The Protective Role of Chinese Prescription Kangen-karyu Extract on Diet-Induced Hypercholesterolemia in Rats

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This study was carried out to investigate the protective potential of Chinese prescription Kangen-karyu, comprising six crude drugs, on coronary heart disease which is the principal cause of morbidity and mortality worldwide. The diet-induced hypercholesterolemic rat model, which shows an elevation in low density lipoprotein (LDL) cholesterol and atherosclerosis, was employed. The control rats fed a diet of 1% cholesterol and 0.5% cholic acid showed the highest cholesterol levels in serum and feces relative to those fed a normal diet, however, the rats administered Kangen-karyu extract showed reductions in these levels without changes in liver cholesterol, indicating that the reduction of serum total cholesterol by Kangen-karyu extract probably arises from an increase in cholesterol excretion. Furthermore, the administration of Kangen-karyu extract significantly prevented the elevation of serum aspartate aminotransferase and alanine aminotransferase, known as marker enzymes of liver damage. The elevated serum levels of LDL cholesterol were lowered, however, the high density lipoprotein cholesterol level was significantly elevated by Kangen-karyu extract and these were dose-dependent decreases in the atherogenic index to 15.2, 8.8 and 7.5 at oral doses of 50, 100 and 200 mg from the 19.4 control value, respectively. In addition, Kangen-karyu extract inhibited LDL oxidation in a dose-dependent manner, and the elevated level of thiobarbituric acid-reactive substances in control rats showed a decline by the administration of Kangen-karyu extract. The present study suggests that Kangen-karyu could play a protective role against hypercholesterolemia through the regulation of cholesterol levels and inhibition of lipid peroxidation.

Key words Kangen-karyu; cholesterol; thiobarbituric acid-reactive substance; atherogenic index; coronary heart disease

Hypercholesterolemia increases the risk of cardiovascular diseases which are the principal causes of mortality as well as morbidity. Coronary heart disease (CHD) is the most common cardiovascular disease and atherosclerosis is considered the most frequent cause of CHD. Epidemiological, clinical, genetic and experimental studies indicate that high serum levels of low density lipoprotein (LDL) cholesterol are associated with atherosclerosis and an increased risk of CHD.[1—3] On the other hand, a growing body of evidence suggests that high density lipoprotein (HDL) cholesterol exerts an antiatherogenic effect by counteracting LDL oxidation.[4—7] Numerous clinical trials of lipid-lowering interventions through diet, drugs and surgery have been well established and diet treatment or drug therapy to regulate HDL and LDL cholesterol levels promises to reduce subsequent CHD-associated pathological conditions.[1,2,8,9] Based on the evidence, great strides have been made to reduce the risk of CHD worldwide by the control of cholesterol levels through diet and drug therapy.

Chinese traditional herbs or medicines have recently attracted a great deal of attention as alternative therapies for various pathological conditions. Those with beneficial effects against human diseases have been developed as a result of clinical experience accumulated over time and have been used widely for the treatment of a variety of inflammatory conditions, cardiovascular disorders and other ailments.[10,11] Kangen-karyu, a Chinese prescription drug comprising six crude drugs, is commonly used to treat symptoms related to blood circulation deficiencies, and is well known to reduce blood and plasma viscosity and thus improve microcirculation.[12] In addition, it has received much attention due to its numerous biological activities, such as inhibition of platelet aggregation, suppression of hypertension and anti-aging.[13—17] On the basis of these studies, Kangen-karyu would also be expected to play a protective role against hypercholesterolemia. The induction of hypercholesterolemia by a high cholesterol diet in experimental animals including rats has been suggested.[18,19] In addition, diet-induced hypercholesterolemia has long been useful for the assessment of agents with beneficial effects on cholesterol regulation. Therefore, the present study was carried out to evaluate the protective potential of Kangen-karyu extract on CHD in the hypercholesterolemia rat model.

