Protective Effects of Morroniside Isolated from Corni Fructus against Renal Damage in Streptozotocin-Induced Diabetic Rats

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In our previous study, we reported the renoprotective effect of Hachimi-jio-gan, a Chinese traditional prescription consisting of eight medicinal plants, and also reported the effect of Corni Fructus (*Cornus officinalis* SIEB. *et* ZUCC.), a component of Hachimi-jio-gan, on diabetic nephropathy using diabetic rats. In this study, we investigated the effects of morroniside isolated from Corni Fructus on renal damage in streptozotocin-treated diabetic rats. Oral administration of morroniside at a dose of 20 or 100 mg/kg body weight/d for 20 d to diabetic rats resulted in significant decreases in increasing serum glucose and urinary protein levels. Moreover, the decreased levels of serum albumin and total protein in diabetic rats were significantly increased by morroniside administration at a dose of 100 mg/kg body weight/d. In addition, morroniside significantly reduced the elevated serum urea nitrogen level and showed a tendency to reduce creatinine clearance. Morroniside also significantly reduced the enhanced levels of serum glycosylated protein, and serum and renal thiobarbituric acid-reactive substances. Protein expressions related to the advanced glycation endproduct (AGE) level and actions, oxidative stress such as N^{ε}-(carboxyethyl)lysine, as well as receptors for AGE and heme oxygenase-1 were increased in diabetic rats, but the levels were also significantly decreased by the administration of morroniside. This suggests that morroniside exhibits protective effects against diabetic renal damage by inhibiting hyperglycemia and oxidative stress. These results indicate that morroniside is one component partly responsible for the protective effects of Corni Fructus and Hachimi-jio-gan against diabetic renal damage.

Key words morroniside; hyperglycemia; oxidative stress; diabetic renal damage

Corni Fructus (*Cornus officinalis* SIEB. *et* ZUCC.), a traditional medicine, is considered one of the 25 plant-based drugs most frequently used in China, Japan, and Korea. Recent studies have reported that Corni Fructus has a plasma glucose-lowering action in normal rats, along with anti neoplastic and anti-microbial effects.[1—3] Moreover, Vareed *et al.*[4] reported that Corni Fructus has been used for improving liver and kidney functions. The components are total iridoid glycosides such as morroniside and loganin and also a few polyphenols such as cornusiin A, B, and C, monomeric and trimeric hydrolysable tannins, and so on.[5,6] Among them, the total iridoid glycosides are well-known as the main active constituents of Corni Fructus. The total iridoid glycosides of Corni Fructus containing loganin and morroniside inhibit oxidative stress[7] and prevent the renal overdeposition of fibronectin and laminin in an experimental diabetic rat model.[8] In particular, morroniside, one of the most abundant iridoid glycosides in Corni Fructus, has been proven effective in preventing diabetic complications such as diabetic angiopathy and the early stages of diabetic nephropathy by regulating renal mesangial cell growth through inhibiting oxidative stress.[9,10] In addition, it exhibits a neuroprotective effect by providing cytoprotection against H_2O_2-toxicity in SH-SY5Y cells.[11]

Diabetes mellitus is characterized by excessive glucose production. An abnormally elevated blood glucose level leads to oxidative stress and the formation of advanced glycation endproduct (AGE), which have been closely linked to diabetic complications such as neuropathy, retinopathy, and nephropathy.[12,13] Especially, diabetics are at increased risk for several types of kidney disease, and the predominant cause of end-stage renal disease in this disorder is diabetic nephropathy.[14,15] However, clinical trials suggest that there is no effective treatment for diabetic nephropathy.[16] Therefore, prevention of the occurrence and progression of diabetic nephropathy has become a very important issue.

