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Anti-hypercholesterolaemia, Antioxidant Activity and Free Radical Scavenger Effects of Traditional Chinese Medicine Prescriptions Used for Stroke

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Abstract

The generation of oxygen radicals and lipid peroxidation may be factors in the cerebral damage secondary to ischaemia of the cerebrovascular disease, as in stroke. Five traditional Chinese medicinal prescriptions were investigated for their antioxidant activity: Shiee Fuu Jwu Iu Tang (TCMP1), Oh Yaw Shuen Chin Saan (TCMP2), Buu Yang Hwan Wuu Tang (TCMP3), Sheau Shiuh Ming Tang (TCMP4), and Chir Hwu Jia Long Guu Muu Lih Tang (TCMP5).

Anti-lipid peroxidation, anti-superoxide formation and free radical scavenger activity were determined by the $FeCl_2$—ascorbic acid-induced lipid peroxidation effects on lipids in-vitro, xanthine oxidase inhibition, cytochrome C system and an electron spin resonance spectrometer, respectively. The results showed that TCMP5 had greater anti-lipid peroxidation and anti-superoxide formation activity than the other prescriptions. TCMP4 had the greatest free radical scavenging effect, TCMP5 showed the greatest superoxide radical scavenger activity and TCMP3 showed the greatest hydroxyl radical scavenger activity. Tests were also performed to evaluate the effects of the five prescriptions on blood lipid in-vivo. The test showed that the prescriptions decreased the level of total cholesterol and LDL-cholesterol in serum in high cholesterol-fed rats.

From these results, it seems probable that these prescriptions may be effective in the prevention and therapy of stroke and ischaemia.

Traditional Chinese medicinal prescriptions have been found to be very effective for the treatment of variety of diseases due to their unique biocompatibility. Shiee Fuu Jwu Iu Tang (TCMP1), Oh Yaw Shuen Chin Saan (TCMP2), Buu Yang Hwan Wuu Tang (TCMP3), Sheau Shiuh Ming Tang (TCMP4) and Chir Hwu Jia Long Guu Muu Lih Tang (TCMP5) are used clinically for patients with cardiovascular disease, such as hypercholesterolaemia, arteriosclerosis and stroke (Chen & Yang 1991). The prescriptions are used in a concentrated powder form in a dose of approximately 3–5 g (1 g = 4·25 g prescription) taken after meals.

Reactive oxygen species such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot -}$) and hydroxyl radical ($^{\cdot}OH$), contribute to a variety of diseases, including ischaemia—reperfusion injury, diabetes mellitus, coronary arteriosclerosis, cardiovascular diseases, cancer, arthritis and immunodeficiencies (Bulkely 1983; Dormandy 1983). Biochemical damage produced by reactive oxygen species has emerged as a fundamental final common pathway of tissue injury in a wide variety of disparate disease processes. Therefore, chemical scavengers, which can eliminate or decompose pathogenic free radicals, would be expected to work as therapeutic agents in these conditions. Some Oriental herbal medicines or Chinese medicinal prescriptions have been found to have similar effects (Krieglstein et al 1986; Erben-Russ et al 1987; Iwata et al 1987; Uchida et al 1987). In this study, we evaluated five traditional Chinese medicinal prescriptions to determine their pharmacological effects on anti-hypercholesterolaemia, anti-

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lipid peroxidation, anti-superoxide formation effects and active oxygen scavenging activity.

Materials and Methods

Traditional Chinese medicinal prescriptions

Crude ingredients of TCMP1, TCMP2, TCMP3, TCMP4 and TCMP5, were purchased from a local herb store. The composition of the prescriptions is shown in Table 1.

An extract of each prescription was prepared according to the proportions given in Table 1. Each recipe (200 g) was decocted three times with 1 L boiling distilled water for 2 h. The decoction was filtered, collected, concentrated, and lyophilized.

