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중국처방전 팔미지황환과 구성생약인 산수유의 당뇨병성 신증에 대한 보호 효과

박찬흠* · 최재수** · 요코자와 타카코***†
*농촌진흥청 국립원예특작과학원 인삼특작부, **부경대학교 수산과학대학, ***토야마대학 이공학연구부

Therapeutic Potential of Chinese Prescription Hachimi-Jio-Gan and Its Crude Drug Corni Fructus against Diabetic Nephropathy

Chan Hum Park*, Jae Sue Choi** and Takako Yokozawa***†
*Department of Herbal Crop Research, NIHHS, RDA, Eumseong 27709, Korea.
**Department of Food and Life Science, Pukyong National University, Busan 48513, Korea.
***Graduate School of Science and Engineering for Research, University of Toyama, Toyama 930-8555, Japan.

ABSTRACT

Background: Traditional plant drugs, are less toxic and free from side effects compared to general synthetic drugs. They have been used for the treatment of diabetes and associated renal damage. In this study, we evaluated effect of Hachimi-jio-gan against diabetic renal damage in a rat model of type 1 diabetic nephropathy induced by subtotal nephrectomy plus streptozotocin (STZ) injection, and in Otsuka Long-Evans Tokushima Fatty (OLETF) rats and *db/db* mice as a model of human type 2 diabetes, and its associated complications. To explore the active components of Hachimi-jio-gan, the antidiabetic effect of corni fructus, a consituent of Hachimi-jio-gan, and 7-*O*-galloyl-$_D$-sedoheptulose, a phenolic compound isolated from corni fructus, were investigated.
Methods and Results: We conducted an extensive literature search, and all required data were collected and systematically organized. The findings were reviewed and categorized based on relevance to the topic. A summary of all the therapeutic effects were reported as figures and tables.
Conclusions: Hachimi-jio-gan serves as a potential therapeutic agent to against the development of type 1 and type 2 diabetic nephropathy. From the results of characterization active components of corni fructus, 7-*O*-galloyl-$_D$-sedoheptulose is considered to play an important role in preventing and/or delaying the onset of diabetic renal damage. 7-*O*-Galloyl-$_D$-sedoheptulose is expected to serve as a novel therapeutic agent against the development of diabetic nephropathy.

***Key Words*:** Corni Fructus, Diabetic Nephropathy, Hachimi-Jio-Gan, 7-*O*-Galloyl-$_D$-Sedoheptulose

INTRODUCTION

Diabetic nephropathy is one of the major diabetic microvascular complications, along with retinopathy and neuropathy, and it is characterized by albuminuria, hypertension, a decline of the glomerular filtration rate (GFR), and glomerular sclerosis (Remuzzi and Bertani, 1998). During its development, glucose exerts toxic actions as a result of processes that are activated within the diabetic kidney, that is, the accumulation of advanced glycation endproducts (AGEs), increase in oxidative stress, abnormal polyol metabolism, and synthesis of growth factors (Lehmann and Schleicher, 2000).

For two decades, clinical and experimental studies have provided evidence of various beneficial effects of glycemic control, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, and antihypertensive drugs against diabetic nephropathy (UKPDS, 1998; HOPE, 2000;

†Corresponding author: (Phone) +08-76-445-6667 (E-mail) yokozawa@inm.u-toyama.ac.jp
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Brenner *et al.*, 2001; Lewis *et al.*, 2001). However, large numbers of patients in many countries are still suffering from diabetic nephropathy.

Traditionally, in Japan, China, and Korea, some Kampo prescriptions that are usually derived from natural sources have long been used for the improvement of subjective symptoms of diabetes and its complications. In particular, Hachimi-jio-gan ameliorates hyperglycemia, so it is used clinically to improve several disorders associated with diabetes (Goto *et al.*, 1989; Furuya *et al.*, 1999), and it has been used widely for the treatment of renal dysfunction in human subjects (Yamada, 1992). Furthermore, Hachimi-jio-gan has long been used widely to treat several chronic diseases, including chronic nephritis, sterility, and vegetative ataxia (Huang, 1997), although scientific evidence supporting a pharmacological basis for its therapeutic effects has yet to be published. Previously, we performed *in vitro* and *in vivo* studies using twelve Chinese prescriptions to investigate the possibility of curing diabetic nephropathy (Yokozawa *et al.*, 2001a), and demonstrated the effects of the oral administration of four Kampo prescriptions: Ompi-to (Wen-Pi-Tang), Keishi-bukuryo-gan (Gui-Zhi-Fu-Ling-Wan), Sairei-to (Chai-Ling-Tang), and Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan), in an animal model of diabetic nephropathy by measuring biochemical parameters that are affected by persistent hyperglycemia (Nakagawa *et al.*, 2001). Among these prescriptions, Hachimi-jio-gan is expected to provide a novel therapeutic approach against diabetic nephropathy.

