Beneficial Effect of Corni Fructus, a Constituent of Hachimi-jio-gan, on Advanced Glycation End-product-Mediated Renal Injury in Streptozotocin-Treated Diabetic Rats

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Previous investigations have demonstrated that Hachimi-jio-gan, a Chinese prescription consisting of eight crude drugs, has a therapeutic potential in diabetes and diabetic nephropathy, using these model rats. To add to these findings, we performed this study to assess whether one of the crude drugs, Corni Fructus (Cornus officinalis Sieb. et Zucc.), had an effect on streptozotocin-induced diabetic rats as a major active constituent, compared with an inhibitor of advanced glycation end-product (AGE) formation, aminoguanidine. Diabetic rats were orally administrated Corni Fructus extract (50, 100, 200 mg/kg body weight/d) or aminoguanidine (100 mg/kg body weight/d). Treatment with Corni Fructus for 10 d suppressed hyperglycemia, proteinuria, renal AGE formation, and related protein expressions, i.e., receptor for AGES, nuclear factor-κB, transforming growth factor-β1, and Nε-(carboxymethyl)lysine, in the same way as with aminoguanidine. However, improvement of renal function, shown via serum creatinine (Cr) and Cr clearance, was superior to aminoguanidine treatment. In conclusion, the present study supported the hypothesis that Corni Fructus plays an important role against diabetic pathogenesis, i.e., reducing glucose toxicities, up-regulating renal function, and consequently ameliorating glycation-associated renal damage; thus, this study may provide a new recognition of crude drugs to clarify the mechanisms of Chinese prescriptions.

Key words Corni Fructus; aminoguanidine; advanced glycation end-product; renal damage; streptozotocin

Diabetes is a disorder of excessive urine excretion and chronic hyperglycemia, and glucose participates in diabetic complications such as atherosclerosis, cardiac dysfunction, and nephropathy. Chronic hyperglycemia accelerates activation of the formation of advanced glycation end-products (AGEs), oxidative stress, the polyol pathway, and protein kinase C pathway. These metabolic factors are synergistically correlated with one another; therefore, an effective treatment with wide-spread effects continues to be required.

Up to now, there have been many experiments focusing on the treatment of diabetes and its complications with traditional medicines including Chinese prescriptions because of their absence of toxic and/or side-effects. In our previous study, we reported that the Chinese prescriptions Hachimi-jio-gan and Keishi-bukuryo-gan had potential therapeutic effects against diabetic nephropathy, and had different functions in terms of their effects on metabolic disorders, especially on AGE formation in Hachimi-jio-gan and oxidative stress in Keishi-bukuryo-gan.1–3 In addition, we also clarified that administration of dried Rehmanniae Radix (Rehmannia glutinosa Libosch. var. purpurea Makino) extract, which is the main constituent of Hachimi-jio-gan, attenuates renal dysfunction in diabetic nephropathy mainly due to its suppression of oxidative stress5; however, for the analysis of this prescription, further characterization of the other constituents is needed. According to the three-dimensional HPLC profile, as previously shown,2,4) morroniside, loganin, and paeoniflorin were detected as the major compounds in Hachimi-jio-gan. Morroniside and loganin are the components of Corni Fructus (Cornus officinalis Sieb. et Zucc.) and paeoniflorin is the component of Moutan Cortex (Paeonia suffruticosa Andrews) common in Keishi-bukuryo-gan. Therefore, in order to clarify the source of a particular action of Hachimi-jio-gan, we chose to evaluate the usefulness of one of the crude drugs, Corni Fructus.

Corni Fructus has been used as a traditional medicine in Japan and China, and the components of this plant are iridoid total 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.6–7) Recently, it has been reported that Corni Fructus has a plasma glucose-lowering action in normal rats, along with anti-neoplastic and anti-microbial effects.8–10) Moreover, Vareed et al.11) also reported that Corni Fructus has been used for improving liver and kidney functions, and iridoid total glycosides has the effect of preventing the overexpression of transforming growth factor (TGF)-β1 and matrixes in glomeruli with a diabetic model.12) However, the mechanisms of Corni Fructus against glucose-associated metabolic disorders in diabetes have yet to be explored. To determine whether Corni Fructus possesses the principal role in Hachimi-jio-gan, which has a strong effect on AGE formation in diabetes and/or diabetic nephropathy, we examined the effect of Corni Fructus in streptozotocin (STZ)-induced diabetic rats, comparing it with the inhibitor of AGE formation, aminoguanidine.

