Review

Bioactive constituents of Corni Fructus: The therapeutic use of morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose as renoprotective agents in type 2 diabetes

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ABSTRACT: Corni Fructus, the fruit of *Cornus officinalis Sieb. et Zucc.* (Cornaceae), is an important crude herb used in Chinese medicine to exhibit several biological activities, including hypoglycemic, antineoplastic, and antimicrobial effects, and to improve liver and kidney functions. We have been investigating the mechanism and bioactive constituents of Corni Fructus using diabetic animal models. Morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose, the main active compounds of Corni Fructus, exhibit the same lowering effects of elevated triglyceride, oxidative stress and advanced glycation endproduct (AGE) formation in the kidney of *db/db* mice. The effects of morroniside and 7-*O*-galloyl-D-sedoheptulose were mediated through modulation by renal sterol regulatory element binding proteins and nuclear factor-kappa B expression, but the effect of loganin was presumably mediated by hypoglycemic and antioxidant effects in the kidney, and also indirectly by the amelioration of metabolic disorders in other organs such as the liver. These findings led us to conclude that morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose would synergistically contribute to the inhibition of metabolic disorders (hyperglycemia and dyslipidemia), oxidative stress, inflammation, as well as AGE formation in the diabetic kidney.

Keywords: Corni Fructus, morroniside, loganin, 7-*O*-galloyl-D-sedoheptulose, *db/db* mice

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1. Introduction

Diabetes mellitus is a major cause of mortality and morbidity worldwide, and its prevalence is increasing at an alarming rate. The prevalence of type 2 diabetes mellitus has been predicted to increase markedly during the next few years, reaching 300 million by 2025 (*1*). The increasing prevalence of diabetes is largely due to the rapid spread of obesity, which is considered the most important risk factor for type 2 diabetes mellitus (*2*). Type 2 diabetes, a predominant type of diabetes mellitus accounting for 90% of cases, is characterized by abnormal insulin secretion caused by impaired pancreatic β-cell function and insulin resistance in hepatic, adipose, and peripheral tissues (*3*). As a result of insulin resistance, aggravations of hyperglycemia and dyslipidemia occur, and, consequently, progressive damage to various tissues is induced in type 2 diabetes. Chronic hyperglycemia and dyslipidemia cause oxidative stress and inflammatory responses through the formation of advanced glycation endproducts (AGEs) (*4,5*), activation of the protein kinase C pathway (*6,7*), increased glucose flux through the polyol pathway (*8*), and the accelerated generation of reactive oxygen species (ROS) (*9,10*). The resulting glycative, glycoxidative, and carbonyl lipotoxicity and oxidative stress can play a key role in the pathogenesis of diabetes (*11-14*). Therefore, the attenuation of oxidative stress and regulation of hyperlipidemia have been considered as ways to alleviate diabetes and diabetic complications.

Clinical evidence has suggested that the appropriate use of traditional Chinese medicines with modern Western medicinal, or mainstream antidiabetic drugs, can prevent or ameliorate the development of diabetic complications. Many diabetic patients choose alternative therapeutic approaches such as herbal or traditional Chinese medicine along with mainstream antidiabetic drugs, thus making alternative therapy for diabetes very popular (*15*). However, these medicines

usually have an insufficient scientific basis and the exact mechanisms behind their beneficial effects are unknown. Therefore, recently, based on a large number of chemical and pharmacological research studies, numerous bioactive compounds have been identified in Chinese medicinal plants for diabetes (*16*), and we have investigated the mechanism and bioactive constituents of Corni Fructus, the fruit of *Cornus officinalis Sieb. et Zucc.* (Cornaceae), in diabetic animal models.

Corni Fructus is an important crude herb used in Chinese medicine. It is considered to be one of the 25 plant-based drugs most frequently used in China, Japan, and Korea. It is known to exhibit several biological activities, including hypoglycemic, antineoplastic, and antimicrobial effects, and to improve liver and kidney functions (*17-19*). We previously reported that treatment with Corni Fructus for 10 days suppressed hyperglycemia, proteinuria, renal AGE formation, and related protein expressions, *i.e.*, receptor for AGEs (RAGE), nuclear factor-kappa B (NF-κB), transforming growth factor-beta1, and N^{ε}-(carboxymethyl)lysine (CML), in the same way as with aminoguanidine. However, improvement of the renal function, shown *via* serum creatinine and creatinine clearance, was superior to aminoguanidine treatment (*20*). In addition, the administration of Corni Fructus inhibited the elevation of both systolic and diastolic blood pressures, and lowered serum total cholesterol levels with a decrease in esterified cholesterol in the diet-induced hypercholesterolemia rat model (*21*). Moreover, the atherogenic index was decreased in a dose-dependent manner, suggesting its protective role against cardiovascular disease through regulating cholesterol and lipoprotein levels (*21*). Therefore, Corni Fructus was suggested to have beneficial effects on diabetes and diabetic complications.

The discovery of efficacious components is essential for clarification of the precise mechanisms of herbal medicines. However, studies on the biological activities of the active components in Corni Fructus are limited. Therefore, we have isolated the major active components of Corni Fructus by employing activity-guided fractionation (Figure 1), and the effects of morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose (Figure 2) were assessed on glucose metabolism, AGE formation, oxidative stress, and inflammation in type 2 diabetic kidney damage to identify their effects and mechanism of action in type 2 diabetes. This paper gives a review of our recent findings, with emphasis on the therapeutic potential of the active constituents of Corni Fructus against diabetic renal damage.

2. Effect of morroniside on renal damage in type 2 diabetic mice

To investigate the effect of morroniside on type 2 diabetic renal damage, we employed *db/db* mice. As an experimental model of obesity-associated type 2 diabetes mellitus, *db/db* mice are widely used and well-established (*22,23*). C57BLKS/J *db/db* mice develop diabetes due to a mutation of the mouse diabetes (*db*) gene that encodes a receptor for leptin. The lack of leptin-receptor signaling results in increased food intake in combination with a phenotype of reduced energy expenditure, reminiscent of the neuroendocrine starvation response (*24*). Consequently, homozygotes (*db/db*) after birth show uncontrolled eating behavior, become obese, and by 3-6 months after birth, develop severe insulin resistance associated with hyperinsulinemia, hyperglycemia, and hyperlipidemia. The *db/db* vehicle-treated group (n = 10) was orally administered water, while the other two groups (n = 10 per group) were orally administered morroniside at a dose of 20 or 100 mg per kg body weight per day for 8 weeks, respectively. The non-diabetic *m/m* mice (n = 6) as a normal group were compared with the diabetic groups.

Consistent with an earlier report (*25*), the body weight, food intake, and water intake of *db/db* mice in this study were markedly higher than those of *m/m* mice due to augmented food consumption in *db/db* mice. The administration of morroniside for 8 weeks led to no difference in body weight and food intake; however, the water intake was significantly reduced in morroniside 100 mg/kg-treated mice (Table 1). These results suggest that the oral administration of morroniside may improve the typical diabetic symptom, an excessive intake of water. The serum glucose, triglyceride, and total cholesterol levels of *db/db* mice were markedly higher than those of *m/m* mice, but no significant changes in the glucose and total cholesterol levels were shown on morroniside administration (Table 2). On the other hand, the elevated serum triglyceride level was significantly decreased in morroniside-treated *db/db* mice in a dose-dependent manner (Table 2).

