JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 1271–1278

Received March 17, 2007
Accepted May 27, 2007
DOI 10.1211/jpp.59.9.0012
ISSN 0022-3573

The protective role of Kangen-karyu against fructose-induced metabolic syndrome in a rat model

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Abstract

The protective effect of Kangen-karyu extract and its mechanisms against fructose-induced metabolic syndrome have been investigated using a rat model. Male Wistar rats were fed a high fructose (65%) diet or standard chow for one week, and for two subsequent weeks were treated with 50 or 100 mg kg^{-1} body weight/day Kangen-karyu extract or vehicle. Serum glucose, glycosylated protein, triglyceride (TG), total cholesterol, and blood pressure levels of high-fructose-fed rats were increased compared with those of normal rats. However, Kangen-karyu extract ameliorated the high-fructose-induced metabolic syndrome including hyperglycaemia and hypertriglyceridaemia. In addition, the increase of hepatic TG content in rats given the high fructose diet was significantly inhibited with the regulation of sterol regulatory element-binding protein (SREBP)-1 expression by Kangen-karyu extract. On the other hand, peroxisome proliferator-activated receptor α and SREBP-2 protein levels were not affected by the feeding of the high fructose diet or Kangen-karyu extract. Moreover, Kangen-karyu extract administration to high-fructose-fed rats markedly reduced the thiobarbituric acid-reactive substance levels in serum, hepatic homogenate, and mitochondria. Furthermore, it inhibited the increase of cyclooxygenase (COX)-2 with the regulation of nuclear factor-kappa B (NF-κB) and bcl-2 proteins in the liver, suggesting that the protective potential of Kangen-karyu extract against metabolic syndrome would be attributed to the regulation of COX-2, NF-κB, and bcl-2 signalling pathways. This study indicated that Kangen-karyu extract significantly improved high-fructose-induced metabolic syndrome such as hyperglycaemia, hyperlipidaemia, and hypertension through the reductions of TG and cholesterol contents with the regulation of hepatic SREBP-1 protein and the NF-κB signalling pathway.

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Introduction

Current interest in fructose metabolism has arisen because of its increased use as a sweetener in the food industry. The hepatic metabolism of fructose has important effects on glucose and lipid metabolism. Dietary fructose is rapidly taken up by the liver and the regulatory step by phosphofructokinase in glycolysis is bypassed, consequently resulting in larger increases of glucose, glycogen, lactate, and pyruvate than in the consumption of glucose (Hallfrisch 1990; Mayes 1993). The possibility that dietary fructose facilitates metabolic derangement and induces oxidative damage is supported by numerous recent studies (Catena et al 2003; Kelley et al 2004; Ackerman et al 2005; Basciano et al 2005). Therefore, a high fructose diet leads to well-characterized metabolic syndrome, typically resulting in hyperinsulinaemia, insulin resistance, hypertension, hypertriglyceridaemia, dyslipidaemia, and a decline in the level of high density lipoprotein cholesterol. Furthermore, such metabolic modifications have been associated with multiplex risk factors for cardiovascular diseases (Fried & Rao 2003; Eckel et al 2005). On the other hand, recent findings have also shown that the metabolic syndrome induced by a high fructose diet can be attenuated by phytochemicals from herbal medicines and foods (Wu et al 2004; Girard et al 2006).

Kangen-karyu, a Chinese prescription comprising six crude drugs, has received much attention due to its variety of biological activity, such as the inhibition of platelet aggregation, suppression of hypertension, and anti-ageing (Takahashi 1991; Gao et al 2001; Makino et al 2002). Our previous studies also showed that Kangen-karyu extract inhibited the ageing process induced by oxidative stress under in-vitro and in-vivo conditions, and it

protected against hypercholesterolaemia through the regulation of cholesterol levels and inhibition of lipid peroxidation (Satoh et al 2004a, b, 2005; Yokozawa et al 2006). Based on this evidence, we have evaluated the effect of Kangen-karyu extract and its related mechanisms against fructose-induced metabolic syndrome in a rat model.

Materials and Methods

Materials

Nonidet P-40 (NP-40), phenylmethane sulfonyl fluoride (PMSF), 2-amino-2-hydroxymethyl-1,3-propanediol (Tris (hydroxymethyl)aminomethane), protease inhibitor mixture dimethylsulfoxide solution, and skim milk powder were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Dithiothreitol (DTT) was purchased from BioVision Inc. (Mountain View, CA). The Bio-Rad protein assay kit and Laemmli sample buffer were purchased from Bio-Rad Laboratories (Tokyo, Japan). The polyclonal antibodies to peroxisome proliferator-activated receptor α (PPARα), sterol regulatory element-binding protein (SREBP)-1 and SREBP-2, nuclear factor-kappa B (NF-κB) p65, inhibitor binding protein kappa B-α (IκB-α), bax, monoclonal antibodies to cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), bcl-2, and goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase (HRP)-conjugated or goat anti-rabbit IgG HRP-conjugated secondary antibody were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Enhanced chemiluminescence Western blotting detection reagents were purchased from GE Healthcare (Piscataway, NJ). The other chemicals and reagents used were of high quality and obtained from commercial sources.