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)), 5-hydroxymethylfurural (5-HMF), oxalic acid, NADPH, sulfanilamide, naphthylethylene diamine dihydrochloride, EDTA, nitro blue tetrazolium (NBT), NADH, phenazine methosulfate (PMS), sodium nitrite, bovine serum albumin (BSA), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris(hydroxymethyl)aminomethane), Tween 20, glycerol, phenyl-methyl sulfonyl fluoride (PMSF), protease inhibitor mixture

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DMSO solution, and skim milk powder. The Bio-Rad protein assay kit was purchased from Bio-Rad Laboratories, Japan. Polyclonal anti nuclear factor-κB (NF-κB) p65 antibody (sc-109), polyclonal anti inhibitor binding protein κB-α (IκB-α) antibody (sc-371), polyclonal anti receptor for AGE (RAGE) antibody (sc-5563), polyclonal anti TGF-β1 antibody (sc-146), goat anti-rabbit IgG horseradish peroxidase (HRP) conjugated secondary antibody (sc-2005), and goat anti-mouse IgG HRP conjugated secondary antibody (sc-2005) were purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, U.S.A. Polyclonal anti Nε-(carboxymethyl)lysine (CML) antibody was kindly provided by Dr. R. Nagai (Kumamoto University, Japan). STZ, aminoguanidine hydrochloride, nitrate reductase, 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 Amersham Bioscience, Piscataway, NJ, U.S.A.

**Corni Fructus Extract** Corni Fructus extract used in this experiment was produced by Tsuma Juntendo, Inc., Tokyo, Japan. A voucher specimen is deposited in the Institute of Natural Medicine, University of Toyama, Japan. The extract gave a dark blue coloration with the FeCl₃ reagent suggesting presence of tannins. The HPLC-DAD analysis showed major peaks arising from gallic acid (8.24 min), morroniside (18.24 min), and lotin (22.9 min), which were identified by comparison of t₁R and UV absorptions with those of authentic samples.

**Experimental Design** 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 chow and water. After several days of adaptation, the rats were injected with citrate buffer without STZ. The blood glucose level was determined using the methods of Naito and Yamanaka. Serum glycosylated protein was measured by the method of Nakayama et al. Serum nitrite/nitrate level was determined using the method of Mabley et al. In brief, the serum was mixed with nitrate reductase and NADPH, which were both dissolved in 40 mM Tris, pH 7.6, and incubated at room temperature for 3h. Following this period, Griess reagent was added and incubated for another 10 min at room temperature. The absorbance of the samples was read at 550 nm. The concentration of nitrite/nitrate was determined from a standard curve of sodium nitrite.

**Antioxidative Capacity in Serum** The serum Superoxide dismutase (SOD) activity was determined using a method based on that of Ewing and Janero. The serum was mixed with 0.125 mM EDTA, 62 µM NBT, 98 µM NADPH, and 33 µM PMS in 50 mM phosphate buffer, pH 7.4, and then the reduced NBT level was measured at 550 nm.

**AGE Level in Kidney** The renal AGE level was determined by the method of Nakayama et al. Mined kidney tissue was delipidated with chloroform and methanol (2:1, v/v) overnight. After washing, the tissue was homogenized in 0.1 N NaOH, followed by centrifugation at 8000×g for 15 min at 4°C. The amounts of AGEs in these alkali-soluble samples were determined by measuring the fluorescence at an emission wavelength of 440 nm and an excitation wavelength at 370 nm. A native BSA preparation (1 mg/ml of 0.1 N NaOH) was used as a standard, and its fluorescence intensity was defined as one unit of fluorescence. The fluorescence values of samples were measured at a protein concentration of 1 mg/ml and expressed in AU compared with a native BSA preparation.

