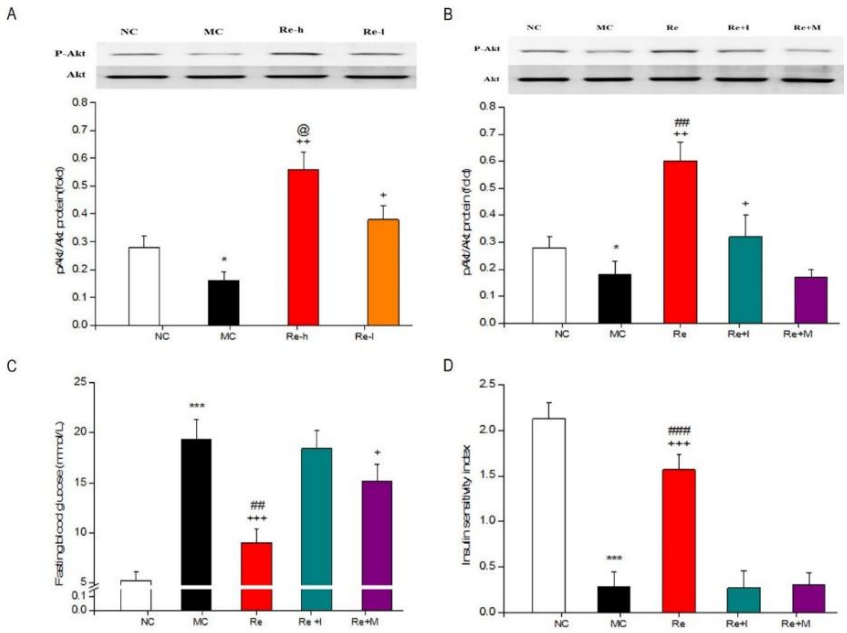

Fig. 6. Re induces Akt phosphorylation and effects of ICI182,780 and MK-2206. A. Representative western blots and densitometric analysis of Akt phosphorylation by Re (N=3). B. Effects of ICI182,780 and MK-2206 on Akt phosphorylation by Re (N=3). C. Effects of ICI182,780 and MK-2206 on lowering FBG by Re (N=10). D. Effects of ICI182,780 and MK-2206 on improving insulin sensitivity by Re (N=10). NC: normal control group; MC: type 2 diabetes (T2D) model control group; Re-l: T2D rats treated by low dose of Re group; Re-h: treated by high dose of Re; Re+I: treated by Re-h plus ICI182,780; Re+M: treated by Re-h plus MK-2206. Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001; compared with MC, ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001; compared with Re-h plus various inhibitors, respectively, $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001; compared with Re-l, $^{@}P$ <0.05.

3.8 Regulative Effects of Ginsenoside Re on Insulin and Glucagon in T2D Rats

Interestingly, it has been demonstrated that estrogen and ER signaling pathway regulate devers targets molecules and signaling pathway including insulin ang glucagon-like peptide 1 (GLP-1) that paly important roles in the development of T2D and insulin resistence [16]. These findings make us to speculate that whether Re could mimic the regulative effects of estrogen on insulin and GLP-1. To examine the speculations, we, first, proceeded Quantitative Real-Time PCR analysis to determine regulative effects of Re on insulin and glucagon (Gcg) mRNA expression in pancreas and measured pancreatic insulin content (PINS). As shown in Fig. 8A-C, compared with NC rats, the rats in the MC had very significant lower insulin mRNA expression and PINS (*P*<0.001), and very higher Gcg mRNA expression (*P*<0.001); indicating that not only insulin gene expression, but also Gcg gene expression were dysregulated in pancreas of T2D rats. Compared with MC, Re-h treated very significantly reverse the impaired insulin mRNA expression and decreased PINS (*P*<0.01) as well as the increased Gcg mRNA expression (*P*<0.01). ICI-182,780 treated very significantly inhibited Re-induced enhancement of insulin mRNA and PINS (*P*<0.01) as well as suppression of Gcg mRNA (*P*<0.01). These results indicate that Re normalize dysregulated insulin and Gcg via ER signaling in pancreas. Second, we analyzed GLP-1content in ileum and colon of T2D rats and observed effects of Re on GLP-1. Compared with NC, MC has very significant lower GLP-1 concentrations (*P*<0.01); while treated with Re-h and E2, respectively, compared with MC, Re very significantly increased GLP-1 concentrations (*P*<0.01), more ovious increasement observed in E2 treated (*P*<0.001); plus ICI-182,780 in the Re treatment, very significantly decreased Re-induced enhancement of GLP-1 concentrations (*P*<0.01) (Fig..8D). These results indicate that Re reverse impaired GLP-1 through ER signaling.

Overall, these data led us to speculate that not only NO (release from eNOS) acts as a main effector, but also alternative effectors or signal molecules such as insulin, Gcg, and GLP-1 participated in ER signaling pathway that mediated potent antidiabetic effects of Re.

