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Protective effects of the Chinese prescription Hachimi-jio-gan against diabetic oxidative stress

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Abstract

We used rats with streptozotocin (STZ)-induced diabetes to investigate the effects of Hachimi-jio-gan on diabetic oxidative stress. Oral administration of Hachimi-jio-gan, at a dose of 50, 100 or 200 mg kg^{-1} (body weight) daily, for 10 days to rats with STZ-induced diabetes resulted in significant dose-dependent decreases in serum levels of glucose and glycosylated protein, implying that Hachimi-jio-gan improves the abnormal glucose metabolism that leads to oxidative stress. Hachimi-jio-gan also showed a tendency to reduce the urine volume and significantly reduced the elevated urinary protein level. Moreover, rats with STZ-induced diabetes had high serum levels of superoxide and nitrite/nitrate. However, the administration of Hachimi-jio-gan dose-dependently reduced the overproduction of radicals associated with diabetes, suggesting the role of Hachimi-jio-gan as a radical scavenger that could protect against diabetic oxidative stress. Furthermore, thiobarbituric acid-reactive substance levels in serum and hepatic and renal mitochondria were dose-dependently lower in the Hachimi-jio-gan-treated groups than in the control diabetic group, which implies that Hachimi-jio-gan would alleviate the oxidative stress associated with diabetes through the inhibition of lipid peroxidation. These results indicate that Hachimi-jio-gan is a potential therapeutic agent that will reduce the damage caused by oxidative stress involved in diabetes.

Introduction

Diabetes is a general term referring to disorders characterized by excessive urine excretion and a metabolic disorder induced by high blood glucose levels. Diabetes is primarily characterized by hyperglycaemia that is mainly attributed to diabetic oxidative stress caused by several factors. Hyperglycaemia leads to the overproduction of free radicals by the non-enzymatic glycation of proteins through Maillard's reaction and the free radicals exert deleterious effects on the function of β-cells making them vulnerable to oxidative stress (Brownlee et al 1984; Njoroge & Monnier 1989). In addition, hyperglycaemia can degrade antioxidant enzyme defences, thereby allowing reactive oxygen species to cause cellular and tissue damage. In recent years, several workers have suggested that oxygen free radicals are generated as a result of hyperglycaemia and cause various complications of diabetes, such as nephropathy, retinopathy and neuropathy (Baynes 1991; Halliwell et al 1992; Giugliano et al 1996; Baynes & Thorpe 1999). In particular, hyperglycaemia increases the activity of protein kinase C in vascular smooth muscle and endothelial cells, which may also contribute to diabetic nephropathy (Larkins & Dunlop 1992). Therefore, attenuation of oxidative stress by modulating hyperglycaemic conditions and decreasing reactive oxygen free radical production may prevent or reverse abnormalities associated with diabetes mellitus and its complications.

Hachimi-jio-gan, a traditional Kampo prescription composed of eight constituents, has long been used in Japan and China for the alleviation of subjective symptoms of diabetes and its complications. In particular, Hachimi-jio-gan ameliorates hyperglycaemia, so it is used clinically to improve several disorders associated with diabetes (Goto et al 1989; Furuya et al 1999). In addition, it has been widely used to treat renal dysfunction in man (Yamada 1992) and several chronic diseases, including chronic nephritis, sterility and vegetative ataxia (Huang 1997). Furthermore, in a previous study, we measured the effects of the administration of four Kampo prescriptions,

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Ompi-to, Keishi-bukuryo-gan, Sairei-to and Hachimi-jio-gan, for 5 weeks in an animal model of diabetic nephropathy by evaluating biochemical processes induced by persistent hyperglycaemia (Nakagawa et al 2001). On the basis of the above reports, Hachimi-jio-gan is expected to be a potential novel therapeutic agent for diabetes and its complications. Although several factors have been suggested as the protective mechanism against diabetic pathological conditions, we could hypothesize the amelioration of diabetic oxidative stress as the crucial protective mechanism. However, studies on the effects of Hachimi-jio-gan on oxidative stress related to diabetes and hyperglycaemia have not been carried out yet.

In this study, we investigated the effects of Hachimi-jio-gan on diabetic oxidative stress by determining whether it ameliorated hyperglycaemia and inhibited free radical production and lipid peroxidation in a diabetic animal model induced by streptozotocin (STZ) that destroys β-cells as a result of damage caused by radicals and a deficit in antioxidative defences.