Therefore, we evaluated the effects of Hachimi-jio-gan and identified its active compound, ameliorating diabetic renal damage using streptozotocin (STZ)-induced type 1 diabetes, a type 1 diabetic nephropathy rat model which underwent a subtotal nephrectomy plus STZ injection, and Otsuka Long-Evans Tokushima Fatty (OLETF) rats and *db/db* mice as a model of human type 2 diabetes and its complications.

MATERIALS AND METHODS

1. Hachimi-jio-gan

The composition of Hachimi-jio-gan was as follows: rehmanniae radix (*Rehmannia glutinosa* Libosch. Var. *purpurea* Makino) 6 g, corni fructus (*Cornus officinalis* Sieb. et Zucc.) 3 g, dioscoreae rhizoma (*Dioscorea japonica* Thunb.) 3 g, alismatis rhizoma (*Alisma orientale* Juzep.) 3 g, hoelen (*Poria cocos* Wolf) 3 g, moutan cortex (*Paeonia suffruticosa* Andrews) 2.5 g, cinnamomi cortex (*Cinnamomum cassia* Blume) 1.0 g, and aconiti tuber (*Aconitum carmichaeli* Debx.) 0.5 g. The above-mentioned crude drugs were boiled together gently in ten times their volume of water for 60 min, filtered, and the filtrate was spray-dried to obtain the extract at a yield of about 10%,

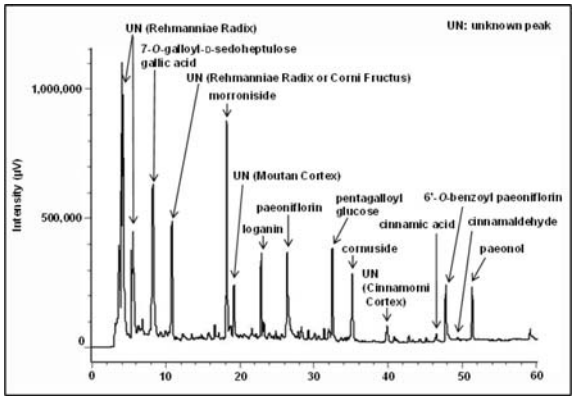

Fig. 1. HPLC profile of Hachimi-jio-gan extract.

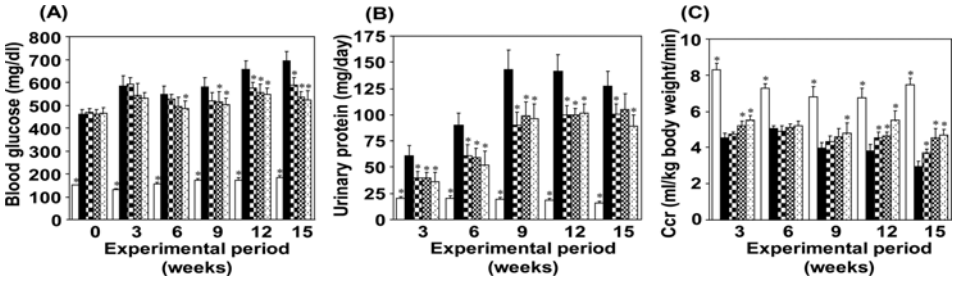

Fig. 2. Blood glucose (A), urinary protein (B) and CCr (C) in normal rats (□) and in diabetic nephropathy rats treated with either Hachimi-jio-gan. ▩; 50 ㎎/㎏ body weight, ▩;100 ㎎/㎏ body weight/day, ▩; 200 ㎎/㎏ body weight/day or control (■) for 15 weeks. Data are the means ± SEM. *$p < 0.05$ vs. control rats with diabetic nephropathy. Figure was taken from Yokozawa *et al.* (2004a).

by weight, of the original preparation. For the analysis of the components of Hachimi-jio-gan, HPLC chromatography was performed. As shown in Fig. 1, 7-*O*-galloyl-$_D$-sedoheptulose, gallic acid, morroniside, loganin, paeoniflorin, pentagalloyl glucose, cornuside, cinnamic acid, 6'-*O*-benzoyl paeoniflorin, cinnamaldehyde, and paeonol were detected as the major components of Hachimi-jio-gan.

2. Effect of Hachimi-jio-gan on type 1 diabetic nephropathy

Diabetic nephropathy is characterized as advanced kidney disease caused by longitudinal hyperglycemia and its metabolic abnormalities. Thus, this study involved long-term administration in order to show the effect of Hachimi-jio-gan on advanced kidney disease in diabetic nephropathy. The rats receiving sub-total nephrectomy and STZ injection showed metabolic abnormalities and renal lesions resembling diabetic nephropathy in humans (Yokozawa *et al.*, 2001b). During the experimental period, the serum glucose and urinary protein excretion levels were remarkably higher in the rat model employed in this study than in normal rats (Fig. 2A, B), indicating that disorders of glucose metabolism and changes in the capillary filtration barrier result in the increased permeability of the glomerular basement membrane. In addition, this rat model showed a significant decrease in creatinine clearance (CCr) (Fig. 2C), which is an effective index for expressing the GFR (Bell, 1991). However, the present investigation demonstrated that the administration of Hachimi-jio-gan reduced the serum glucose and urinary protein excretion levels, but increased CCr (Fig. 2A-C), suggesting that the effective control of glucose metabolism is a therapeutic target for preventing diabetic complications including diabetic nephropathy. Moreover, the decreased serum albumin level in this animal model was reversed by the administration of Hachimi-jio-gan. On the basis of these results, it was found that STZ injection to sub-totally nephrectomized rats resulted in progressive diabetic nephropathy, and Hachimi-jio-gan prevented or delayed it (Yokozawa *et al.*, 2004a).