**Mitochondrial TBA-Reactive Substance Level in Kidney** Mitochondria were prepared from kidney homogenate by differential centrifugation (8000×g and 12000×g, respectively) at 4°C according to the methods of Johnson and Lardy and Jung and Bergade, respectively, with minor modifications. Each pellet was resuspended in preparation medium and the concentration of TBA-reactive substance was determined by the method of Uchiyama and Mihara. The protein level was examined by the method of Izhaki and Gill with BSA as the standard.
Protein Extraction and Western Blot Analyses Renal cortical sections were homogenized with ice-cold lysis buffer (pH 7.5) containing 137 mM NaCl, 20 mM Tris–HCl, 1% Tween 20, 10% 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 RAGE, NF-κBp65, IκB-α, TGF-β₁, and CML protein expressions, 30 μg protein of each sample was electrophoresed through 8, 12, and 15% sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE). Separated proteins were electrophoretically transferred to a nitrocellulose membrane, blocked with 5% skim milk solution for 1 h, and then incubated with primary antibodies to RAGE, NF-κBp65, IκB-α, TGF-β₁, CML, 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, MD) and quantified as the ratio to β-actin. The evaluation of these protein levels at mean values against control rats is represented as 1 and the corresponding values for the diabetic rats are expressed as the ratios of these values.

Statistical Analysis The results are presented as the mean±S.E. The effect of Corni Fructus on each parameter was examined using the one way Analysis of Variance. Individual differences among groups were analyzed by Dunnett’s test and significance was accepted at $p<0.05$.

RESULTS

Body Weight Change and Serum Glucose Level A significant decrease in body weight during the 10 d was observed in the diabetic control rats compared with control rats, showing no difference between initial and final values; however, administration of Corni Fructus at doses of 100 and 200 mg to diabetic rats increased body weight gain significantly, similarly to aminoguanidine-treated diabetic rats (Fig. 1A).

Figure 1B shows serum glucose levels over the 10-d administration period. In contrast with body weight gain, diabetic control rats showed an increase from the initial value (from 524.1 to 577.2 mg/dl) and the final value was 5.6-fold higher than that of control rats. On the other hand, 50, 100, and 200 mg Corni Fructus-treated diabetic rats showed a significant decrease compared with diabetic control rats (498.3 mg/dl, 499.3 mg/dl, and 460.8 mg/dl, respectively), and oral administration of 100 mg aminoguanidine led to a reduction to 472.0 mg/dl.

Serum Constituents Table 1 shows the serum constituents except for the glucose level at the end of this experiment. The serum glycosylated protein and total cholesterol levels of diabetic control rats were significantly increased by about 1.81- and 1.88-fold, respectively, relative to the control levels, while Corni Fructus extract decreased these levels significantly, and the effects in the Corni Fructus 100 mg group were nearly the same as in the 100 mg aminoguanidine-treated group. Diabetes led to significant increases in TBA-reactive substance and decreases in albumin levels, while Corni Fructus extract decreased these levels significantly, and there was only a tendency of a decrease in the TBA-reactive substance level by treatment with Corni Fructus and aminoguanidine, and no effect on the albumin level in Corni Fructus-treated groups except for a slight increase without

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (n=5)</th>
<th>Diabetic control (n=8)</th>
<th>Diabetic +CF50 (n=8)</th>
<th>Diabetic +CF100 (n=8)</th>
<th>Diabetic +CF200 (n=8)</th>
<th>Diabetic +AG100 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosylated protein (nmol/mg protein)</td>
<td>12.2±0.2</td>
<td>22.1±0.8⁻ᵃ</td>
<td>21.1±0.7⁻ᵇ</td>
<td>19.4±0.8⁻ᵇ</td>
<td>18.5±1.0⁻ᵇ</td>
<td>20.2±1.1⁻ᵇ</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.22±0.03</td>
<td>2.93±0.06⁻ᵃ</td>
<td>2.88±0.04⁻ᵇ</td>
<td>2.91±0.02⁻ᵇ</td>
<td>2.88±0.03⁻ᵇ</td>
<td>3.01±0.08⁻ᵇ</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>42.8±1.4</td>
<td>80.5±5.4⁻ᵃ</td>
<td>67.9±5.1⁻ᵇ</td>
<td>65.4±3.8⁻ᵇ</td>
<td>62.6±3.7⁻ᵇ</td>
<td>64.2±4.7⁻ᵇ</td>
</tr>
<tr>
<td>TBA-reactive substance (nmol/ml)</td>
<td>2.47±0.26⁻ᵃ</td>
<td>5.57±0.45⁻ᵃ</td>
<td>5.20±0.51⁻ᵇ</td>
<td>5.03±0.40⁻ᵇ</td>
<td>4.96±0.54⁻ᵇ</td>
<td>4.71±0.60⁻ᵇ</td>
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</tbody>
</table>

CF50, 100 or 200: Corni Fructus (50, 100 or 200 mg/kg, p.o.); AG100, aminoguanidine (100 mg/kg, p.o.). Results are shown as the means±S.E. (n=5 or 8). a $p<0.001$ vs control rats; b $p<0.05$; c $p<0.001$ vs diabetic control rats.
significance in the aminoguanidine-treated group.