Preparation of Kangen-karyu extract

The composition of Kangen-karyu used in this study was 2.25 g Paeoniae Radix (a root of *Paeonia lactiflora* Pallas), 2.25 g Cnidii Rhizoma (a rhizome of *Cnidium officinale* Makino), 2.25 g Carthami Flos (a petal of *Carthamus tinctorius* L.), 1.125 g Cyperi Rhizoma (a rhizome of *Cyperus rotundus* L.), 1.125 g Aucklandiae Radix (a root of *Aucklandia lappa* Dcne.), and 4.5 g Salviae Miltiorrhizae Radix (a root of *Salvia miltiorrhiza* Bunge). These herbs were extracted with 25 vol water at 100°C for 1 h. After filtration, the solution was evaporated under reduced pressure to give an extract at a yield of 44%, by weight, of the starting materials. For analysis of the components of Kangen-karyu, the aqueous extract was dissolved in aqueous ethanol (50% v/v) with sonication, and filtered through a Cosmonice filter (PVDF, 0.45 μm, Nakarai Tesque, Inc.). Reverse-phase HPLC analysis was performed using a Cosmosil 5C_{18}-AR II column (250 × 4.6 mm i.d., Nakarai Tesque, Inc.) with elution gradients of 4–30% v/v (39 min) and 30–75% v/v (15 min) CH_3CN in 50 mM H_3PO_4 at a flow rate of 0.8 mL min^{-1}. The ultraviolet (UV) absorbance from 200 to 400 nm was monitored and the three-dimensional data were processed by a JASCO photodiode array detector MD-910. All assigned peaks were identified by carrying out co-injection tests with authentic samples and compared with the UV spectral data. The major compounds of Kangen-karyu detected were paeoniflorin, pentagalloyl glucose, rosmarinic acid, lithospermic acid, and lithospermic acid B (Figure 1).

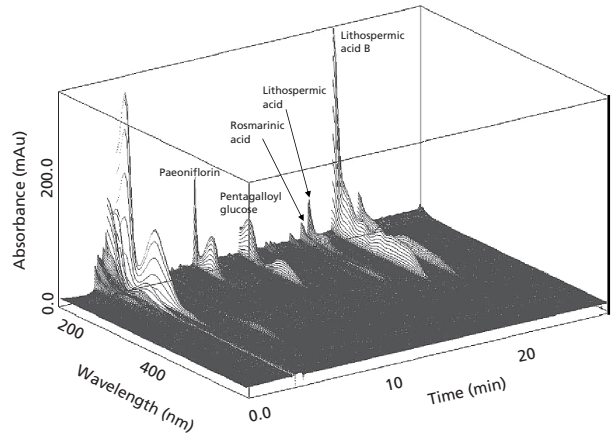

Figure 1 HPLC chromatograph.

Animals and treatments

The Guidelines for Animal Experimentation approved by the University of Toyama were followed during these experiments. Wistar male rats (Japan SLC Inc., Hamamatsu, Japan) were maintained with water and food freely available at a constant humidity and temperature with a 12-h light/dark cycle. After acclimation, the animals (217 ± 6 g) were randomized into two groups: a normal group without supplementary fructose and a high-fructose-supplemented group (65% in diet for one week, Table 1). After approximately one week on the high fructose diet, rats were screened for the induction of hypertriglyceridaemia (serum triglyceride (TG) concentration >130 mg dL^{-1}) by obtaining blood from the tail vein. Rats were divided into three groups by body weight and serum TG levels. For the subsequent two weeks, the three groups were supplemented with the high fructose diet and Kangen-karyu extract at 50 or 100 mg kg^{-1}/day was administered by oral gavage. A normal group of rats was included. Each experimental group contained seven to eight rats. During the experimental period, dietary consumption was kept at

Table 1 Composition of the diets

Ingredients	Normal diet (g/100 g)	High fructose diet (g/100 g)
Casein	20	20
Corn starch	65	–
Fructose	–	65
Corn oil	5	5
Salt mixture	4	4
Vitamin mixture	1	1
Cellulose powder	4.5	4.5
DL-Methionine	0.3	0.3
Choline bitartrate	0.2	0.2

the same level (16 g/rat). At the end of the study, blood samples were obtained from the abdominal aorta under pentobarbital anaesthesia (50 mg kg^{-1}, i.p.), and the serum was immediately separated from the blood samples by centrifugation at 1000 *g* for 15 min at 4°C. After renal perfusion through the renal artery with ice-cold 0.9% NaCl solution, each tissue was removed, weighed, and stored at –80°C until analysis.