Materials and Methods

Preparation of Hachimi-jio-gan extract

The Hachimi-jio-gan extract used was produced by Tsumura Juntendo Inc. (Tokyo, Japan). 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 gently in 10 times their volumes of water for 60 min and then filtered. The filtrate was spray-dried to obtain the extract at a yield of about 10%, by weight, of the original preparation. For the analysis of Hachimi-jio-gan's components, the aqueous extract (0.5 g) was extracted with 20 mL methanol under ultrasonication for 30 min. The solution was filtered through a membrane filter (450 nm) and then subjected to HPLC analysis, which was performed using a TSK-GEL ODS-80TS column ($\phi 4.6 \times 250$ mm; TOSOH, Japan) with an LC $10AD_{vp}$ pump and an SPD-$M10A_{vp}$ absorbance detector. The solvent for elution was (A) 50 mM AcOH–$AcONH_4$ and (B) CH_3CN, and the column was eluted with a linear gradient of 90% A and 10% B changing over 60 min to 100% B. The flow rate was 1.0 mL min^{-1} and the effluent from the column was monitored and the three-dimensional data was processed by an SPD-M10A array detector. All assigned peaks were identified by carrying out a co-injection test with authentic samples and compared with the UV spectral data using the CLASS LC-10 Ver. 1.62 software (Shimadzu, Japan). The three-dimensional HPLC profile of Hachimi-jio-gan extract is shown in Figure 1. Morroniside, loganin and paeoniflorin were detected as the major compounds of Hachimi-jio-gan; penta-*O*-galloylglucose, benzoylmesaconine, benzoylpaeoniflorine, 16-ketoalisol A, paeonol, cinnamic acid and cinnamaldehyde were also observed.

Animal experiment

Animal preparation

The Guidelines for Animal Experimentation, approved by Toyama Medical and Pharmaceutical University, were followed in these experiments. Male Wistar rats (120–130 g) from Japan SLC, Inc. (Hamamatsu, Japan) were used. They were kept in a wire-bottomed cage under a conventional lighting regimen with a dark night. The room temperature (about 25°C) and humidity (about 60%) were controlled automatically. They were allowed free access to laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water. After several days of adaptation, STZ (Sigma Chemical Co., St Louis, MO) dissolved in citrate buffer (pH 4.5) was injected intraperitoneally at a dose of 50 mg kg^{-1} following overnight fasting. One week after the injection, the glucose level of blood from the tail vein was determined and the rats were divided into 4 groups ($n = 7$ per group), avoiding any inter-group differences in blood glucose levels. The control group was given saline (vehicle), while the other groups were given orally the Hachimi-jio-gan extract at a dose of 50, 100 or 200 mg kg^{-1} daily using a stomach tube. After administration for 10 consecutive days, the rats were killed by decapitation, blood samples were collected and serum was separated immediately by centrifugation. The liver and kidneys were removed, rinsed with cold saline, frozen and stored at −80°C until they were assayed.

Assays of serum and urine samples

The glucose level was measured using a commercial kit (Glucose CII-Test Wako; Wako Pure Chemical Industries, Osaka, Japan). The creatinine (Cr) level was determined using a commercial reagent (CRE-EN Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan). Glycosylated protein level was determined using a modified thiobarbituric acid (TBA) assay of Fluckiger & Winterhalter (1976), in which non-enzymatically bound glucose is released as 5-hydroxymethylfurfural (5-HMF) and quantified colorimetrically. Briefly, serum (100 μL) was diluted to 1.0 mL with H_2O and mixed with 500 μL oxalic acid (1 M), hydrolysed for 4.5 h at 100°C and then, after reaction with TBA, glycosylated haemoglobin was quantified by measuring the absorbance at 443 nm. TBA-reactive substance level was measured using the method of Naito & Yamanaka (1978). Protein level was quantified using a commercial protein assay kit (A/G B-Test Wako; Wako Pure Chemical Industries) with bovine serum albumin as the standard. Superoxide (O_2^-) level was measured using a method based on that of Ewing & Janero (1995). Twenty microlitres of serum was pipetted into a microwell containing 200 μL freshly prepared 33 μM phenazine methosulfate in 50 mM phosphate buffer, pH 7.4. After incubation at room temperature for