**Antioxidative Activity in Serum** Serum nitrite/nitrate levels, indicative of nitric oxide formation and SOD activity, represented by the inhibition of NBT reduction, indicative of superoxide anion \((O_2^-)\) generation, were increased in diabetic control rats; however, treatment with Corni Fructus affected these incidences dose-dependently, and especially, the Corni Fructus 200 mg group and aminoguanidine group showed strongly reduced nitrite/nitrate levels compared to the control group (Table 2).

**Renal Function** Table 3 shows the renal function parameters of the control and experimental groups. In the diabetic state, serum urea-N and Cr levels significantly increased 1.82- and 1.18-fold, respectively, while Corni Fructus treatment from the lowest dose of 50 mg caused a significant decrease in these levels, and these effects were superior to treatment from the lowest dose of 50 mg Corni Fructus-treated group was the same as in the aminoguanidine-treated group. Conversely, there was a significant decrease in the Cr\(_r\) level in the diabetic control rats compared with the control rats; however, Corni Fructus-treated groups at all doses, particularly the highest dose group of 200 mg, improved further than the control level, while improvement was poor in the aminoguanidine-treated group. The urinary protein excretion level was increased in the diabetic control rats, without significance, relative to the control rats, but both Corni Fructus- and aminoguanidine-administered rats showed larger decreases in the level compared to control rats.

**Renal Weight, AGE, and Mitochondrial TBA-Reactive Substance Levels** The renal weights of diabetic rats treated with Corni Fructus and aminoguanidine decreased approximately to the same level, while diabetic control rats showed a significant 1.46-fold increase compared to the control rats (Table 4). The renal levels of AGES and mitochondrial TBA-reactive substance were increased 1.85- and 1.55-fold, respectively, in diabetic control rats as compared to the control rats.

**DISCUSSION**

The role of tight glycemic control, which was performed by The Diabetes Control and Complication Trial Research Group,\(^3\) has been emphasized and is still supported as being important to reduce diabetic microvascular disease in type 1 (insulin-dependent) diabetes, and for more than two decades, many researchers have discussed the pathological features of diabetes and/or its complications which are caused by the hyperglycemia-accelerated formation of AGEs in tissue. On the other hand, aminoguanidine, the prototype AGE inhibitor, has been effective in retarding the full range of diabetic complications, such as nephropathy, neuropathy, retinopathy, and vasculopathy. According to studies in STZ diabetic rats, administration of aminoguanidine attenuates the accumulation values (Table 4). These levels were equally decreased by the administration of Corni Fructus dose-dependently and of aminoguanidine; additionally, the effect observed in the 200 mg Corni Fructus-treated group was the same as in the aminoguanidine-treated group.

**Western Blotting of Renal Cortex** For further investigation, the protein levels of RAGE, NF-\(\kappa B\), 1\(-\beta\)-\(\tau\), TGF-\(\beta\), and CML in the renal cortex were determined by Western blot analyses, and the results are presented in Fig. 2. Based on band densities, renal RAGE, NF-\(\kappa B\), TGF-\(\beta\), and CML, which was estimated to be a 50 kDa molecule, CML-modified, and also anti-CML antibody-reactive protein, were significantly elevated in diabetic control rats compared with the control rats, but no significant changes were observed in 1\(-\beta\)-\(\tau\). However, down-regulation of these protein expressions was shown in the rats given high dose Corni Fructus but also aminoguanidine, and the levels of RAGE and NF-\(\kappa B\) observed in the Corni Fructus 200 mg group were more prominent than those of the aminoguanidine-treated group.