Chemicals

Cholesterol, ferrous sulphate, ferrous chloride, hydrogen peroxide (H_2O_2), and L(+)-ascorbic acid, were purchased from Wako Pure Chemical Ins., Ltd (Osaka, Japan). cholic acid, cytochrome C, diethylenetriamine pentaacetic acid (DETAPAC), ethylenediamine tetraacetic acid (EDTA), hypoxanthine (HPX), sodium dodecyl sulphate (SDS), thiobarbituric acid (TBA), Tris-HCl and xanthine were obtained from Sigma Chemical Co. 5,5-Dimethyl-1-pyrr-loine-1-oxide (DMPO) was supplied by Labotec Co., Ltd, and xanthine oxidase (XOD) was from Boehringer Mannheim. Superoxide dismutase (SOD) was obtained from Toyobo Co., Ltd, and *n*-butanol was from Merck Chemical Co. All other chemicals used were of the highest grade commercially available.

Anti-hypercholestaerolaemia activity

The effect of five prescriptions on rats fed on a high cholesterol diet was determined by the method described by Yokozawa et al (1997). Male LWH Wistar rats, 150–160 g, were purchased from the National Cheng Kung University Laboratory Animals Center. They were kept in a wire-bottomed cage under a conventional lighting regimen with darkness at night. The room temperature (approx. 25°C) and humidity (approx. 60%) were

Table 1. Composition of the five dried traditional Chinese medicinal prescriptions.

Shiee Fuu Jwu Iu Tang (TCMP1)	
Prunus persica (L.) Batsch.	4 g
Angelicae sinensis (Oliv.) Diels.	3 g
Rehmanniae glitinosa (Gaertn.) Libosch.	3 g
Panax pseudoginseng Wall. var. notoginseng	3 g
Crocus sativus L. (Iridaceae)	3 g
Poncirus trifoliata (L.) Raf.	2 g
Paeonia lactiflora Pall.	2 g
Glycyrrhiza uralensis Fisch.	1·5 g
Platycodon grandiflorum (Jacq.) A.DC.	1·5 g
Ligusticum wallichii Franch.	1·5 g
Bupleurum chinense DC.	1 g

Buu Yang Hwan Wuu Tang (TCMP3)	
Astragalus membranaceus (Fisch) Bge.	40 g
Angelicae sinensis (Oliv.) Diels.	2 g
Paeonia veitchii Lynch.	1·5 g
Ligusticum wallichii Franch.	1 g
Prunus persica (L.) Batsch.	1 g
Pheretima aspergillum E. perrier	1 g
Crocus sativus L.	1 g

Chir Hwu Jia Long Guu Muu Lih Tang (TCMP5)	
Bupleurum chinense. DC.	5 g
Pinelliae ternate (Thunb.) Breit.	4 g
Poria cocos (Schw.) Wolf.	3 g
Cinnamomum cassia Presl.	3 g
Scutellaria baicalensis Georgi.	2·5 g
Zizyphus jujuba Mill.var. inermis (Bge.) Rehd.	2·5 g
Zingiber officinale Rosc.	2·5 g
Panax ginseng C.A.Mey.	2·5 g
Fossilia ossis Mastodi.	2·5 g
Ostrea rivularis Gould.	2·5 g
Rheum palmatum L.	1 g

Oh Yaw Shuen Chin Saan (TCMP2)	
Lindera strychnifolia (Sieb.et Zucc.) Villar.	2·5 g
Citrus tangerina Hort. et Tanaka.	2·5 g
Bombyx mori L.	2·5 g
Ephedra sinica Stapf.	2·5 g
Ligusticum wallichii Franch.	2·5 g
Platycodon grandiflorum (Jacq.) A.DC.	2·5 g
Zingiber officinale Rosc.	2 g
Poncirus trifoliate (L.) Raf.	2 g
Angelica anomala Lallem.	1·5 g
Glycyrrhiza uralensis Fisch.	1·5 g