4. DISCUSSION

In the present study, our data indicate that Re produces potent antidiabetic effects that characterized in integrated multi-targeted actions, integrating of the multi-targeted actions of Re mediated by ER signaling pathway. These findings revealed a novel molecular mechanism that activation of ER/Akt/eNOS/NO signaling pathway by Re was mainly responsible for the multi-targeted potent antidiabetic effects of Re on T2D (Fig. 8E).

4.1 Ginsenoside Re Produces Potent Antidiabetic Effects

More than 40 different ginsenosides that have been identified and isolated from the root of *Panax ginseng* were generally divided into two major groups panaxadiols (e. g. Rb1, Rb2, Rc, Rd, Rg3, Rh2, Rh3) and panaxatriols (e. g. Re, Rf, Rg1, Rg2, Rh1) based on their chemical structure [32]. In the present study, we firstly evaluated antidiabetic efficacies of five tapes of representative panaxadiols (Rb1, Rd, Rg3) and panaxatriols (Re, Rg1) on T2D in the same experimental system, and the Re was identified more effective component. The five tapes of ginsenosides that are quantitatively the most important derivatives among the all ginsenosides in the ginseng root, have the higher absolute bioavailability in the oral administration of ginseng, as well as the commonly studied ginsenosides derivatives regarding metabolic syndromes including diabetes [24,33]. We evaluated the effects of five tapes of ginsenosides on 2TD with examine the main indexes including FBG, ISI, HbA1c, TC, TG, LDL-C, FFA, SOD, MDA, GSH-Px, TNF-α, NF-κB and IL-6 could reflect the main aspects of pathogenesis and therapeutic targets for T2D [1,4,5,6]. Results of comprehensive analysis from the main indexes indicated that the Re has the potent antidiabetic effects that characterized in the most significantly lowering blood glucose and glucagon, improving insulin sensitivity, anti-inflammation, and anti-oxidative stress, compared with the others tested (Figs. 1-4); whereas regulative actions of Rb1and Rg3 on the glucose metabolic disorder and inflammation were less powerful, compared with the Re , the powerful actions of Rb1and Rg3 characterized in the antihyperlipidemic efficacies (Fig. 3), it is supported by previous reports [34, 35, 36]. Rd, a main component of panaxadiols, has weaker efficacies on anti-hyperglycemia and anti-hyperlipidemia. Rg1, a main component of panaxatriols, exhibits various biological activities [37], characteristics of the actions are similar the actions of ginsenoside Re, but the potency of actions was weaker than that of ginsenoside Re (Fig. 1-4). Our study also found the common feature in the actions of panaxadiols and panaxatriols are significant anti-oxidative stress. Sum of above, we reasoned that Re was the most effective component for T2D among the panaxadiols and panaxatriols derivatives were tested.

4.2 The Potent Antidiabetic Effects of Ginsenoside Re Characterized by Multi-targeted Therapeutic Actions

Our data not only confirmed Re exhibiting pharmacological effects of anti-hyperglycemia, anti-hyperlipidemia and anti-oxidative stress on metabolic syndrome including diabetes [38, 39], but also firstly showed more potent efficacies of Re among the representative panaxadiols and panaxatriols derivatives on T2D (Figs 1-4). These results are supported by several studies [27, 28]. Moreover, our data also firstly showed Re decrease serum IL-6, TNF-α and NF-κB levels in T2D rats (Figure 4D-F), and provide more evidences for Re possesses significant anti-inflammatory actions. These results are supported by independent reports that Re inhibits NF-κB and antagonizes TNF-α in cultured 3T3-L1 cells [40,41]. In addition, our experiments firstly explored the anti-apoptotic activities of Re that characterized in the up regulation of Bcl-2 mRNA and down regulation of Bax and caspase-3 mRNA expression in the pancreas of T2D rats (Fig. 5A-D). It has demonstrated that Re has anti-apoptotic effects via regulation of Bcl-2, Bax, inducible nitric oxide synthase (iNOS) and caspase-3 in substantia nigra neurons of Parkinson's disease mouse model [42]. More importantly, our results also first time showed antidiabetic effects of Re involve NO (release from eNOS) actions (Fig.e 7), reports from our