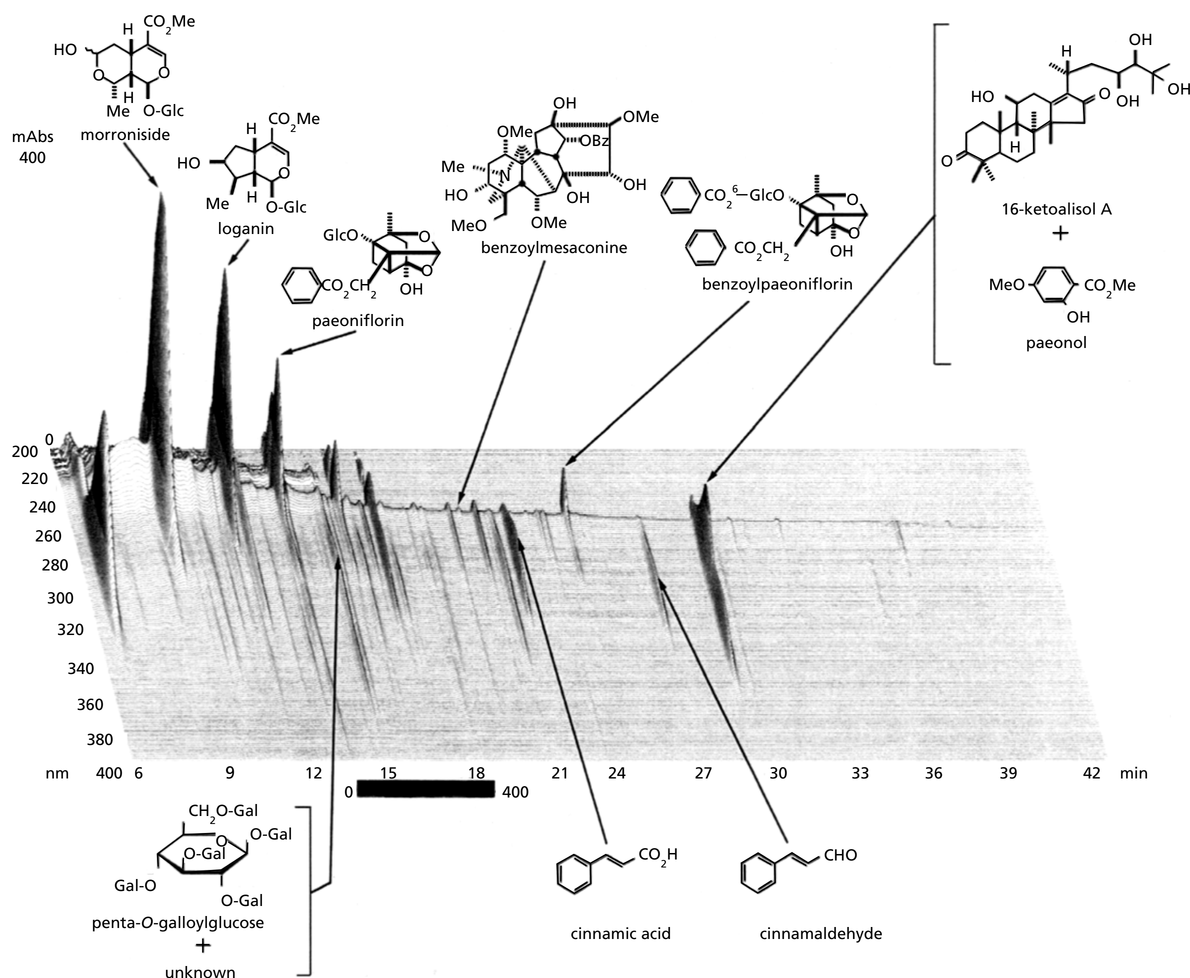

Figure 1 Three-dimensional HPLC profile of Hachimi-jio-gan extract.

5 min, the O_2^- level was measured at 540 nm using a Microplate Reader, Model 3550-UV (Bio-Rad, Tokyo, Japan). The nitrite and nitrate level was measured primarily by following the method of Misko et al (1993). Briefly, serum was filtered through an Ultrafree-MC microcentrifuge filter unit (Millipore, Bedford, MA) for 1 h at 17 300 *g* to remove haemoglobin released by cell lysis. As nitrite in serum was mostly oxidized to nitrate by reacting with the iron-haem center of haemoglobin, the resulting nitrate was first reduced to nitrite by incubation with nitrate reductase and nitrite/nitrate level was measured by a microplate assay method based on the Griess reaction (Green et al 1982). Urinary protein level was determined by the sulfosalicylic acid method (Sakagishi 1968).

