Anti-diabetic Action of 7-*O*-Galloyl-D-sedoheptulose, a Polyphenol from Corni Fructus, through Ameliorating Inflammation and Inflammation-Related Oxidative Stress in the Pancreas of Type 2 Diabetics

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Compelling evidence indicates that polyphenolic antioxidants show protective effects against diabetic complications. We investigated the effects of a polyphenolic compound, 7-*O*-galloyl-D-sedoheptulose (GS), from Corni Fructus on a type 2 diabetic *db*/*db* mouse model. After 6 weeks of GS treatment, the effects of GS on serum and pancreatic biochemical factors were investigated. To define the underlying mechanism of these effects, we examined several key inflammatory markers, and inflammation-related oxidative stress markers. The results showed that levels of glucose, leptin, insulin, C-peptide, resistin, tumor necrosis factor-α, and interleukin-6 in serum were down-regulated, while adiponectin was augmented by GS treatment. In addition, GS suppressed reactive oxygen species and lipid peroxidation in the pancreas, but increased the pancreatic insulin and pancreatic C-peptide contents. Moreover, GS modulated protein expressions of pro-inflammatory nuclear factor-kappa Bp 65, cyclooxygenase-2, inducible nitric oxide synthase, c-Jun N-terminal kinase (JNK), phospho-JNK, activator protein-1, transforming growth factor-β_1, and fibronectin. Based on these results, we conclude that a plausible mechanism of GS's anti-diabetic action may well be its anti-inflammatory property and anti-inflammatory-related anti-oxidative action. Thus, further investigation of GS as an effective anti-diabetic treatment for type 2 diabetes is warranted.

Key words 7-*O*-galloyl-D-sedoheptulose; type 2 diabetes; pancreas; inflammation; oxidative stress; fibrosis

Insulin resistance is a primary defect that is a characteristic feature of type 2 diabetes.[1,2] The state of insulin resistance leads to increased insulin secretion by pancreatic β-cells and compensatory hyperinsulinemia. As long as compensatory hyperinsulinemia is sufficient to overcome the insulin resistance, fasting glycemia and glucose tolerance remain relatively normal. In patients predestined to progress to type 2 diabetes, β-cell compensation efficiency declines and relative insulin insufficiency develops, leading to impaired glucose tolerance and, eventually, type 2 diabetes. Consequently, type 2 diabetes results from the progressive failure of pancreatic β-cells in a setting of chronic insulin resistance.[3–5]

Reactive oxygen species (ROS) play an important role in insulin resistance and pancreatic β-cell dysfunction, a highly prevalent condition implicated in the development of type 2 diabetes.[6–10] Under a diabetic condition, chronic hyperglycemia may induce large amounts of ROS that are responsible for the progressive dysfunction of β-cells, worsening insulin resistance and further promoting relative insulin deficiency ROS.[11] β-Cells, in particular, are particularly sensitive to ROS because they are low in free-radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase, and superoxide dismutase.[12] The ROS formed may also indirectly damage cells by activating a variety of stress-sensitive intracellular signaling pathways, including nuclear factor-κB (NF-κB), mitogen-activated protein kinase p38, c-Jun part of activator protein-1 (AP-1), hexosamines, protein kinase C, and the polyol pathway.[9,13] Also, ROS-mediated activation of NF-κB and AP-1, two redox-sensitive transcription factors, are evolutionarily conserved and involved in a wide variety of pro-inflammatory and fibrosis genes including cytokines, chemokines, adhesion molecules, and inducible effector enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).[14]

The C57BL/KsJ-*db*/*db* mouse model (*db*/*db* mouse) is a useful model in the study of type 2 diabetes because it manifests many of the characteristics of the human disease including hyperphagia, hyperglycemia, insulin resistance, and obesity. The use of polyphenolic compounds have been growing in interest as anti-diabetic agents for their ability to protect against early-stage diabetes and the development of complications.[15] We reported that polyphenolic compounds show anti-diabetic effects in high-glucose-treated renal cells,[16,17] streptozotocin-induced diabetic rats,[18] and in a mouse model of type 2 diabetes.[19] We recently reported in a series of articles that the polyphenolic compound 7-*O*-galloyl-D-sedoheptulose (GS) from Corni Fructus (*Cornus officinalis* Sieb. *et* Zucc.) has a structure that is protective against hypertriglycemia,[20] an anti-diabetic effect in streptozotocin-induced diabetic rats,[21] and an anti-oxidative action against oxidative stress by suppressing oxidative modifications of lipids and the formation of advanced glycation endproducts.[22] However, the protective molecular mechanisms of anti-inflammatory GS against type 2 diabetes remain to be elucidated. In this experiment, we investigated the effects of GS on oxidative stress-related inflammatory mediators and pro-inflammatory-induced insulin secretory functional changes in pancreatic islet cells of type 2 diabetic *db*/*db* mice.

MATERIALS AND METHODS

Materials Protease inhibitor mixture solution, 4,6-dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid, TBA), and 10% neutral-buffered formalin were purchased from Wako

The authors declare no conflict of interest.