Determination of serum component levels

The levels of serum glucose, TG, and total cholesterol were determined using a commercial reagent (Glucose C II-Test Wako, Triglyceride E-Test Wako, and Cholesterol E-Test Wako obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan). Serum glycosylated protein and thiobarbituric acid (TBA)-reactive substance levels were measured using the methods of McFarland et al (1979) and Naito & Yamanaka (1978), respectively.

Measurement of hepatic TG and total cholesterol contents

The liver of each rat was homogenized, and total lipids were extracted with a mixture of chloroform and methanol (2:1, v/v) according to the method of Folch et al (1957). TG and total cholesterol content were determined using the Wako kit described above.

Measurement of blood pressure

At the end of the experiment, blood pressure was measured by the tail-cuff method using an automatic blood pressure monitoring system (UR-5000, UETA, Tokyo, Japan). The animals were kept at 37°C for 30 min before blood pressure measurement. The average of five consecutive readings for accurate measurement was used for blood pressure evaluation.

Isolation of hepatic mitochondria and measurement of TBA-reactive substance levels

Liver was homogenized with a nine-fold vol ice-cold 0.9% NaCl solution. Mitochondria were prepared from hepatic homogenates by differential centrifugation (800 *g* and 12 000 *g* at 4°C for 15 min) according to the methods of Johnson & Lardy (1967), and Jung & Pergande (1985) with slight modifications. Each pellet was resuspended in preparation medium and the TBA-reactive substance concentration was determined by the method of Buege & Aust (1978). Briefly, resuspension of 250 μL of each pellet or a working standard was added to 750 μL TBA-TCA-HCl solution (0.4% TBA, 15% TCA and 2.5% HCl) and it was heated at 95–100°C for 20 min and cooled on an ice-bath. The samples were centrifuged at 1000 *g* at room temperature for 10 min to transfer supernatants from the denatured protein precipitate. TBA-reactive substance was determined by measuring the absorbance at 532 nm. The TBA-reactive substance value was expressed in nmol malondialdehyde (MDA) (mg protein)$^{-1}$ by a calibration curve constructed from MDA (0–25 nmol mL^{-1}) in 1,1,3,3-tetramethoxypropane. The protein level was evaluated by the method of Itzhaki & Gill (1964) with bovine serum albumin as the standard.

Homogenization, isolation of cytosol and nuclear extracts

Each liver was homogenized by a Potter-Elvehjem homogenizer in 4 vol (w/v) buffer A containing (in mM) 25 Tris-HCl (pH 7.5), 250 NaCl, 5 EDTA, 1 PMSF, 1 DTT, and protease inhibitor mixture (in mM: 100 4-(2-aminoethyl) benzenesulfonyl fluoride, 0.08 aprotinin, 2 leupeptin, 5 bestatin, 1 pepstatin A, and 1.5 E-64). Homogenates were incubated for 15 min on ice, 10% NP-40 was added, and then they were centrifuged at 4000 *g* at 4°C for 5 min. Supernatants were used for iNOS, COX-2, bax, and bcl-2 protein determination. Nuclear extracts were isolated using the Sakurai et al (1996) method. Briefly, liver was homogenized by a Potter-Elvehjem homogenizer in 4 vol (w/v) buffer B containing (in mM) 10 2-[4-(2-hydroxyethyl)-1-piperazyl] ethanesulfonic acid (HEPES) (pH 7.9), 10 KCl, 0.1 EDTA, 1 DTT, 0.5 PMSF, and protease inhibitor mixture as above. Homogenates were incubated for 15 min on ice, 10% NP-40 was added, and then they were centrifuged at 4000 *g* for 5 min at 4°C. Supernatants were used for IκB-α protein determination, and pellets were resuspended in 2 vol buffer C containing (in mM) 20 HEPES, 400 NaCl, 1 EDTA, 1 DTT, 1 PMSF, and the protease inhibitor mixture. Homogenates were kept for 15 min at 4°C and then centrifuged at 14 000 *g* for 5 min at 4°C. Supernatants were collected in microcentrifuge tubes, and used for PPARα, SREBP-1/2, and NF-κB protein determination. The protein concentration of homogenates and nuclear extracts were determined by Bio-Rad protein assay. Each sample (30 μg protein/lane) was denatured by boiling in Laemmli sample buffer and stored at –80°C until the assay.