Diabetic nephropathy is also well-known as a chronic disease which causes a non-enzymatic glycation reaction, an abnormal polyol pathway, and increases oxidative stress, which all synergistically affect renal mesangial cells, progressing glomerulosclerosis through some steps (Brownlee *et al.*, 1984; Cooper *et al.*, 1998; Lehmann and Schleicher, 2000). The glycosylated serum protein level increased in the animal model we used, which suggests that sugar oxidation was stimulated, increasing damage to both sugars and proteins in the circulation and vascular walls, continuing and reinforcing the cycle of oxidative stress and damage. In addition, the accumulation of AGEs in the kidney was also observed. However, Hachimi-jio-gan decreased the levels of glycosylated serum proteins and AGEs significantly and dose-dependently, suggesting that it can inhibit oxidative and irreversible renal damage caused

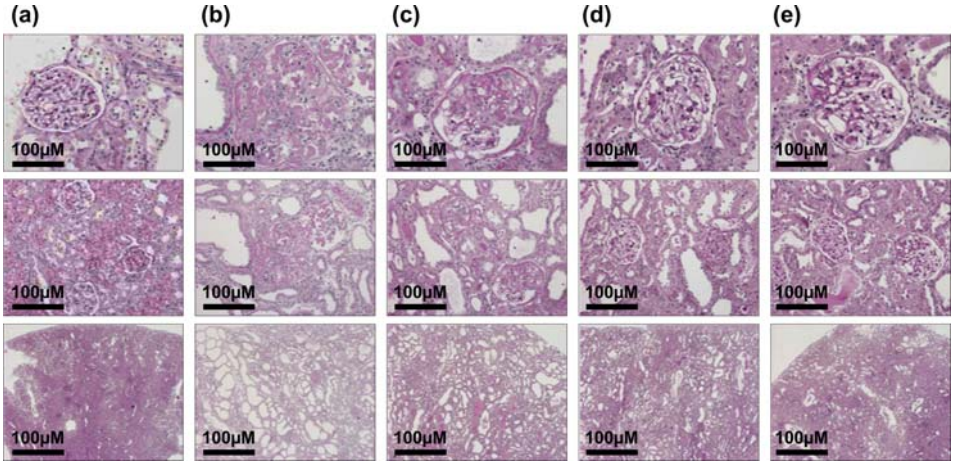

Fig. 3. Photomicrographs of the glomeruli (upper panel, × 200), tubulus (middle panel, × 100) and interstitium (lower panel, × 20) obtained from normal rats (a), diabetic nephropathy rats in the control (b) and Hachimi-jio-gan-treated [50 ㎎/㎏ body weight/day (c), 100 ㎎/㎏ body weight/day (d) and 200 ㎎/㎏ body weight/day (e)] groups. Figure was taken from Yokozawa *et al.* (2004a).