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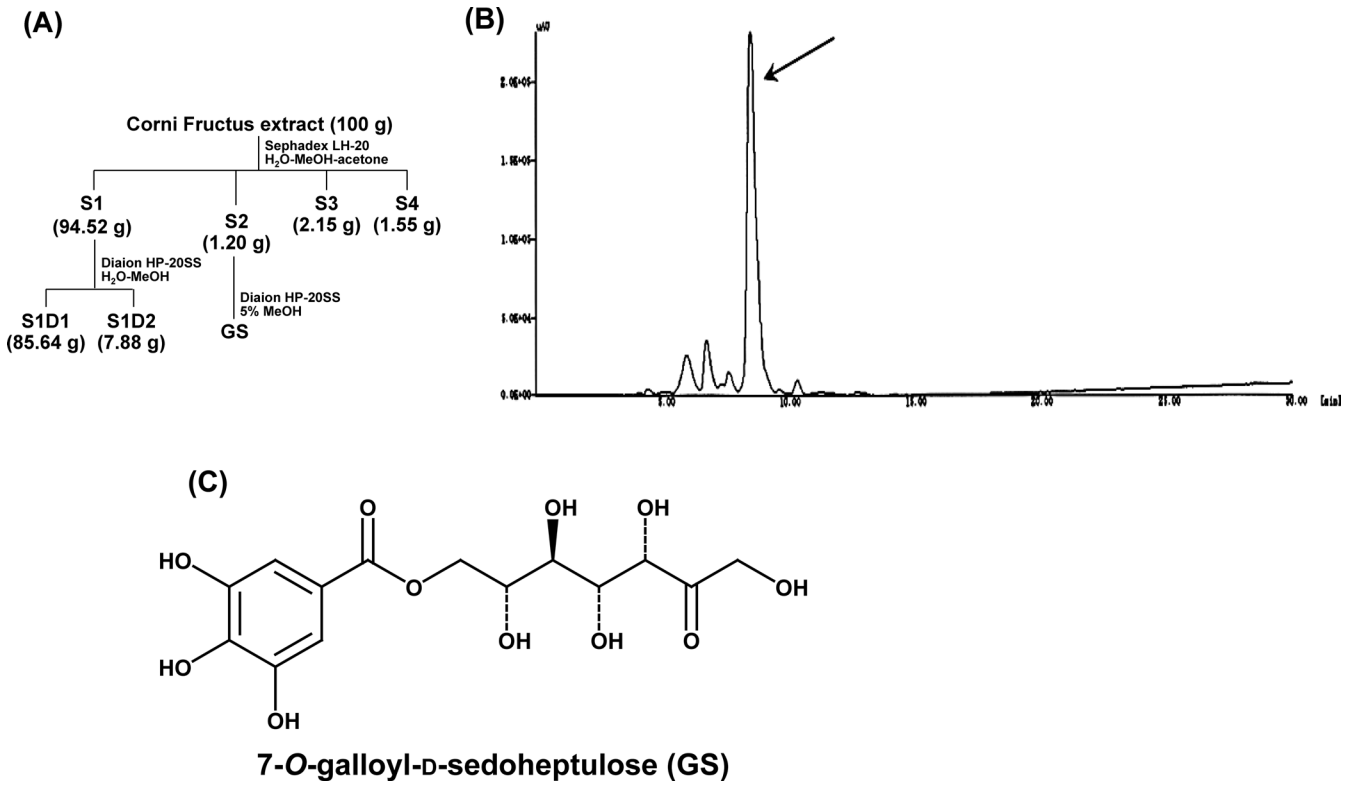

Fig. 1. Fractionation of Corni Fructus, HPLC Profile of GS, and Its Structure

(A) Fractionation of Corni Fructus was performed as described in Materials and Methods. (B) HPLC profile. The large peak shown by the arrow is the structure of GS, as described in (C).

Pure Chemical Industries, Ltd. (Osaka, Japan). 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was purchased from Molecular Probes (Eugene, OR, U.S.A.). The Bio-Rad protein assay kit and pure nitrocellulose membrane were purchased from Bio-Rad Laboratories (Tokyo, Japan). β-Actin and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Rabbit polyclonal antibodies against NF-κB p65, transforming growth factor-β_1 (TGF-β_1) and fibronectin, and mouse monoclonal antibodies against COX-2, iNOS, and histone were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). Poly anti c-Jun N-terminal kinase (JNK), phospho (p)-JNK, and mono anti AP-1 (c-Jun) were purchased from Cell Signaling Technology (Danvers, MA, U.S.A.). Goat anti-rabbit and goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. ECL Western Blotting Detection Reagents were purchased from GE Healthcare (Piscataway, NJ, U.S.A.). All other chemicals and reagents were purchased from Sigma Chemical Co.

Preparation of *Cornus officinalis* Fractions and Purification of GS The extract of *Cornus officinalis* (100 g), which was produced by Tsumura & Co. (Tokyo, Japan) was fractionated by Sephadex™ LH-20 column chromatography (32×5 cm) with water containing increasing proportions of methanol (0–100%, 10% stepwise gradient elution) and finally 60% acetone to yield four fractions: S1 (94.52 g), S2 (1.20 g), S3 (2.15 g), and S4 (1.55 g). The fraction S1 was further separated by Diaion™ HP-20SS column chromatography (28×5 cm) with water-methanol (0–100%, 10% stepwise gradient elution) to yield S1D1 (85.64 g) and S1D2 (7.88 g). TLC and HPLC analyses, which were performed as mentioned above, showed that S1D1 and S1D2 mainly contained sugars and iridoid glycosides, and S2, S3, and S4 contained phenolic substances (Fig. 1A). A portion of S2 (150 mg) was further purified by MCI-gel CHP20P column chromatography (28×2 cm) with 0–10% MeOH to yield GS (98 mg), as shown in Fig. 1B. A white amorphous powder, high resolution (HR) FAB-MS *m/z*: 363.0903, $C_{14}H_{19}O_{11}$ [M+H] requires 363.0927. ^{1}H-NMR (acetone-d_6+D_2O) of the major anomer δ: 7.13 (s, galloyl-H), 4.36 (m, H-4, H-7a), 4.23 (dd, J=6.6, 11.7 Hz, H-7b), 4.09 (d, J=6.4 Hz, H-3), 4.05 (m, H-6), 3.88 (t, J=5.5, H-5), 3.50 (2H, br s, H-1), ^{13}C-NMR (acetone-d_6+D_2O) of the major anomer δ: 167.0 (galloyl C-7), 145.9 (galloyl C-3,5), 138.7 (galloyl C-4), 121.5 (galloyl C-1), 109.8 (galloyl C-2,6), 103.7 (C-2), 83.3 (C-5), 78.0 (C-3), 77.1 (C-4), 71.1 (C-6), 66.2 (C-7), 64.4 (C-1). Other anomeric carbon signals are observed at δ 98.2, 103.7, and 109.0. Assignments of the signals were achieved by correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond connectivity (HMBC) spectral analysis. The structure was further confirmed by the formation of an osazone derivative: a mixture of the compound (10 mg), phenylhydrazine hydrochloride (20 mg), and sodium acetate (30 mg) in water (0.5 mL) was heated at 80°C for 25 min, and the resulting precipitates were collected by filtration. The ^{1}H-NMR spectral data (in DMSO-d_6) and $[\alpha]_D$ value coincided with the data for the osazone derivative of GS.

Experimental Animals and Treatment Animal experiments were carried out according to the 'Guidelines for Animal Experimentation' approved by the Ethics Committee of the University of Toyama (Registration No.: S-2006 INM-22).