MATERIALS AND METHODS

Preparation of Kangen-karyu Extract The composition of Kangen-karyu used in this study was 2.25 g Paeoniae Radix (*Paeonia lactiflora* PALLAS root), 2.25 g Cnidii Rhizoma (*Cnidium officinale* MAKINO rhizome), 2.25 g Carthami Flos (*Carthamus tinctrius* L. petal), 1.125 g Cyperi Rhizoma (*Cyperus rotundus* L. rhizome), 1.125 g Aucklandiae Radix (*Aucklandia lappa* DCNE. root) and 4.5 g Salviae Miltiorrhizae Radix (*Salvia miltiorrhiza* BUNGE root). These herbs were extracted with 25 volumes of 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% (w/w), by weight, of the starting materials. For the analysis of Kangen-karyu's components, the aqueous extract was dissolved in 50% aqueous ethanol (v/v) with sonication, and filtered through a Cosmonice filter (PVDF, 0.45 μm, Nacalai Tesque, Inc.). Reverse-phase HPLC analysis was performed using a

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Cosmosil 5C_{18}-AR II column (250×4.6 mm i.d., Nacalai 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. The ultraviolet (UV) absorbance from 200 to 380 nm was monitored and the three-dimensional data was processed by a JASCO photodiode array detector MD-910. All assigned peaks were identified by carrying out a co-injection test with authentic samples and comparing with the UV spectral data. Lithospermic acid B, paeoniflorin, pentagalloyl glucose, lithospermic acid and rosmarinic acid were the major compounds of Kangen-karyu, and albiflorin, carthamin, cyperol and α-cyperone were also observed.

Animals and Diets Thirty-seven male Wistar rats aged 5 weeks (120—130 g) were obtained from Japan SLC Inc. (Hamamatsu, Japan). They were kept under a conventional light regimen with a dark night at room temperature (about 23 °C) and humidity (about 60%). The rats were divided into 5 groups, avoiding any intergroup differences in body weight. The basal diet (cholesterol- and cholic acid-free, $n=5$) was fed to the normal group and the high cholesterol diet (contained 1% cholesterol and 0.5% cholic acid) was fed to the control and treated groups (Kangen-karyu extract, $n=8$ for each dose) according to a pair-feeding schedule for the experimental period. Kangen-karyu extract was dissolved in water and given orally to rats at doses of 50, 100 and 200 mg/kg body weight/d for 20 d using a stomach tube. Weight gain was measured twice a week. The feces were collected during the last two days and stored frozen at 4 °C until analysis. At the end of the experimental period, the rats were decapitated, their blood was collected and serum for the measurement of cholesterol and thiobarbituric acid (TBA)-reactive substance levels was obtained immediately by centrifugation. Livers were removed, dried on tissue paper, weighed and stored at −80 °C until analysis.

Measurement of Cholesterol Levels and Atherogenic Index Serum total and free cholesterol levels were determined using commercial kits of Cholesterol and Free Cholesterol E-Test Wako (Wako, Osaka, Japan). Serum esterified cholesterol levels were calculated by subtracting the free cholesterol levels from the total cholesterol levels. Serum LDL cholesterol and serum HDL cholesterol levels were determined according to the method of Noma *et al*.[20,21] The liver and feces of each rat were homogenized, the total cholesterol was extracted with a mixture of chloroform and methanol (2 : 1, v/v) and the amounts of total cholesterol were determined using the Wako kit described above. Atherogenic index was calculated as follows: atherogenic index=(total cholesterol−HDL cholesterol)/HDL cholesterol.

Isolation of Lipoproteins Lipoproteins were isolated from serum using density-gradient ultracentrifugation, as described by Havel *et al*.[22] Lipoprotein fractions were isolated from 4 ml of serum using a Beckman Optima XL-70 ultracentrifuge and a 70.1 Ti rotor operating at 160000×***g***. Serum was transferred to a tube and density was adjusted to 1.006, 1.019 and 1.063 g/ml with the same volume of KBr solution. Serum was divided into three lipoprotein classes by density: very low density lipoprotein (VLDL, d 1.006); intermediate density lipoprotein (IDL, $1.006<d<1.019$); LDL ($1.019<d<1.603$). The appropriate times were calculated to be 16 h for VLDL, 18 h for IDL, and 20 h for LDL isolation at 4 °C.

LDL Oxidation Before oxidation, LDL was diluted with 0.15 M NaCl (pH 7.4) to a concentration of 300 μg of protein/ml. LDL protein levels were determined according to the method of Lowry *et al*.[23] Oxidation of LDL was carried out by incubation with freshly prepared 20 μM $CuSO_4$ at 37 °C for 4 h in a shaking water bath, immediately after which the extent of lipid peroxidation was determined by measuring the amount of TBA-reactive substance formed.[24]

Serum TBA-Reactive Substance Levels The serum TBA-reactive substance levels were measured using the method of Naito and Yamanaka.[25]

Activities of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) The serum AST and ALT activities were determined using commercial kits of GOT-UV and GPT-UV Test Wako (Wako).