Our previous investigations have demonstrated that Hachimi-jio-gan, a Chinese prescription consisting of eight crude drugs, owes it therapeutic potential to ameliorating functional and structural features associated with experimental types 1 and 2 diabetic nephropathy rat models.[17,18] Hachimi-jio-gan showed a preventive effect against kidney damage by reducing renal oxidative injury and the expression of fibronectin and transforming growth factor-β_1 (TGF-β_1) protein, involved in the pathophysiology of diabetic nephropathy in diabetic rats, and effectively inhibited the progression of chronic renal failure through decreasing uremic toxins, elevating antioxidant enzymes such as superoxide dismutase and catalase activities, and ameliorating histopathological lesions in the kidney.[19,20] In addition, this traditional prescription also alleviated diabetic oxidative stress through the inhibition of lipid peroxidation.[21] Cornus Fructus, one of eight medicinal plants comprising the Hachimi-jio-gan prescription, showed a beneficial effect on AGE-mediated renal injury in diabetic rats, and one of its major components, 7-*O*-galloyl sedoheptulose, exhibited an antidiabetic effect.[22,23] From our ongoing project to identify active components protecting against diabetic renal damage, we investigated the effects of morroniside, the main iridoid component of Corni Fructus, on renal damage in streptozotocin (STZ)-induced diabetic rats.

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MATERIALS AND METHODS

Materials The following reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan): 4,6-dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid (TBA)), oxalic acid, bovine serum albumin (BSA), 2-amino-2-hydroxymethyl-1,3-propadiol(Tris(hydroxymethyl)aminomethane), Tween 20, phenylmethyl sulfonyl fluoride (PMSF), protease inhibitor mixture DMSO solution, and skim milk powder. Dithiothreitol (DTT) was purchased from BioVision Inc. (Mountain View, CA, U.S.A.). The Bio-Rad protein assay kit was purchased from Bio-Rad Laboratories (Tokyo, Japan). Polyclonal anti-receptor for AGE (RAGE) antibody (sc-5563), rabbit polyclonal IgG heme oxygenase-1 (HO-1) antibody (sc-10789), goat anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibody (sc-2004), and goat anti-mouse IgG HRP conjugated polyclonal anti-receptor secondary antibody (sc-2005) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, U.S.A.). Monoclonal anti-N^{ε}-(carboxyethyl)lysine (CEL) antibody and polyclonal anti-N^{ε}-(carboxymethyl)lysine (CML) antibody were kindly provided by Dr. R. Nagai (Kumamoto University, Japan). STZ, glycerol, Nonidet P 40 (NP-40), and anti-mouse β-actin antibody were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). ECL Western blotting detection reagents were purchased from GE Healthcare (Piscataway, NJ, U.S.A.).

Isolation of Morroniside from Corni Fructus The Corni Fructus extract (100 g) was purchased from Tsumura & Co. (Tokyo, Japan) and applied to Sephadex LH-20 (Sigma, St. Louis, U.S.A.) column (5×32 cm) chromatography using a water–methanol mixture (100 : 0→0 : 100) and 60% acetone as an elution solvent to obtain four fractions (CF1—4). The CF1 fraction (94.52 g) was rechromatographed with Diaion HP-20SS (Mitsubishi Chemical Ind., Co., Ltd., Tokyo, Japan) column (5×8 cm) eluted with a water–methanol mixture (100 : 0→0 : 100) to divide it into two fractions (CF1A and B). Then, 1 g of the CF1B fraction (7.88 g) was further divided into five fractions (CF1B1—5) by MCI-gel CHP20 (Mitsubishi Chemical Ind., Co., Ltd., Tokyo, Japan) column (2.8×26 cm) chromatography employing washing with methanol (0→30%). Finally, the CF1B4 fraction (405.2 mg) was subjected to ODS (Fuji Silysia Chemical Ltd., Aichi, Japan) column chromatography and elution with methanol (0→21%) to obtain two fractions (CF1B4A and B). In the CF1B4A fraction (167 mg), morroniside (Fig. 1) was obtained and its purity (>95%) was confirmed by ^{1}H- and ^{13}C-NMR spectral data.[24]