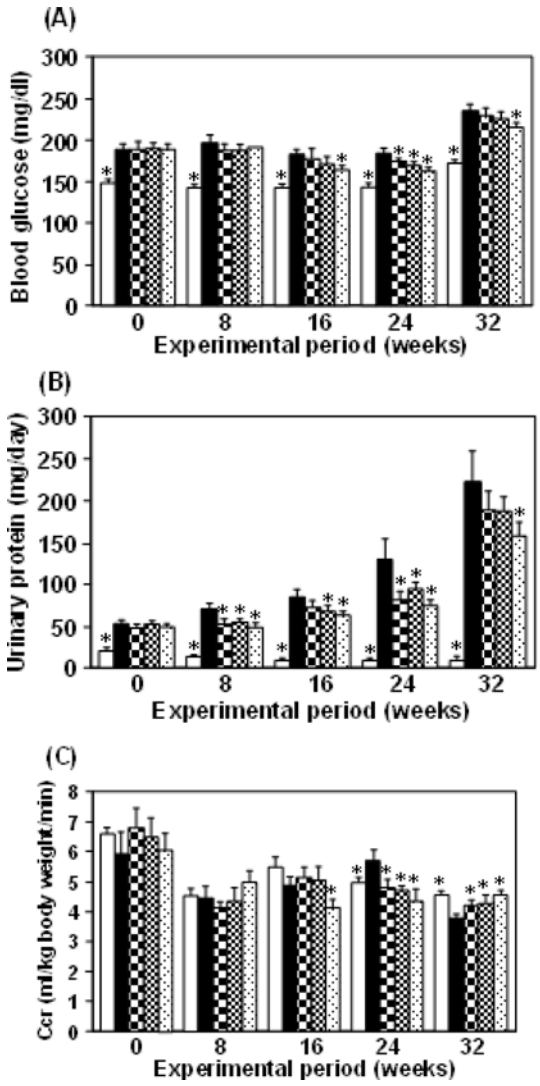

Fig. 4. Blood glucose (A), urinary protein (B) and CCr (C) in LETO rats (□) and in OLETF rats treated with either Hachimi-jio-gan. ▩; 50 mg/kg body weight, ▩; 100 mg/kg body weight/day, ▩; 200 mg/kg body weight/day or control (■) for 32 weeks. Data are the means ± SEM. *$p < 0.05$ vs. untreated OLETF rats. Figure was taken from Yamabe and Yokozawa (2006).

by protein glycation reactions. Also, our study showed that renal sorbitol levels were markedly elevated in rats with diabetic nephropathy compared with normal rats, but the administration of Hachimi-jio-gan significantly decreased the sorbitol level, suggesting that the disturbance of the glucose-dependent metabolic pathway and irreversible tissue damage caused by such disturbance under conditions of diabetic nephropathy would be ameliorated by decreasing the activity of the polyol pathway and inhibiting the protein glycation reaction. Moreover, the serum and renal thiobarbituric acid (TBA)-reactive substance levels were measured to determine the effects of Hachimi-jio-gan on oxidative stress in relation to the development of diabetic nephropathy. Lipid peroxidation levels in the serum and kidney were significantly elevated in rats with diabetic nephropathy compared with normal rats, while the administration of Hachimi-jio-gan reduced these levels. These findings suggest that the administration of Hachimi-jio-gan would ameliorate the oxidative stress associated with diabetic nephropathy through the inhibition of lipid peroxidation and, thus, it would result in the improvement of renal lesions caused by oxidative stress (Yokozawa *et al.*, 2004a).

The clinical manifestations of diabetic nephropathy, notably proteinuria, hypertension, and renal insufficiency, are closely associated with the severity of renal lesions (Winetz *et al.*, 1982; Adler, 1997). In the animal model used, the histopathological features of diabetic nephropathy, glomerular sclerosis, and tubulointerstitial lesions were observed. A substantial body of evidence from cell culture experiments and experimental models of diabetic nephropathy suggests that progressive renal damage is the ultimate expression of the pathological consequences of accumulating abnormalities of the glomerulus and tubulointerstitium (Winetz *et al.*, 1982; Yaqoob *et al.*, 1994; Adler, 1997). Hachimi-jio-gan had a significant protective effect on the renal lesions, as demonstrated by the histopathological evaluations, and Fig. 3 shows typical photomicrographs of kidney tissue obtained from each group, suggesting that Hachimi-jio-gan improves the renal dysfunction associated with renal lesions. Therefore, the results of the present study confirm that Hachimi-jio-gan has a protective effect in diabetic nephropathy rats through the amelioration of metabolic disorders, oxidative stress, and renal dysfunction associated with renal lesions (Yokozawa *et al.*, 2004a).

3. Effect of Hachimi-jio-gan on type 2 diabetic nephropathy

OLETF rats were used as a model of human type 2 diabetes (Kawano *et al.*, 1992), and Hachimi-jio-gan was orally administered for 32 weeks. Male OLETF diabetic rats compared with Long-Evans Tokushima Otsuka (LETO) control rats over the time course maintained a higher

body weight as well as food and water consumption levels, but Hachimi-jio-gan-treated groups did not show any differences, while Hachimi-jio-gan reduced the increase of the serum glucose level in OLETF diabetic rats from the latter half of the administration period (Fig. 4A). On the other hand, OLETF control rats showed marked proteinuria, but the oral administration of Hachimi-jio-gan markedly reduced the urinary protein excretion rates from an early stage and this was maintained up to the end of the experimental period (Fig. 4B). In addition, Ccr levels were higher in untreated OLETF rats than LETO or Hachimi-jio-gan-treated OLETF rats at 24 weeks of administration, indicating glomerular hyperfiltration, which led to a decline in their renal function at 32 weeks. However, the abnormal renal function was normalized by the administration of Hachimi-jio-gan (Fig. 4C), suggesting that long-term treatment with Hachimi-jio-gan may counteract renal damage and delay end-stage renal disease (Yamabe and Yokozawa, 2006).