Preparation of mitochondria and measurement of TBA-reactive substance level

Mitochondria were prepared from liver and kidney homogenates by differential centrifugation (800 *g* and 12 000 *g*, respectively) in a refrigerated centrifuge (4°C) according to the methods of Johnson & Lardy (1967) and Jung & Pergande (1985), respectively, with slight modifications. Each pellet was resuspended in preparation medium and the TBA-reactive substance concentration was determined by the method of Uchiyama & Mihara (1978). Protein level was evaluated by the method of Itzhaki & Gill (1964) with bovine serum albumin as the standard.

Data analysis

Results are expressed as means ± s.e. The effect on each parameter was examined using the one-way analysis of variance. Individual differences between groups were evaluated using Dunnett's test and those at $P < 0.05$ were considered to be statistically significant.

Results

Table 1 shows the effects of Hachimi-jio-gan on the changes in body and tissue weights of rats with STZ-induced diabetes over the 10-day experimental period. The body weight gain of STZ-induced diabetic rats was significantly lower than that of normal rats. The

Table 1 Effect of Hachimi-jio-gan on body and tissue weights of rats with streptozotocin-induced diabetes

Group	Dose (mg kg^{-1} daily)	Body weight			Liver weight (g (100 g B.W.)$^{-1}$	Kidney weight (g (100 g B.W.)$^{-1}$
		Initial (g)	Final (g)	Gain (g/10 days)		
Normal rats	—	220.8 ± 3.8	284.4 ± 8.9	63.6 ± 5.6	3.51 ± 0.09	0.70 ± 0.01
Diabetic rats						
Control	—	177.0 ± 3.1	205.4 ± 8.3	28.4 ± 6.1[a]	4.30 ± 0.09[a]	0.99 ± 0.03[a]
Hachimi-jio-gan	50	172.7 ± 4.3	205.0 ± 8.3	28.8 ± 5.1[a]	4.12 ± 0.06[a,b]	0.99 ± 0.03[a]
Hachimi-jio-gan	100	188.0 ± 2.4	213.8 ± 5.5	25.8 ± 4.8[a]	4.10 ± 0.04[a,b]	0.98 ± 0.03[a]
Hachimi-jio-gan	200	178.3 ± 2.6	209.6 ± 6.1	31.3 ± 3.8[a]	4.00 ± 0.09[a,c]	0.98 ± 0.02[a]

[a]$P < 0.001$ vs normal rats; [b]$P < 0.01$, [c]$P < 0.001$ vs diabetic control rats.

Table 2 Effect of Hachimi-jio-gan on serum constituent levels of rats with streptozotocin-induced diabetes

Group	Dose (mg kg^{-1} daily)	Glucose (mg dL^{-1})	Glycosylated protein (nmol (mg protein)$^{-1}$)	Cr (mg dL^{-1})
Normal rats	—	166.7 ± 2.2	6.71 ± 0.09	0.332 ± 0.025
Diabetic rats				
Control	—	560.0 ± 28.4[a]	22.02 ± 1.57[a]	0.349 ± 0.026
Hachimi-jio-gan	50	521.0 ± 17.5[a,b]	21.57 ± 0.91[a]	0.340 ± 0.019
Hachimi-jio-gan	100	497.4 ± 14.9[a,c]	20.37 ± 1.10[a]	0.326 ± 0.027
Hachimi-jio-gan	200	464.5 ± 13.3[a,c]	18.17 ± 0.90[a,c]	0.309 ± 0.019

[a]$P < 0.001$ vs normal rats; [b]$P < 0.05$, [c]$P < 0.001$ vs diabetic control rats.

Hachimi-jio-gan-treated groups had slightly higher body weight gains than control groups, but these changes were not significant. In addition, the liver weight of diabetic rats showed significant increase compared with that of normal rats, while Hachimi-jio-gan treatment for 10 days attenuated the abnormal increase significantly. On the other hand, Hachimi-jio-gan extract did not have any effect on the increase in the kidney weight of diabetic control rats.