### Table 2. Antioxidative Effects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Nitrite/nitrate ((\mu M))</th>
<th>Inhibition of NBT reduction (% of diabetic control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>15.7±1.9</td>
<td>84.7±2.1</td>
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<tr>
<td>Diabetic control (n=8)</td>
<td>32.6±5.7 (^{E})</td>
<td>100.0±5.7 (^{E})</td>
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<tr>
<td>Diabetic+CF50 (n=8)</td>
<td>26.4±7.4 (^{a})</td>
<td>97.5±2.1 (^{E})</td>
</tr>
<tr>
<td>Diabetic+CF100 (n=8)</td>
<td>19.0±3.4 (^{a})</td>
<td>93.7±1.0 (^{a})</td>
</tr>
<tr>
<td>Diabetic+CF200 (n=8)</td>
<td>10.5±0.5 (^{a})</td>
<td>91.5±3.0 (^{a})</td>
</tr>
<tr>
<td>Diabetic+AG100 (n=5)</td>
<td>8.3±0.3 (^{a})</td>
<td>91.1±5.5 (^{a})</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (g/100 g B.W.)</th>
<th>AGE (AU)</th>
<th>Mitochondrial TBA-reactive substance (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=5)</td>
<td>0.72±0.03</td>
<td>3.35±0.48</td>
<td>1.72±0.09</td>
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<tr>
<td>Diabetic control (n=8)</td>
<td>1.05±0.03 (^{a})</td>
<td>6.19±0.29 (^{a})</td>
<td>2.66±0.19 (^{a})</td>
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<tr>
<td>Diabetic+CF50 (n=8)</td>
<td>0.98±0.01 (^{a})</td>
<td>6.05±0.36 (^{a})</td>
<td>1.79±0.13 (^{a})</td>
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<tr>
<td>Diabetic+CF100 (n=8)</td>
<td>0.97±0.02 (^{a})</td>
<td>5.32±0.46 (^{a})</td>
<td>1.51±0.08 (^{a})</td>
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<tr>
<td>Diabetic+CF200 (n=8)</td>
<td>0.98±0.01 (^{a})</td>
<td>4.92±0.27 (^{a})</td>
<td>1.47±0.09 (^{a})</td>
</tr>
<tr>
<td>Diabetic+AG100 (n=5)</td>
<td>0.99±0.03 (^{a})</td>
<td>4.93±0.81 (^{a})</td>
<td>1.45±0.12 (^{a})</td>
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</table>

### Table 3 Renal Function Parameters

<table>
<thead>
<tr>
<th>Items</th>
<th>Control (n=5)</th>
<th>Diabetic control (n=8)</th>
<th>Diabetic+CF50 (n=8)</th>
<th>Diabetic+CF100 (n=8)</th>
<th>Diabetic+CF200 (n=8)</th>
<th>Diabetic+AG100 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Urea-N (mg/dl)</td>
<td>18.5±0.7</td>
<td>33.6±2.1 (^{E})</td>
<td>27.8±1.5 (^{E})</td>
<td>23.1±1.6 (^{E})</td>
<td>20.9±1.1 (^{E})</td>
<td>24.7±3.1 (^{E})</td>
</tr>
<tr>
<td>Serum Cr (mg/dl)</td>
<td>0.341±0.010</td>
<td>0.404±0.021 (^{E})</td>
<td>0.348±0.012 (^{E})</td>
<td>0.351±0.015 (^{E})</td>
<td>0.338±0.017 (^{E})</td>
<td>0.364±0.007 (^{E})</td>
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<tr>
<td>Ccr (ml/kg B.W./min)</td>
<td>7.68±0.14</td>
<td>5.27±0.59 (^{E})</td>
<td>6.28±0.63 (^{E})</td>
<td>7.47±0.48 (^{E})</td>
<td>8.92±0.22 (^{E})</td>
<td>5.73±1.28 (^{E})</td>
</tr>
<tr>
<td>Urine protein (mg/dl)</td>
<td>13.3±0.9</td>
<td>16.8±2.2</td>
<td>12.5±3.1 (^{E})</td>
<td>12.1±2.7 (^{E})</td>
<td>10.3±0.9 (^{E})</td>
<td>9.7±1.1 (^{E})</td>
</tr>
</tbody>
</table>

**Notes:** CF50, 100 or 200: Corni Fructus (50, 100 or 200 mg/kg, p.o.); AG100, aminoguanidine (100 mg/kg, p.o.). Results are shown as the means±S.E. (n=5 or 8). a) \(p<0.05\), b) \(p<0.01\), c) \(p<0.001\) vs. control rats; d) \(p<0.05\), e) \(p<0.01\), f) \(p<0.001\) vs. diabetic control rats.