We clarified that Hachimi-jio-gan ameliorated the oxidative and irreversible renal damage caused by protein glycation reactions using a type 1 diabetic nephropathy rat model (Yokozawa *et al.*, 2004a). A number of mechanisms contribute to the development of kidney damage in diabetes, that is, glucotoxicity, oxidative stress, AGE accumulation, fibrogenesis, and cytokine production such as transforming growth factor-β (TGF-β), which may be influenced by activation of the islet rennin-angiotensin system (Fukami *et al.*, 2004). OLETF rats showed significantly elevated serum glycosylated protein and renal AGE levels, but Hachimi-jio-gan treatment significantly and dose-dependently reduced them to below the levels of non-diabetic LETO rats. In addition, we investigated the TBA-reactive substance levels in the serum and kidney to clarify the influence of Hachimi-jio-gan on oxidative stress. The long-term administration of Hachimi-jio-gan reduced renal TBA-reactive substance levels significantly, despite only showing a tendency to reduce serum levels without significance. These findings suggest that the effect of Hachimi-jio-gan in reducing oxidative stress caused by the binding activity of AGEs, plasma proteins, and receptors for AGEs (RAGE) in glomerular mesangial cells or macrophages is superior to its effect against markers of oxidative stress in the serum. Furthermore, Hachimi-jio-gan suppressed not only TGF-β_1 and fibronectin protein synthesis but also nuclear factor-kappa B (NF-κB)-induced immune and inflammatory factors, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (Cox-2) (Yamabe and Yokozawa, 2006). Taken together, Hachimi-jio-gan may ameliorate functional abnormalities in association with the angiotensin II-TGF-β signaling pathway in mesangial cells induced by AGE-RAGE-mediated reactive oxygen species, which cause fibronectin overexpression in mesangial cells, leading to glomerular sclerosis.

4. Which is the main contributor among the eight crude drugs in Hachimi-jio-gan?

There have been many experiments focusing on the treatment of diabetes and its complications with herbal medicines including traditional Chinese prescriptions. According to our previous study, it was discovered that Hachimi-jio-gan had effects on metabolic disorders, especially on AGE formation and elevated oxidative stress in diabetic nephropathy (Nakagawa *et al.*, 2001), while we have demonstrated that Keishi-bukuryo-gan showed potential therapeutic effects on diabetic nephropathy via reducing oxidative stress (Nakagawa *et al.*, 2003). In addition, we also clarified that the administration of dried rehmanniae radix extract, which is the main constituent of Hachimi-jio-gan, attenuates renal dysfunction in diabetic nephropathy mainly due to its suppression of oxidative stress (Yokozawa *et al.*, 2004b); However, for the analysis of this prescription, further characterization of the other constituents is needed. According to the HPLC profile, 7-*O*-galloyl-$_D$-sedoheptulose, gallic acid, morroniside, loganin, paeoniflorin, pentagalloyl glucose, cornuside, cinnamic acid, 6'-*O*-benzoyl paeoniflorin, cinnamaldehyde, and paeonol were detected as the major components of Hachimi-jio-gan (Fig. 1). 7-*O*-Galloyl-$_D$-sedoheptulose, morroniside, and loganin are components of corni fructus (Wang *et al.*, 2003; Park *et al.*, 2012), and paeoniflorin is the component of moutan cortex common in Keishi-bukuryo-gan (Kaneda *et al.*, 1972). Therefore, on the assumption that corni fructus is a major contributor to the effect of Hachimi-jio-gan, the following study was carried out.

5. Corni fructus

Corni fructus has been used as a traditional medicine in China, Japan, and Korea. It has been reported that corni fructus has several biological activities, including

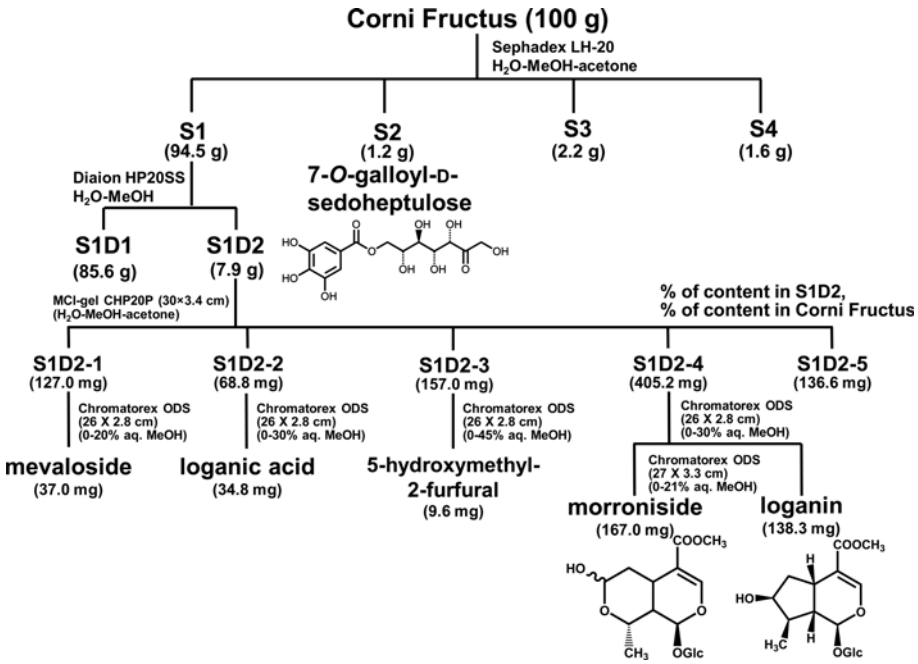

Fig. 5. Fractionation and isolation of compounds from corni fructus.