Six-week-old male *db*/*db* and age-matched non-diabetic *m*/*m* mice were purchased from Japan SLC Inc. (Hamamatsu, Japan). Mice were maintained under a 12-h light/dark cycle, and housed at a controlled temperature (23±3°C) and humidity (about 60%). The mice were allowed free access to laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan, comprising 24.0% protein, 3.5% lipids, and 60.5% carbohydrate) and water *ad libitum*. After adaptation (at 9 weeks of age), blood glucose levels taken from the tail vein were estimated, and then *db*/*db* mice were divided into three groups (n=10/group). GS was orally administered every day at a dose of 20 or 100 mg/kg body weight, respectively, while vehicle-treated *db*/*db* mice were given water orally. The non-diabetic *m*/*m* mice (n=6), as a normal control group, were used for comparisons with the diabetic groups. The body weight, food intake, and water intake were determined every day during the administration period. After 6 weeks of administration, blood samples were collected by cardiac puncture from anesthetized mice. The serum was separated instantly from blood samples by centrifugation. Successively, mice were perfused with ice-cold physiological saline after cardiac puncture, and the pancreas was harvested, snap-frozen in liquid nitrogen, and stored at −80°C until analyses.

Estimation of Serum Parameters Serum glucose was determined using a commercial kit (Glucose CII-Test from Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum leptin and insulin (Morinaga Institute of Biological Science, Yokohama, Japan), C-peptide (Shibayagi Co., Ltd., Gunma, Japan), adiponectin (CycLex Co., Ltd., Nagano, Japan), resistin (R&D Systems, Inc., Minneapolis, MN, U.S.A.), tumor necrosis factor-α (TNF-α) (Endogen, Woburn, MA, U.S.A.), and interleukin-6 (IL-6) (eBioscience; San Diego, CA, U.S.A.) levels were estimated based on enzyme-linked immunosorbent assays. The serum ROS level was investigated by employing the method of Ali *et al.*,[23] and the TBA-reactive substance (TBARS) level was determined using the method of Naito and Yamanaka.[24]

Measurement of Insulin and C-Peptide Contents in the Pancreas The insulin and C-peptide contents of pancreatic tissue were estimated using the method of Portha *et al.*[25] In brief, the pancreas was homogenized for 1 min by ultrasonic disintegration at 4°C in acid-alcohol solution (75% ethanol, 1.5% 12 mol/L HCI 23.5% distilled water). After 1 night at −20°C, the extracts were centrifuged, and the insulin and C-peptide concentrations of the supernatants were estimated with the immunosorbent assay kit.

Determination of ROS Generation and TBARS Levels in the Pancreas ROS generation was measured employing the method of Ali *et al.*[23] Pancreatic tissues were homogenized on ice with 1 mM ethylenediamine tetraacetic acid (EDTA)–50 mM sodium phosphate buffer (pH 7.4), and then 25 mM DCFH-DA was added to homogenates. After incubation for 30 min, the changes in fluorescence values were determined at an excitation wavelength of 486 nm and emission wavelength of 530 nm. The pancreatic TBARS content was determined using the method of Mihara and Uchiyama.[26] Serum ROS generation was measured using the method of Ali *et al.*,[23] and the serum TBARS level was determined employing the method of Naito and Yamanaka.[24]

Preparation of Nuclear and Post-Nuclear Fractions Nuclear protein extraction was carried out according to the method of Komatsu.[27] Briefly, pancreatic tissue was homogenized with ice-cold lysis buffer containing 5 mM Tris–HCl (pH 7.5), 2 mM $MgCl_2$, 15 mM $CaCl_2$, and 1.5 M sucrose, and then 0.1 M dithiothreitol (DTT) and protease inhibitor mixture solution were added. After centrifugation (10500×g for 20 min at 4°C), the pellet was suspended with extraction buffer containing 20 mM 2-[4-(2-hydroxyethyl)-1-piperazyl]ethanesulfonic acid (pH 7.9), 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, and 25% (v/v) glycerol, and then 0.1 M DTT and protease inhibitor mixture solution were added. The mixture was placed on ice for 30 min, and then the nuclear fraction was prepared by centrifugation at 20500×g for 5 min at 4°C. The post-nuclear fraction was extracted from the pancreas of each mouse as described below. In brief, pancreatic tissue was homogenized with ice-cold lysis buffer (pH 7.4) containing 137 mM NaCl, 20 mM Tris–HCl, 1% Tween 20, 10% glycerol, 1 mM PMSF, and protease inhibitor mixture solution. The homogenate was then centrifuged at 2000×g for 10 min at 4°C. The protein concentration in each fraction was determined using a Bio-Rad protein kit (Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Immunoblotting Analyses For the estimation of AP-1 and NF-κB p65, 15 μg of protein from each nuclear fraction was electrophoresed through an 8% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE). Separated proteins were transferred to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and then incubated with primary antibodies to AP-1, NF-κB p65, and histone, respectively, overnight at 4°C. After the blots were washed, they were incubated with anti-rabbit or anti-mouse IgG HRP-conjugated secondary antibody for 1.5 h at room temperature. Also, 15 μg of protein of each post-nuclear fraction of JNK, p-JNK, COX-2, iNOS, TGF-β_1, and fibronectin was electrophoresed through 8–15% SDS-PAGE. Each antigen-antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with LAS-4000 (FUJIFILM, Tokyo, Japan). Band densities were measured using ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to histone and/or β-actin. The protein levels of groups are expressed relative to those of *m*/*m* mice (represented as 1).

Histological Analysis The excised parts of pancreatic tissue were immediately fixed in 10% neutral-buffered formalin, processed as paraffin-embedded sections, and stained with Azan. Microscopic examination was carried out by a pathologist who was unaware of the groups of mice.

Statistical Analysis The data are expressed as means± S.E.M. Significance was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test (SPSS 11.5.1 for Windows, 2002, SPSS Inc., U.S.A.). Values of $p<0.05$ were considered significant.