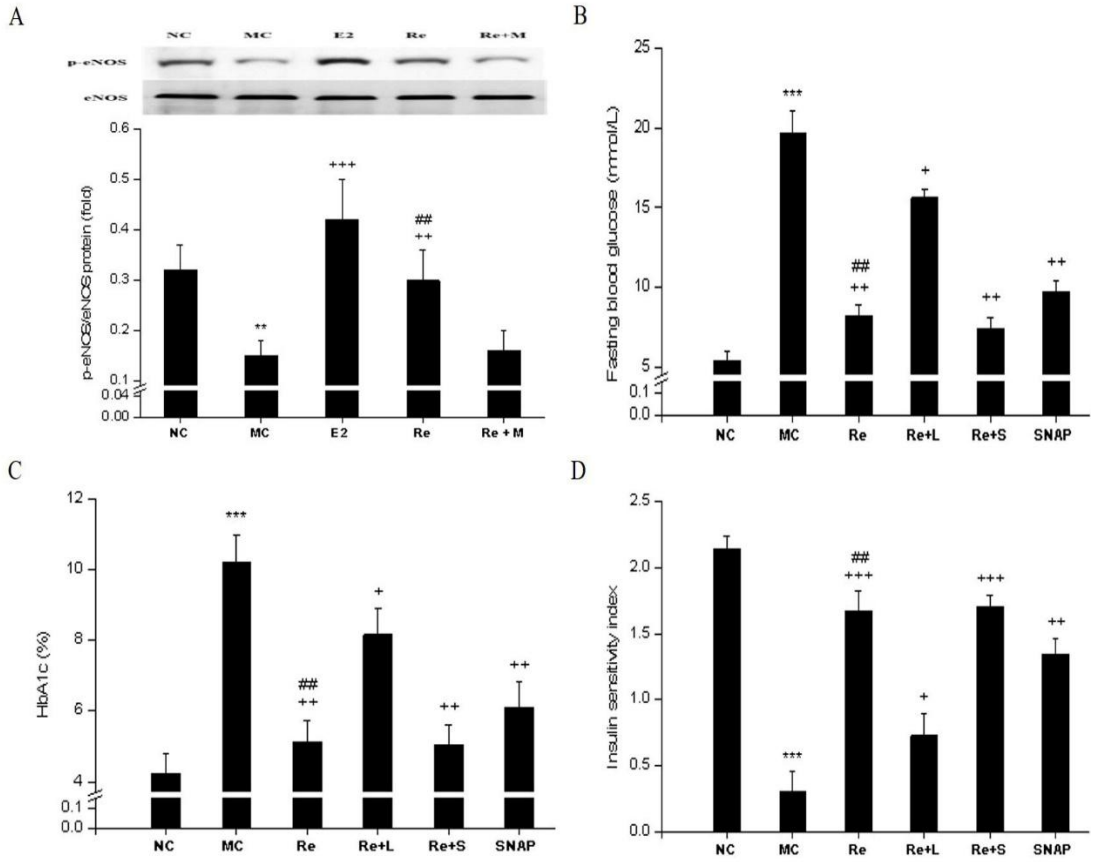

Fig.7. Effects of Re on eNOS phosphorylation and effects of L-NIO and SNAP.
A. Representative western blots and densitometric analysis of eNOS phosphorylation by Re (N=3). B, C, and D, effects of L-NIO and SNAP on Re-induced lowering FBG, decreasing serum HbA1c, and increasing ISI, respectively. NC: normal control group; MC: type 2 diabetes (T2D) model control group; Re: T2D rats treated by high dose of Re (Re-h); E2: treated by17β-estradiol; Re+M: treated by Re-h plus MK-2206; Re+L: treated by Re-h plus L-NIO (eNOS inhibitor); Re+S: treated by Re-h plus SNAP (NO donor); SNAP: treated by SNAP (NO donor). Compared with NC, $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001(N=10); compared with MC, ^{+}P <0.05, ^{++}P <0.01, ^{+++}P <0.001(N=10); compared with Re + M and Re+L treated, respectively, $^{\#}P$ <0.05, $^{\#\#}P$ <0.01(N=10).

previous study and others have demonstrated the NO actions involve beneficial effects of Re in several other tissues [30,43]. Our data also confirmed Re enhances GLP-1 secretion in ileum and colon of T2D rats (Fig.8D) [44]. It should be noted that our data also firstly showed Re normalized the dysregulated insulin and Gcg gene expression, and reverse impaired insulin content in pancreas of T2D rats (Fig. 8A-C). Collectively, our study demonstrated that Re produces multiple therapeutic effects on T2D , such as anti-oxidative stress, anti-inflammation , anti-apoptosis, restoration of pancreatic β-cell as well as α-cell functions , and normalizing dysregulated insulin and Gcg, results in improving insulin sensitivity, anti-hyperglycemia, and anti-hyperlipidemia; in which Re regulates multiple target molecules including ER, Akt, eNOS, NO, insulin , Gcg, GLP-1 , Bcl-2, SOD, TNF-α, IL-6 that were closely associated with pathogenesis and remission of 2TD.

4.3 Integrating of the Multi-targeted Potent Antidiabetic Effects of Ginsenoside Re Mediated by ER Signaling Pathway

It has demonstrated that several signaling molecules including AMPK, GLUT4, GLP-1, PPAR-γ, SOD, Bcl-2, TNF-α, and NF-κB act as potential targets for antidiabetic effects of Re [38-