Abnormal renal lipid metabolism is a major symptom of type 2 diabetes (*26*), and the renal glucose uptake is also markedly increased in type 2 diabetes (*27*). This could explain the accumulation of glucose and fatty acids noted in diabetic kidneys, and may play a role in the development of diabetic nephropathy (*27*). Sun *et al.* (*28*) reported that sterol regulatory element binding protein-1 (SREBP-1) expression was increased in the kidney cortex, resulting in the up-regulation of enzymes responsible for fatty acid synthesis and a high renal triglyceride content as a consequence, which was associated with mesangial expansion and glomerulosclerosis. The transcriptional activation of SREBP-1 can be up-regulated by insulin (*29*), glucose (*10*), and liver X receptor (*30*). The treatment of morroniside led to significant reductions of renal glucose, triglyceride, and total cholesterol contents in *db/db* mice (Figures 3A-3C), which suggested that morroniside effectively prevented the excessive

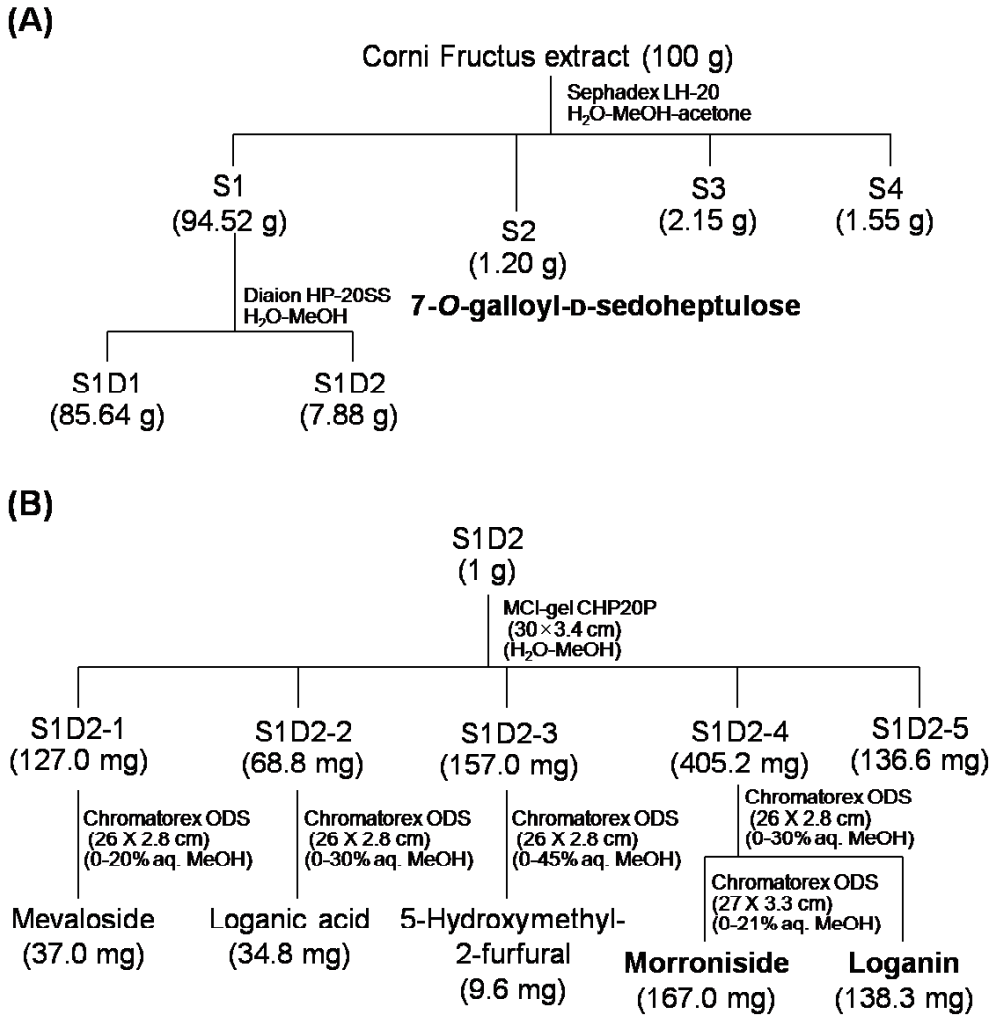

Figure 1. Isolation of morroniside, loganin and 7-*O*-galloyl-D-sedoheptulose from (A) Corni Fructus extract and (B) fraction S1D2. The extract of *Cornus officinalis* (100 g), which was produced by Tsumura & Co. (Tokyo, Japan) was fractionated by Sephadex™ LH-20 column chromatography (32 × 5 cm) with water containing increasing proportions of methanol (0-100%, 10% stepwise gradient elution) and finally 60% acetone to give 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 give S1D1 (85.64 g) and S1D2 (7.88 g). The structures of morroniside, loganin and 7-*O*-galloyl-D-sedoheptulose were confirmed by the further purification and spectrometric identification.

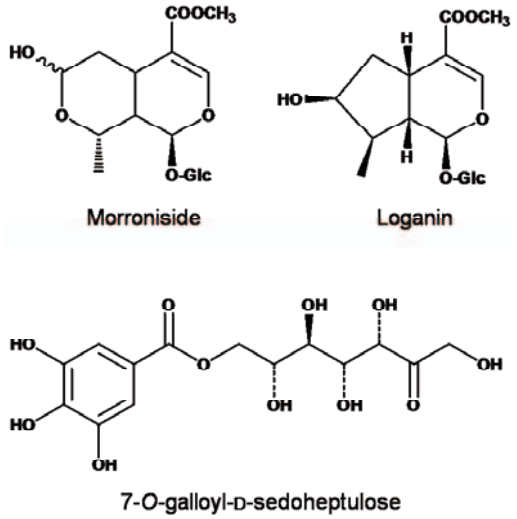

Figure 2. Chemical structure of morroniside, loganin and 7-*O*-galloyl-D-sedoheptulose.

glucose supply and abnormal lipid accumulation in the kidney. Compared with vehicle-treated *db/db* mice, no alteration in PPARα expressions of renal tissue was shown in *m/m* mice (Figure 3D). However, morroniside administration significantly lowered the expression of proteins associated with lipid homeostasis, SREBP-1 and SREBP-2, in the kidney of *db/db* mice (Figures 3E and 3F).

Subsequently, the effects of morroniside on factors related to ROS and inflammation in renal tissues were investigated. Increased thiobarbituric acid-reactive substance (TBARS) formation and oxidative stress induced by ROS production and a reduced ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) are known to decrease insulin sensitivity and increase renal inflammation (*31*). In this study, TBARS and ROS levels also markedly increased in vehicle-treated *db/db* mice, and these biochemical

Table 1. Body weight, food intake and water intake

Group	Body weight			Food intake (g/day)	Water intake (mL/day)
	Initial (g)	Final (g)	Gain (g/8 weeks)		
m/m	18.6 ± 1.8***	25.4 ± 0.9**	6.4 ± 0.1**	2.7 ± 0.2**	4.1 ± 0.2**
db/db					
Veh	41.4 ± 0.3	55.2 ± 2.4	13.8 ± 1.2	7.0 ± 0.2	15.4 ± 1.2
M-20	42.5 ± 0.7	57.9 ± 1.3	15.4 ± 0.7	7.4 ± 0.1	15.4 ± 0.5
M-100	41.6 ± 0.4	57.5 ± 1.3	15.9 ± 0.5	6.6 ± 0.2	11.6 ± 0.9*

m/m, misty; Veh, vehicle-treated *db/db* mice; M-20, morroniside 20 mg/kg body weight-treated *db/db* mice; M-100, morroniside 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs*. vehicle-treated *db/db* mice values.