Sheau Shiuh Ming Tang (TCMP4)	
Saposhnikovia divaricata (Turcz.) Schischk.	3 g
Cinnamomum cassia Presl.	2 g
Ephedra sinica Stapf.	2 g
Prunus armeniaca L.	2 g
Ligusticum wallichii Franch.	2 g
Paeonia lactiflora Pall.	2 g
Glycyrrhiza uralensis Fisch.	2 g
Scutellaria baicalensis Georgi.	2 g
Stephania tetrandra S.Moore.	2 g
Zingiber officinale Rosc.	2 g
Aconiti Carmichael Debx.	1 g
Zizyphus jujuba Mill. var. inermis (Bge.) Rehd.	1 g

automatically controlled. The rats were fed a high cholesterol diet containing 1% cholesterol, 0·5% cholic acid and 5% pork fat. The prescription extracts were dissolved in water, and given to the rats orally every day for 30 days. The dose was adjusted to 0·5 g or 1·0 mg kg^{-1} by regulating the concentration in response to water consumption by the rats. Control rats were given access to water alone. Six rats were used in each experimental group. After 30 days, the rats were killed by decapitation between 13 00 and 14 00 h to avoid any effect of circadian variation. The blood samples were allowed to clot at 4°C, and then centrifuged. The plasma obtained was used for the determination of various chemical parameters.

Anti-lipid peroxidation activity

According to the method of Ohkawa et al (1979), the reaction mixture comprised 0·25 mL liver homogenate, 0·1 mL Tris-HCl buffer (pH 7·2), and 0·05 mL of the test prescription extracts. The mixture was incubated at 37°C for 1 h in a capped tube, and then 0·5 mL 0·1 M HCl, 0·2 mL 9·8% SDS, 0·9 mL distilled water and 2 mL 0·6% TBA were added to each tube and vigorously shaken. The tubes were placed in a boiling water bath (100°C) for 30 min. After cooling, the flocculent precipitate was removed by adding 5 mL *n*-butanol and centrifuged at 3000 rev min^{-1} for 25 min. The potency of the supernatant was measured using a Hitachi U-2000 spectrophotometer at 532 nm. The rat liver homogenate was induced with Fe^{2+}/ascorbic acid ($FeCl_2$–ascorbic acid) to cause nonenzymatic lipid peroxidation. To determine the effect of the five prescriptions on lipid peroxidation, test agents were incubated with a rat liver homogenate in the presence of $FeCl_2$–ascorbic acid. The lipid peroxide concentration was determined by MDA–TBA adduct (complexion of malondialdehyde with thiobarbituric acid) at 532 nm (Mihara et al 1980; Wong et al 1987).

Anti-superoxide formation

Anti-superoxide formation was determined in the xanthine oxidase inhibition test. Xanthine oxidase activity was evaluated by spectrophotometric measurement of the formation of uric acid from xanthine (Chang et al 1994). Samples were dissolved in and diluted with 50 mM KH_2PO_4 buffer (pH 7·8) to obtain each concentration. Superoxide formation was calculated by measuring uric acid production by spectrophotometry at 295 nm. The IC50 value of each prescription was then calculated.

Free radical scavenging activity

Free radical scavenging activity was determined in the cytochrome C test. Superoxide anion was assayed spectrophotometrically by a cytochrome C reduction method described by McCord & Fridovich (1969). Xanthine oxidase converts xanthine to uric acid and yields superoxide anions, and these radicals directly reduce ferri-cytochrome C to ferro-cytochrome C. Superoxide anion production was calculated from the increase in absorption at 550 nm which is the maximum absorption of reduced cytochrome C. Therefore, superoxide anion scavenging activity was evaluated by spectrophotometric measurement of the ferri-cytochrome C. When test prescriptions showed superoxide scavenging activity, there was a decrease in the reduction of ferri-cytochrome C.