41,44-46]. However, signaling pathway by which Re regulate the multiple signaling molecules is not yet clearly elucidated. Accumulating evidence has implicated that many biological actions of ginsenosides are mediated by plasma membrane receptors /or intracellular receptors signaling, particularly steroid receptors, and some ginsenosides including Re have been shown to exhibit estrogen-like activity via acting as a functional ligand of ER [29,31]. Re acts as full agonist for sex steroid receptors including ER in several biological systems [29, 47]. In the present study, our data show that Re mimic the multi-targeted antidiabetic activities and insulinotropic properties of estrogen [12,16,48,49]; functionally, the activities of anti-hyperglycemia and improving insulin sensitivity induced by Re were inhibited by the ERα inhibitor (ICI-182,780) (Fig.5); indicating that the actions of Re are mediated by ERα. Structurally, Re and its metabolic derivatives in the intestines possesses potent affinities to bind human ERα [25,29, 31]. Consequently, we confirmed Re activates Akt that was a critical node cascade in the downstream of ER_{α} signaling pathway; functionally, the actions of Re were inhibited by Akt inhibitor (Fig. 6). These results are strongly supported by several reports that Re activates ER/or Akt signaling pathway in several other biological systems and pathophysiological conditions including diabetic complication [29,50,51]. Thus, we reasoned that ERα/Akt signaling pathway appears to be a main signaling pathway that is responsible for the antidiabetic effects of Re. Furthermore, our data showed Re activates eNOS via ERα/Akt signaling, and Re-induced antidiabetic actions are inhibited by a relatively selective inhibitor (L-NIO) of eNOS, and NO donor (SNAP) treatment did not affect Re-induced antidiabetic actions (Fig. 7); suggesting that the antidiabetic effects of Re involve NO (release from eNOS) actions. Re induce release of NO from eNOS via activation of ERα /Akt signaling pathway in several other tissues [29,43,51]. These evidences encouraged us to speculate that the NO might be a main effector that is mainly responsible for the antidiabetic effects of Re in the ERα/Akt signaling pathway. This concept could be further supported by diverse biological actions of NO that play very important role in the pathogenesis and remission of T2D [52], ERα/ Akt /eNOS/NO signaling pathway that was a main classical mechanism for estrogen actions [53], and Akt /eNOS/NO signaling pathway is essential for IR, obesity, and diabetes [52]. In addition, our data also speculated that several candidate molecules such as insulin, Gcg, and GLP-1 regulated by Re through ERα signaling. We did not test whether NO that released by Re via ERα/ Akt /eNOS signaling pathway regulates insulin, Gcg, and GLP-1. It has been demonstrated that ERα signaling and NO (release from eNOS) regulate many signaling cascades including insulin, AMPK, PPAR-γ, GLUT4, GLP-1, ATP sensitive potassium channel (K_{ATP}), Bcl-2, TNFα and cGMP that are closely associated to obesity and 2TD [16, 52,54]. Therefore, NO release from eNOS act as multi-targeted effector and messenger in the regulation of glucose homeostasis and insulin sensitivity, represents the potential clinical therapeutic importance of NO-based interventions and NO targeted therapies that can hopefully facilitate the development of new treatments named “NO therapy” for T2D. Altogether, we reasoned that activation of ERα/Akt/eNOS/NO signaling pathway by Re appears to be the main mechanism for the multi-targeted antidiabetic effects of Re.

4.4 Ginsenoside Re is an Alternative Multi-targeted Therapy for T2D

Developing multi-targeted effective therapeutic measures for multifactorial T2D are on the hunt, estrogen therapy and NO therapy could be imaging as potential multi-targeted therapeutic strategy for T2D [52,54,55]. However, systemic delivery of estrogen /or NO agent potentially increase its serious adverse effects of estrogen/or NO agent toxicity [17,56]. Thus, it is potentially important in the clinical setting that Re produces multi-targeted potent antidiabetic effects via activation of ERα signaling pathway in the NO dependent manner described in the present study. It is supported by estrogen induces eNOS-NO has potential therapeutic importance [57]. Moreover, Re did not stimulate proliferation of estrogen-responsive human breast cancer cell line MCF-7; rather, Re partially inhibited E2-induced MCF-7 proliferation [29], and it has anticancer effects that without mediated by ER [58]. In addition, our data imply that Re is a naturally harvested, mechanisms specific agonist of ERα. Thus, we expect that Re is a novel effective and safety multi-targeted therapeutic for T2D, particularly, a novel candidate for replacement of both estrogen therapy and NO therapy in the prevention and treatment of T2D. It was further supported by Re has many beneficial effects on the diabetic complications [38,51,59].