The serum levels of glucose and glycosylated protein were markedly elevated in rats with STZ-induced diabetes (Table 2). However, the administration of Hachimi-jio-gan reduced these levels significantly and dose-dependently. The glucose level of control rats increased from 166.7 to 560.0 mg dL^{-1}, but it decreased in rats given Hachimi-jio-gan at doses of 50, 100 and 200 mg kg^{-1} daily to 521.0, 497.4 and 464.5 mg dL^{-1}, respectively. Moreover, the elevation of the glycosylated protein concentration in diabetic control rats from 6.71 to 22.02 nmol (mg protein)$^{-1}$ was reduced significantly in rats given Hachimi-jio-gan at a dose of 200 mg kg^{-1} daily to 18.17 nmol (mg protein)$^{-1}$. The serum Cr levels of the groups given Hachimi-jio-gan were slightly, but not significantly, lower than the control value.

The urine volume and urinary protein levels of control rats with diabetes were much higher than those of normal rats (Table 3). However, the urine volume of rats with STZ-induced diabetes given Hachimi-jio-gan orally for 10 days showed a tendency to decline without significance.

Table 3 Effect of Hachimi-jio-gan on urine volume and urinary protein of rats with streptozotocin-induced diabetes

Group	Dose (mg kg^{-1} daily)	Urine volume (mL day^{-1})	Urinary protein (mg day^{-1})
Normal rats	—	12.3 ± 2.6	1.02 ± 0.27
Diabetic rats			
Control	—	97.3 ± 9.4[a]	9.25 ± 0.93[a]
Hachimi-jio-gan	50	96.4 ± 6.3[a]	7.09 ± 0.97[a,b]
Hachimi-jio-gan	100	91.2 ± 12.3[a]	6.88 ± 0.49[a,c]
Hachimi-jio-gan	200	90.8 ± 6.7[a]	6.17 ± 1.08[a,c]

[a]$P < 0.001$ vs normal rats; [b]$P < 0.01$, [c]$P < 0.001$ vs diabetic control rats.

In addition, Hachimi-jio-gan inhibited urinary protein excretion significantly in a dose-dependent manner. The urinary protein level of the diabetic control rats was 9.25 mg day^{-1}, but those of diabetic rats given 50, 100 and 200 mg kg^{-1} Hachimi-jio-gan daily were 7.09, 6.88 and 6.17 mg day^{-1}, respectively.

Table 4 shows the inhibitory effects of Hachimi-jio-gan on serum levels of O_2^- and nitrite/nitrate, an indicator of nitric oxide (NO) production. The rats with diabetes induced by STZ had higher serum levels of O_2^- than normal rats. However, the administration of Hachimi-jio-gan at doses of 50, 100 and 200 mg kg^{-1} daily to

Table 4 Effect of Hachimi-jio-gan on serum superoxide and nitrite/nitrate levels of rats with streptozotocin-induced diabetes

Group	Dose (mg kg^{-1} daily)	O_2^- (absorbance)	Nitrite/nitrate (μM)
Normal rats	—	0.312 ± 0.026	2.07 ± 0.07
Diabetic rats			
Control	—	0.383 ± 0.034^b	2.32 ± 0.27
Hachimi-jio-gan	50	0.381 ± 0.009^b	2.13 ± 0.14
Hachimi-jio-gan	100	0.340 ± 0.015^c	$1.71 \pm 0.15^{a,e}$
Hachimi-jio-gan	200	0.332 ± 0.009^d	$1.79 \pm 0.17^{a,e}$

$^aP < 0.05$, $^bP < 0.001$ vs normal rats; $^cP < 0.05$, $^dP < 0.01$, $^eP < 0.001$ vs diabetic control rats.

diabetic rats resulted in significant decreases in the serum levels of O_2^- and NO compared with those of diabetic control rats. The O_2^- level increased from 0.312 to 0.383, whereas the oral administration of Hachimi-jio-gan at doses of 50, 100 and 200 mg kg^{-1} daily led to decreases to 0.381, 0.340 and 0.332, respectively. In addition, the serum nitrite/nitrate level of rats given 200 mg kg^{-1} daily Hachimi-jio-gan decreased from 2.32 μM to 1.79 μM.