hypoglycemic, antineoplastic, and antimicrobial effects, and to improve liver and kidney functions (Chang *et al.*, 2004; Liou *et al.*, 2004; Vareed *et al.*, 2006). We previously reported that treatment with corni fructus ameliorated hyperglycemia, proteinuria, renal AGE formation, and related protein expressions, *i.e.*, RAGE, NF-κB, TGF-β_1, and N^{ε}-(carboxymethyl)lysine (CML), in the same way as aminoguanidine. However, improvement of the renal function, shown *via* serum Cr and CCr, was superior to that with aminoguanidine treatment (Yamabe *et al.*, 2007). 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 (Park *et al.*, 2009). Moreover, the atherogenic index was decreased in a dose-dependent manner, suggesting its protective role against cardiovascular disease through regulating cholesterol and lipoprotein levels (Park *et al.*, 2009). Thus, corni fructus may be helpful to prevent and/or delay the onset of diabetes and diabetic complications.

6. Efficacy-based identification of active component of corni fructus

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 very poorly. Hence, we have isolated the active components of corni fructus by employing activity-guided fractionation (Fig. 5). The major components of morroniside, loganin, or 7-*O*-galloyl-$_D$-sedoheptulose are considered to be a beneficial effect on diabetes and diabetic complications. 7-*O*-galloyl-$_D$-sedoheptulose is only detected as a compound from corni fructus, and its biological activity has not known until now except for our previous research. For these reasons, we further clarified the mechanisms of 7-*O*-galloyl-$_D$-sedoheptulose acting against diabetic kidney disease in expectation of identifying it as the novel active contributor in corni fructus.

7. 7-*O*-Galloyl-$_D$-sedoheptulose

To investigate the effect of 7-*O*-galloyl-$_D$-sedoheptulose, *db/db* mice were used. Being a spontaneous mutant strain, the C57BLKS/J *db/db* mouse, has the *db* mutation, a splicing mutation caused by a point mutation in the downstream intron of the leptin receptor gene, and so it is unresponsive to leptin. Leptin is a peptide hormone secreted by adipocytes and is involved in eating behavior and energy homeostasis. For this reason, after birth,

Table 1. Analysis of serum glucose, leptin, insulin, and C-peptide.

Item	*m/m*[1]	*db/db*		
		Vehicle[2]	GS20[3]	GS100[4]
Glucose (㎎/㎗)	186 ± 25*	791 ± 42	745 ± 31	683 ± 41
Leptin (ng/㎗)	2.30 ± 0.32*	20.24 ± 0.29	18.51 ± 0.75	17.57 ± 0.87*
Insulin (ng/㎖)	1.82 ± 0.06*	3.72 ± 0.45	2.68 ± 0.11*	2.40 ± 0.04*
C-peptide (pg/㎖)	177 ± 15*	1,983 ± 277	1,135 ± 139*	970 ± 142*

[1]*m/m*; misty, [2]Vehicle; vehicle-treated *db/db* mice, [3]GS20; 7-*O*-galloyl-$_D$-sedoheptulose 20 ㎎/㎏ body weight-treated *db/db* mice, [4]GS100; 7-*O*-galloyl-$_D$-sedoheptulose 100 ㎎/㎏ body weight-treated *db/db* mice. Data are the means ± SEM. *$p < 0.05$ vs. vehicle-treated *db/db* mouse values. Table was taken from Park *et al*. (2013).

homozygous diabetic (*db/db*) mice show unrepressed eating behavior, become obese, and develop severe insulin resistance associated with hyperinsulinemia and hyperglycemia (Sharma *et al.*, 2003). In the present study, *db/db* mice showed the symptoms of diabetes, such as hyperglycemia, hyperleptinemia, and hyperinsulinemia, compared with homozygous control (*m/m*) mice, as shown in Table 1.

7-*O*-Galloyl-$_D$-sedoheptulose treatment significantly decreased serum leptin and insulin levels at a dose of 100 ㎎/㎏, while the serum glucose level was slightly decreased without significance. The serum C-peptide level was compared as an indirect biomarker of insulin secretion. As expected, there was a significant increase in the serum C-peptide level in the vehicle-treated *db/db* group, which was closely associated with the increased removal of blood glucose (Park *et al.*, 2013). Thus, 7-*O*-galloyl-$_D$-sedoheptulose administration prevents diabetes in *db/db* mice, as evidenced by improved insulin sensitivity through the maintenance of normal insulin and glucose levels and the preservation of insulin and C-peptide levels in the serum, meaning that 7-*O*-galloyl-$_D$-sedoheptulose can ameliorate impaired glucose and insulin tolerance in *db/db* mice.