RESULTS

Body Weight Gain, Food Intake, and Water Intake As Table 1 shows, the initial, final, and gain in body weights, and the amounts of food and water intake in *db*/*db* mice were significantly higher than those in *m*/*m* mice. Compared to the vehicle-treated *db*/*db* mice, the body weights were not changed by GS treatment throughout the experimental periods. However, the administration of GS led to a significant decrease of food intake in a dose-dependent manner. The water intake

showed a tendency toward a slight decrease (without significance) by 20 and 100 mg/kg of GS treatment for 6 weeks.

Hematological Analyses Table 2 shows the effect of GS on the serum constituents. GS administration significantly reduced serum leptin and insulin levels at a dose of 100 mg/kg, and the C-peptide level at doses of 20 and 100 mg/kg, while the serum glucose level was slightly decreased without significance. In addition, resistin, TNF-α, and IL-6 levels were increased in the *db*/*db* control group compared to the *m*/*m* group, and reduced by GS administration. Regarding the adiponectin level, the oral administration of GS at a dose of 100 mg to *db*/*db* mice significantly increased the decreased level. Meanwhile, the level of ROS and TBARS in *db*/*db* mice treated orally with 20 and 100 mg/kg body weight of GS decreased in a dose-dependent manner.

Pancreatic Insulin and C-Peptide Contents The levels of insulin and C-peptide in the pancreas of vehicle-treated *db*/*db* mice were significantly decreased compared to those of *m*/*m* mice (Fig. 2). GS administration at doses of 20 and 100 mg/kg led to a significant increase in the insulin level in the pancreas, and the decreased C-peptide level was significantly increased in GS-treated *db*/*db* mice at a dose of 100 mg/kg.

Biomarkers Associated with Oxidative Stress in the Pancreas As shown in Fig. 3, the levels of ROS and TBARS in the pancreas of vehicle-treated *db*/*db* mice were higher than those of *m*/*m* mice, whereas these enhanced levels were significantly reduced by GS treatment nearly to the level of *m*/*m* mice.

Pancreatic Oxidative Stress-Related Protein Expressions We performed Western blot analyses to clarify the role of GS in pancreatic oxidative damage by JNK, p-JNK, and AP-1. As shown in Fig. 4, these proteins showed significantly elevated expressions in vehicle-treated *db*/*db* mice in comparison with *m*/*m* mice. Up-regulated pancreatic JNK, p-JNK, and AP-1 protein expressions were reduced by 20 and 100 mg/kg GS treatment, nearly to the levels of *m*/*m* mice (Figs. 4A–C).

Pancreatic Inflammation-Related Protein Expressions Expression levels of inflammation-related protein were enhanced in the pancreas of *db*/*db* mice, and the results are presented in Fig. 5. Regarding NF-κB p65 protein expression, the oral administration of GS at a dose of 100 mg to *db*/*db* mice significantly decreased the enhanced expression (Fig. 5A). In the case of COX-2 protein, groups administered GS showed decreased expressions but without significance (Fig. 5B), while 100 mg GS treatment significantly reduced the level of iNOS protein (Fig. 5C).

Pancreatic Fibrosis-Related Protein Expressions To assess the effect of GS on pancreatic fibrosis, we quantified the fibrosis-related protein TGF-β_1 and fibronectin expressions. As shown in Fig. 6A, the elevated expression of TGF-β_1 protein in vehicle-treated *db*/*db* mice was down-regulated by the administration of GS. In particular, a marked down-regulation of TGF-β_1 protein was observed in the group administered 100 mg/kg. Fibronectin protein expression was markedly higher in vehicle-treated *db*/*db* mice than *m*/*m* mice, but both 20 and 100 mg GS-treated mice showed significantly reduced expressions compared to those of vehicle-treated *db*/*db* mice,

Table 1. Body Weight, Food Intake, and Water Intake

Group	Dose (mg/kg body weight/d)	Body weight			Food intake (g/d)	Water intake (mL/d)
		Initial (g)	Final (g)	Gain (g/6 weeks)		
m/*m*	—	21.6±0.5**	25.0±0.6**	3.4±0.1**	3.2±0.2**	3.8±0.3**
db/*db*						
Vehicle	—	40.2±0.7	47.0±0.9	6.8±0.7	7.0±0.1	19.4±4.0
GS	20	40.2±0.6	45.6±1.4	5.4±0.8	6.2±0.1*	13.2±0.1
GS	100	39.9±0.6	44.4±1.2	4.5±1.0	5.7±0.1*	12.8±0.1

m/m, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated. *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.05$, **$p<0.001$ *vs.* vehicle-treated *db*/*db* mouse values.

Table 2. Hematological Analyses

Group	Dose (mg/kg body weight/d)	Glucose (mg/dL)	Leptin (ng/dL)	Insulin (ng/mL)	C-Peptide (pg/mL)	Adiponectin (ng/mL)
m/*m*	—	170±25***	2.14±0.31***	1.94±0.13**	165±17***	6.10±0.31***
db/*db*						
Vehicle	—	774±40	19.85±0.35	3.51±0.44	2240±283	3.26±0.12
GS	20	758±30	19.21±0.67	2.57±0.11	1489±259*	3.61±0.12
GS	100	696±38	17.39±1.07*	2.45±0.08*	1338±206**	4.45±0.18***
Group	Dose (mg/kg body weight/d)	Resistin (pg/mL)	TNF-α (pg/mL)	IL-6 (ng/mL)	ROS (fluorescence/min/mL)	TBARS (nmol/mL)
m/*m*	—	529±9***	149±12*	9.24±1.25**	577±79***	12.16±1.55***
db/*db*						
Vehicle	—	561±16	243±30	19.34±2.16	1399±127	21.51±1.05
GS	20	376±20***	166±32**	15.88±2.38	853±93**	12.97±1.71***
GS	100	348±17***	144±14**	12.35±1.50*	789±77***	9.01±0.99***

m/m, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated. *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.05$, **$p<0.01$, ***$p<0.001$ *vs.* vehicle-treated *db*/*db* mouse values.