The diabetic rats had higher lipid peroxidation levels in serum, liver and kidney than those of normal rats (Table 5). However, the administration of Hachimi-jio-gan reduced these levels significantly. As the dose administered increased, the TBA-reactive substance levels decreased significantly. The 50, 100 and 200 mg kg^{-1} daily doses resulted in decreases of the serum TBA-reactive substance level from 0.166 nmol (mg protein)$^{-1}$ to 0.083, 0.081 and 0.073 nmol (mg protein)$^{-1}$, respectively. In addition, in rats given Hachimi-jio-gan 200 mg kg^{-1} daily orally, the TBA-reactive substance levels of the hepatic and renal mitochondria declined from 0.596 to 0.344 nmol (mg protein)$^{-1}$ and from 1.860 to 1.414 nmol (mg protein)$^{-1}$, respectively.

Discussion

Diabetes mellitus is the most common endocrine disorder characterized by hyperglycaemia and long-term complications affecting the eyes, kidneys, nerves and blood vessels. The underlying mechanism responsible for its complications, as well as for diabetes itself, remains unclear, though possible events such as activation of protein kinase C, the polyol pathway, non-enzymatic glycation and oxidative stress have been suggested (Giugliano et al 1996; King 1996; Cooper et al 1997; Williams et al 1997; Lu et al 1998; Sharpe et al 1998; Ceriello 2000). Recently, much attention has been focused on the role of oxidative stress and it has been suggested that oxidative stress may constitute the key and common events in the pathogenesis of different diabetic complications (Ceriello 2000). Therefore, amelioration of diabetic oxidative stress may prevent or reverse abnormalities associated with diabetes and its complications.

Hachimi-jio-gan, a traditional Kampo prescription composed of eight constituents, has long been used in Japan and China for the alleviation of subjective symptoms of diabetes and its complications. In addition, it has been widely used to treat renal dysfunction in man (Yamada 1992) and several chronic diseases, including chronic nephritis, sterility and vegetative ataxia (Huang 1997), although the pharmacological basis for its therapeutic effects has not been established. On the basis of the above reports, Hachimi-jio-gan may be a potential novel therapy for diabetes and its complications. In addition, the therapeutic role of Hachimi-jio-gan is considered to be attributed to the amelioration of diabetic oxidative stress. Therefore, in this study we used the STZ-induced diabetic rat model to investigate the effects of Hachimi-jio-gan on diabetic oxidative stress.

The abnormalities of energy utilization and metabolism responsible for the destruction of β-cells and disorder of insulin secretion in the diabetic state could cause the abnormal changes in body weight gain and tissue weight. The rats with diabetes induced by STZ showed loss of body weight gain and increased liver and kidney weights. However, the administration of Hachimi-jio-gan attenuated the physiological changes associated with diabetes, implying that Hachimi-jio-gan normalized the energy utilization and metabolism.

To investigate the effect of Hachimi-jio-gan on abnormal glucose metabolism, we determined the levels of glucose and glycosylated protein in serum. Hyperglycaemia, the primary clinical manifestation of diabetes, is associated

Table 5 Effect of Hachimi-jio-gan on TBA-reactive substance levels of rats with streptozotocin-induced diabetes

Group	Dose (mg kg^{-1} daily)	Serum (nmol (mg protein)$^{-1}$)	Hepatic mitochondria (nmol (mg protein)$^{-1}$)	Renal mitochondria (nmol (mg protein)$^{-1}$)
Normal rats	—	0.055 ± 0.006	0.421 ± 0.014	1.743 ± 0.083
Diabetic rats				
Control	—	0.166 ± 0.034^a	0.596 ± 0.052^a	1.860 ± 0.072^a
Hachimi-jio-gan	50	$0.083 \pm 0.005^{a,b}$	$0.512 \pm 0.026^{a,b}$	$1.653 \pm 0.123^{a,c}$
Hachimi-jio-gan	100	$0.081 \pm 0.005^{a,c}$	$0.412 \pm 0.034^{a,c}$	$1.527 \pm 0.062^{a,c}$
Hachimi-jio-gan	200	$0.073 \pm 0.008^{a,c}$	$0.344 \pm 0.017^{a,c}$	$1.414 \pm 0.074^{a,c}$

$^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ vs normal rats; $^dP < 0.01$, $^eP < 0.001$ vs diabetic control rats.

with the development of certain diabetic complications (Brownlee & Cerami 1981). In addition, hyperglycaemia and glycation of proteins are associated with the development of diabetic complications, resulting in the generation of oxygen free radicals (Brownlee & Cerami 1981). Indeed, excessive glycation of many proteins is detected in humans with diabetes. 5-HMF is involved in the non-enzymatic browning process and non-enzymatically bound glucose in serum is released as 5-HMF (Bunn et al 1978; McFarland et al 1979). Therefore, we evaluated 5-HMF levels to determine the extent of glycosylation of serum protein. The effects on glucose and glycosylated protein levels indicated that the administration of Hachimi-jio-gan might prevent the pathogenesis of diabetic complications caused by impaired glucose metabolism and glycosylation of serum proteins, eventually resulting in improvement of the diabetic pathological condition.