Diabetic renal damage is known to involve hyperglycemia-induced oxidative stress. Increased oxygen and peroxy radicals worsen tissue oxidative stress, which affects the oxidation of important macromolecules including proteins, lipids, carbohydrates, and DNA chains. Moreover, reactive oxygen species (ROS) activate the signal transduction cascade and transcription factors and overexpression of genes and proteins in glomerular mesangial and tubular epithelial cells, leading to pathological changes in the kidney (Lee *et al.*, 2003). Therefore, we investigated the effect of 7-*O*-galloyl-$_D$-sedoheptulose on the oxidative stress and ROS-related factors involved in the development of diabetes-induced renal damage using *db/db* mice. As shown in Fig. 6, 7-*O*-galloyl-$_D$-sedoheptulose effectively attenuated oxidative stress via a decrease in ROS and TBA-reactive substance levels as well as an enhanced reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio. In addition, increased serum urea nitrogen and creatinine levels associated with an abnormal renal function were significantly lowered by 7-*O*-galloyl-$_D$-sedoheptulose treatment (Table 2) (Park *et al.*, 2012).

Although the origin of increased ROS generation in renal disease is multifactorial, recent studies have focused on the fact that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase mainly participates in the process of ROS generation (Geiszt *et al.*, 2000; Nath and Norby, 2000; Shiose *et al.*, 2001). Renal NADPH oxidase expression was reported to be enhanced in glomeruli and distal tubules in the presence of diabetic nephropathy (Gill and Wilcox, 2006). Structurally, NADPH oxidase comprises the membrane-associated cytochrome b_{558}, composed of one $p22^{phox}$ and one $gp91^{phox}$ subunit and at least four cytosolic subunits ($p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and the small GTP_{ase} *rac*1 or *rac*2) (Babior *et al.*, 2002). In a rodent model of type 2 diabetes (*db/db* mouse), the renal expression of Nox-4 and $p22^{phox}$ was increased, and this was associated with ROS-induced renal damage (Sedeek *et al.*, 2010). Hence, we examined the renal protein expression of Nox-4 and $p22^{phox}$, subunits of NADPH oxidase, to identify the exact mechanism behind the reduction of renal ROS levels

Table 2. Kidney functional parameters.

Item	*m/m*[1]	*db/db*		
		Vehicle[2]	GS20[3]	GS100[4]
Urea nitrogen (㎎/㎗)	20.7 ± .9*	26.6 ± 1.1	26.3 ± 1.0	21.3 ± 1.1*
Creatinine (㎎/㎗)	0.52 ± 0.04*	1.24 ± 0.07	1.21 ± 0.06*	0.97 ± 0.04*

[1]*m/m*; misty, [2]Vehicle; vehicle-treated *db/db* mice, [3]GS20; 7-*O*-galloyl-$_D$-sedoheptulose 20 ㎎/㎏ body weight-treated *db/db* mice, [4]GS100; 7-*O*-galloyl-$_D$-sedoheptulose 100 ㎎/㎏ body weight-treated *db/db* mice. Data are the means ± SEM. *$p < 0.05$ vs. vehicle-treated *db/db* mouse values. Table was taken from Park *et al*. (2012).

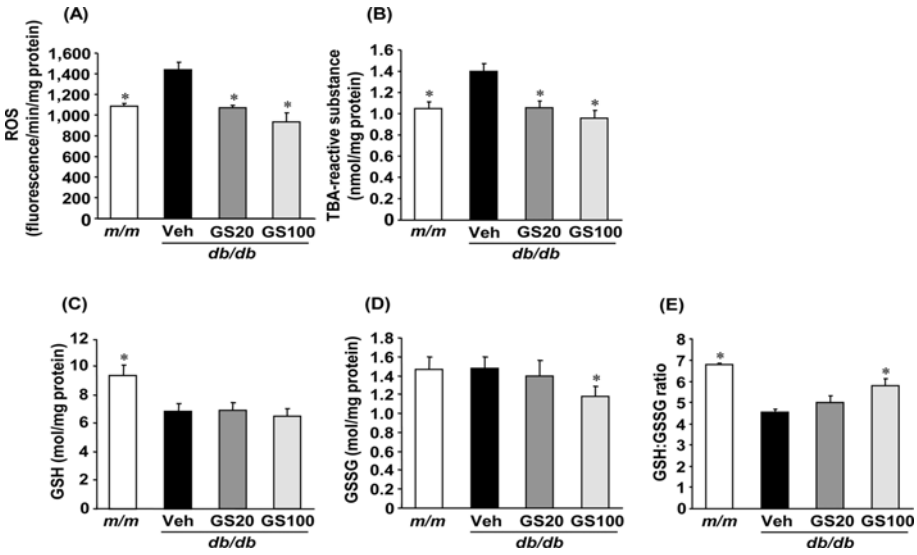

Fig. 6. ROS (A), TBA-reactive substance (B), GSH (C), GSSG (D) and GSH/GSSG (E) levels in the kidney of type 2 diabetic *db/db* mice treated with 7-*O*-galloyl-$_D$-sedoheptulose for 8 weeks. *m/m*; misty, Veh; vehicle-treated *db/db* mice, GS20; 7-*O*-galloyl-$_D$-sedoheptulose 20 ㎎/㎏ body weight-treated *db/db* mice, GS100; 7-*O*-galloyl-D-sedoheptulose 100 ㎎/㎏ body weight-treated *db/db* mice. Data are the means ± SEM. **p* < 0.05 vs. vehicle-treated *db/db* mouse values. Figure was taken from Park *et al.*, (2012).