**Notes:** CF50, 100 or 200: Corni Fructus (50, 100 or 200 mg/kg, p.o.); AG100, aminoguanidine (100 mg/kg, p.o.). Results are shown as the means±S.E. (n=5 or 8). a) \(p<0.05\), b) \(p<0.01\), c) \(p<0.001\) vs. control rats; d) \(p<0.05\), e) \(p<0.01\), f) \(p<0.001\) vs. diabetic control rats.
of renal AGEs, as well as retarding the development of albu-
minuria and mesangial expansion,24) and thus, we carried out
this experiment with aminoguanidine to clarify whether
Corni Fructus had an anti-diabetic effect through inhibiting
AGE formation.

Our results indicated that diabetic rats induced by STZ did
not show body weight changes during the 10-d experimental
period, suggesting that these animals were undergoing
growth retardation due to the obstruction of glucose uptake
caused by the lack of insulin following STZ injection, but
treatment with Corni Fructus increased the body weight from
the initial value as well as that of aminoguanidine. On the
contrary, the serum glucose level observed in diabetic control
rats was increased beyond the initial level; however, Corni
Fructus- and aminoguanidine-treated rats showed significant
reductions in these levels compared with diabetic control
rats, and these levels were all lower than the initial levels.
Therefore, we supposed that Corni Fructus could show an
anti-hyperglycemic effect, having a correlation with body
weight gain.

In the present study, typical characteristics of diabetes
were shown. First is the increase of serum glycosylated pro-
tein, which is a parameter caused by glucose and other reduc-
ing sugars such as ribose and fructose reacting with the
amino residues of proteins to form Amadori products, for in-
stance, glycosylated hemoglobin (HbA1c), and the O2 is also
generated in the process of AGE formation. The next is ab-
normal lipid metabolism, which can lead to lipid peroxida-
tion with reactive oxygen species (ROS) and renal lipid accu-
mulation, playing a role in the pathogenesis of diabetic
nephropathy. In addition, diabetes also shows renal dysfunc-
tion, i.e., increased serum Cr, urea-N and proteinuria and de-
creased Ccr levels, reflecting a decline in the glomerular fil-
tration rate. However, the rats given Corni Fructus showed
improved glycation and total cholesterol levels, and a strong
renal function, with certain antioxidative activities, while
there was only a tendency to reduce the TBA-reactive sub-
stance level. On the other hand, aminoguanidine had almost
the same effects as those of Corni Fructus except concerning
renal function, shown by the serum Cr and Ccr levels, and
these differences might reflect AGE clearance; that is, the re-
duction of serum glycosylated protein was contrary to the
glycemic control shown in 100 mg of Corni Fructus and
aminoguanidine treated groups. Aminoguanidine had been
reported to inhibit nitric oxide production and to trap reactive
di-carbonyls, impeding conversion to AGEs, prevent cross-
linking, inhibit free radical formation, and lower total choles-
sterol and lipid peroxidation independent of glycemic
control.25–27) Hence, there are still uncertain mechanisms of
these medicinal efficacies, but we may hypothesize that dif-
ferences between Corni Fructus and aminoguanidine are that
Corni Fructus can improve AGE clearance with activated
renal function, while aminoguanidine mainly inhibits AGE
formation via its antioxidant properties.

Several lines of studies have provided substantial evidence
that multiple factors caused by hyperglycemia contribute to
the development of diabetic kidney disease. Among them,
the impacts of AGEs have been recognized over a wide
range, resulting in the expression and activation of patho-
genic mediators implicated in the development of diabetic
nephropathy, such as extracellular matrix, oxidative stress,
cytokines, and growth factors, via receptor-dependent and/or
independent pathways. Therefore, we first demonstrated
renal AGE accumulation and the mitochondrial lipid peroxi-
dation level. As a result, diabetic control rats showed in-
creased kidney weight and AGE accumulation significantly,
indicating renal hypertrophy, and also showed an increased
level of TBA-reactive substance. On the other hand, oral ad-
mistration of Corni Fructus ameliorated these changes.
Particularly, Corni Fructus successfully reduced the AGE
and TBA-reactive substance levels at the dose of 100 mg, and
the effect on the latter was strong compared with the control
level, suggesting that Corni Fructus suppressed the state of
oxidative stress. In contrast, the results of aminoguanidine re-
sembled those of Corni Fructus 200 mg treatment.