Animals and Treatment The Guidelines for Animal Experimentation approved by the University of Toyama were followed in all experimental studies. Five-week-old, male Wistar rats (120—130 g) were obtained from Japan SLC, Inc. (Hamamatsu, Japan), kept in wire-bottomed cages, and exposed to a 12-h light/dark cycle. The room temperature and humidity were maintained automatically at about 25 °C and 60%, respectively. They 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. After several days of adaptation, the rats were injected intraperitoneally with STZ (50 mg/kg body weight) in 10 mM citrate buffer (pH 4.5). Ten days after the injection, the body weights were measured and blood samples were obtained between 10 a.m. and 11 a.m., avoiding the influence of food consumption, to determine glucose levels, and the rats with glucose levels >400 mg/dl were randomly divided into three experimental groups containing eight rats each. The diabetic control group was given water, while the other diabetic groups were orally administered 20 or 100 mg/kg daily of morroniside dissolved in water *via* gavage. The rats which underwent a sham injection of citrate buffer without STZ were also used as normal controls containing five rats. After 20 d of the administration period, 24-h urine samples were collected using metabolic cages and blood samples were obtained between 10 a.m. and 11 a.m. from the abdominal aorta under pentobarbital anesthesia (50 mg/kg body weight, intraperitoneally), and then the serum was immediately separated from the blood samples by centrifugation. After renal perfusion through the renal artery with ice-cold physiological saline, the kidneys were removed from each rat and frozen at −80 °C until analysis.

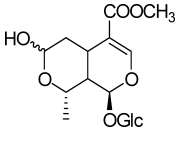

Fig. 1. Chemical Structure of Morroniside

Clinical Characteristics of Animals Serum levels of glucose, albumin, total protein, urea nitrogen, and creatinine (Cr) were examined using commercial reagents (Glucose CII-Test and A/G B-Test obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); BUN Kainos and CRE-EN Kainos were obtained from Kainos Laboratories, Inc. (Tokyo, Japan)). Serum glycosylated protein was colorimetrically measured by determining 5-hydroxymethyl furfural (5-HMF) formation from glucose according to the TBA assay.[25] TBA-reactive substance levels were determined using the methods of Naito and Yamanaka.[26] Urine component levels were determined as follows: protein by the sulfosalicylic acid method, and Cr using a commercial reagent (CRE-EN Kainos). Cr clearance (Ccr) was calculated on the basis of urinary Cr, serum Cr, urine volume, and body weight using the following equation: Ccr (ml/min/kg body weight)=[urinary Cr (mg/dl)×urinary volume (ml)/serum Cr (mg/dl)]×[1000/body weight (g)]×[1/1440 (min)].

Mitochondrial TBA-Reactive Substance Level in Kidney Mitochondria were prepared from kidney homogenate by differential centrifugation (800×***g*** and 12000×***g***, respectively) at 4 °C according to the methods of Johnson and Lardy[27] and Jung and Pergande,[28] respectively, with minor modifications. Each pellet was resuspended in preparation medium and the concentration of TBA-reactive substance was determined by the method of Mihara and Uchiyama.[29] The protein level was examined by the method of Itzhaki and Gill[30] with BSA as the standard.

Protein Preparation and Western Blot Analyses Renal cortical sections in all experimental rats were homogenized with ice-cold lysis buffer (pH 7.5) containing 137 mM NaCl, 20 mM Tris–HCl, 1% (v/v) Tween 20, 10% (v/v) glycerol, 1 mM PMSF, and protease inhibitor mixture DMSO solution. After centrifugation (2000×***g*** at 4 °C) to ensure equal loading among lanes, the protein concentration of each tissue was

determined using a Bio-Rad protein assay kit and BSA as a standard, and then immunoblotting was carried out.

For the determination of CML, CEL, RAGE, and HO-1 protein expressions, 30 μg of protein of each sample was electrophoresed through 8 or 10% sodium dodecylsulfate polyacrylamide gels (SDS-PAGE). Separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and then incubated with primary antibodies to CML, CEL, RAGE, HO-1, and β-actin, respectively, overnight at 4 °C. After the blots were washed, they were incubated with goat anti-rabbit and/or goat anti-mouse IgG HRP conjugated secondary antibody for 90 min at room temperature. Each antigen–antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with LAS-1000 plus (Fujifilm, Japan).