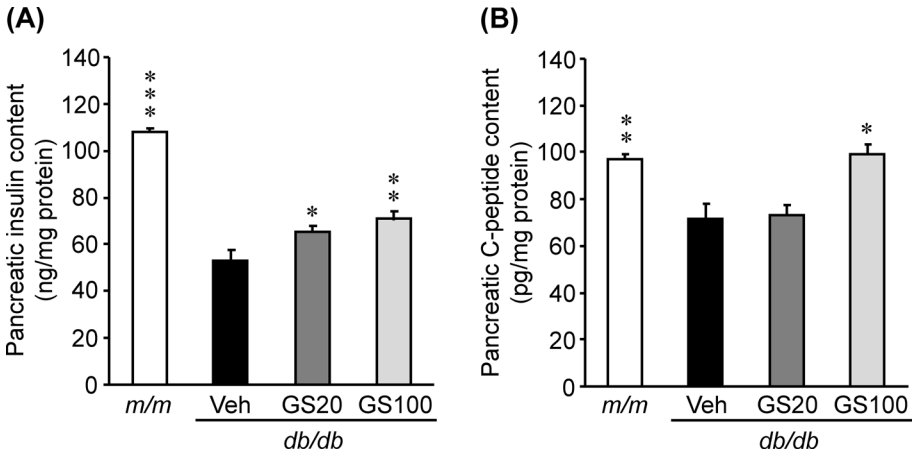

Fig. 2. Insulin (A) and C-Peptide (B) Contents in the Pancreas

m/*m*, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.05$, **$p<0.01$, ***$p<0.001$ *vs.* vehicle-treated *db*/*db* mouse values.

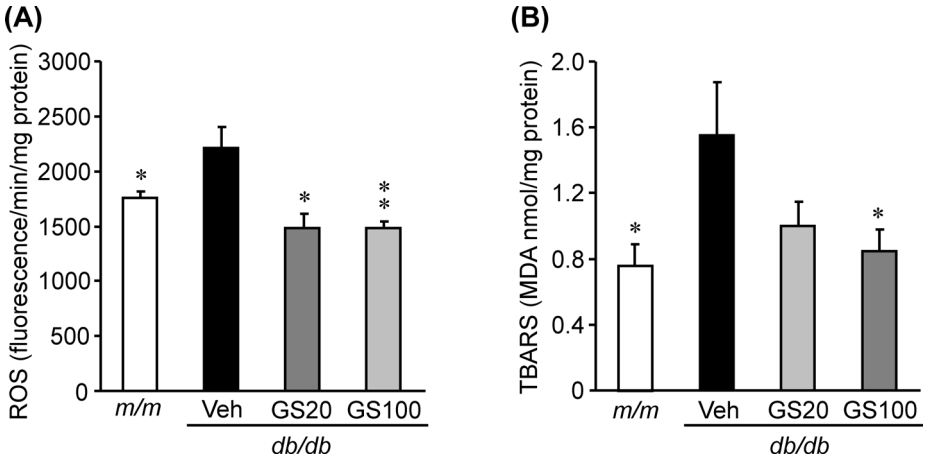

Fig. 3. ROS (A) and TBARS (B) Levels in the Pancreas

m/*m*, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.05$, **$p<0.01$ *vs.*. vehicle-treated *db*/*db* mouse values.

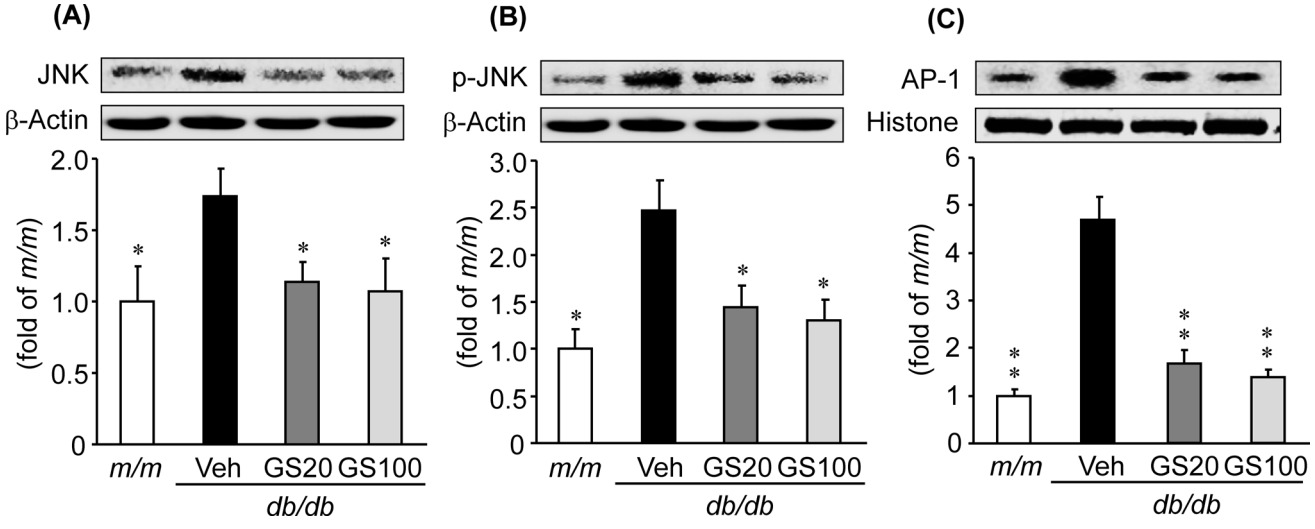

Fig. 4. JNK (A), p-JNK (B), and AP-1 (C) Protein Expressions in the Pancreas

m/*m*, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.05$, **$p<0.01$ *vs.* vehicle-treated *db*/*db* mouse values.