Western blot analysis

Western blot analysis was carried out using 30 μg homogenates for iNOS, COX-2, bcl-2, and bax, cytosol extract for IκB-α, and crude nuclear extract for PPARα, SREBP-1/2, and NF-κB from the liver. The proteins were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (10% w/v), and the separated proteins were blotted onto nitrocellulose. Blots were blocked overnight at 4 °C with 5% skim milk in TBS-T (25 mM Tris-HCl (pH 8.3), 140 mM NaCl, 2 mM KCl, and 0.1% Tween 20). Membranes were then incubated for 3 h at 4°C with the primary polyclonal antibody raised against NF-κB, IκB-α, bax, PPARα, and SREBP-1/2 (dilution 1:1000), and monoclonal antibodies against iNOS, COX-2, bcl-2 (1:1000), and β-actin (1:5000). After extensive washing, incubation with the secondary antibody (rabbit polyclonal or mouse monoclonal antibody) at a dilution of 1:1000 was also performed for 40 min at room temperature. Specific protein was visualized using enhanced chemiluminescence Western blotting detection reagents and detected by chemiluminescence with LAS-1000 Plus (Fujifilm, Tokyo, Japan).

Statistical analysis

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

Results

Characteristics of experimental animals

Table 2 shows the effect of a high fructose diet and Kangen-karyu extract on changes in body and tissue weight. Body weight gain was slightly increased in the high-fructose-fed control rats compared with normal rats, but it was not significantly different between the groups. On the other hand, the liver weight of high-fructose-fed control rats was significantly increased compared with that of normal rats, while the oral administration of Kangen-karyu extract to high-fructose-fed rats led to a significant decrease in comparison with control rats. Epididymal fat weight was significantly increased by Kangen-karyu extract at an oral dose of 100 mg kg^{-1}/day. In addition, the high fructose diet caused significant increases in serum glucose, glycosylated protein, TG, and total cholesterol levels. However, the administration of Kangen-karyu extract led to a significant decrease in these levels in a dose-dependent manner. Systolic blood pressure was significantly elevated in rats fed the high fructose diet, whereas rats given Kangen-karyu extract showed lowered values compared with control rats. Moreover, the administration of Kangen-karyu extract led to a lowering effect on diastolic blood pressure. The heart rate was not significantly different between the rats fed normal and high fructose diets.

TG and total cholesterol contents in liver

The effect of Kangen-karyu extract on TG and total cholesterol in the livers of rats fed the high fructose diet is shown in Table 2. Hepatic TG contents in high-fructose-fed control rats increased 1.6-fold compared with the normal rats. However, the administration of Kangen-karyu extract significantly lowered the TG contents in a dose-dependent manner compared with fructose-fed control rats. In addition, the elevation of hepatic total cholesterol contents in rats given the high fructose diet was significantly reduced by the administration of Kangen-karyu extract.

Protein expression of PPARα, SREBP-1, and SREBP-2

To investigate the effects of Kangen-karyu extract on lipid metabolism in the liver, the protein levels of PPARα and SREBP-1/2 were determined using Western blot analyses (Figure 2). The protein expression of PPARα and SREBP-2 was not significantly different among the experimental groups. On the other hand, SREBP-1 protein in high-fructose-fed control rats was significantly increased by 30% compared with normal rats. The oral administration of Kangen-karyu extract at oral doses of 50 or 100 mg kg^{-1}/day significantly decreased the level of SREBP-1 protein by 21% and 30%, respectively, compared with high-fructose-fed control rats.

TBA-reactive substance levels in serum and liver

TBA-reactive substance levels in serum and liver are summarized in Table 3. The serum level of high-fructose-fed control rats was significantly elevated to 2.50 nmol mL^{-1} in comparison with that of normal rats (1.58 nmol mL^{-1}). However, the

Table 2 Characteristics of experimental animals and TG and total cholesterol content in liver