Table 2. Hematological analyses

Item	*m/m*	*db/db*		
		Veh	M-20	M-100
Glucose (mg/dL)	219.7 ± 12.0***	765.2 ± 44.8	753.8 ± 34.2	713.6 ± 32.3
Triglyceride (mg/dL)	89.6 ± 5.7***	298.7 ± 24.6	229.8 ± 24.8*	175.4 ± 19.1**
Total cholesterol (mg/dL)	74.0 ± 1.3***	168.5 ± 8.3	160.4 ± 11.2	173.8 ± 4.2

m/m, misty; Veh, vehicle-treated *db/db* mice; M-20, morroniside 20 mg/kg body weight-treated *db/db* mice; M-100, morroniside 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs*. vehicle-treated *db/db* mice values.

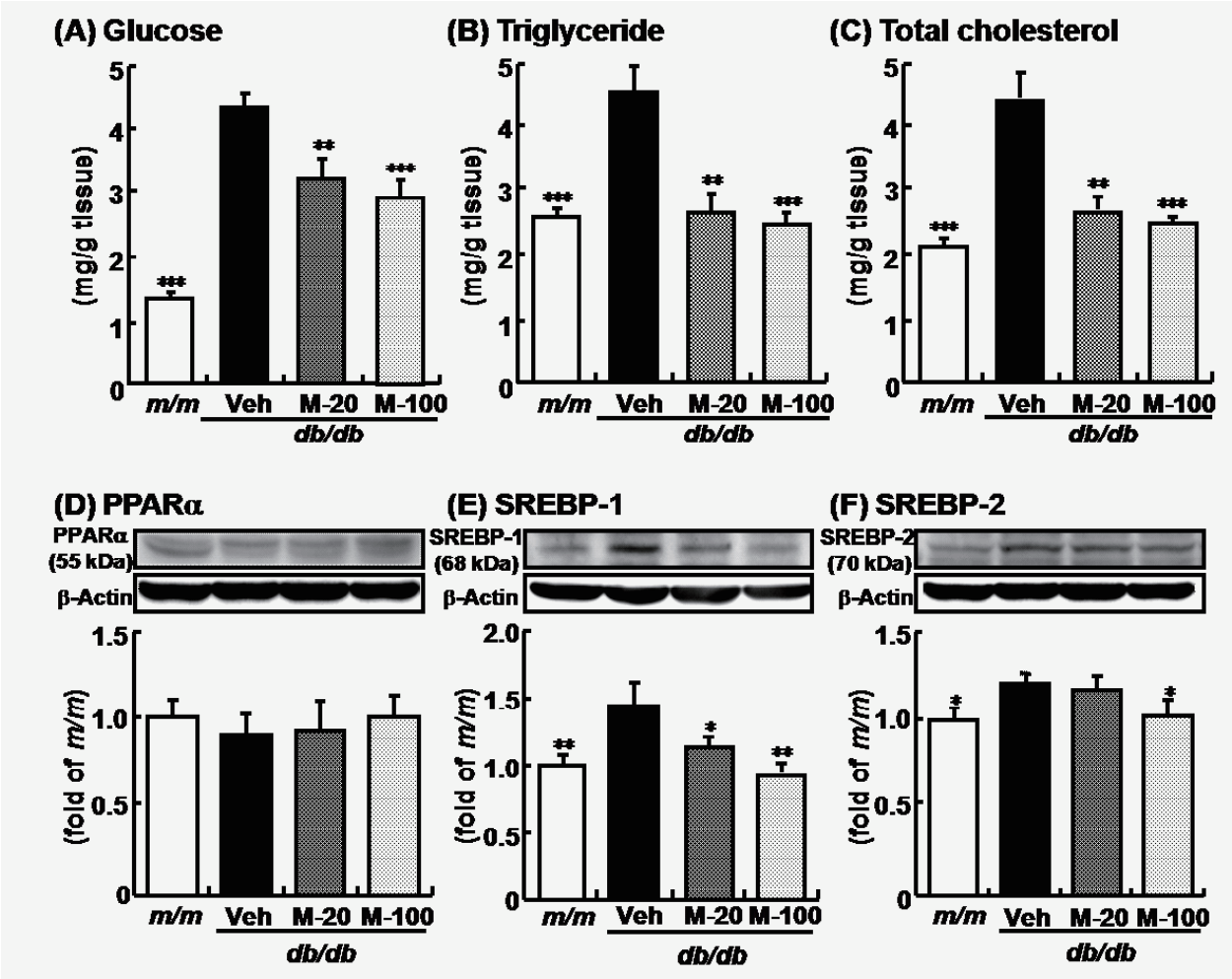

Figure 3. The glucose, triglyceride and total cholesterol contents and the protein expressions related to lipid metabolism in the kidney. (A) Glucose content, **(B)** triglyceride content, **(C)** total cholesterol content, **(D)** PPARα expression, **(E)** SREBP-1 expression, **(F)** SREBP-2 expression. *m/m*, misty; Veh, vehicle-treated *db/db* mice; M-20, morroniside 20 mg/kg body weight-treated *db/db* mice; M-100, morroniside 100 mg/kg body weight-treated *db/db* mice. The results are presented as the means ± S.E. ($n = 6$ or 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

factors were significantly reduced by oral morroniside administration in a dose-dependent manner (Table 3). Also, the reduced ratio of GSH/GSSG in vehicle-treated *db/db* mice in the kidney was increased by morroniside treatment almost to the level of *m/m* normal control mice (Table 3).

Hyperglycemia also causes oxidative stress due to the increased mitochondrial production of superoxide, including the depletion of NADPH and consequent disturbance of glutathione and nitric oxide metabolism. These oxidative stresses are responsible for the regulation of the transcriptional pathways of NF-κB (*32*), which is a transcription factor thought to play an important role in the onset of inflammation (*33*). NF-κB activation can lead to the enhanced expression of proinflammatory cytokines, chemokines, adhesion molecules, inflammatory receptors, and inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (*34-36*). Therefore, the modulation of NF-κB activation may provide a direct way of inhibiting inflammatory mediators (*37*). From the analysis of renal protein expression, the administration of morroniside could reduce the elevated renal NF-κBp65, COX-2, and iNOS levels (Figure 4). These results suggest that the administration of morroniside can alleviate renal damage induced by ROS through the deactivation of NF-κB and subsequent restoration of the antioxidative state.

In summary, morroniside has beneficial effects against type 2 diabetic renal damage mediated by a decrease in augmented concentrations of glucose, triglyceride, and cholesterol *via* the down-regulation of SREBP-1 and SREBP-2 proteins in the kidney of *db/db* mice. Also, morroniside ameliorated oxidative stress and its related inflammation in the kidney. Consequently, the protective role of morroniside against type 2 diabetic renal damage was suggested to be mediated by the amelioration of metabolic disorders including dyslipidemia, oxidative stress, and the inflammatory response.