Superoxide anion and hydroxyl radicals scavenging activity

Superoxide radicals were generated from a hypoxanthine–xanthine oxidase reaction system, trapped by DMPO, and the spin adduct of $O_2^{\cdot -}$ (DMPO-OOH) was analysed using an electron spin resonance (ESR) spectrometer (McCord & Fridovich 1969; Mitsuta et al 1990; Kohno et al 1991). A solution of 2·0 mM HPX/PBS (A), 5·5 mM DETAPAC/PBS (B), various concentrations of crude drug extracts or SOD (C), and 0·4 units mL^{-1} XOD/PBS (D), was prepared before use. The XOD solution was stored in an ice bath to prevent inactivation of enzyme. Then 50 μL of A, 35 μL of B, 50 μL of C and 15 μL of DMPO were transferred into a test tube. To the mixed solution, 50 μL of D was added. Hydroxyl radicals were generated as follows: a solution of 1 mM ferrous sulphate (A), 5·5 mM DETAPAC/PBS (B), various concentrations of crude drug extracts or ascorbic acid (C), and 1 mM hydrogen peroxide (D) was prepared before use. Then 37·5 μL of A, 37·5 μL of B, 50 μL of C and 20 μL DMPO (0·092 M) were pipetted into a test tube and 75 μL of D was added to the mixed solution. Analysis of spin adduct (DMPO-OH) was performed using an ESR spectrometer.

The reaction mixture was stirred and transferred to a quartz-analysing cell and placed into the cavity of an ESR spectrometer (JEOL-JES-FR80, JEOL Ltd, Tokyo, Japan). At 40 s after the addition of XOD or hydrogen peroxide, samples were analysed and the relative intensity of the signal of DMPO-OOH or DMPO-OH spin adduct was measured as the ratio to the intensity of Mn^{2+} signal. ESR spectra were recorded at 37°C with a field set at 335·4 ± 5·0 mT, modulation frequency 100 kHz, modulation amplitude 0·79 × 0·1 mT, response time

0·1 s, sweep time 2 min, microwave power 8·0 mW (9·416 GHz), receiver gain 2×100 when superoxide radicals were trapped, and at 1×100 when hydroxyl radicals were trapped.

Results

Table 2 compares the plasma cholesterol concentrations in treated rats and control rats in the anti-hypercholesterolaemia activity test. The total cholesterol, and the LDL cholesterol concentrations in control rats fed a high-cholesterol diet increased significantly compared with normal rats, reflecting hypercholesterolaemia. Administration of the prescriptions tended to decrease the levels of total cholesterol and LDL cholesterol. However, the effect was moderate and similar for all prescriptions except for TCMP4, which was ineffective at 0·5 g.

The antioxidant activity (IC50) of the five prescriptions is summarized in Table 3. In the anti-lipid peroxidation activity test, the IC50 ranged from 2·33 to 7·93 mg mL^{-1}. All of the prescriptions showed anti-lipid peroxidation activity, with TCMP5 displaying the most potent activity. In the xanthine oxidase inhibitor test, the IC50 ranged from 0·33 to 0·80 mg mL^{-1}, and both TCMP4 and TCMP5 showed strong anti-superoxide formation activity. In the cytochrome C test, the IC50 ranged from 0·048 to 0·384 mg mL^{-1}, and TCMP2 and TCMP4 had the strongest superoxide scavenger activity.

The ESR spin-trapping technique is a powerful tool for investigating superoxide and hydroxyl radicals scavenging potency (Lin et al 1995). When DMPO was added to a solution of the HPX-XOD reaction system, the spin adduct DMPO-OOH was formed. When SOD of various concentrations was added to the system, the signal intensity of DMPO-OOH decreased with an increase in the SOD concentration (Table 4). This can be explained by the reaction between $O_2^{\cdot -}$ and DMPO, which is inhibited by SOD. The same result occurred on addition of the extracts of the five prescriptions, and the inhibition ratio using the standard SOD was calculated. The linear calibration curve ($y = 0{\cdot}209x - 0{\cdot}019$; $r = 0{\cdot}998$) using a standard SOD (0–16·98 units mL^{-1}) solution was determined with peak intensities of the internal standard signal in MnO, which was set in the ESR spectrometer and the superoxide radical. With the addition of the five prescription extracts, a response, similar to that which happened with the addition of SOD, occurred. The scavenging activity of the prescriptions was obtained from the calibration curve by comparing the averaged relative peak height of extracts and that of standard SOD (SOD-like activity). The results comparing the averaged relative peak heights of standard and extracts are showed in Table 5. The SOD-like activity and IC50 values (the amount of SOD that causes 50% inhibition of the ESR signal intensity of DMPO-OOH) of TCMP1, TCMP2, TCMP3, TCMP4, and