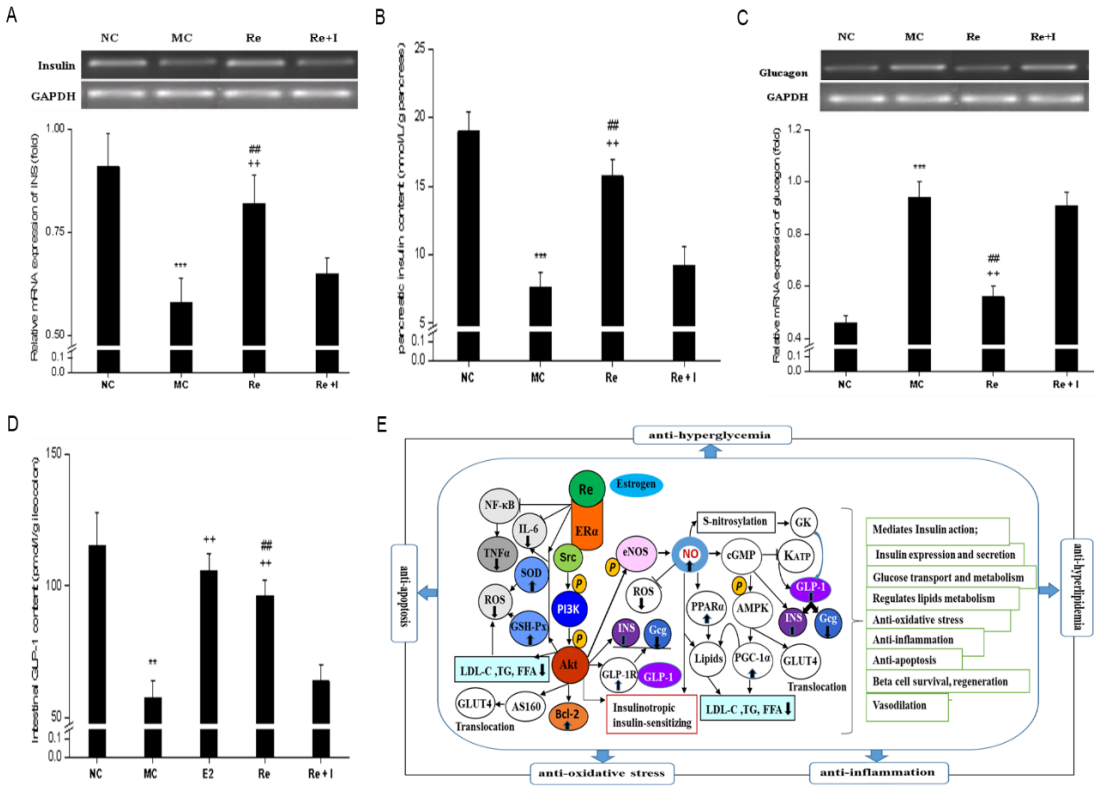

Fig. 8. Regulative effects of Re on insulin and glucagon in type 2 diabetes rats, and proposed schematic model for regulatory mechanisms of Re on ER signaling pathway.

A. Effect of Re on insulin mRNA expression in the pancreas of type 2 diabetes (T2D) rats (N=3). B. Enhancement of pancreatic insulin content by Re (N=10). C. Effect of Re on glucagon mRNA expression in the pancreas of T2D rats (N=3). D. Re reverse impaired ileocolonic GLP-1 in T2D rats. NC: normal control group; MC: T2D model control group; Re: T2D rats treated by high dose of Re (Re-h); E2: treated by17β-estradiol; Re+I: treated by Re-h plus ICI182,780. Compared with NC, * P<0.05, ** P<0.01, *** P<0.001; compared with MC, $^{+}$P <0.05, $^{++}$ P <0.01, $^{+++}$P <0.001; compared with Re plus ICI182,780 (ER inhibitor), $^{\#}$ P <0.05, $^{\#\#}$ P <0.01. E. Proposed schematic model for regulatory mechanisms of Re on ER signaling pathway. Re acts as a structural and functional agonist for ERα, and activates ERα, sequentially activate, Akt and eNOS, release NO that acts a main effector inducing multi-targeted biological actions closely associated to pathogenesis and remission of T2D; may also acts as signals regulates multiple targets molecules, such as GLP-1, insulin, and glucagon. Integrating of the multi-targeted actions of Re mediated by ER signaling pathway, results in protection of β-cell as well as α-cell function, restoration of dysregulated insulin as well as glucagon, reducing IR, and regulation of glucose and lipid homeostasis; these contribute to Re producing multi-targeted potent antidiabetic effects. Akt: Threonine kinase; AMPK: AMP-activated protein kinase; AS160: Akt substrate of 160 kDa; BcL-2: B-cell lymphoma-2; cGMP: Cyclic guanosine monophosphate; eNOS: Endothelial nitric oxide synthase; ERα: Estrogen receptor alpha; FFA: Free fatty acid; Gcg: Glucagon; GLP-1:Glucagon-like peptide 1; GLP-1R:Glucagon-like peptide 1 receptor; GLUT4: Glucose transporter-4; GK: glucokinase; GSH-Px: Glutathione peroxidase; K_{ATP}: ATP sensitive potassium channel; IL-6: Interleukin 6; INS: Insulin; IR: Insulin resistance; LDL-C: Low density lipoprotein cholesterol; NO: Nitric oxide; NF-κB: Nuclear factor κB; PI3K: Phosphatidylinositol 3-kinase; ROS: Reactive oxygen species; P: Phosphorylation; PPAR-γ: Peroxisome proliferator-activated receptor gamma; PGC-1α: peroxisome proliferator-activated receptor-γ coactivator1α; SOD: Superoxide dismutase; TG: Triglyceride; TNF-α: Tumor necrosis factor alpha.