Statistical Analysis The results are presented as the mean±S.E. The effect of Kangen-karyu extract on each parameter was examined using one-way analysis of variance. Individual differences among groups were analyzed by Dunnett's test and those at $p<0.05$ were accepted as significant.

RESULTS

Changes of Body and Liver Weights As shown in Table 1, the rats fed a 1% cholesterol and 0.5% cholic acid diet showed a decrease in body weight gain compared with normal rats from 85.6 to 76.8 g and an increase in liver weight from 2.92 to 3.53 g. On the other hand, the administration of Kangen-karyu extract did not show any significant effects on these parameters as compared with the control rats.

Serum Cholesterol Levels Table 2 shows the effect of Kangen-karyu extract administration for 20 d on serum cholesterol levels. The rats fed a high cholesterol diet had markedly high serum levels of total, free and esterified cholesterols relative to those fed a normal diet. These levels in

Table 1. Body and Liver Weights in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	Body weight			Liver weight (g/100 g B.W.)
		Initial (g)	Final (g)	Gain (g/20 d)	
Normal rats	—	135.6±1.5	221.2±3.2	85.6±3.1	2.92±0.06
Hypercholesterolemic rats					
Control	—	135.8±1.2	212.5±2.9**	76.8±3.3**	3.53±0.07†
Kangen-karyu extract	50	135.8±1.3	210.0±2.3†	74.3±1.6†	3.45±0.08†
Kangen-karyu extract	100	135.4±1.5	213.8±3.6*	78.4±3.7*	3.55±0.06†
Kangen-karyu extract	200	135.6±1.4	210.5±3.1†	74.9±2.5†	3.45±0.08†

*$p<0.05$, **$p<0.01$, $^{\dagger}p<0.001$ *vs*. normal rats.

Table 2. Serum Cholesterol Levels in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Esterified cholesterol (mg/dl)
Normal rats	—	62.8±3.3	18.8±0.7	38.4±5.3
Hypercholesterolemic rats				
Control	—	183.2±19.7*	41.1±2.4*	144.5±21.3*
Kangen-karyu extract	50	164.9±17.1*	34.5±2.3*,**	127.0±14.7*
Kangen-karyu extract	100	125.9±9.9*,**	33.0±1.6*,**	94.4±9.6*,**
Kangen-karyu extract	200	137.4±9.6*,**	25.7±0.8*,**	101.1±8.4*,**

* $p<0.001$ *vs*. normal rats; ** $p<0.001$ *vs*. hypercholesterolemic control rats.

Table 3. Serum Lipoprotein Cholesterol Levels in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	IDL cholesterol (mg/dl)
Normal rats	—	24.6±2.0	18.2±1.3	9.9±0.8	3.4±0.4
Hypercholesterolemic rats					
Control	—	9.6±0.7*	91.4±10.2*	36.9±0.6*	33.8±1.5*
Kangen-karyu extract	50	10.7±0.6*	79.5±9.3*	28.4±0.1*,**	23.5±1.0*,**
Kangen-karyu extract	100	13.2±1.0*,**	61.9±5.8*,**	19.6±1.4*,**	19.1±0.4*,**
Kangen-karyu extract	200	15.8±1.1*,**	58.8±4.8*,**	20.3±0.2*,**	22.2±0.4*,**

* $p<0.001$ *vs*. normal rats; ** $p<0.001$ *vs*. hypercholesterolemic control rats.

rats given Kangen-karyu extract were, however, significantly reduced as compared with control rats. The rats administered Kangen-karyu extract at 100 mg/kg body weight for 20 d showed reductions in the levels of total, free and esterified cholesterol to 68.7%, 80.3% and 65.3%, respectively.

Serum Lipoprotein Cholesterol Levels and Atherogenic Index The effect of Kangen-karyu extract on serum lipoprotein cholesterol levels is shown in Table 3. The control rats showed low levels of HDL cholesterol compared with normal rats, but the administration of Kangen-karyu extract elevated HDL cholesterol in a dose-dependent manner. HDL cholesterol was increased from 9.6 mg/dl to 15.8 mg/dl by 200 mg of Kangen-karyu extract. On the other hand, the oral dose of 200 mg led to significant reductions in the levels of LDL, VLDL and IDL cholesterol to 64.3%, 55.0% and 65.7%, respectively. We also calculated the atherogenic index of each experimental group. The atherogenic index of normal rats was 1.6, but that of control rats fed the high cholesterol diet was significantly elevated to 19.4. However, the administration of Kangen-karyu extract led to dose-dependent decreases in atherogenic index to 15.2, 8.8 and 7.5 at oral doses of 50, 100 and 200 mg, respectively.