3. Effect of loganin on renal damage in type 2 diabetic mice

To identify the effect of loganin on type 2 diabetic renal damage, *db/db* mice (n = 10 per group) were orally

Table 3. Biomarkers associated with oxidative stress in kidney

Item	*m/m*	*db/db*		
		Veh	M-20	M-100
TBARS (nmol/mg protein)	1.24 ± 0.03***	1.90 ± 0.09	1.54 ± 0.07**	1.30 ± 0.06*
ROS (Fluorescence/min/mg protein)	2,168 ± 33***	3,086 ± 185	2,017 ± 93***	1,769 ± 53*
GSH (μmol/mg protein)	7.44 ± 0.25***	4.45 ± 0.15	6.61 ± 0.30**	7.41 ± 0.26***
GSSG (μmol/mg protein)	6.29 ± 0.43*	5.12 ± 0.31	6.24 ± 0.19*	6.33 ± 0.10*
GSH/GSSG	1.20 ± 0.05***	0.89 ± 0.05	1.07 ± 0.06**	1.17 ± 0.03***

m/m, misty; Veh, vehicle-treated *db/db* mice; M-20, morroniside 20 mg/kg body weight-treated *db/db* mice; M-100, morroniside 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

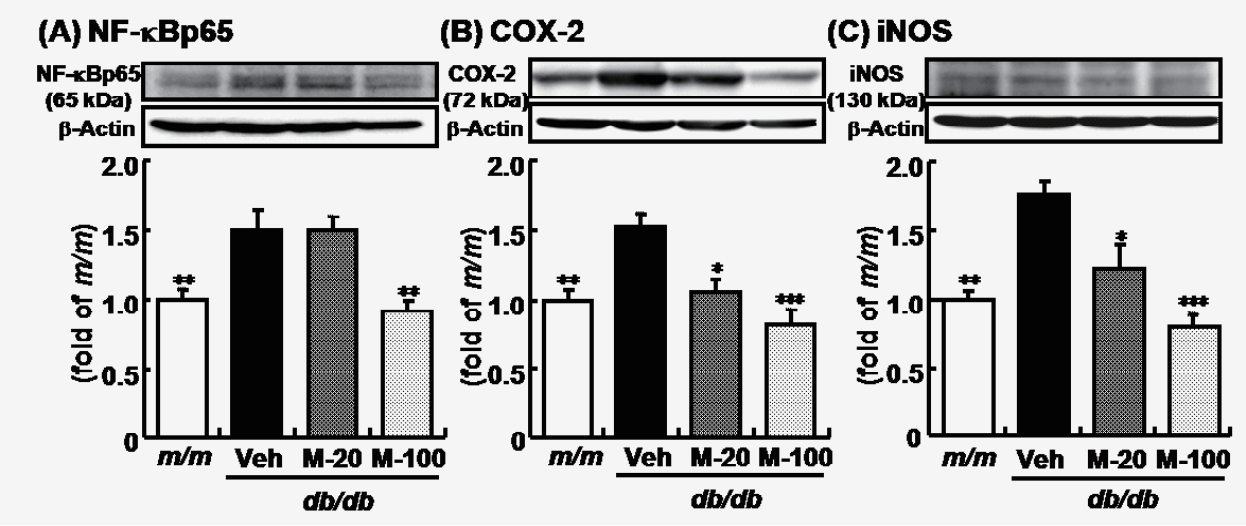

Figure 4. The protein expressions related inflammation in the kidney. (A) NF-κBp65 expression, **(B)** COX-2 expression, **(C)** iNOS expression. *m/m*, misty; Veh, vehicle-treated *db/db* mice; M-20, morroniside 20 mg/kg body weight-treated *db/db* mice; M-100, morroniside 100 mg/kg body weight-treated *db/db* mice. The results are presented as the means ± S.E. (n = 6 or 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

administered loganin at a dose of 20 or 100 mg per kg body weight per day for 8 weeks, respectively. The non-diabetic *m/m* mice ($n = 6$) as a normal group were compared with the diabetic groups.

The *db/db* mice displayed typical phenotypes of obesity-induced diabetes with a marked increase in body weight gain, and food and water intakes. Although there were no changes in the body weight and water intake, food consumption was significantly reduced in loganin-treated groups after an 8-week experimental period (Table 4). The serum glucose, triglyceride and total cholesterol levels of *db/db* mice were markedly higher than those of *m/m* mice (Table 5). The loganin 100 mg/kg-treated *db/db* mice showed a decrease in serum glucose (Table 5) presumably caused by the reduced food intake. Several studies have shown that just few days of caloric restriction can induce marked improvements in glycemic control (*39*). Furthermore, the 8-week administration of loganin to *db/db* mice significantly improved the serum lipid profile with dose-dependent reductions of triglyceride; however, the total cholesterol level remained unchanged (Table 5). The levels of glucose, triglyceride, and total cholesterol in the kidney of vehicle-treated *db/db* mice were significantly elevated compared to those of *m/m* mice (Figures 5A-5C), but loganin administration at 100 mg/kg led to a marked decrease in the triglyceride level in the kidney of *db/db* mice (Figure 5B). However, compared with vehicle-treated *db/db* mice, no alteration in PPARα expressions of renal tissue was shown in *m/m* mice (Figure 5D). SREBP-1 and SREBP-2 protein expressions were markedly elevated in the kidney of vehicle-treated *db/db* compared with *m/m* mice (Figures 5E and 5F), but there were no changes on loganin treatment. These results suggest that the lipid-lowering effect of loganin may be mediated by its effect on other organs such as the liver.

As shown in Table 6, the levels of TBARS and ROS in the kidney of vehicle-treated *db/db* mice were higher than those of *m/m* mice, whereas these enhanced levels were significantly reduced by loganin treatment nearly to the level of *m/m* mice. The *db/db* vehicle group showed significantly decreased GSH/GSSG ratios in the kidney compared with the *m/m* group, which resulted from the decreased GSH and increased GSSG, but this reduction in the GSH/GSSG ratio recovered nearly to the level of *m/m* mice on loganin treatment.

The two distinctive AGEs, CML and N^{ε}-(carboxyethyl)lysine (CEL), are formed on proteins by glycoxidation and/or lipid peroxidation pathways. CML accumulates with TBARS in glomerular lesions, resulting in structural and functional alterations in extracellular matrix proteins (*39*). In addition, RAGE is activated by AGEs, and AGE-RAGE interaction increases ROS formation, with the subsequent activation of NF-κB and release of pro-inflammatory cytokines (*40*). In the present study, the enhanced renal protein expressions of NF-κB, COX-2, and iNOS in the kidney of *db/db* mice remained unchanged on loganin administration in *db/db* mice (Figures 6A-6C). The protein expressions of AGE-related proteins were enhanced in the kidneys of *db/db* mice at the age of 17 weeks, but the oral administration of loganin attenuated the increase in CML accumulation (Figure 6E). Therefore, loganin was suggested to have no effect on the inflammatory damage in the kidney, but inhibited AGE accumulation possibly through hypoglycemic effect.

In summary, loganin has a milder effect than morroniside against type 2 diabetic renal damage.

Table 4. Body weight, food intake and water intake

Group	Body weight			Food intake (g/day)	Water intake (mL/day)
	Initial (g)	Final (g)	Gain (g/8 weeks)		
m/m	21.4 ± 0.5**	27.1 ± 0.9**	5.2 ± 0.6**	3.2 ± 0.2**	3.8 ± 0.3**
db/db					
Veh	39.6 ± 0.3	52.7 ± 1.6	13.7 ± 1.1	7.0 ± 0.1	19.4 ± 4.0
L-20	38.6 ± 0.6	52.2 ± 1.4	13.5 ± 0.9	6.2 ± 0.1*	13.2 ± 0.1
L-100	38.5 ± 0.4	50.9 ± 0.9	11.6 ± 0.6	5.7 ± 0.1*	12.8 ± 0.1

m/m, misty; Veh, vehicle-treated *db/db* mice; L-20, loganin 20 mg/kg body weight-treated *db/db* mice; L-100, loganin 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.001$ *vs*. vehicle-treated *db/db* mice values.