Band densities were determined by Scion image software (Scion Corporation, Frederick, Maryland, U.S.A.) and quantified as the ratio to β-actin. The evaluation of these protein levels at mean values against normal rats is represented as 1, and the corresponding values for the diabetic rats are expressed as the ratios of these values.

Statistical Analysis The effect of morroniside on each parameter was examined using one-way Analysis of Variance. Individual differences among groups were analyzed by Dunnett's test. $p<0.05$ was considered significant. The results are expressed as means±S.E.M.

RESULTS

Changes in Serum Glucose Level and Body Weight Figure 2 shows the effect of morroniside (20 or 100 mg/kg body weight/d) on the serum glucose level and body weight in STZ-induced diabetic rats during the 20-d experimental period. The glucose level in STZ-induced diabetic rats was significantly higher than that of normal rats and was gradually increased with diabetic period, but the administration of morroniside prevented this increasing serum glucose level (Fig. 2A), while there were no changes in body weight in diabetic rat groups (Fig. 2B).

Food Intake, Water Intake, Urine Volume, and Kidney

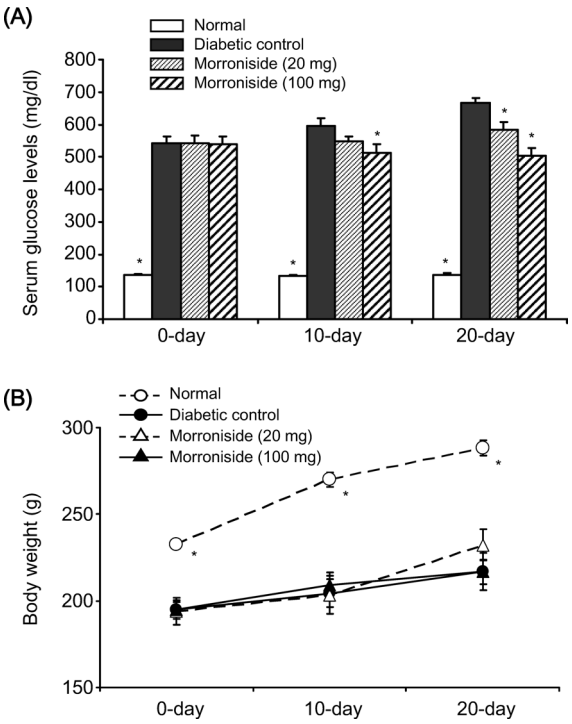

Fig. 2. Changes of Serum Glucose Level (A) and Body Weight (B)
Significance: $*p<0.05$ *vs.* each diabetic control value.

Table 1. Characteristics of Experimental Rats

Group	Dose (mg/kg BW/d)	Food intake (g/d)	Water intake (ml/d)	Urine volume (ml/d)	Kidney weight (g/100 g BW)
Normal rats	—	19.1±0.5*	38.9±4.6*	15.0±3.2*	0.669±0.018*
Diabetic rats					
Control	—	32.3±0.5	158.5±10.0	127.1±8.9	1.079±0.014
Morroniside	20	30.8±0.8	145.8±12.7	113.3±9.6	1.019±0.013*
Morroniside	100	28.9±0.2*	137.3±7.5	102.9±8.6	1.031±0.024

Significance: $*p<0.05$ *vs.* each diabetic control value.