Oxidized LDL Level Table 4 shows the peroxidation levels of LDL isolated from the blood of normal and cholesterol-fed rats. Administration of Kangen-karyu extract markedly inhibited this peroxidation reaction: even the lowest dose of 50 mg reduced the level to 16.3 nmol/mg protein compared with the control value, 17.4 nmol/mg protein. Similarly, the oral doses of 100 and 200 mg of Kangen-karyu extract reduced the level to 15.2 nmol/mg protein, and the stronger inhibition to 15.0 nmol/mg protein was observed with the 200 mg dose.

Serum TBA-Reactive Substances Level The effect of Kangen-karyu extract on lipid peroxidation in rats fed a hypercholesterolemic diet is presented in Table 5. The oral administration of Kangen-karyu extract at dose of 200 mg/kg body weight/d for 20 d significantly reduced the serum TBA-

Table 4. LDL Oxidation in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	Oxidized LDL (MDA nmol/mg protein)
Normal rats	—	13.6±0.1
Hypercholesterolemic rats		
Control	—	17.4±1.3†
Kangen-karyu extract	50	16.3±0.1**
Kangen-karyu extract	100	15.2±0.1*,††
Kangen-karyu extract	200	15.0±0.1§

* $p<0.05$, ** $p<0.01$, † $p<0.001$ *vs*. normal rats; †† $p<0.05$, § $p<0.01$ *vs*. hypercholesterolemic control rats.

Table 5. Serum TBA-Reactive Substance Levels in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	TBA-reactive substance (nmol/ml)
Normal rats	—	3.03±0.23
Hypercholesterolemic rats		
Control	—	4.01±0.21*
Kangen-karyu extract	50	3.88±0.27*
Kangen-karyu extract	100	3.63±0.10*
Kangen-karyu extract	200	3.08±0.12**

* $p<0.001$ *vs*. normal rats; ** $p<0.001$ *vs*. hypercholesterolemic control rats.

Table 6. Feces Cholesterol Levels in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	Total cholesterol (mg/g feces)
Normal rats	—	1.7±0.1
Hypercholesterolemic rats		
Control	—	17.7±0.5*
Kangen-karyu extract	50	19.6±0.5*,**
Kangen-karyu extract	100	20.9±0.6*,**
Kangen-karyu extract	200	20.6±0.5*,**

* $p<0.001$ *vs*. normal rats; ** $p<0.001$ *vs*. hypercholesterolemic control rats.

Table 7. Liver Cholesterol Levels in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	Total cholesterol (mg/liver/100 g B.W.)	Free cholesterol (mg/liver/100 g B.W.)	Esterified cholesterol (mg/liver/100 g B.W.)
Normal rats	—	9.1±2.5	12.2±1.3	6.8±1.4
Hypercholesterolemic rats				
Control	—	106.2±3.7*	51.5±3.6*	54.6±3.9*
Kangen-karyu extract	50	90.5±6.4*	46.9±3.0*	43.7±6.9*
Kangen-karyu extract	100	104.7±7.2*	52.8±3.3*	51.9±6.5*
Kangen-karyu extract	200	102.9±4.3*	48.8±2.9*	54.1±2.3*

* $p<0.001$ *vs*. normal rats.

Table 8. Hepatic Function Parameters in Hypercholesterolemic Rats Treated with Kangen-karyu Extract

Group	Dose (mg/kg B.W./d)	AST (IU/l)	ALT (IU/l)
Normal rats	—	68.9±2.4	10.5±0.4
Hypercholesterolemic rats			
Control	—	80.0±2.6*	12.2±0.4*
Kangen-karyu extract	50	79.5±2.0*	12.7±0.8**
Kangen-karyu extract	100	78.7±1.4*	12.6±0.6**
Kangen-karyu extract	200	74.4±2.4*,†	10.2±0.5§

* $p<0.01$, ** $p<0.001$ *vs*. normal rats; † $p<0.01$, § $p<0.001$ *vs*. hypercholesterolemic control rats.

reactive substances level from 4.01 nmol/ml to 3.08 nmol/ml.

Feces Cholesterol Level The feces total cholesterol level in rats fed the 1% cholesterol and 0.5% cholic acid diet was significantly higher than that of rats fed the normal diet, whereas the level was significantly elevated by the 50 and 200 mg administrations of Kangen-karyu extract to 19.6 mg/g feces and 20.6 mg/g feces from 17.7 mg/g feces, respectively (Table 6).