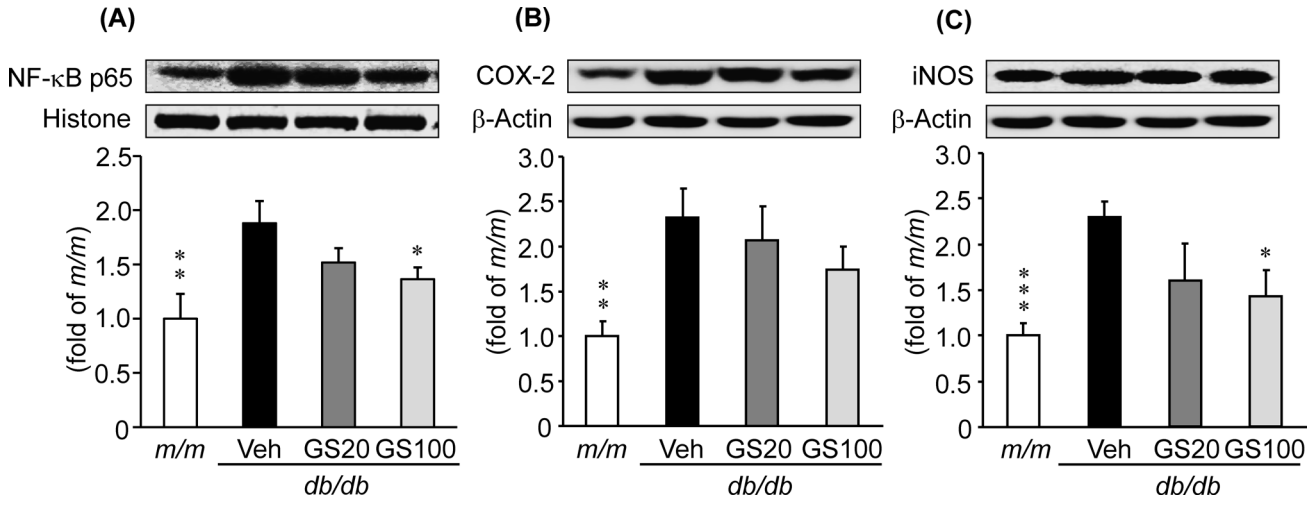

Fig. 5. NF-κB p65 (A), COX-2 (B), and iNOS (C) Protein Expressions in the Pancreas

m/*m*, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.05$, **$p<0.01$, ***$p<0.001$ *vs.* vehicle-treated *db*/*db* mouse values.

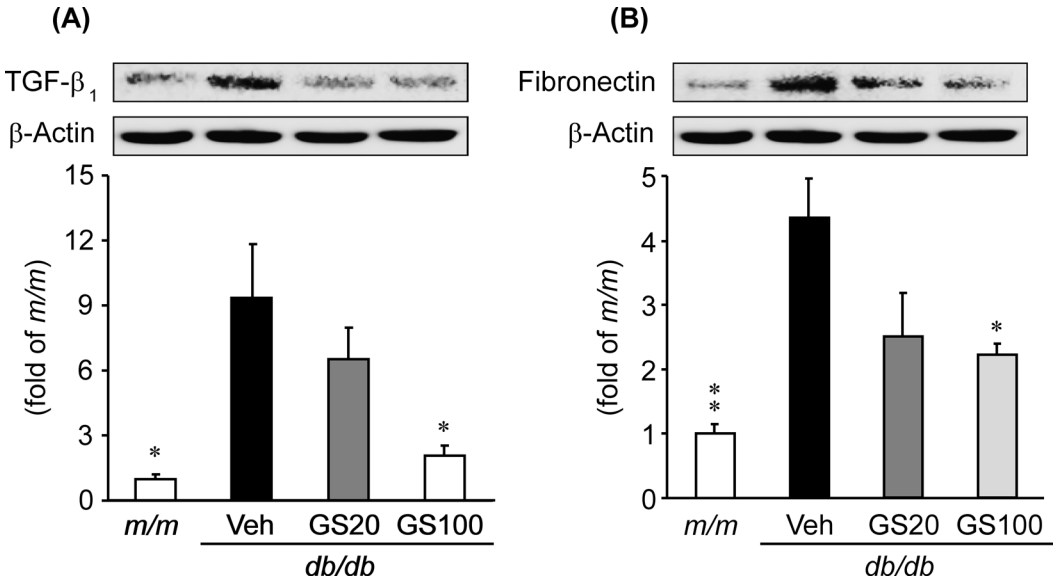

Fig. 6. TGF-β_1 (A) and Fibronectin (B) Protein Expressions in the Pancreas

m/*m*, misty; Veh, vehicle-treated *db*/*db* mice; GS20, GS 20 mg/kg body weight-treated *db*/*db* mice; GS100, GS 100 mg/kg body weight-treated *db*/*db* mice. The results are presented as the means±S.E.M. *$p<0.01$, **$p<0.001$ *vs.* vehicle-treated *db*/*db* mouse values.

respectively (Fig. 6B).

Histology To evaluate pancreatic fibrosis, sections of pancreatic tissue obtained from *m*/*m* and *db*/*db* mice were stained with Azan. Figures 7B–D show representative blue-stained fibrotic tissue sections from vehicle-treated *db*/*db* and GS-treated mice, respectively, while Fig. 7A shows a relative *m*/*m* mouse. The administration of GS showed a reduction of the blue-stained section.

DISCUSSION

Phenolic compounds are phytochemicals widely distributed in the human diet through the intake of plant-derived products that are abundant in fruit, vegetables, cereals, cocoa derivatives, and nuts, as well as in beverages such as tea, coffee, soy milk, and red wine.[28–30] These phenolic compounds are the most beneficial antioxidants in the human diet.[31] Many studies have discovered that the intake of natural antioxidants is correlated with a low occurrence of cancer, heart disease, diabetes, and age-related diseases, but there are still controversial opinions.[32–34] We previously reported that GS, a bioactive phenolic compound isolated from the fruit of Corni Fructus, exhibited hepato- and reno-protective effects in insulin-resistant type 2 diabetic *db*/*db* mice. It showed lipid-lowering and -ameliorating effects *via* down-regulating the expression of sterol regulatory element binding protein-1 through increased serum adiponectin secretion, protective effects against oxidative stress, and advanced glycation endproduct formation, and protective potential through the inhibition of oxidative stress-sensitive mechanisms of the pro-inflammatory response and apoptosis by decreased serum cytokines such as TNF-α and IL-6 levels in the liver and kidney of those with type 2 diabetes.[22,35,36] However, the effects of GS on inflammation linked to oxidative stress damage and the development of pancreatic