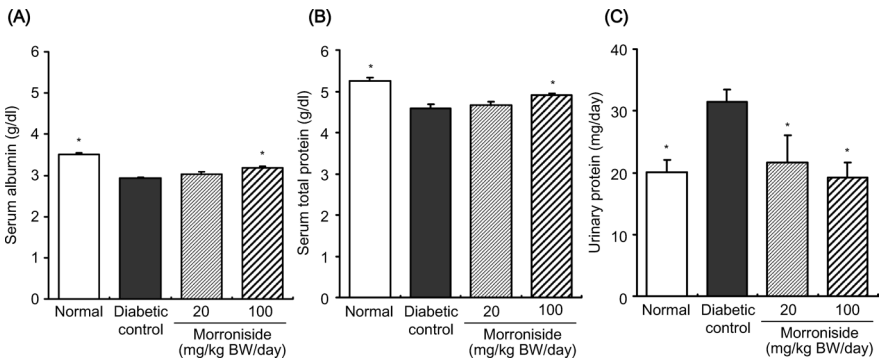

Fig. 3. Serum Albumin (A), Total Protein (B), and Urinary Protein (C) Levels
Significance: $*p<0.05$ *vs.* each diabetic control value.

Weight Table 1 shows the effects of morroniside on the changes in food and water intake, urine volume, and kidney weight. In diabetic control rats, the food and water intake, urine volume, and kidney weight were significantly higher than in normal rats, but the administration of morroniside significantly decreased the food intake and kidney weight. In addition, morroniside administration led to a tendency toward a decrease in the elevated water intake and urine volume.

Serum Albumin, Total Protein, and Urinary Protein Levels The levels of serum albumin and total protein were significantly decreased in STZ-treated diabetic rats, while the level of urinary protein was increased (Fig. 3). However, morroniside significantly increased the levels of serum albumin and total protein (Figs. 3A, B). Moreover, the urinary protein level was markedly reduced by morroniside in a dose-dependent manner (Fig. 3C).

Serum Urea Nitrogen and Ccr Levels Figure 4 shows the effect of morroniside on renal function parameters in diabetic rats. As shown in Fig. 4A, the serum urea nitrogen level in STZ-induced diabetic rats was significantly elevated, but the level was reduced by oral morroniside administration at a dose of 100 mg/kg body weight/d. There were no significant changes in the Ccr level among the normal, diabetic control, and morroniside-administered groups (Fig. 4B).

Serum Glycosylated Protein Level The serum glycosylated protein level was significantly increased under diabetes (Fig. 5). The elevated glycosylated protein level in serum was slightly reduced by the administration of morroniside at a dose of 20 mg/kg body weight/d, but showed a significant decrease on 100 mg administration.

Serum and Renal Mitochondrial TBA-Reactive Substance Levels Figure 6 shows that the serum and renal mitochondrial TBA-reactive substance levels were significantly increased about 2.17- and 1.27-fold, respectively, in STZ-induced diabetic control rats compared to the normal values. The elevated serum TBA-reactive substance level was significantly lowered by the oral administration of morroniside (Fig. 6A), and the renal mitochondrial TBA-reactive substance level was also reduced to nearly normal values in morroniside-administered rats at a dose of 100 mg/kg body weight (Fig. 6B).

Western Blotting in Renal Cortex To evaluate the effects of morroniside on AGE- and oxidative stress-related proteins expressions in the renal cortex of diabetic rats, we performed Western blot analyses with CML, CEL, RAGE,

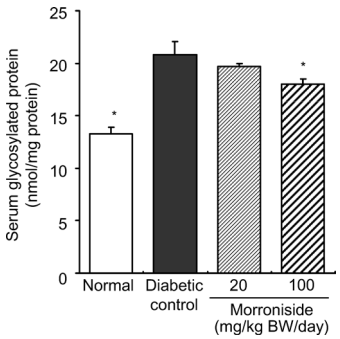

Fig. 5. Serum Glycosylated Protein Level

Significance: $*p<0.05$ *vs.* each diabetic control value.