Table 5. Hematological analyses

Item	*m/m*	*db/db*		
		Veh	L-20	L-100
Glucose (mg/dL)	206.2 ± 6.5**	854.9 ± 24.3	809.5 ± 25.9	739.8 ± 38.1*
Triglyceride (mg/dL)	87.4 ± 5.4**	303.4 ± 24.5	163.2 ± 16.7**	151.8 ± 14.2**
Total cholesterol (mg/dL)	82.4 ± 6.2**	165.0 ± 11.2	174.6 ± 9.7	156.9 ± 4.9

m/m, misty; Veh, vehicle-treated *db/db* mice; L-20, loganin 20 mg/kg body weight-treated *db/db* mice; L-100, loganin 100 mg/kg body weight-treated *db/db* mice. * $p < 0.01$, ** $p < 0.001$ *vs*. vehicle-treated *db/db* mice values.

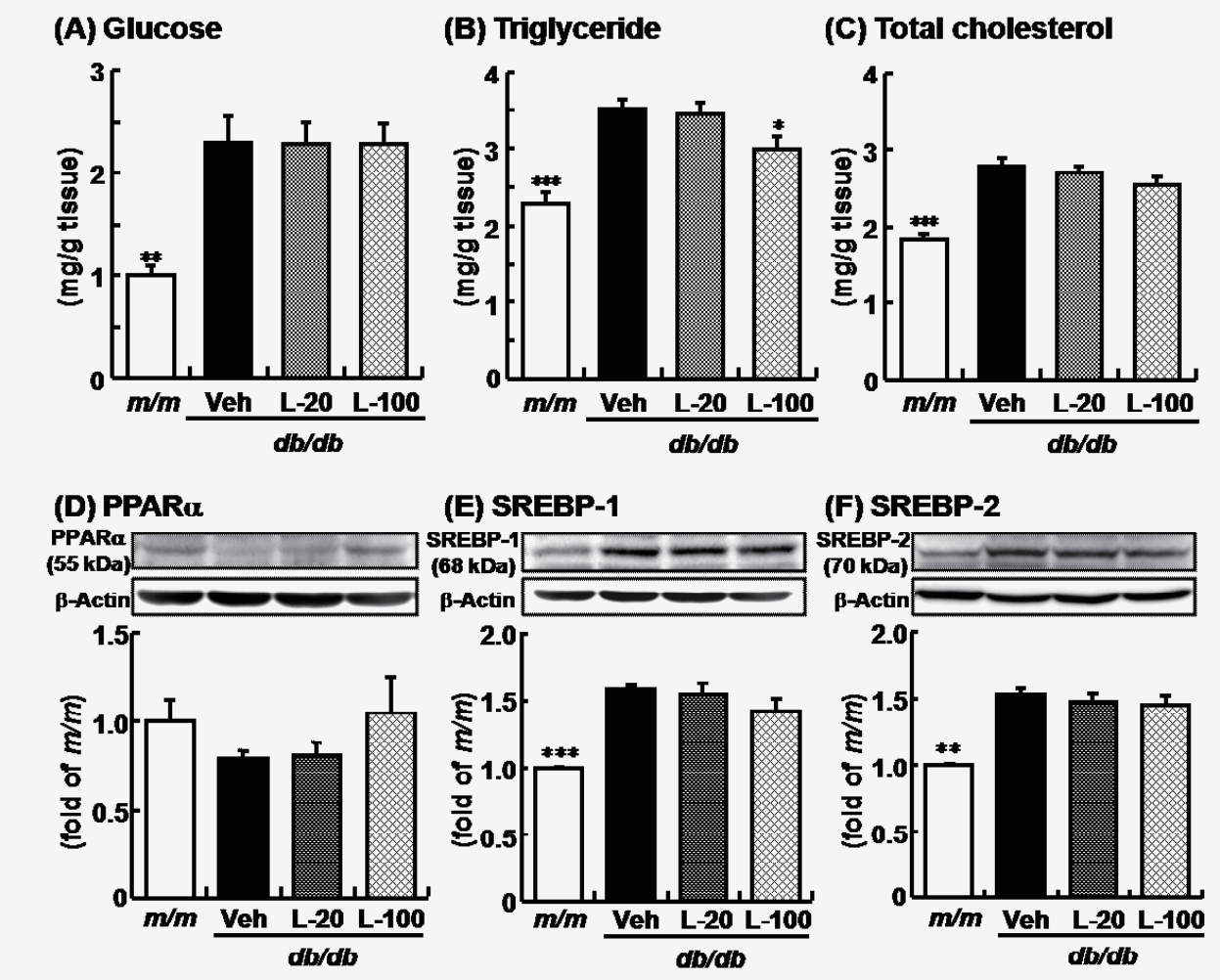

Figure 5. The glucose, triglyceride and total cholesterol contents and the protein expressions related to lipid metabolism in the kidney. (A) Glucose content, **(B)** triglyceride content, **(C)** total cholesterol content, **(D)** PPARα expression, **(E)** SREBP-1 expression, **(F)** SREBP-2 expression. *m/m*, misty; Veh, vehicle-treated *db/db* mice; L-20, loganin 20 mg/kg body weight-treated *db/db* mice; L-100, loganin 100 mg/kg body weight-treated *db/db* mice. The results are presented as the means ± S.E. (n = 6 or 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

Table 6. Biomarkers associated with oxidative stress in kidney

Item	*m/m*	*db/db*		
		Veh	L-20	L-100
TBARS (nmol/mg protein)	1.20 ± 0.02***	1.37 ± 0.01	1.29 ± 0.05	1.27 ± 0.03*
ROS (Fluorescence/min/mg protein)	2,056 ± 34*	2,913 ± 216	1,704 ± 132***	1,745 ± 142***
GSH (μmol/mg protein)	8.45 ± 0.18*	7.66 ± 0.24	8.32 ± 0.19*	8.62 ± 0.18**
GSSG (μmol/mg protein)	2.83 ± 0.08*	3.22 ± 0.11	2.73 ± 0.16*	2.86 ± 0.10*
GSH/GSSG	3.00 ± 0.11***	2.39 ± 0.09	3.13 ± 0.17**	3.04 ± 0.11***

m/m, misty; Veh, vehicle-treated *db/db* mice; L-20, loganin 20 mg/kg body weight-treated *db/db* mice; L-100, loganin 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

However, the administration of loganin led to the suppression of hyperglycemia through the inhibition of food intake. The effect of loganin on type 2 diabetic renal damage was suggested to be mediated by hypoglycemic and antioxidant effects in the kidney, and also indirectly by the amelioration of metabolic disorders including dyslipidemia, oxidative stress, and the inflammatory response in other organs such as the liver.

4. Effect of 7-*O*-galloyl-D-sedoheptulose on renal damage in type 2 diabetic mice

To identify the effect of 7-*O*-galloyl-D-sedoheptulose on type 2 diabetic renal damage, *db/db* mice (n = 10 per group) were orally administered 7-*O*-galloyl-D-sedoheptulose at a dose of 20 or 100 mg per kg body weight per day for 8 weeks, respectively. The non-diabetic *m/m* mice (n = 6) as a normal group were

compared with the diabetic groups.