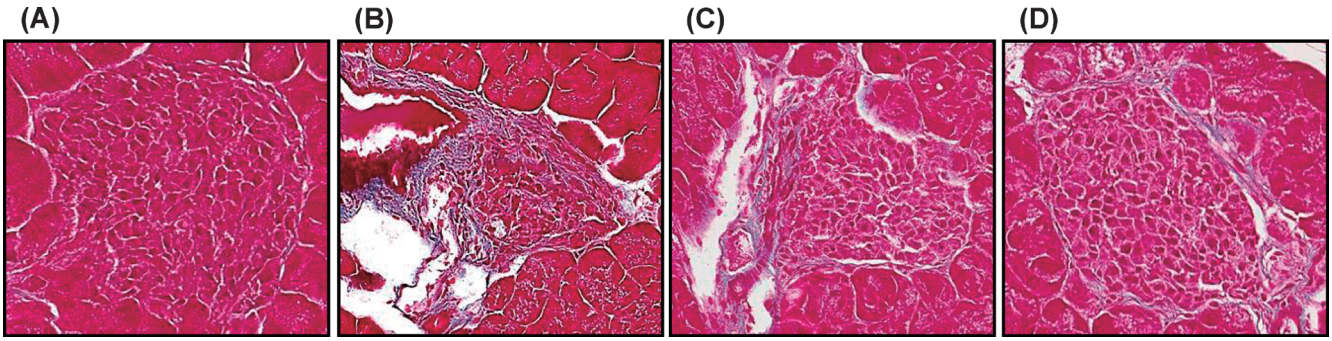

Fig. 7. Azan Staining of Pancreatic Tissue

(A) Misty, (B) Vehicle-treated *db*/*db* mice, (C) GS 20 mg/kg body weight-treated *db*/*db* mice, and (D) GS 100 mg/kg body weight-treated *db*/*db* mice. ×20.

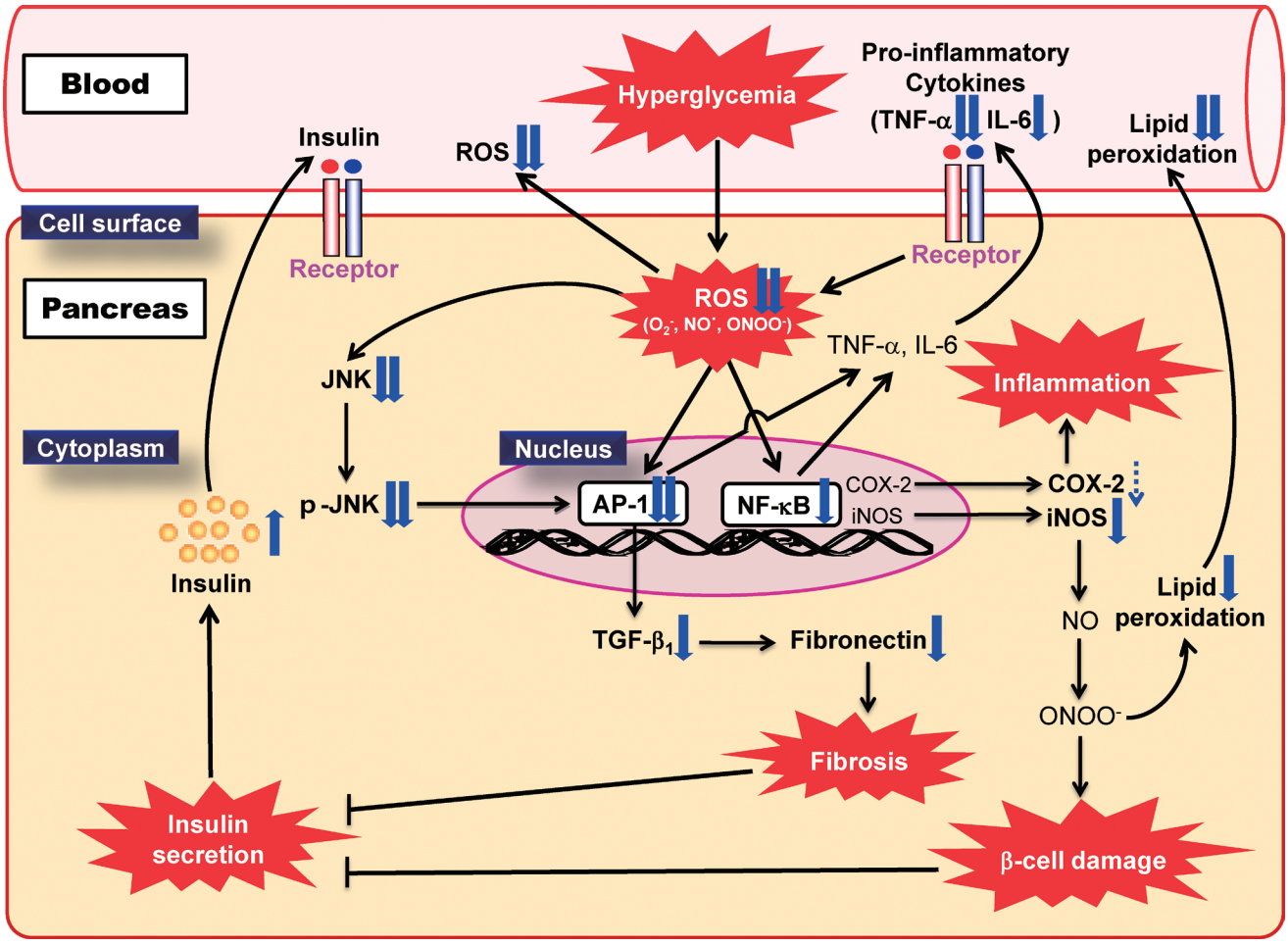

Fig. 8. Predictable Mechanisms in Pancreatic Tissues Administered 7-*O*-Galloyl-D-sedoheptulose

disorders have not been investigated.

In this study, we assessed the protective effects of GS against type 2 diabetes by investigating pinpointing markers in the serum and pancreas of *db*/*db* mice, thereby highlighting GS as a promising anti-diabetic agent for type 2 diabetes. To the best of our knowledge, our present work provides the first evidence that GS derives its anti-diabetic actions from its ability to suppress inflammation and inflammation-related oxidative stress in the serum and pancreas, as shown in the type 2 diabetic experimental mouse model we employed.