5. CONCLUSION

In summary, in the present study, identified the multi-targeted potent antidiabetic effects of Re, demonstrated a novel pharmacological mechanism that activating ERα/ Akt /eNOS/NO signaling pathway by Re was mainly responsible for the integrated multi-targeted antidiabetic effects of Re. Our data provide the first evidence for the potential use of Re, as a multi-targeted synergistic therapeutic for T2D, particularly, a novel candidate for replacement of estrogen therapy and NO therapy in diabetes. Further studies are necessary to clarified crosstalk mechanism between ERα signaling and G protein-coupled estrogen receptor GPER (formerly GPR30) signaling underlying the antidiabetic effects of Re.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study followed the guideline to the Declaration of Helsinki, animal care and experiments were approved by the Animal Ethics Committee of Inner Mongolia Medical University, which abides by the law of the State (the People's Republic of China) Administration of Experimental Animals（ethical clearance number, YKD2017150）.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat. Med. 2017;23:804–814.
2. Jung HO, Soo HK, Young MC, Soo L, Jang HC, Kyong SP, Nam H C. 10-year trajectory of β-cell function and insulin sensitivity in the development of type 2 diabetes: a community-based. Lancet Diabetes & Endocrinology. 2016;4:27-34.
3. Jun SM, Kyu CW. Pancreatic α-Cell Dysfunction in Type 2 Diabetes: Old Kids on the Block. Diabetes & Metabolism Journal. 2015;39:1-9
4. Aastha M, Rajesh KM, Sarama S, Praveen KS, Sanjay KB, Satya VR. Oxidative stress and inflammatory markers in type 2 diabetic patients. Eur J Clin Invest. 2020;50:e13238.
5. Del PS. Role of glucotoxicity and lipotoxicity in the pathophysiology of Type 2 diabetes mellitus and emerging treatment strategies. Diabetic Medicine. 2009;26:1185-1192.
6. Xu Q, Wang L, Luo J, Shi D. The Hot and Potential Targets of Type 2 Diabetes Mellitus Treatment in Recent Decade. Current Drug Targets. 2018;19:55-69.
7. Holst JJ, Meier JJ. Loss of inverse relationship between pulsatile insulin and glucagon secretion in patients with type 2 diabetes. Diabetes. 2011;60:2160–2168.
8. Chung I, Rajakumar G, Subramanian U, Venkidasamy B, Khanna VG, Thiruvengadam M. Insights on the current status and advancement of diabetes mellitus type 2 and to avert complications: An overview. Biotechnology and Applied Biochemistry. 2019;920-928.
DOI: 10.1002/bab.1853
9. Nauck MA, Wefers J, Meier JJ. Treatment of type 2 diabetes: challenges, hopes, and anticipated successes.The Lancet

Diabetes & Endocrinology. 2021; published; 2021.
DOI: 10.1016/S2213-8587(21) 00113-3.

10. Israel H. For debate: Pharmacological priorities in advanced type 2 diabetes. Journal of Diabetes and its Complications. 2020;34:107510.
11. Kawakami M, Yokota-Nakagi N, Uji M, Yoshida KI, Tazumi S, Takamata A, Uchida Y, Morimoto K. Estrogen replacement enhances insulin-induced AS160 activation and improves insulin sensitivity in ovariectomized rats. AJP - Endocrinology and Metabolism. 2018;315: E1296-E1304.
12. Kumar R, Balhuizen A, Amisten S, Lundquist I, Salehi SA. Insulinotropic and Antidiabetic Effects of 17{beta}-Estradiol and the GPR30 Agonist G-1 on Human Pancreatic Islets. Endocrinology. 2011; 152:2568-2579.