	Normal rats	High-fructose-fed rats		
		Control	Kangen-karyu (50 mg)	Kangen-karyu (100 mg)
Body weight (g)	244.0 ± 4.3	253.8 ± 3.0	256.7 ± 3.5[d]	257.2 ± 5.8
Liver weight (g/100 g body weight)	3.36 ± 0.09	4.60 ± 0.15[c]	4.41 ± 0.07[c,d]	4.28 ± 0.01[c,f]
Epididymal fat weight (g/100 g body weight)	1.07 ± 0.05	1.06 ± 0.05	1.13 ± 0.09	1.23 ± 0.06[a,e]
Fluid intake (ml day^{-1})	29.5 ± 1.5	24.6 ± 2.0[b]	21.0 ± 1.5[c,d]	23.7 ± 1.7[c]
s-Glucose (mg dL^{-1})	158.5 ± 4.7	201.0 ± 6.8[c]	186.2 ± 7.5[c,d]	178.2 ± 10.9[a,e]
s-Glycosylated protein (nmol (mg protein)$^{-1}$)	30.9 ± 0.8	35.6 ± 1.1[c]	34.3 ± 1.0[c]	32.5 ± 0.4[f]
s-Triglyceride (TG) (mg dL^{-1})	56.4 ± 5.6	188.3 ± 17.5[c]	151.5 ± 13.8[c,e]	117.4 ± 17.9[c,f]
s-Total cholesterol (mg dL^{-1})	63.4 ± 1.9	83.5 ± 5.2[c]	82.2 ± 4.6[c]	78.1 ± 3.4[c]
Systolic blood pressure (mmHg)	111.0 ± 2.6	132.8 ± 2.4[c]	116.6 ± 2.0[b,f]	113.2 ± 1.7[f]
Diastolic blood pressure (mmHg)	62.9 ± 4.2	77.8 ± 4.4	57.6 ± 2.9[f]	62.3 ± 3.2[d]
Heart rate (beats min^{-1})	445.0 ± 13.4	474.1 ± 11.3	461.9 ± 21.9	483.1 ± 13.7[a]
Hepatic TG content				
mg/liver	201.1 ± 9.3	314.6 ± 21.4[c]	308.2 ± 3.8[c]	281.6 ± 13.5[c,d]
(mg/liver)/100 g body weight	81.4 ± 1.3	133.0 ± 1.4[c]	118.8 ± 2.1[c,f]	115.3 ± 2.2[c,f]
Hepatic total cholesterol content				
mg/liver	94.9 ± 3.7	134.6 ± 4.2[c]	112.3 ± 5.5[c,f]	104.1 ± 3.8[a,f]
(mg/liver)/100 g body weight	36.4 ± 2.2	52.0 ± 0.8[c]	43.5 ± 0.2[c,f]	42.3 ± 0.7[c,f]

[a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ vs normal rats; [d]$P<0.05$, [e]$P<0.01$, [f]$P<0.001$ vs high-fructose-fed control rats.

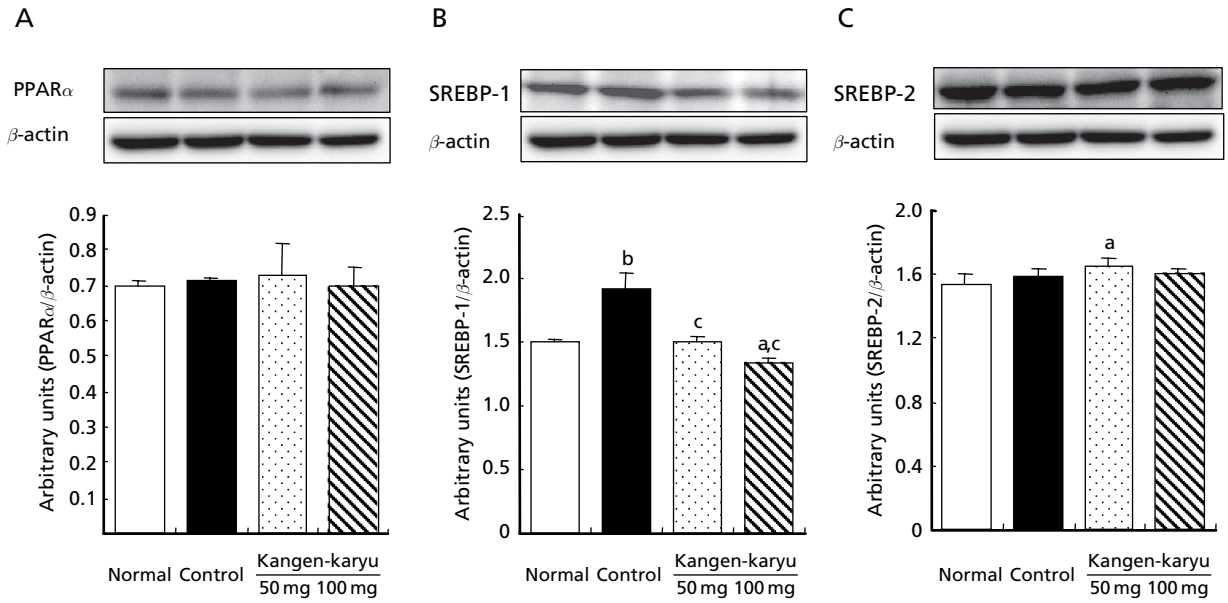

Figure 2 Western blot analyses of PPARα, SREBP-1, and SREBP-2 protein expression levels in the liver. [a]$P < 0.05$, [b]$P < 0.001$ vs normal rats; [c]$P < 0.001$ vs high-fructose-fed control rats.