Table 3. Summary of the multiple antioxidant effects of five traditional Chinese medicinal prescriptions.

Group	IC50 ($mgmL^{-1}$)		
	Lipid peroxidation	Xanthine oxidase inhibition test	Cytochrome C test
TCMP1	3·87	0·67	0·319
TCMP2	3·87	0·80	0·071
TCMP3	3·23	0·74	0·384
TCMP4	7·93	0·43	0·048
TCMP5	2·33	0·33	0·193

Table 2. Effects of five traditional Chinese medicinal prescriptions on serum lipids of rats fed on a high-cholesterol diet.

Group	Dose (g kg^{-1} day^{-1})	Total cholesterol (mg dL^{-1})	LDL (mg dL^{-1})
Control	–	79·62 ± 10·45	54·90 ± 7·69
Cholesterol diet	–	156·06 ± 3·99**	131·12 ± 21·62**
TCMP1	0·5	118·68 ± 11·74†	97·72 ± 10·46
	1·0	87·20 ± 5·37†	70·32 ± 6·23†
TCMP2	0·5	117·04 ± 6·49†	88·56 ± 6·79†
	1·0	95·26 ± 10·27†	98·52 ± 5·12†
TCMP3	0·5	116·18 ± 4·73†	98·72 ± 3·67†
	1·0	104·02 ± 13·84†	87·46 ± 10·76†
TCMP4	0·5	134·44 ± 16·34†	110·50 ± 15·27
	1·0	113·08 ± 3·77†	95·36 ± 1·73†
TCMP5	0·5	115·80 ± 9·97†	100·16 ± 12·91
	1·0	102·68 ± 8·16†	90·34 ± 8·60†

Each value represents mean ± s.d., $n = 6$. **$P < 0{\cdot}01$ significantly different from control group (Student's *t*-test). †$P < 0{\cdot}05$ significantly different from cholesterol diet group (Student's *t*-test).

TCMP5 were $9{\cdot}36\times10^{-4}$, $1{\cdot}16\times10^{-4}$, $7{\cdot}72\times10^{-4}$, $1{\cdot}35\times10^{-4}$, $9{\cdot}54\times10^{-5}$ g mL^{-1}, respectively. TCMP5 had a more direct superoxide radical scavenging effect than other prescriptions. The spin adduct DMPO-OH, was formed when DMPO was added to a solution of the ferrous sulphate-hydrogen peroxide reaction system. Ascorbic acid, used as a scavenger of hydroxyl radical, was added to the reaction system. Then the signal decayed with increasing ascorbic acid concentration (0–1·0 mM) (Table 6). The calibration curve was determined with peak intensities of the internal standard signal of Mn^{2+} in MnO and the hydroxyl radical. Table 7 shows the relationship between the signal intensity of DMPO-OH and the concentration of the five prescriptions added. The hydroxyl radical scavenging activity of the extracts of the five prescriptions was obtained from the calibration curve. The hydroxyl radical scavenging activity and IC50 (the amount of ascorbic acid that causes 50% inhibition of the ESR signal intensity of DMPO-OH) of TCMP1, TCMP2, TCMP3, TCMP4, and TCMP5 were $1{\cdot}23\times10^{-2}$, $9{\cdot}87\times10^{-3}$, $8{\cdot}98\times10^{-3}$, $1{\cdot}01\times10^{-2}$, $1{\cdot}36\times10^{-2}$ g mL^{-1}, respectively. TCMP4 showed the greatest direct-hydroxyl radical scavenging effect.