13. Joseph P and Franck MJ. Importance of oestrogen receptors to preserve functional β-cell mass in diabetes. Nature Reviews Endocrinology. 2012;8:342–351.
14. Cahua-Pablo JÁ, Flores-Alfaro E, Cruz M. Estrogen receptor alpha in obesity and diabetes. Revista Médica del Instituto Mexicano del Seguro Social. 2016;54: 521-30.
15. Mauvais-Jarvis F. Estrogen and androgen receptors: regulators of fuel homeostasis and emerging targets for diabetes and obesity. Trends Endocrinol. Metab. 2011; 22:24–33.
16. Rodrigo PAB, Jan-Ake G. Estrogen Receptors and the Metabolic Network. Cell Metabolism. 2011;14:289-299.
17. Mehta J, Kling JM, Manson JAE. Risks, Benefits, and Treatment Modalities of Menopausal Hormone Therapy: Current Concepts. Frontiers in Endocrinology. 2021; 12: 564781-564781.
18. Paloma Alonso-Magdalena, Ana B. Ropero, Marta García-Arévalo, Sergi Soriano, Iván Quesada, Sarheed J. Muhammed, Albert Salehi, Jan-Ake Gustafsson, Ángel Nadal. Antidiabetic Actions of an Estrogen Receptor β Selective Agonist. Diabetes. 2013; 62:2015-2025.
19. Sandipan C, Biswas PK. Structural insights into selective agonist actions of tamoxifen on human Estrogen Receptor alpha. J Mol Model. 2014; author manuscript available in PMC; 2015.
20. Cho WCS, Yue KKM, Leung AWN. An outline of diabetes mellitus and its treatments by traditional Chinese medicine and acupuncture. J. Chin. Med. (UK). 2005;78:29–37.
21. Kim OS, Choi JH, Soung YH, Lee SH, Lee JH, Ha JM, Ha BJ, Heo MS, Lee SH. Establishment of in vitro test system for the evaluation of the estrogenic activities of natural products. Arch Pharm Res (NY). 2004;27:906–911.
22. Wei Chen, Prabhu Balan, David G Popovich. Review of Ginseng Anti-Diabetic Studies. Molecules. 2019;24:5401.
23. Gui QF, Xu ZR, Xu KY, Yang YM. The efficacy of ginseng-related therapies in type 2 diabetes mellitus: an updated systematic review and meta-analysis. Medicine. 2016;95:e2584.
24. Litao B, Jialiang G, Fan W, Jing Z, Danwei W, Junping W. Therapeutic Potential of Ginsenosides as an Adjuvant Treatment for Diabetes. Frontiers in Pharmacology. 2018;9:423.
25. Tiehua Z, Shuning Z, Ligang H, Yongjun W, Xiao JX, Tianzhu G, et all. Computational and experimental characterization of estrogenic activities of 20(S, R)-protopanaxadiol and 20(S, R)-protopanaxatriol. Journal of Ginseng Research. 2020;44:690-696.
26. Gillis CN. Panax ginseng pharmacology: a nitric oxide link? Biochem Pharmacol. 1997;54:1–8.
27. Attele AS, Zhou YP, Xie JT, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan CS. Antidiabetic effects of Panax ginseng berry extract and the identification of an effective component. Diabetes. 2002; 5:1851–1858.
28. Xie JT, Mehendale SR, Li X, Quigg R, Wang X, Wang CZ, Wu JA, Aung HH, P AR, Bell GI, Yuan CS. Anti-diabetic effect of ginsenoside Re in ob/ob mice. Biochim Biophys Acta. 2005;1740 :319-325.
29. Tetsushi Furukawa, Chang-Xi Bai, Asami Kaihara, Eri Ozaki, Takashi Kawano, Yutaka Nakaya, Muhammad Awais, Moritoshi Sato, Yoshio Umezawa, and Junko Kurokawa. Ginsenoside Re, a Main Phytosterol of Panax ginseng, Activates Cardiac Potassium Channels via a Nongenomic Pathway of Sex Hormones. Mol Pharmacol. 2006;70:1916–1924.
30. Bai C-X, Takahashi K, Masumiya H, Sawanobori T, Furukawa T. Nitric oxide-dependent modulation of the delayed