As shown in the results, the initial, final, and gain of body weights, and the levels of food and water intake in *db/db* mice were significantly higher than those in *m/m* mice (Table 7). Compared with the vehicle-treated *db/db* mice, the levels of body weight and food and water intake were not changed by 7-*O*-galloyl-D-sedoheptulose treatment throughout the experimental period. The oral administration of 7-*O*-galloyl-D-sedoheptulose affected its favorable influences on the serum lipid profile and on renal glucose and triglyceride (Table 8, Figures 7A and 7B). The effect of 7-*O*-galloyl-D-sedoheptulose treatment on renal functional parameters (creatinine and urea nitrogen) are summarized in Table 9. The serum levels of creatinine and urea nitrogen in *db/db* mice were significantly higher than in *m/m* mice. However, these elevated renal dysfunction parameters in *db/db* mice were

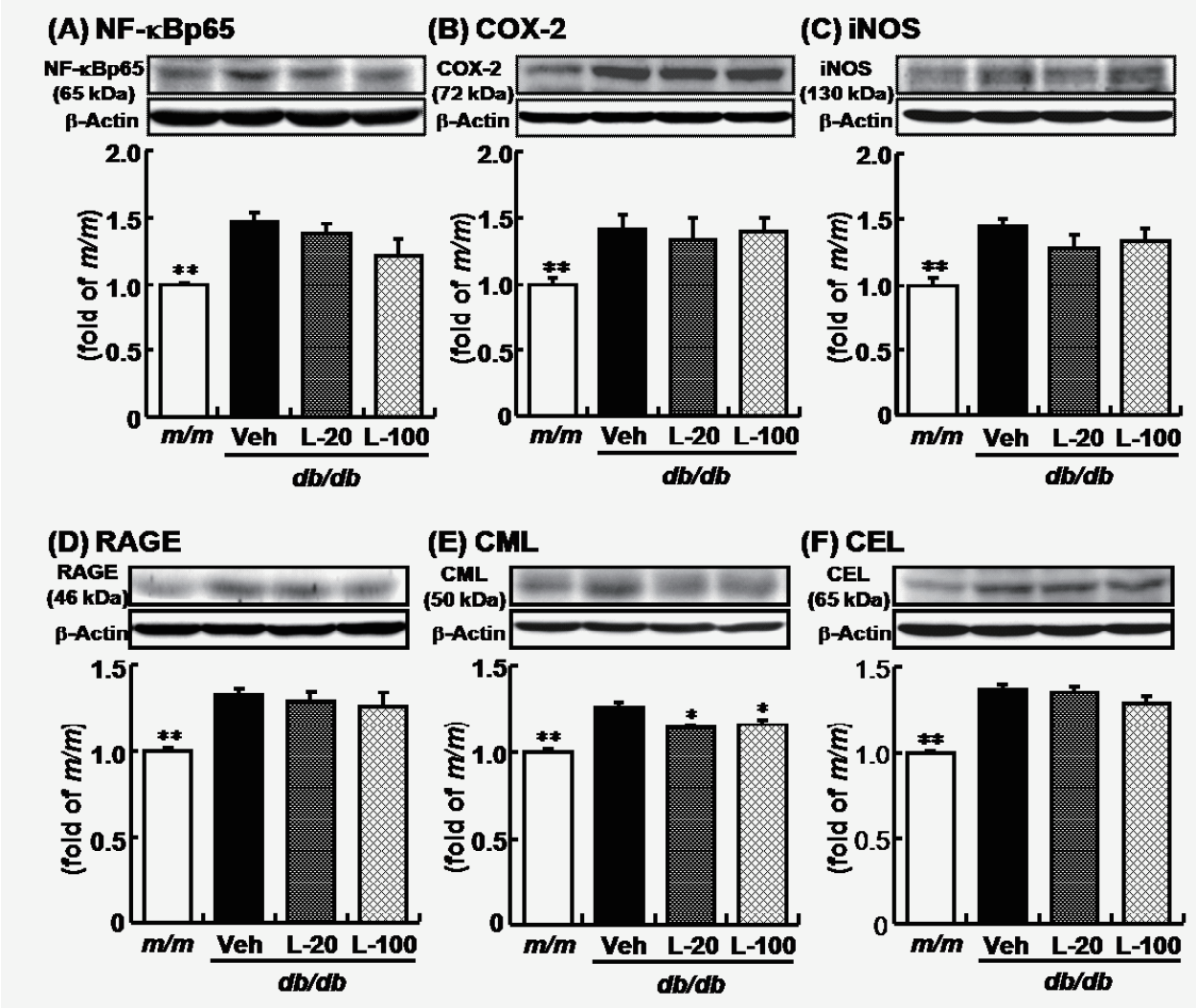

Figure 6. The protein expressions related inflammation and AGEs in the kidney. (A) NF-κBp65 expression, **(B)** COX-2 expression, **(C)** iNOS expression, **(D)** RAGE expression, **(E)** CML expression, **(F)** CEL expression. *m/m*, misty; Veh, vehicle-treated *db/db* mice; L-20, loganin 20 mg/kg body weight-treated *db/db* mice; L-100, loganin 100 mg/kg body weight-treated *db/db* mice. The results are presented as the means ± S.E. ($n = 6$ or 10). * $p < 0.05$, ** $p < 0.01$ *vs.* vehicle-treated *db/db* mice values.

Table 7. Body weight, food intake and water intake

Group	Body weight			Food intake (g/day)	Water intake (mL/day)
	Initial (g)	Final (g)	Gain (g/8 weeks)		
m/m	22.1 ± 0.5**	25.8 ± 0.8**	3.7 ± 0.3**	2.9 ± 0.1**	3.9 ± 0.3*
db/db					
Veh	40.0 ± 0.8	49.2 ± 1.3	9.2 ± 0.5	5.6 ± 0.2	13.2 ± 0.8
GS-20	39.5 ± 0.9	45.9 ± 2.5	6.4 ± 1.6	5.4 ± 0.2	13.4 ± 0.7
GS-100	40.2 ± 0.8	49.7 ± 1.8	9.6 ± 1.1	5.5 ± 0.1	12.8 ± 0.8

m/m, misty; Veh, vehicle-treated *db/db* mice; GS-20, 7-*O*-galloyl-D-sedoheptulose 20 mg/kg body weight-treated *db/db* mice; GS-100, 7-*O*-galloyl-D-sedoheptulose100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.01$ *vs.* vehicle-treated *db/db* mice values.

Table 8. Hematological analyses

Item	*m/m*	*db/db*		
		Veh	GS-20	GS-100
Glucose (mg/dL)	204.5 ± 13.3**	753.2 ± 33.9	722.9 ± 54.6	775.6 ± 35.0
Triglyceride (mg/dL)	56.8 ± 3.1**	242.6 ± 17.0	181.5 ± 14.7*	145.1 ± 13.6**
Total cholesterol (mg/dL)	115.9 ± 5.9**	183.2 ± 11.3	186.7 ± 14.2	171.0 ± 9.4*

m/m, misty; Veh, vehicle-treated *db/db* mice; GS-20, 7-*O*-galloyl-D-sedoheptulose 20 mg/kg body weight-treated *db/db* mice; GS-100, 7-*O*-galloyl-D-sedoheptulose 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