Table 3 TBA-reactive substance in serum and liver

Group	Dose (mg kg^{-1}/day)	TBA-reactive substance		
		Serum (nmol mL^{-1})	Hepatic homogenate (nmol (mg protein)$^{-1}$)	Hepatic mitochondria (nmol (mg protein)$^{-1}$)
Normal rats	–	1.58 ± 0.05	0.477 ± 0.011	0.267 ± 0.005
High-fructose-fed rats				
Control	–	2.50 ± 0.23[b]	0.555 ± 0.027[b]	0.459 ± 0.016[b]
Kangen-karyu	50	2.14 ± 0.17[a,c]	0.490 ± 0.021[d]	0.347 ± 0.015[b,d]
Kangen-karyu	100	1.90 ± 0.19[d]	0.484 ± 0.021[d]	0.339 ± 0.020[b,d]

[a]$P < 0.01$, [b]$P < 0.001$ vs normal rats; [c]$P < 0.05$, [d]$P < 0.001$ vs high fructose-fed control rats.

administration of Kangen-karyu extract at 50 or 100 mg led to reductions to 2.14 and 1.90 nmol mL^{-1}, respectively. In addition, the increased hepatic levels were significantly decreased with dose-dependence; in particular, at 100 mg Kangen-karyu extract, hepatic homogenate and mitochondria levels declined from 0.555 to 0.484 nmol (mg protein)$^{-1}$ and 0.459 to 0.339 nmol (mg protein)$^{-1}$, respectively.

Protein levels involved in inflammatory status of liver

Figure 3 represents the effects of Kangen-karyu extract on the inflammatory status of the liver against high-fructose-induced metabolic syndrome. NF-κB protein in high-fructose-fed rats significantly increased compared with normal rats, whereas the oral administration of Kangen-karyu extract led to a decline in the expression by 22% and 38% at oral doses of 50 and 100 mg kg^{-1}/day, respectively. The level of IκB-α protein was slightly increased in rats fed 50 mg Kangen-karyu extract. In addition, the level of iNOS protein was not significantly different among the experimental groups. Moreover, COX-2 protein in rats fed the high fructose diet was significantly increased compared with normal rats; however, the oral administration of Kangen-karyu extract led to a significant decrease of COX-2 protein expression. On the other hand, a change in the expression of bax protein was not observed among the experimental groups. The expression of bcl-2 protein was decreased in the high-fructose-fed control rats, but Kangen-karyu extract resulted in a slight increase in the expression.

Discussion

Animal models fed a high fructose diet are regarded as limited models of metabolic syndrome and have been widely employed for the study of this syndrome, since the diet is