Table 2 shows that GS ameliorates enhanced blood glucose in vehicle-treated *db*/*db* mice, data that are in agreement with our previous study revealing that *db*/*db* mice showed a diabetic status[19] and further showing that the increased leptin in vehicle-treated *db*/*db* mice indicated a deficiency in leptin receptors in *db*/*db* mice.[37] This current study shows that the serum insulin level was significantly higher in the vehicle-treated *db*/*db* group compared to the *m*/*m* group. The serum C-peptide level was compared as an indirect biomarker of insulin secretion. As expected, there was a significant increase in the serum C-peptide level in the vehicle-treated *db*/*db* group, which was closely associated with the increased removal of blood glucose. Thus, GS treatment prevents diabetes in *db*/*db* mice, as evidenced by improved insulin sensitivity through

the maintenance of normal insulin and glucose levels and the preservation of insulin and C-peptide levels in the pancreas (Fig. 2), meaning that GS can ameliorate impaired glucose and insulin tolerance in *db*/*db* mice. These results correlate with a previous report showing that anti-diabetic treatment advanced insulin sensitivity and the protection of insulin and C-peptide levels in the pancreas of *db*/*db* mice.[38)]

Oxidative stress is produced under diabetic conditions and is likely implicated in the development of pancreatic β-cell dysfunction found in diabetes as pancreatic β-cells are vulnerable to oxidative stress. For example, when pancreatic β-cells were exposed to oxidative insult, insulin gene expression was markedly suppressed, and when *db*/*db* mice were treated with antioxidants, glucose tolerance was diminished.[39)] As shown in Table 2 and Fig. 3, GS treatment suppressed serum and pancreatic ROS and lipid peroxidation, indicating that GS treatment protects against oxidative insults in the serum and pancreas of *db*/*db* mice. These results further support our previous study,[19)] showing that an anti-oxidative action is an important mechanism in protection against type 2 diabetes.[22)] In addition, our current study also provided a new revelation regarding the anti-diabetogenic action of GS by documenting that it suppressed several key pro-inflammatory molecular mediators, like TNF-α, IL-6, NF-κB p65, COX-2, JNK, p-JNK, and TGF-β_1, as shown in Table 2 and Figs. 4–6.

Previously, we proposed that the suppression of inflammation is possibly linked to anti-diabetic effects,[18)] and other studies have reported that type 2 diabetes can occur through mechanisms related to the inflammatory state.[2)] As inflammation is considered a major factor contributing to type 2 diabetes,[2)] we examined pro-inflammatory markers including TNF-α and IL-6 in the serum, and found that GS treatment inhibited serum TNF-α and IL-6 (Table 2). Our data on the suppression of serum TNF-α and IL-6 are associated with our previous report presenting data that the anti-diabetic action occurs from an inhibition of inflammation.[18)]

We further examined pro-inflammatory NF-κB p65, COX-2, and TGF-β_1 protein levels in the pancreas of *db*/*db* mice, and found that GS treatment down-regulated levels of NF-κB p65, COX-2, and TGF-β_1, suggesting that GS treatment had anti-diabetic effects due to its anti-inflammatory actions (Figs. 5, 6). These results showing the amelioration of pro-inflammatory markers, *i.e.*, NF-κB p65, COX-2, and TGF-β_1 protein expressions, are in parallel with a recent report showing enhanced COX-2 protein expression due to NF-κB activation.[40)] These results also confirm our previous work[18)] that the suppression of inflammation by the modulation of NF-κB p65 and COX-2 is a critical factor contributing to an anti-diabetic action.

It also has been shown that polyphenolic compounds can modulate inflammatory responses *via* the inhibition of COX-2 protein expression through the suppression of JNK activation and inhibition of pro-inflammatory mediators, like TNF-α, by attenuation of the NF-κB and JNK pathways.[41)] As shown in Fig. 4, GS modulated the activation of the JNK and NF-κB pathways. These data are consistent with a previous report showing that not only the modulation of oxidative stress and consequent activation of the JNK pathway but also the suppression of inflammation are implicated in the development of β-cell dysfunction found in diabetes, which, therefore, would make these useful therapeutic targets against diabetes.[42)]

Adipocytes, particularly those located within visceral fat, are known as major secretors of both pro- and anti-inflammatory factors, often referred to as adipokines, and several inflammatory biomarkers secreted by adipose tissue, such as IL-6, IL-1β, and TNF-α, have been cited as independent predictors of diabetes.[43)] As Table 2 shows, GS produces its anti-inflammatory effects *via* the regulation of adiponectin, IL-6, and TNF-α, indicating that the anti-inflammatory properties of GS result in protection against insulin resistance, results which are related to a previous report,[43)] revealing that the suppression of inflammation *via* the modulation of adiponectin, IL-6, and TNF-α is an important protective factor against insulin resistance. It was reported that NF-κB results in insulin resistance by activating pro-inflammatory cytokines like TNF-α, IL-6, IL-1β, and resistin, which consequently activates the JNK and NF-κB pathways to create a vicious cycle that will exacerbate tissue damage.[44)]

One of our significant findings in this study is GS's suppression of diverse pro-inflammatory cytokines such as TNF-α, IL-6, resistin, and TGF-β_1 that activate the JNK and NF-κB pathways and pro-inflammatory COX-2 protein expression. In particular, our data showing the suppression of both oxidative stress and inflammation by GS treatment are consistent with our previous report,[18)] presenting a close relationship between anti-oxidative and anti-inflammatory actions in diabetes. Thus, based on the results from both our previous and current works, we suggest a possible mechanism by which the anti-diabetic action of GS mediates type 2 diabetes through its dual suppression of oxidative stress and inflammation, as shown in our experiments with *db*/*db* mice. Consecutively, GS could reduce the increased level of TGF-β_1 in the pancreas, and could show a reduction in pancreatic fibrosis by means of histological evaluation and fibronectin (Figs. 6, 7). These findings suggest that the hyperglycemic control of GS may, at least in part, be derived from the amelioration of pancreatic disorders such as pancreatic fibrosis.

Our study revealed that GS suppresses type 2 diabetes in *db*/*db* mice. An important mechanism of GS's anti-diabetic effect is its capacity to reduce the oxidative stress state by diminishing ROS generation and lipid peroxidation in the pancreas. Our data further suggest that another critical mechanism of GS's anti-diabetic property is its ability to ameliorate inflammation and fibrosis through modulation of the serum TNF-α and IL-6 levels and the pancreatic protein expressions of JNK, p-JNK, AP-1, NF-κB p65, COX-2, iNOS, TGF-β_1, and fibronectin (Fig. 8).

In conclusion, we suggest that GS treatment protects against type 2 diabetes by its dual ameliorating effect on oxidative stress, inflammation and fibrosis. Our present work documents experimental evidence of GS as an anti-diabetic agent, and warrants further clinical investigation.

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