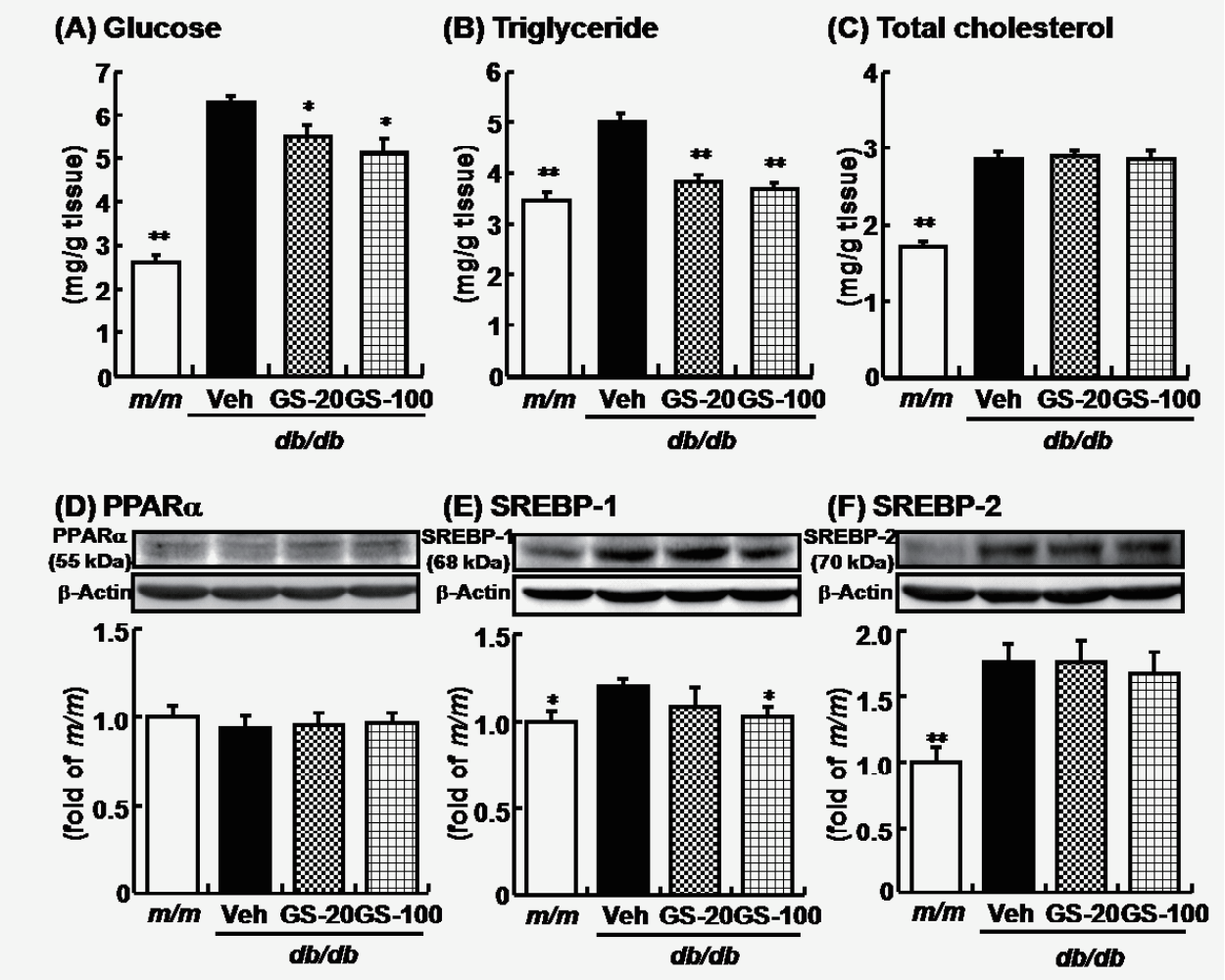

Figure 7. The glucose, triglyceride and total cholesterol contents and the protein expressions related to lipid metabolism in the kidney. (A) Glucose content, **(B)** triglyceride content, **(C)** total cholesterol content, **(D)** PPARα expression, **(E)** SREBP-1 expression, **(F)** SREBP-2 expression. *m/m*, misty; Veh, vehicle-treated *db/db* mice; GS-20, 7-*O*-galloyl-D-sedoheptulose 20 mg/kg body weight-treated *db/db* mice; GS-100, 7-*O*-galloyl-D-sedoheptulose 100 mg/kg body weight-treated *db/db* mice. The results are presented as the means ± S.E. ($n = 6$ or 10). * $p < 0.05$, ** $p < 0.01$ *vs.* vehicle-treated *db/db* mice values.

efficiently reduced by the 7-*O*-galloyl-D-sedoheptulose treatments. These results suggested the amelioration of renal dysfunction in *db/db* mice by 7-*O*-galloyl-D-sedoheptulose treatment. Compared with *db/db* vehicle-treated mice, no alteration in PPARα expressions of renal tissue were shown in *m/m* and 7-*O*-galloyl-D-sedoheptulose-treated mice (Figure 7D). However, SREBP-1 and SREBP-2 protein expressions were markedly elevated in the kidney of vehicle-treated *db/db* compared with *m/m* mice (Figures 7E and 7F). The administration of 7-*O*-galloyl-D-sedoheptulose of 100 mg/kg completely normalized the increased expressions of renal SREBP-1; however, SREBP-2 protein expressions remained unchanged in the kidney tissues (Figures 7E and 7F).

Besides the beneficial effects on lipid metabolism, 7-*O*-galloyl-D-sedoheptulose administration exerted an antioxidant effect. The elevated renal ROS and TBARS levels in *db/db* mice were lowered nearly to the level of *m/m* mice by 7-*O*-galloyl-D-sedoheptulose

Table 9. Renal functional parameters

Item	*m/m*	*db/db*		
		Veh	GS-20	GS-100
Creatinine (mg/dL)	0.31 ± 0.01*	0.46 ± 0.04	0.38 ± 0.02*	0.36 ± 0.02*
Urea-N (mg/dL)	27.5 ± 0.8**	40.3 ± 1.9	40.1 ± 1.6	38.5 ± 1.1*

m/m, misty; Veh, vehicle-treated *db/db* mice; GS-20, 7-*O*-galloyl-D-sedoheptulose 20 mg/kg body weight-treated *db/db* mice; GS-100, 7-*O*-galloyl-D-sedoheptulose 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.001$ *vs*. vehicle-treated *db/db* mice values.

Table 10. Biomarkers associated with oxidative stress in kidney

Item	*m/m*	*db/db*		
		Veh	GS-20	GS-100
TBARS (nmol/mg protein)	1.03 ± 0.06***	1.62 ± 0.05	1.45 ± 0.04*	1.18 ± 0.08***
ROS (Fluorescence/min/mg protein)	1,798 ± 149***	3,338 ± 222	2,552 ± 124**	2,529 ± 105**
GSH (μmol/mg protein)	12.06 ± 0.72*	9.92 ± 0.36	10.69 ± 0.17	10.62 ± 0.35
GSSG (μmol/mg protein)	2.30 ± 0.15	2.58 ± 0.12	2.55 ± 0.02	2.40 ± 0.09
GSH/GSSG	5.01 ± 0.22**	4.07 ± 0.08	4.05 ± 0.09	4.28 ± 0.22

m/m, misty; Veh, vehicle-treated *db/db* mice; GS-20, 7-*O*-galloyl-D-sedoheptulose 20 mg/kg body weight-treated *db/db* mice; GS-100, 7-*O*-galloyl-D-sedoheptulose 100 mg/kg body weight-treated *db/db* mice. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs*. vehicle-treated *db/db* mice values.

treatment at 100 mg/kg (Table 10). The *db/db* vehicle mice showed significantly decreased GSH/GSSG ratios compared with the *m/m* group, which resulted from the decreased GSH and increased GSSG in the kidney, but the reduction of the GSH/GSSG ratio in the kidney of *db/db* mice was not recovered on 7-*O*-galloyl-D-sedoheptulose treatment (Table 10). 7-*O*-galloyl-D-sedoheptulose administration showed inhibitory effects on the expression of an oxidative stress-induced transcriptional factor, NF-κB, in the kidney with the down-regulation of COX-2 and iNOS (Figures 8A-8C). These results showed that the anti-inflammatory effects of 7-*O*-galloyl-D-sedoheptulose may be associated with the down-regulation of COX-2 and iNOS followed by the inhibition of NF-κB transcription stimulated by oxidative stress in the kidney of type 2 diabetic mice.