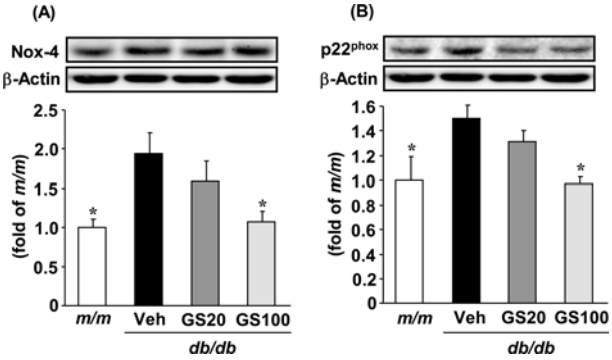

Fig. 7. Nox-4 (A) and p22^{phox} (B) protein expressions in the kidney of type 2 diabetic *db/db* mice treated with 7-*O*-galloyl-$_D$-sedoheptulose for 8 weeks. *m/m*; misty, Veh;vehicle-treated *db/db* mice, GS20; 7-*O*-galloyl-$_D$-sedoheptulose 20 ㎎/㎏ body weight-treated *db/db* mice, GS100; 7-*O*-galloyl-$_D$-sedoheptulose 100 ㎎/㎏ body weight-treated *db/db* mice. Data are the means ± SEM. **p* < 0.05 vs. vehicle-treated *db/db* mouse values. Figure was taken from Park *et al.* (2012).

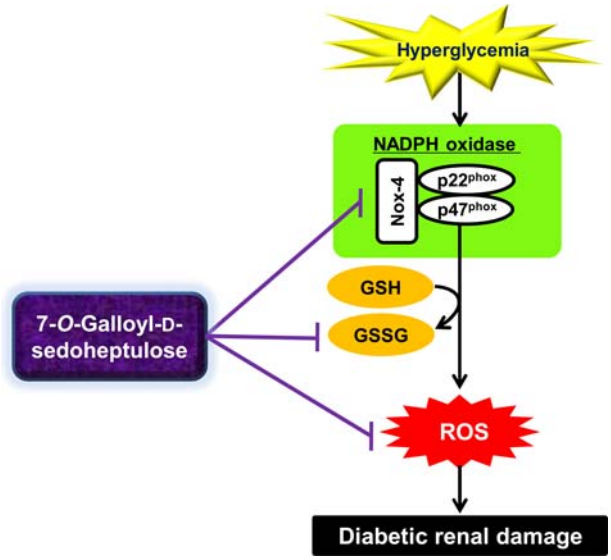

Fig. 8. Possible anti-diabetic nephropathy mechanism of 7-*O*-galloyl-$_D$-sedoheptulose.

in the 7-*O*-galloyl-$_D$-sedoheptulose-treated group. In western blot analysis, Nox-4 and p22^{phox} protein expressions were significantly upregulated in the type 2 diabetic kidney; however, 7-*O*-galloyl-$_D$-sedoheptulose administration at 100 ㎎/㎏ significantly normalized the increased subunits of NADPH oxidase (Fig. 7) (Park *et al.*, 2012). These results indicate that the inhibitory effect of 7-*O*-galloyl-$_D$-sedoheptulose on ROS generation was due to the downregulated expression of NADPH oxidase in *db/db* mice.

This study supports the concept that, in hyperglycemia, enhanced oxidative stress and the upregulation of NADPH oxidase are associated with renal damage in type 2 diabetes. 7-*O*-Galloyl-$_D$-sedoheptulose administration

effectively alleviated these unfavorable responses in the presence of diabetic injury of the kidney, as shown in Fig. 8. Therefore, the present study can accumulate the 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.

RESULTS AND DISCUSSION

We have studied Hachimi-jio-gan in order to establish a therapeutic strategy for the prevention of diabetic nephropathy using rat models. These results indicate that Hachimi-jio-gan could have a protective role either in early or advanced stages of type 1 and 2 diabetic nephropathy via the amelioration of several metabolic disorders caused by hyperglycemia and also a renoprotective effect. To add to these findings, we clarified that corni fructus (*Cornus officinalis* Sieb. et Zucc.) has similar effects to Hachimi-jio-gan, and also successfully identified the most important contributor to the effect of Hachimi-jio-gan, *i.e.*, 7-*O*-galloyl-$_D$-sedoheptulose, which was isolated from corni fructus. This component is expected to become a novel therapeutic agent against the development of diabetic nephropathy.

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