Liver Cholesterol Level The liver cholesterol profiles of rats fed the hypercholesterol diet are shown in Table 7. While the rats fed the high cholesterol diet showed markedly higher liver total, free and esterified cholesterol levels relative to those fed a normal diet, the administration of Kangen-karyu extract did not show any significant effects on the liver cholesterol profiles compared with the control rats.

Activities of AST and ALT Table 8 shows the effect of Kangen-karyu extract on hepatic function with the measurement of AST and ALT levels. The control rats fed the high cholesterol diet showed significant elevations in AST and ALT levels, however the oral dose of Kangen-karyu extract at 200 mg led to significant reductions in the levels of AST and ALT.

DISCUSSION

Although several factors such as life style, a diet high in saturated fat and cholesterol, family history, age, hypertension and diabetes mellitus, have been reported to cause heart failure,[26—28] high levels of cholesterol, particularly LDL cholesterol, in the blood are mainly responsible.[4,29,30] Several studies have indicated that diet treatment or drug therapy to regulate cholesterol levels can reduce subsequent CHD-associated morbidity and mortality.[1,8] Based on the evidence, great efforts have been made to reduce the risk of CHD through the regulation of cholesterol; thus, the therapeutic benefits of plant extracts without side effects have been the focus of many extensive dietary studies.[19,31,32]

While numerous trials of diet- or drug-based cholesterol reduction have provided compelling evidence that reducing cholesterol levels decrease the incidence of CHD,[1,2,8,9] the therapeutic or improving effects of Chinese traditional medicines including Kangen-karyu against pathological conditions caused by hypercholesterolemia have rarely been studied. In the present study, we investigated the effects of Kangen-karyu extract on hypercholesterolemia using a hypercholesterolemic rat model. The elevated cholesterol especially LDL cholesterol level plays crucial role in atherosclerosis and intimal lesions that progress from fatty streaks to ulcerated plaques.[28,33] In addition, these studies also support the idea that lowering total and LDL cholesterol concentrations reduces the incidence of CHD events.

The administration of Kangen-karyu extract reduced the elevated levels of serum cholesterol caused by the hypercholesterolemic diet. In addition, the decrease of total serum cholesterol induced by Kangen-karyu extract was ascribed to decreases in both free and esterified cholesterol levels. A small fraction of the cholesterol in the liver is incorporated into the membranes of hepatocytes, but most of it is exported. Esterified cholesterol is one of the exported forms and it is formed in the liver through the action of acyl-CoA-cholesterol acyl transferase and lecithin-cholesterol acyl transferase, which catalyzes the conversion of cholesterol into a more hydrophobic form of cholesterol ester that is either transported in secreted lipoprotein particles to other tissues that use cholesterol, or it is stored in the liver. Thus, a reduction in esterified cholesterol indicates that the cholesterol was used for the synthesis of vital molecules in tissues, including the liver.

The liver is generally considered as the primary organ responsible for maintaining cholesterol homeostasis, and therefore the activities of serum AST and ALT, which are known to be marker enzymes for liver damage, were evaluated. They were markedly elevated in hyperlipidemic animals compared to normal rats, which is consistent with other studies,[19,34,35] whereas administration of Kangen-karyu extract markedly prevented elevations in serum AST and ALT. We also measured total cholesterol in liver and feces to investigate whether the cholesterol lowering effect of Kangen-karyu extract was due to the reduction of cholesterol synthesis or the enhancement of its excretion. The elevation of fecal total cholesterol without changes in liver cholesterol indicates that the reduction of serum total cholesterol by Kangen-karyu extract is probably caused by the inhibition of dietary cholesterol absorption.

The crucial risk factor for CHD includes a low level of HDL cholesterol. The association between a low level of HDL cholesterol and an increased risk of cardiovascular disease has been well established through epidemiological and clinical studies.[4,6,7,36] Since low HDL cholesterol plays a direct role in the atherogenic process and is acknowledged as a strong predictor of CHD, therapeutic intervention to raise HDL cholesterol together with other risk factors is widely encouraged. The administration of Kangen-karyu extract led to the significant elevation of HDL cholesterol, indicating its promising protective potential against cardiovascular disease. Furthermore, administration of Kangen-karyu reduced the atherogenic index dose-dependently, which is commonly used as an index of risk for CHD, mainly by increasing the HDL cholesterol level.