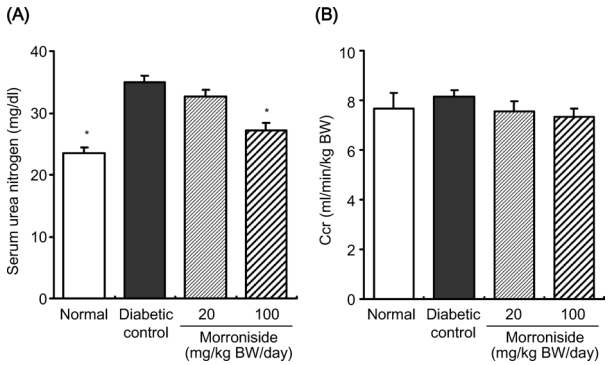

Fig. 4. Serum Urea Nitrogen (A) and Ccr (B) Levels

Significance: $*p<0.05$ *vs.* each diabetic control value.

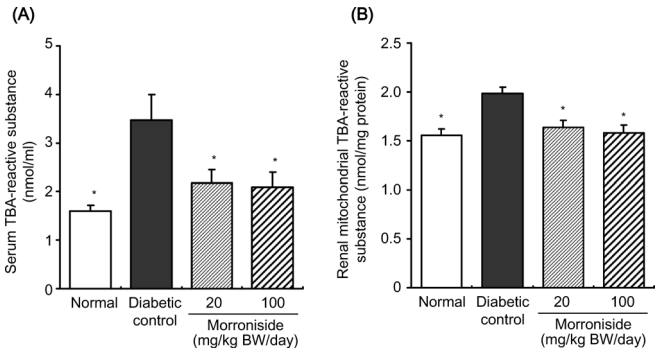

Fig. 6. Serum (A) and Renal Mitochondrial TBA-Reactive Substance (B) Levels

Significance: $*p<0.05$ *vs.* each diabetic control value.

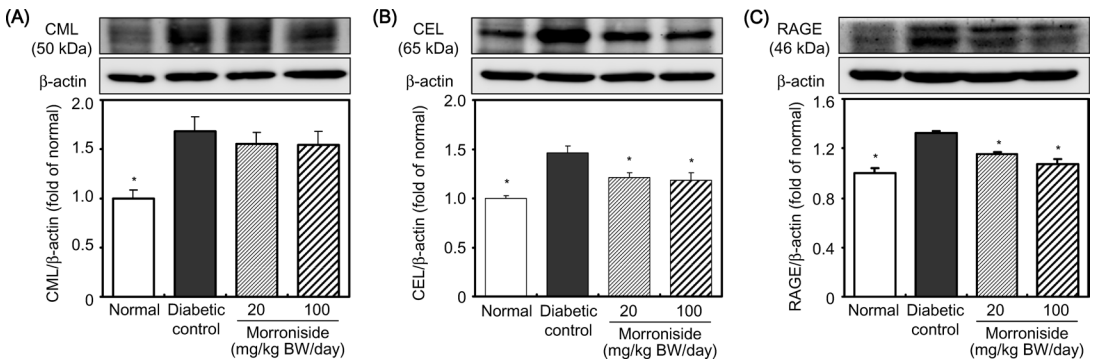

Fig. 7. CML (A), CEL (B), and RAGE (C) Levels in Renal Cortex

Significance: $*p<0.05$ *vs.* each diabetic control value.

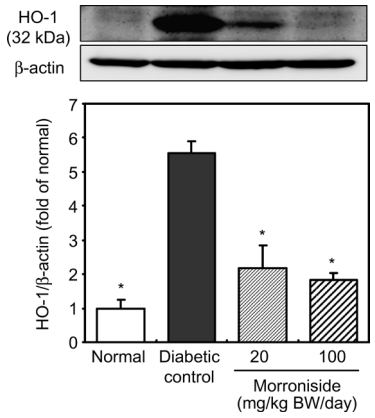

Fig. 8. HO-1 Level in Renal Cortex

Significance: $*p<0.05$ *vs.* diabetic control value.

and HO-1 antibodies. As shown in Fig. 7, the expression levels of CML, CEL, and RAGE in diabetic rats were significantly increased compared to normal rats. However, the elevated expressions of CEL and RAGE were markedly reduced by morroniside administration in a dose-dependent manner. There was only a tendency toward a decrease in the CML expression level *via* treatment with morroniside. Moreover, the expression volume of HO-1 was markedly elevated 5.56-fold in STZ-induced diabetic rats compared with normal rats, but the oral administration of morroniside at doses of 20 and 100 mg/kg a day decreased the expression level compared to normal rats (Fig. 8).