rectifier K^+ current and the L-type Ca 2^+ current by ginsenoside Re, an ingredient of Panax ginseng, in guinea-pig cardiomyocytes. Br J Pharmacol. 2004; 142:567–575.

31. Bae EA, Shin JE, Kim DH. Metabolisms of ginsenoside Re by human intestinal microflora and its estrogenic effects. Biol Pharm Bull. 2005;28:1903-1908.
32. Wang X, Sakuma T, Asafu-Adjaye E, Shiu GK. Determination of ginsenosides in plant extracts from Panax ginseng and Panax quinquefolius L. by LC/MS/MS. Anal Chem. 1999;71:1579-1584.
33. Jian-Ming L, Qizhi Y, and Changyi C. Ginseng Compounds: An Update on Their Molecular Mechanisms and Medical Applications. Curr Vasc Pharmacol. 2009; 7:293–302.
34. Ye X, Ling S, Kristina JL, Patrick T, Yuqing X, Wang Gj, et all. Antiobesity and Antihyperglycemic Effects of Ginsenoside Rb1 in Rats. Diabetes. 2010;59:2505–2512.
35. Hye-Min Lee, Ok-Hwan Lee, Boo-Yong Lee. Effect of Ginsenoside Rg3 and Rh2 on Glucose Uptake in Insulin-resistant Muscle Cells. J. Korean Soc. Appl. Biol. Chem. 2010;53:106-109.
36. Kim KS, Yang HJ, Lee IS, Kim KH, Park J, Jeong HS, et al. The glycone of ginsenoside Rg3 enables glucagon-like peptide-1 secretion in enteroendocrine cells and alleviates hyperglycemia in type 2 diabetic mice. Sci.Rep. 2015;5:18325. DOI: 10.1038/srep18325.
37. Tian W, Chen L, Zhang L, Wang B, Li XB, Fan KR, Ai CH, Xia X, Li SD, Li Y. Effects of ginsenoside Rg1 on glucose metabolism and liver injury in streptozotocin-induced type 2 diabetic rats. Genet. Mol. Res. 2017;16, gmr16019463.
38. Youjin Hao, Zhimou Liu, Yongsheng Huang, Yan Wang, Jing-Tian Xie. Antihyperglycemic Effect of Ginsenoside Re and Its Possible Mechanisems. International Journal of Biomedical and Pharmaceutical Sciences. 2012;6(Special Issue 1):84-89.
39. William CSC, Chung WS, Lee SKW, Albert WN Leung, Christopher HK Chengc, Kevin KM Yue. Ginsenoside Re of Panax ginseng possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats. European Journal of Pharmacology. 2006; 550:173–179.
40. Zhiguo Z, Xiaoying L, Wenshan L, Yisheng Y, Hong G, Jun Y, Yun S, Guang N. Ginsenoside Re Reduces Insulin Resistance through Inhibition of c-Jun NH 2 -Terminal Kinase and Nuclear Factor-κB. Molecular Endocrinology. 2008;22:186–195.
41. Gao Y, Yang MF, Su YP, Jiang HM, You XJ, Yang YJ,et all. Ginsenoside Re reduces insulin resistance through activation of PPAR-γ pathway and inhibition of TNF-α production. Journal of Ethnopharmacology. 2013;147:509-16.
42. Xu BB, Liu CQ, Gao X, Zhang WQ, Wang SW, Cao YL. Possible mechanisms of the protection of ginsenoside Re against MPTP-induced apoptosis in substantia nigra neurons of Parkinson's disease mouse model. Journal of Asian Natural Products Research. 2005; 7: 215-224.
43. Gao Y, Zhu P, Xu SF , Li YQ, Deng J, Yang DL. Ginsenoside Re inhibits PDGF-BB-induced VSMC proliferation via the eNOS/NO/cGMP pathway. Biomedicine & Pharmacotherapy. 2019;115:108934.
44. Gao J, Lu SS, Zhang L, Zhou N. Ginsenoside Re promotes glucagons-like-peptide-1 amide secretion in diabetic rats. Chinese Remedies & Clinics. 2011; 11:1383-1385.
45. Han DH, Kim SH, Kazuhiko H, Jung SR, Kenneth SP, Samuel K, et all. Ginsenoside Re Rapidly Reverses Insulin Resistance in Muscles of High-fat Diet Fed Rats. Metabolism. 2012;61:1615–1621.
46. Jung MS, Chung SH. AMP-activated Protein Kinase: A Potential Target for Ginsenosides? Arch Pharm Res. 2011;34:1037-1040.
47. 47. Leung KW, Leung FP, Huang Y, Mak NK, Wong RN. Non-genomic effects of ginsenoside Re in endothelial cells via glucocorticoid receptor. FEBS Lett. 2007; 581:2423–8.
48. Cedric LM, Khoi C, Hu M, Christina SO, Evan RS, Kenneth SK, et all. Estrogens protect pancreatic β-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. Proc. Natl Acad. Sci. USA. 2006;103:9232–9237.
49. 49. Yamabe N, Kang KS, Zhu BT. Beneficial effect of 17β-estradiol on hyperglycemia and islet β-cell functions in a streptozotocin-induced diabetic rat model. Toxicol. Appl. Pharmacol. 2010; 249:76–85.

50. Chen RC , Wang J, Yang L, Sun GB, Sun XB. Protective effects of ginsenoside Re on lipopolysaccharide-induced cardiac dysfunction in mice. Food & Function. 2016;7:2278-87.
51. Xie W, Qu M, Dai Z, Zhang X, Zhang C, Dong X, Sun G, Sun X. Ginsenoside Re Attenuates High Glucose-Induced RF/6A Injury via Regulating PI3K/AKT Inhibited HIF-1α/VEGF Signaling Pathway. Frontiers in Pharmacology. 2020;11:695.
52. Zahra B, Parvin M, Asghar G. Role of Nitric Oxide in Insulin Secretion and Glucose Metabolism. Trends in Endocrinology & Metabolism. 2020;31:118-130.
53. Chambliss KL, Yuhanna IS, Mineo C, Liu P, German Z, Sherman TS, Mendelsohn ME, Anderson RG, Shaul PW. Estrogen receptor alpha and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. Circ Res. 2000; 87: e44–e52.
54. Brian ES and Bradford GH. Anti-obesogenic role of endothelial nitric oxide synthase. Vitam Horm. 2014; 96: 323–346.
55. Salpeter SR, Walsh JM, Ormiston TM, Greyber E, Buckley NS, Salpeter EE. Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women. Diabetes Obes. Metab. 2006; 8:538–554.
56. Weinberger B, Laskin DL, Heck DE, Laskin JD. The toxicology of inhaled nitric oxide. Toxicol Sci. 2001;59:5-16.
57. Vera-Arzave C, Pacheco-Yepez J, Mejía-Barradas C M, Cárdenas-Jaramillo L M, Campos-Rodríguez R, Abarca-Rojano E, 17β-estradiol replacement therapy induces eNOS, nNOS and estrogen receptor β in hypophysectomized rats with inflamed footpads. Journal of Biological Regulations & Homeostatic Agents. 2019;33:1395-1403.
58. Jang HJ, Han IH, Kim YJ, et al. Anticarcinogenic Effects of Products of Heat-Processed Ginsenoside Re, a Major Constituent of Ginseng Berry, on Human Gastric Cancer Cells. J Agric Food Chem. 2014;62:2830-2836.
59. 59. Yao WL, Xia Z, Wei L, Qian L, Jian Y Ya QW, Xiao XYi. Ginsenoside Re attenuates diabetes-associated cognitive deficits in rats. Pharmacology Biochemistry and Behavior. 2012;101:93-98.

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