To clarify the effect of Corni Fructus concerning AGE ac-
tion with the receptor-dependent pathway, we performed
Western blot analyses against AGE-related protein expres-
sions in the renal cortex. It has been reported that AGEs trig-
ger the activation of NF-κB by interaction with RAGE, lead-
ing to its translocation to the nucleus where it induces tran-
scription,28) and the promoter region of the RAGE gene con-
tains NF-κB binding sites,29) potentially producing a self-
perpetuating pathway. Moreover, the AGE–RAGE interaction
activates TGF-β-Smad signaling pathways and subsequently
induces mesangial cell hypertrophy by the downstream medi-
ator of p27 and glomerular sclerosis by fibronectin synthesis.
through autocrine angiotensin II generation via ROS overproduction.\textsuperscript{30} In this study, diabetic control rats showed slight but significant increases in RAGE, NF-\(\kappa\)B, and TGF-\(\beta\)
but no change in \(\mathrm{IC}_{50}-\kappa\) protein expressions which are not only
regulated but also regulated by activated NF-\(\kappa\)B and sequestered NF-\(\kappa\)B in the cytoplasm to inactivate the NF-
\(\kappa\)B.\textsuperscript{31} While the 10-d Corni Fructus administration dose-de-
pendently normalized RAGE and NF-\(\kappa\)B proteins, and these
effects in the 200 mg-treated group were superior to
aminoguanidine treatment, Corni Fructus decreased the expression of TGF-\(\beta\) only at 200 mg but it was inferior to
aminoguanidine.

When we consider AGEs from another viewpoint, over a
decade have been identified in tissues including CML, pento-
sidine, and pyranine, and the known AGEs are immunologi-
cally distinct and coexist on different carrier proteins with al-
bumin, hemoglobin, collagens, lens crystalline, and low den-
sity lipoprotein cholesterol.\textsuperscript{32} 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.\textsuperscript{33}
Therefore, CML could serve as a general bio-marker of ox-
idative stress resulting from carbohydrate and lipid oxidation reactions; however, CML is characterized physicochemically
by neither cross-linking nor fluorescence. Therefore, in this
study, we measured renal AGEs using their characteristics of
fluorescence, but also measured the level of CML by Western blot
analysis in order to estimate another type of AGE. As a
result, accumulation of CML in the renal cortex also in-
creased in STZ-induced diabetic rats as well as the above-
mentioned protein expressions, and the effects of Corni Fruc-
tus and aminoguanidine were similar to those of TGF-\(\beta\).
Taking these results into consideration, though these correla-
tions were not clearly described due to the relatively short
period of the examination, we could speculate that Corni Fructus
influenced not only the AGE-RAGE pathway but also the TGF-\(\beta\)
signaling pathway and CML protein to some extent.

As discussed above, it was discovered that Corni Fructus
showed beneficial effects on the hyperglycemic state and
incidental renal damage and had a renoprotective effect, sug-
gest that Corni Fructus may ameliorate the course of dia-
abetic renal complications. However, the mechanisms respon-
sible for the effects of Corni Fructus on glucose-induced tis-
sue damage still need to be discussed. Some researchers have
emphasized the importance of hepatic function as a site of
AGE metabolism.\textsuperscript{34—36} In fact, tissue distribution was
in Hachimi-jio-gan which showed an antidiabetic effect via
reducing hyperglycemia and its related renal damage, as
reported previously.\textsuperscript{24—25} Consequently, Corni Fructus amelo-
rated glucose-associated metabolic disorders as well as
aminoguanidine and a decline in renal function, these effects
being similar to Hachimi-jio-gan. Thus, this study provides
supporting evidence for the therapeutic potential of Hachimi-
jo-gan, and Corni Fructus may also be helpful to prevent
and/or delay the onset of diabetes-induced renal injury; based
upon this, the investigation of major active constituents of
Corni Fructus is in progress.

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