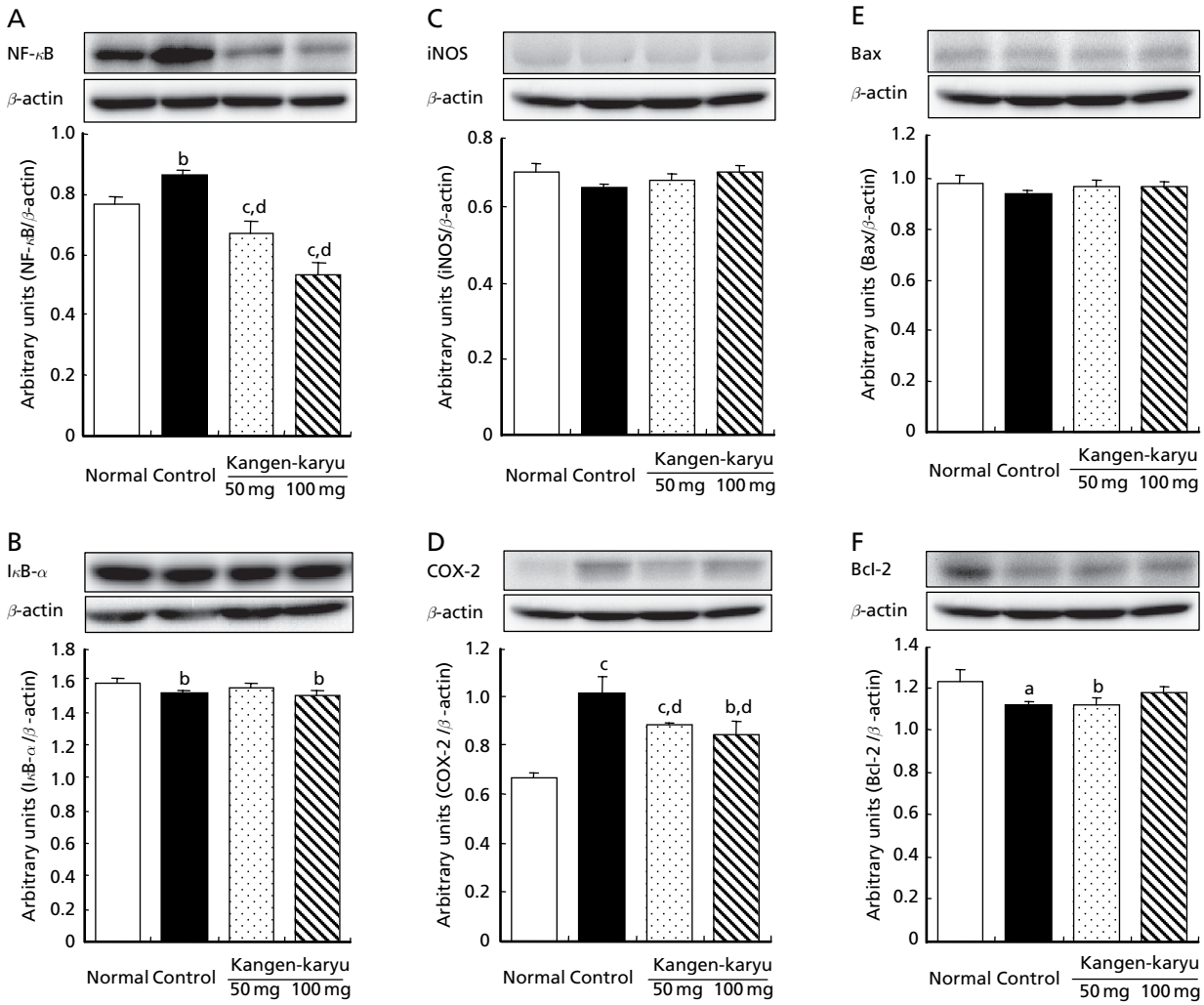

Figure 3 Western blot analyses of protein expression levels involved in the inflammatory status of the liver. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs normal rats; [d]$P < 0.001$ vs high-fructose-fed control rats.

related to the high incidence of several pathological metabolic conditions such as insulin resistance, hypertension, and dyslipidaemia. In addition, high-fructose-fed animals exhibited an alternation of lipid metabolism due to hepatic oxidative stress as a result of the burden of fructose metabolism (Kelley et al 2004), eventually becoming a risk factor for cardiovascular disorders. Consistent with other evidence, in this investigation the high fructose diet led to significant elevations of serum glucose, glycosylated protein, TG, and total cholesterol levels. Moreover, elevations in blood pressure and heart rate were also observed. These results indicated that a high fructose diet would lead to pathological conditions such as diabetes and cardiovascular disease. Therefore, in this study, a rat model with fructose-induced metabolic syndrome was used to investigate the protective role of Kangen-karyu extract against metabolic syndrome.

The administration of Kangen-karyu extract attenuated the increase in liver weight by the high fructose diet in a dose-dependent manner and it increased the epididymal fat weight in rats fed Kangen-karyu extract at an oral dose of 100 mg (Table 2). The slight increase of epididymal adipose tissue by Kangen-karyu extract at a high dose may have probably resulted in a decline of hepatic TG secretion and serum TG concentrations through a reduction in circulating free fatty acid concentrations, leaving less free fatty acids available for hepatic TG synthesis. In addition, the high fructose diet elevated the serum glucose and glycosylated protein levels, which may indicate the progression of insulin resistance. The effect of Kangen-karyu extract to reduce the serum levels would probably play a protective role against the abnormal metabolism of carbohydrate induced by the high fructose diet. Moreover, our results showed that treatment with Kangen-karyu extract ameliorated the increase of hepatic TG and total cholesterol contents with the regulation of blood pressure (Table 2). This suggested that Kangen-karyu would protect against hypertriglycaemia and hypertension induced by a high fructose diet. Furthermore, it inhibited the increase of the total cholesterol level in the liver. Previous studies

demonstrated that Kangen-karyu prevented hypercholesterolaemic atherosclerosis and attenuated the risk of cardiovascular diseases by not only reductions in low density lipoprotein cholesterol and its oxidation, but also the decline in lipid peroxidation (Yokozawa et al 2006). Taken together, Kangen-karyu is expected to play a protective role against metabolic syndrome related to a high fructose diet including hypertriglycaemia, dyslipidaemia, and hypertension.