Over the experimental period, urine volume and urinary protein excretion were markedly elevated in the rat model used, reflecting the physiological abnormalities associated with diabetes. However, the administration of Hachimi-jio-gan for 10 days reduced urinary protein significantly. On the basis of these results, we would expect Hachimi-jio-gan to normalize the physiological changes associated with diabetes and prevent the development of diabetes and its complications.

Under diabetic conditions, free radicals such as O_2^- and NO are produced as a result of the induction of the glycation reaction in β-cells that have been affected by diabetic oxidative stress. It has been well established that the animal model of diabetes induced by STZ injection results in the destruction of β-cells by reactive radicals (Asplund et al 1984; Oberley 1988; Kubisch et al 1997; West 2000). Our results showed that rats with STZ-induced diabetes had high serum levels of O_2^- and NO, indicating that STZ leads to oxidative stress, which will eventually affect the function of β-cells. O_2^- is an attractive candidate for a mediator of endothelial dysfunction in diabetes (Tesfamariam 1994). Under conditions of diabetes, the increased production of O_2^- occurs via hyperglycaemia, auto-oxidation of glucose or non-enzymatic protein glycation. This study showed that Hachimi-jio-gan led to a decrease in the generation of O_2^- induced by diabetes. However, we have to also consider that the O_2^- level may reflect the O_2^- generation activity in the condition of 20% oxygen in the air, not the exact in-vivo O_2^- generation that is quite difficult to measure because of its short half-life. NO is also responsible for deleterious effects on β-cell function and it interacts with O_2^- to form the highly reactive hydroxyl radical that leads to reactive oxidative damage under conditions of diabetes (Oberley 1988; Beckman et al 1990; Mandrup-Poulsen et al 1990). In addition, NO targets intracellular antioxidative enzymes, resulting in the loss of their function (Corbett & McDaniel 1992). Hachimi-jio-gan scavenged O_2^- and NO resulting from diabetic oxidative stress. The free radical scavenging property of Hachimi-jio-gan suggests that Hachimi-jio-gan might protect against diabetic oxidative stress.

Oxidative stress is associated with the peroxidation of lipids, which is determined by measuring TBA-reactive substance levels. The concentration of lipid peroxidation products may also reflect the oxidative stress associated with the diabetic condition. Baynes (1991) and Kakkar et al (1995) reported that tissue and blood malondialdehyde levels of rats with STZ-induced diabetes increased due to lipid peroxidation. Therefore, the measurement of TBA-reactive substance is frequently used to determine the oxidative stress in diabetes. In this study, the TBA-reactive substance concentrations of hepatic and renal mitochondria, as well as serum, significantly increased after the induction of diabetes in rats. Consistent with our results, several other studies in which TBA-reactive substance levels were assayed in human and animal models have also shown increased lipid peroxidation in the plasma, liver and kidneys of diabetic subjects (Sato et al 1979; Karpen et al 1982; Nourooz-Zadeh et al 1997; Stanely et al 2001). The increase of lipid peroxidation in tissues such as the liver and kidneys implies the susceptibility to diabetic oxidative stress, leading to diabetic complications. From this viewpoint, prevention of lipid peroxidation resulting from oxidative stress is considered to play a crucial role in protecting against the disorders involved in diabetes. The administration of Hachimi-jio-gan resulted in the efficient inhibition of lipid peroxidation in the liver and kidneys, as well as in the serum, suggesting the alleviation of oxidative stress of diabetic pathological condition through the inhibition of lipid peroxidation.

The results of this study imply that Hachimi-jio-gan plays a role in ameliorating glucose metabolism and attenuating the oxidative stress under diabetes through scavenging free radicals and inhibiting lipid peroxidation. Hachimi-jio-gan may be a beneficial therapy for the pathological conditions associated with diabetic oxidative stress. On the basis of this study, the protective potential of individual ingredients and major compounds in Hachimi-jio-gan against oxidative stress related to diabetes has to be investigated for the mutual and synergistic protective mechanism among them.

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