Discussion

Oxidant stress has been increasingly implicated as a mechanism of disease, including in cancer, arteriosclerosis and aging. Apart from a few rare genetic defects, dietary factors are thought to play a key role in the regulation of production of reactive oxygen species. An imbalance between nutrients, and in particular those with antioxidant activity, could explain the onset of an increased production of free radicals. Free radicals are involved in the formation of both arteriosclerosis and thrombosis. Therefore, considerable interest has recently been focused on their role in the development of ischaemic cerebral injury (Hillbom 1999). The

Table 4. Electron spin resonance (ESR) signal activity of Mn^{2+} and superoxide radical in various concentrations of superoxide dismutase (SOD).

SOD (units mL^{-1})	ESR signal peak height		Averaged relative peak height
	Mn^{2+}	Radical	
0·00	92·60	287·60	3·11
1·68	88·40	194·60	2·20
4·24	90·60	157·40	1·74
8·49	90·40	101·80	1·13
12·73	90·60	81·60	0·86
16·98	88·20	60·00	0·68

Calibration curve: $y = 0{\cdot}209x - 0{\cdot}019$ ($r = 0{\cdot}998$), where $y = (I_0/I-1)$; x = SOD concn (unit mL^{-1}); I indicates the relative peak height in various concentrations of SOD; and I_0 = without SOD.

Table 6. Electron spin resonance (ESR) signal activity of Mn^{2+} and hydroxyl radical in various concentrations of ascorbic acid.

Ascorbic acid (mM)	ESR signal peak height		Averaged relative peak height
	Mn^{2+}	Radical	
0·000	55·000	548·000	9·964
0·200	52·000	424·400	8·162
0·400	53·800	281·600	5·234
0·600	53·400	187·400	3·509
0·800	55·000	82·000	1·491
1·000	52·800	43·400	0·882

Calibration curve: $y = 0{\cdot}059x^3 - 0{\cdot}362x^2 + 0{\cdot}793x + 0{\cdot}013$, where y = ascorbic acid concn (mM); $x = (I_0/I-1)$; I indicates the averaged relative peak height in various concentrations of ascorbic acid; and I_0 = without ascorbic acid.

Table 5. SOD-like (superoxide radical scavenger) activity assay.

Sample	Concn. (g mL^{-1})	Signal peak height		SOD activity (units g^{-1})	SOD-like activity[a]	IC50[b] (g mL^{-1})
		Mn^{2+}	Radical			
TCMP1	$2{\cdot}01\times10^{-3}$	90·60	88·80	10·45	$5{\cdot}2\times10^{3}$	$9{\cdot}36\times10^{-4}$
TCMP2	$2{\cdot}56\times10^{-4}$	90·60	87·00	10·76	$4{\cdot}2\times10^{4}$	$1{\cdot}16\times10^{-4}$
TCMP3	$1{\cdot}59\times10^{-3}$	91·00	91·60	10·05	$6{\cdot}3\times10^{3}$	$7{\cdot}72\times10^{-4}$
TCMP4	$3{\cdot}15\times10^{-4}$	93·40	86·40	11·35	$3{\cdot}6\times10^{4}$	$1{\cdot}35\times10^{-4}$
TCMP5	$2{\cdot}02\times10^{-4}$	91·80	90·80	10·31	$5{\cdot}1\times10^{4}$	$9{\cdot}54\times10^{-5}$

[a]Calibration curve: $y = 0{\cdot}209x - 0{\cdot}019$, where $y = (I_0/I-1)$; $I_0 = 3{\cdot}106$; I indicates the average relative peak height = radical peak height/Mn^{2+} peak height in various concentrations of the five prescriptions extracts; x = SOD concn (units); SOD-like activity = SOD (units)/ the five prescriptions extracts concentration. [b]IC50 indicates the amount of SOD that causes 50% inhibition of the ESR signal intensity of DMPO-OOH.