DISCUSSION

Hachimi-jio-gan, a Kampo prescription composed of eight constituents (Rehmanniae Radix, Corni Fructus, Dioscoreae Rhizoma, Alismatis Rhizoma, Hoelen, Moutan Cortex, Cinnamomi Cortex, and Aconiti Tuber), has been traditionally used for the alleviation of subjective symptoms of diabetes and its complications in Japan and China. In particular, Hachimi-jio-gan ameliorates hyperglycemia, so it is clinically applied to improve several disorders associated with diabetes.[31,32] In addition, it has been widely used for the treatment of renal dysfunction in humans[33] and several chronic diseases including chronic nephritis, sterility, and vegetative ataxia.[34] We also previously reported that Hachimi-jio-gan ameliorated functional and structural features associated with experimental type 1 and 2 diabetic nephropathy rat models.[17,18]

Corni Fructus, one component of Hachimi-jio-gan, has been used in traditional folk medicine as a treatment for several conditions such as frequent urination, impotence, diabetes, and collapse with profuse sweating, acting on the liver and kidney channels, as well as health foods such as tea and functional beverages.[35] In a previous study, we found that Corni Fructus could ameliorate glucose-associated metabolic disorders, and its mechanisms were intimately related to the formation of AGE, as well as those of Hachimi-jio-gan,[22] and iridoid glycosides from Corni Fructus improve metabolic parameters associated with the development of diabetic renal damage.[23]

According to the three-dimensional HPLC profile, as previously shown,[17,18] morroniside, loganin, and paeoniflorin were detected as the major compounds in Hachimi-jio-gan. Morroniside and loganin are known components of Corni Fructus. It was reported that total iridoid glycosides prevented the glomerular overexpression of TGF-β_1 and matrixes in streptozotocin-induced diabetic rats.[8] On the basis of these facts, we hypothesized that morroniside, an iridoid glycoside, would be a major active ingredient in Corni Fructus, and examined the renoprotective effect of morroniside using STZ-induced diabetic rats to clarify the essential renoprotective compounds in Corni Fructus.

A deficiency or insufficiency of insulin secretion or insulin resistance in the diabetic state usually causes a decrease in body weight gain and increases in food intake, water intake, kidney weight, and urine volume. In our experimental condition, the STZ-induced diabetic rats also showed the same symptoms. However, oral administration of morroniside slightly improved these physico-metabolic abnormalities. In particular, the increasing serum glucose level with diabetic period was significantly prevented by the 100 mg/kg of morroniside administration (Fig. 2). These results suggest that morroniside would improve physiological abnormalities and might prevent the pathogenesis of diabetic complications caused by hyperglycemia under diabetes.

Diabetes also leads to renal dysfunction, *i.e.*, increased proteinuria and serum urea nitrogen, and decreased Ccr levels, reflecting a decline in the glomerular filtration rate. In our diabetic rats, the levels of serum albumin and total protein were significantly decreased, while urinary protein and serum urea nitrogen levels were markedly increased (Figs. 3, 4). However, morroniside administration significantly enhanced the decreased levels of serum albumin and total protein, and alleviated the elevated serum urea nitrogen level at a dose of 100 mg/kg body weight a day. Particularly, the elevated urinary protein level was markedly decreased by the administration of morroniside in a dose-dependent manner. However, there was only a tendency to reduce the Ccr levels. It was considered that the administration period, 20 d, was comparably too short to improve these parameters significantly, or that early diabetic renal changes occurred, not advanced ones, in this study. However, on the basis of these results, we suggest that morroniside delays the progression of diabetic nephropathy by improving renal function.