The AGE-RAGE interaction activates transforming growth factor-beta1 signaling pathways and subsequently induces mesangial cell hypertrophy and glomerular sclerosis through fibronectin synthesis (*41*,*42*). Therefore, AGE accumulation in the kidney has been regarded as an index of progressive renal damage in diabetic complications. CML and CEL are well-characterized compounds that are commonly used as AGE markers (*43*). Particularly, CML is not only referred to as a glycoxidation product similar to pentosidine, but is also formed during the metal-catalyzed oxidation of polyunsaturated fatty acids in the presence of protein (*44*). In the present study, not only the over-expression of AGE (CML and CEL) but also the higher levels of NF-κB in the kidney of *db/db* mice were alleviated by 8-week treatment with 7-*O*-galloyl-D-sedoheptulose (Figures 8A, 8E, and 8F).

In summary, 7-*O*-galloyl-D-sedoheptulose treatment improved the impaired kidney function in type 2 diabetic mice. The renoprotective effects of 7-*O*-galloyl-D-sedoheptulose in diabetes were mediated by the lipid-lowering and anti-inflammatory effects through the modulation of renal SREBP-1 and NF-κB expressions, respectively, and the inhibition of AGE accumulation.

5. Conclusion and Perspectives

The antidiabetic effects and mechanisms of morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose in *db/db* mice, as type 2 diabetic mice, were investigated, with a focus on the kidney damage caused by hyperglycemia, dyslipidemia, inflammation, RAGE activation, and AGE formation. Morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose showed the same lowering effects on elevated triglyceride, oxidative stress (TBARS and ROS) and AGE formation in the kidney of *db/db* mice. The effects of morroniside and 7-*O*-galloyl-D-sedoheptulose were mediated by the modulation of renal SREBP and NF-κB expressions, but the effect of loganin was presumably mediated by the hypoglycemic and antioxidant effects in the kidney, and also indirectly by the amelioration of metabolic disorders in other organs such as the liver.

In conclusion, two iridoid glycosides (morroniside and loganin) and one low-molecular-weight polyphenol (7-*O*-galloyl-D-sedoheptulose), the main active compounds of Corni Fructus, beneficially acted in type 2 diabetic model *db/db* mice through specified mechanisms, as summarized above. These findings allowed us to conclude that morroniside, loganin, and 7-*O*-galloyl-D-sedoheptulose would synergistically contribute to the inhibition of metabolic disorders (hyperglycemia and dyslipidemia), oxidative stress,

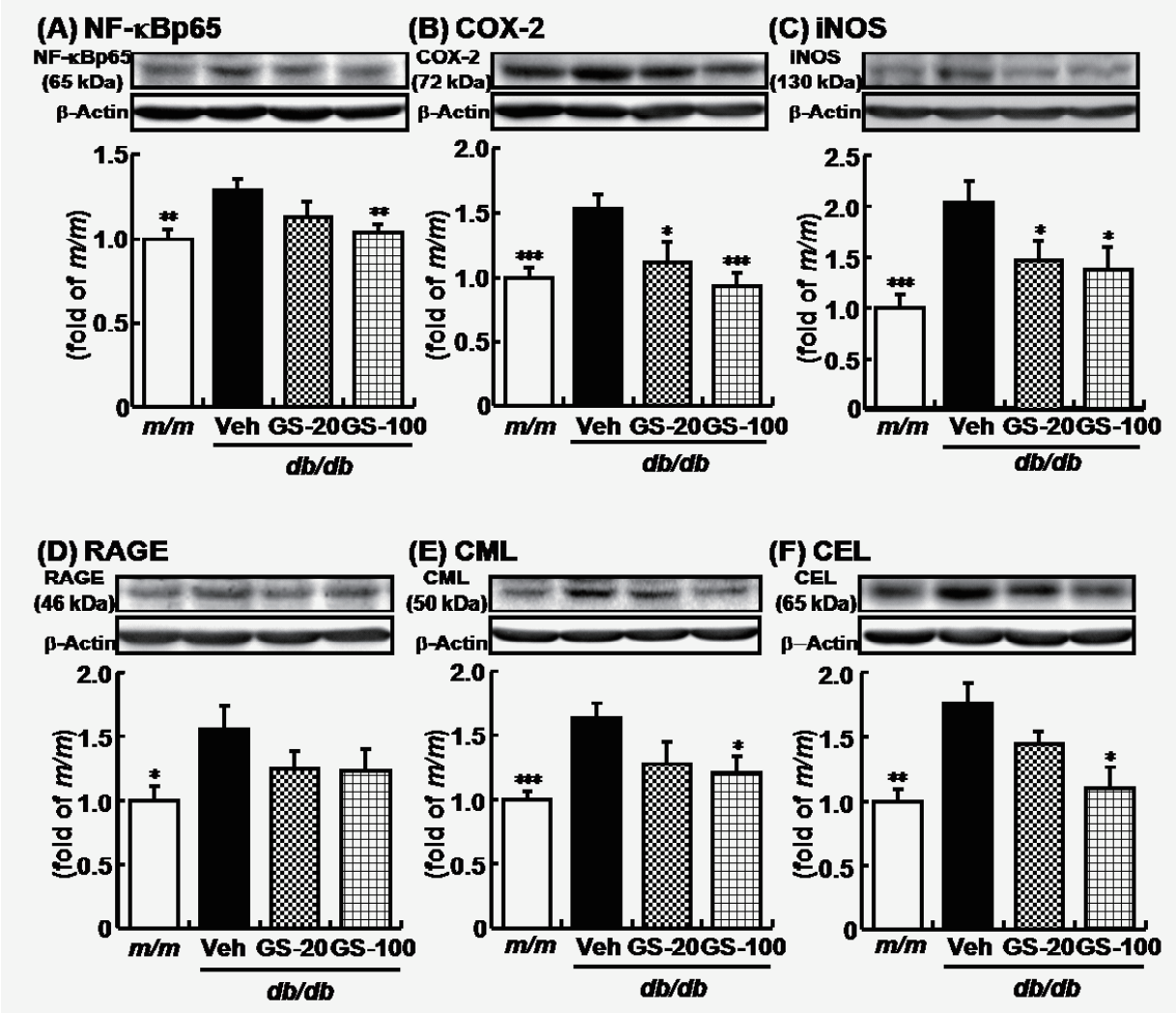

Figure 8. The protein expressions related inflammation and AGEs in the kidney. (A) NF-κBp65 expression, **(B)** COX-2 expression, **(C)** iNOS expression, **(D)** RAGE expression, **(E)** CML expression, **(F)** CEL expression. *m/m*, misty; Veh, vehicle-treated *db/db* mice; GS-20, 7-*O*-galloyl-D-sedoheptulose 20 mg/kg body weight-treated *db/db* mice; GS-100, 7-*O*-galloyl-D-sedoheptulose 100 mg/kg body weight-treated *db/db* mice. The results are presented as the means ± S.E. (n = 6 or 10). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* vehicle-treated *db/db* mice values.

inflammation, as well as AGE formation in the diabetic kidney. The present study advances knowledge on the beneficial effects of bioactive constituents of Corni Fructus, as well as the possible development of therapeutic or preventive agents for diabetic complications.

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