Dietary fructose in the liver is rapidly taken up by the liver, where it can be converted to glycerol-3-phosphate, favouring the esterification of unbound fatty acids to form TGs. On the other hand, fatty acid availability for TG synthesis not only depends on the plasma free fatty acid supply to the liver but also on de-novo fatty acid synthesis and β-oxidation. It is well known that β-oxidation of fatty acids and the synthesis of fatty acids and TG in the liver are regulated by the nuclear receptors PPARα and SREBP-1, respectively (Kersten et al 2000; Roglans et al 2002). In addition, SREBP-2 preferentially activates cholesterol synthesis. PPARα plays an important role in the metabolic homeostasis of fatty acids through its regulation of target genes encoding enzymes for fatty acid β-oxidation and fatty acid transporters (Schoonjans et al 1997; Aoyama et al 1998; Palmer et al 1998; Leone et al 1999). The rats fed a high fructose diet and Kangen-karyu extract did not show an altered expression of PPARα, suggesting no significant role in fatty acid β-oxidation. On the other hand, an increase in hepatic SREBP-1 protein levels without changes of PPARα and SREBP-2 was observed, resulting in the elevation of serum and hepatic TG levels by ingestion of a high fructose diet. Consistent with the present results, Miyazaki et al (2004) reported the induction of hepatic mRNA and protein levels of SREBP-1 and lipogenic gene expression including fatty acid synthase, acetyl-CoA carboxylase, and stearoyl-CoA desaturase, whereas SREBP-2 protein levels were not changed in mice following seven days on a 60% fructose diet. However, Kangen-karyu extract ingestion resulted in suppression of the hepatic SREBP-1 protein level, probably playing a crucial role to decrease hepatic TG contents. These results suggested the possibility that Kangen-karyu would lower the serum and hepatic TG levels through a signalling pathway that regulated TG synthesis but not β-oxidation of fatty acids. On the other hand, although the hepatic total cholesterol content due to high fructose feeding was significantly increased, the protein level of the SREBP-2, a key transcription factor controlling cholesterol biosynthesis, was not affected by high fructose feeding or Kangen-karyu extract administration (Figure 2). This suggested that the reduction of the hepatic total cholesterol content caused by Kangen-karyu extract was not associated with hepatic cholesterol synthesis, but probably involved other mechanisms such as cholesterol excretion.

Although the underlying mechanisms for the detrimental consequences of a high fructose diet are not clear, several reports support that oxidative stress plays a critical role in the pathogenesis of metabolic syndrome (Catena et al 2003; Kelley et al 2004). Oxidative stress induced by a high fructose diet is attributed to metabolic syndrome, since a high fructose diet alters lipid metabolism and leads to dysregulation in the liver. As shown in Table 3, the TBA-reactive substance levels were elevated in rats fed the high fructose diet. However, Kangen-karyu extract decreased the level in serum, hepatic homogenate, and mitochondria, a marker of oxidative stress. Our previous studies also supported these findings that Kangen-karyu extract had an antioxidative activity and protective effect against oxidative stress (Satoh et al 2004a, b, 2005). This suggested the promising role of Kangen-karyu to ameliorate oxidative stress induced by ingestion of a high fructose diet.

The clinical criteria for the diagnosis of metabolic syndrome include the condition of inflammation with abdominal obesity, atherogenic dyslipidaemia, hypertension, insulin resistance, and a prothrombotic state. Therefore, we determined the effect of Kangen-karyu extract on inflammatory protein levels by oxidative stress in the liver in a rat model with high-fructose-induced metabolic syndrome (Figure 3). The increase in NF-κB activity was elicited through the degradation of IκB-α via phosphorylation by IκB kinase (Helenius et al 1996; Kim et al 2000). The activation of NF-κB activity, in turn, up-regulates the synthesis of anti-apoptotic members, the bcl-2 family (Sasaki et al 2001), and increases the transcription of genes that encode protective enzymes such as iNOS and COX-2 (Chung et al 2002). Our data showed that Kangen-karyu extract attenuated the increase of hepatic COX-2 protein by fructose diet ingestion through regulation of the NF-κB signalling pathway. However, Kangen-karyu extract did not alter the protein levels of iNOS and bax. In addition, Kangen-karyu induced the elevation of bcl-2 protein expression, indicating its role to prevent oxidative stress caused by the activation of NF-κB. These results suggested that Kangen-karyu extract may have reduced the severity of hepatic inflammation and liver cell injury induced by a high fructose diet with the regulation of its related protein expression. Several lines of evidence also support the disruption of the NF-κB pathway under hypertriglyceridaemic and hyperglycaemic stress responses by inhibiting oxidative stress and inflammatory responses (Dichtl et al 1999; Kelley et al 2004; Stentz et al 2004). Taken together, the present results indicated that the protective potential of Kangen-karyu extract against metabolic syndrome would be attributed to the regulation of COX-2, NF-κB, and bcl-2 signalling pathways.

Conclusions

The results suggested that Kangen-karyu extract would ameliorate high-fructose-induced metabolic syndrome such as hyperglycaemia, hyperlipidaemia, and hypertension. The administration of Kangen-karyu extract ameliorated metabolic syndrome through the reduction of TG and cholesterol concentrations with regulation of the hepatic SREBP-1 protein level and suppression of inflammation by the regulation of COX-2 and NF-κB protein levels. Since the Chinese prescription Kangen-karyu is composed of several compounds with biological effects, further study of the active components with protective activity against metabolic syndrome is necessary.

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