The protective roles of HDL cholesterol from CHD have been suggested to occur in various ways.[5,7,37—39] HDL exerts part of its antiatherogenic effect by counteracting LDL oxidation and recent studies also showed that HDL promotes the reverse cholesterol transport pathway, in which HDL induces an efflux of excess accumulated cellular cholesterol and prevents the generation of an oxidatively modified LDL. Furthermore, HDL inhibits the oxidation of LDL by transition metal ions, but also prevents 12-lipoxygenase-mediated formation of lipid hydroperoxides.[39] On the basis of these studies, if the investigation on atherosclerotic lesions in blood vessel walls would be supported, Kangen-karyu would be expected to play an antiatherogenic role through the inhibition of LDL oxidation as well as the elevation of HDL cholesterol.

LDL cholesterol is also the primary target of CHD risk-reduction therapy. Moreover, elevations in VLDL and IDL cholesterol levels are also risk factors of CHD. The results of many large scale primary and secondary prevention trials have demonstrated that lowering serum cholesterol and LDL cholesterol can significantly reduce the risk of cardiovascular events.[1,8,40] Kangen-karyu extract lowered the serum LDL, VLDL and IDL cholesterol levels significantly. When there is excess LDL in the blood, it is deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions. Therefore, the serum LDL cholesterol concentration should be used as the basis for initiating and monitoring the treatment of patients with elevated blood cholesterol levels. Experimental animals which consumed high dietary levels of cholesterol developed elevated LDL cholesterol levels and atherosclerosis.[41] Ross[33] also reported a relationship between LDL cholesterol and atherosclerosis, and suggested that the pathological process could be reversed by reducing the serum LDL cholesterol level. In view of our present results with a hypercholesterolemic rat model, we would expect Kangen-karyu to lower serum levels of cholesterol including LDL, VLDL and IDL cholesterols.

The oxidation of LDL cholesterol as well as LDL cholesterol itself plays an important role in the pathogenesis of atherosclerotic conditions. LDL is oxidized by Cu^{2+}, resulting in the formation of peroxides. In addition, oxidized LDL contributes to various atherosclerotic stages, not only foam cell formation *via* scavenger receptor uptake but also direct chemotactic activity toward monocytes, smooth muscle cells, and T-lymphocytes, inhibition of biological activity, production of endothelium-derived nitric oxide, and the induction of cytotoxicity leading to endothelial injury.[42,43] There is no doubt that LDL undergoes oxidative modification *in vivo* and that the inhibition of LDL oxidation is important for the attenuation of atherosclerotic changes. Administration of Kangen-karyu extract inhibits LDL oxidation, which is a key step in atherosclerotic progression due to elevated endogenous antioxidative activity and the subsequent development of atherosclerotic lesions. However, for the clear explanation on the effect on LDL oxidation, the level of triglyceride and types and content of fatty acid have to be considered. Several studies demonstrated that the triglyceride level following the high cholesterol diet was unchanged compared to control animals.[19,44] On the other hand, Fungwe *et al*. documented that rats fed the high cholesterol diet showed the significant increase in triglyceride level.[45]

Hypercholesterolemia leads to the increased production of oxygen free radicals,[46] which exert their cytotoxic effect by causing lipid peroxidation with the formation of malondialdehyde (MDA). The present study showed the elevation of serum MDA level in rats fed the hypercholesterolemic diet. Several investigators have also supported this rise in MDA levels in animals fed high cholesterol diets.[47,48] Elevated levels of lipid peroxidation products may be responsible for some of the pathological effects of hyperlipidemia. However, the administration of Kangen-karyu extract resulted in MDA level reduction and the decrease in lipid peroxidation probably leads to a reduction in the arterial wall cholesterol content that has to be further studied. As the serum TBA-reactive substances level decreased after the administration of Kangen-karyu extract, the present study carefully suggests that Kangen-karyu would play the protective role in progression of atherosclerosis caused by hypercholesterolemia.

Chinese traditional medicines and prescriptions with beneficial effects against various pathological conditions have recently attracted a great deal of attention as alternative therapies. The results of our present study support the hypothesis that the Chinese prescription drug Kangen-karyu would itself be useful as an alternative therapy for hypercholesterolemia. Kangen-karyu led to a decrease in atherogenic index through the elevation of HDL cholesterol. In addition, it resulted in not only reductions in LDL cholesterol and its oxidation, but also a reduction in lipid peroxidation. If the effect of Kangen-karyu on blood vessel in atherosclerotic plaques would be supported, Kangen-karyu would be expected to prevent hypercholesterolemic atherosclerosis and risk of CHD.

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