Table 7. Hydroxyl radical scavenger activity assay.

Sample	Concn. ($g\,mL^{-1}$)	Signal peak height		Ascorbic acid[a] (mM)	$^{\cdot}OH$ scavenger activity[b] (units g^{-1})	IC50[c] ($g\,mL^{-1}$)
		Mn^{2+}	Radical			
TCMP1	$2{\cdot}01\times10^{-3}$	51·40	195·00	0·601	41	$1{\cdot}23\times10^{-2}$
TCMP2	$2{\cdot}56\times10^{-4}$	55·60	217·00	0·594	51	$9{\cdot}87\times10^{-3}$
TCMP3	$1{\cdot}59\times10^{-3}$	56·80	206·04	0·610	56	$8{\cdot}98\times10^{-4}$
TCMP4	$3{\cdot}15\times10^{-4}$	55·00	276·20	0·499	50	$1{\cdot}01\times10^{-2}$
TCMP5	$2{\cdot}02\times10^{-4}$	51·60	272·60	0·473	37	$1{\cdot}36\times10^{-2}$

[a]Calibration curve: $y = 0{\cdot}059x^3 - 0{\cdot}363x^2 + 0{\cdot}794x + 0{\cdot}013$, where y = ascorbic acid concn (mM); $x = (I_0/I-1)$; $I_0 = 9{\cdot}964$; I indicates the averaged relative peak height = radical peak height/Mn^{2+} peak height in various concentrations of the prescription extracts. [b]Where 1 unit means the hydroxyl radical scavenger activity by 1 mM ascorbic acid in this reaction system. [c]IC50 indicates the amount of ascorbic acid that cause 50% inhibition of the ESR signal intensity of DMPO-OH.

initial occurrence in the development of arteriosclerosis is the appearance of foam cells resulting from the accumulation of lipids consisting mainly of cholesterol. However, much remains unclear about how foam cells are produced in LDL hyperlipoproteinaemia and how they induce atheriosclerotic changes. It has been noted that there is a close relationship between arteriosclerosis and the decrease of serum lipids. A body of evidence indicating that the oxidation of LDL plays an important role in the development of arteriosclerosis has led investigators to consider a preventive role for dietary constituents with antioxidant activity. LDL is oxidized before the onset of oxidation of LDL polyunsaturated fatty acid, suggesting a possible role in delaying the onset of LDL oxidation.

The $FeCl_2$–ascorbic acid stimulated lipid peroxidation method is an indirect measure of lipid peroxidation, which is susceptible to interference by endogenous and exogenous substances, and should be regarded as an indication rather than as an absolute measure of total tissue lipid peroxide levels (Draper & Hadley 1990). Xanthine oxidase converts hypoxanthine to xanthine and then xanthine to uric acid in the presence of molecular oxygen to yield superoxide anion and hydrogen peroxide. Xanthine oxidase-derived superoxide anion has been linked to post-ischaemic tissue injury and oedema as well as changes in vascular permeability (McCord & Fridovich 1969). Limitation of superoxide anion regeneration by the enzymatic pathway would be beneficial in ischemia and oedema.

This study showed that superoxide and hydroxyl radicals generated from the HPX-XOD reaction system and Fenton reaction system, respectively, were suppressed by extracts of the five prescriptions. This indicates that these prescriptions contain potent antioxidant activity capable of scavenging superoxide and hydroxyl radicals.

In conclusion, we found that the five traditional Chinese prescriptions tested have powerful anti-hypercholesterolaemia, anti-lipid peroxidation, anti-superoxide anion formation and free radical scavenging activity. The prescriptions could exert a beneficial effect against tissue injury and pathological alterations caused by superoxide and hydroxyl radicals. The results provide information on the mechanisms of dietary antioxidants in preventing oxidant stress.

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