Several studies have provided substantial evidence that multiple factors caused by hyperglycemia contribute to the development of diabetic kidney disease. The hyperglycemic condition results in irreversible tissue damage *via* the protein glycation reaction that leads to the formations of glycosylated protein and AGE.[36,37] Glycosylated serum protein was increased in the present diabetic animal model, which implies that it stimulates the oxidation of sugars, enhancing damage to both sugars and proteins in the circulation and vascular wall, continuing and reinforcing the cycle of oxidative stress and damage. The elevated level of serum glycosylated protein in diabetic rats was significantly reduced by morroniside administration in a dose-dependent manner (Fig. 5). These results indicate that morroniside might prevent the pathogenesis of diabetic complications caused by the glycosylation of serum proteins, eventually resulting in the improvement of diabetic pathological conditions.

The metabolic disorders under diabetic nephropathy and

the protein glycation reaction induce lipid peroxidation caused by oxidative stress, which plays a potential role in diabetic glomerulosclerosis and renal fibrosis.[38,39] In the present study, morroniside was evaluated for its effect on oxidative stress in relation to the development of diabetic nephropathy *via* the measurement of the serum and renal mitochondrial lipid peroxidation level. TBA-reactive substance levels in the serum and kidney were markedly elevated in diabetic control rats as compared with normal rats, while the administration of morroniside decreased the level dose-dependently (Fig. 6). This suggests that the administration of morroniside would ameliorate oxidative stress under the diabetic condition through the inhibition of lipid peroxidation, and, thus, it would result in the improvement of renal lesions caused by oxidative stress.

To investigate protein expressions related to the AGE level and actions, we performed Western blot analyses in the renal cortex, evaluating AGE actions with receptors related to the intracellular responses and the renal AGE level characterized physicochemically by neither cross-linking nor fluorescence, *e.g.*, CML and CEL, identified in aging and diabetes-related diseases. That is, these products are not only derived from glucose-metabolic intermediates and metabolites of glycolysis, but also serve as general bio-markers of oxidative stress resulting from carbohydrate and lipid oxidation reactions.[40—42] It is well-known that RAGE is the best-characterized receptor for AGE, which is composed of multiple members of the immunoglobulin superfamily.[43] The binding of RAGE by AGE activates several intracellular signaling pathways such as mitogen-activated protein kinase, nuclear factor-κB, and AP-1, and increases the production of cytokines such as tumor necrosis factor-α, interleukin-1β, and monocyte chemoattractant protein-1.[44] The binding of AGE-RAGE leads to albuminuria and mesangial expansion, and results in glomerular sclerosis. Therefore, the AGE-RAGE pathway is considered as a candidate molecular target for the prevention and treatment of diabetic nephropathy.[45] CML, a major AGE in diabetic nephropathy, is known to be a parameter of oxidative stress, and its accumulation upregulates RAGE expression on podocytes and precedes changes in the glomerular extracellular matrix in the glomeruli during the course of diabetic nephropathy.[46,47] Inhibition of its accumulation results in the amelioration of glomerular lesions in diabetic rats.[48] Therefore, renal accumulations of CML, CEL, and RAGE expression were investigated in this study. CEL and RAGE levels in diabetic rats were markedly higher than normal, but they were significantly ameliorated in morroniside-administered groups (Fig. 7). HO-1 plays a critical role in the regulation of cellular oxidative stress, maintains normal renal function, and prevents renal tissue injury due to oxidative stress, and the onset of diabetes coincided with an increase in HO-1 protein levels and a paradoxical decrease in HO-1 activity.[49—51] Our present data also showed that HO-1 protein levels were increased in diabetic rats, but the levels were significantly decreased by morroniside administration (Fig. 8). These results suggest that morroniside can prevent diabetic renal damage *via* inhibiting AGE-RAGE binding rather than AGE formation as well as oxidative stress.

In conclusion, morroniside, one of the components of Corni Fructus, showed a protective effect against renal damage in STZ-induced diabetic rats. These results suggest that morroniside is partly responsible for the renoprotective effects of Corni Fructus as well as Hachimi-jio-gan.

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