Curcumin or Saikosaponin a Improves Hepatic Antioxidant Capacity and Protects Against CCl_4-Induced Liver Injury in Rats

Shu-Ju Wu,[1,2] Yun-Ho Lin,[3] Chia-Chou Chu,[1] Ya-Hui Tsai,[1,4] and Jane C.-J. Chao[4,5]

Schools of [1]Pharmacy and [4]Nutrition and Health Sciences and [3]Department of Pathology, School of Medicine, Taipei Medical University; [5]Nutrition Research Center, Taipei Medical University Hospital, Taipei; and [2]Department of Nursing, Chang Gung Institute of Technology, Taoyuan, Taiwan, Republic of China

ABSTRACT Curcumin and saikosaponin a, the bioactive phytochemicals of turmeric and *Bupleurum*, act as antioxidants. This study investigated the effects of supplementation with curcumin and/or saikosaponin a on hepatic lipids and antioxidant status in rats with CCl_4-induced liver injury. Male Sprague-Dawley rats were randomly divided into control, CCl_4, CCl_4 + curcumin (0.005%; CU), CCl_4 + saikosaponin a (0.004%; SS), and CCl_4 + curcumin + saikosaponin a (0.005% + 0.004%; CU+SS) groups. CCl_4 (40% in olive oil) was injected intraperitoneally at a dose of 0.75 mL/kg once a week. Curcumin and/or saikosaponin a was administered orally 1 week before CCl_4 injection for 8 weeks. The pathological results showed that liver fibrosis was ameliorated in the SS and CU+SS groups. After 8 weeks, supplementation with curcumin and/or saikosaponin a significantly decreased plasma alanine aminotransferase and aspartate aminotransferase activities, as well as plasma and hepatic cholesterol and triglyceride levels. The CU+SS group showed reversal of the impaired hepatic superoxide dismutase activity and an increase in total glutathione level. Supplementation with curcumin and/or saikosaponin a significantly improved hepatic antioxidant status and suppressed malondialdehyde formation. Therefore, supplementation with curcumin and/or saikosaponin a protects against CCl_4-induced liver injury by attenuating hepatic lipids and lipid peroxidation and enhancing antioxidant defense. Curcumin and saikosaponin a had no additive effects on hepatoprotection except for greater improvement in the total glutathione level and antioxidant status.

KEY WORDS: • ***antioxidant enzymes*** • ***curcumin*** • ***hepatocellular damage*** • ***lipid peroxidation*** • ***saikosaponin a***

INTRODUCTION

MANY HERBS AND HERBAL EXTRACTS are widely used to protect the liver and as adjunctive therapy for hepatic diseases, including steatosis, hepatitis, and cirrhosis. Bioactive phytochemicals, such as phenols, saponins, glycosides, and alkaloids, in such herbs have been reported to possess hepatoprotective activity.[1] These phytochemicals have been found to protect against oxidative stress. Liver damage can be induced by viral infection, drug intoxication, and exposure to oxidative stress. Oxidative stress is associated with chronic hepatitis and fibrosis,[2] and antioxidants are thought to be beneficial in protecting against liver injury.

Curcumin ($C_{21}H_{20}O_6$), a curcuminoid-containing polyphenolic pigment, is a principal active ingredient of turmeric (*Curcuma longa*). The major curcuminoids are curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin has been studied for its anti-inflammatory, antioxidant, and cholesterol-lowering properties.[3,4] Bupleuri radix (Chinese name, *chai hu*; Japanese name, *sho-saiko-to*) has immunomodulatory, hepatoprotective, antitumor, and antiviral activities[5,6] from saikosaponins. Saikosaponins include saikosaponin a, b, c, and d. Saikosaponin a ($C_{42}H_{68}O_{13}$), a principal active ingredient, exhibits antitumor, anti-inflammatory, and lipid-lowering activities.

The combined effect of curcumin and saikosaponin a on hepatoprotection against CCl_4-induced liver injury has not been studied yet. Therefore, we used pure phytochemical compounds of curcumin and saikosaponin a to demonstrate their hepatoprotective effects on steatosis and hepatic antioxidant status against CCl_4-induced oxidative stress.

MATERIALS AND METHODS

Animals and treatments

Fifty male Sprague-Dawley rats (weighing 200–250 g) were purchased from the National Laboratory Animal Center (Taipei, Taiwan, Republic of China). Rats were individually housed under a 12-hour light/dark cycle (05:00–17:00,

Manuscript received 17 August 2007. Revision accepted 22 January 2008.

Address reprint requests to: Jane C.-J. Chao, School of Nutrition and Health Sciences, Taipei Medical University, 250 Wu-Hsing Street, Taipei, Taiwan 110, Republic of China, E-mail: chenjui@tmu.edu.tw

light) at 22 ± 2°C. After a 1-week adaptation, rats were randomly divided into five groups: control, CCl_4, CCl_4 + curcumin (0.005%; CU), CCl_4 + saikosaponin a (0.004%; SS), and CCl_4 + curcumin + saikosaponin a (0.005% + 0.004%, respectively; CU+SS) groups. Curcumin extract containing a minimum of 95% curcuminoids was isolated from the dried rhizome of *C. longa* L., which was separated into one major (curcumin) and two minor fractions (bisdemethoxycurcumin and demethoxycurcumin) by thin-layer chromatography on silica using chloroform-ethanol (25:1 vol/vol) solvent as the mobile phase. Saikosaponin a (>98%, China Chemical & Pharmaceutical, Taipei) was extracted from *Bupleurum* root and identified by the ratio of fronts value of 0.33 and retention time of 7.02 minutes using a reverse-phase high-performance liquid chromatography analysis. Curcumin, saikosaponin a, or both, mixed into the powdered chow diet (Laboratory Rodent Diet 5001™, PMI Nutritional International, Brentwood, MO), were administered orally at the doses of 0.005% and 0.004%, respectively, 1 week before CCl_4 injection at week 1 for 8 weeks. CCl_4 (40% in olive oil) was injected intraperitoneally at a dose of 0.75 mL/kg of body weight once a week for 8 times to induce liver injury. Rats receiving the vehicle (olive oil, 0.75 mL/kg of body weight) in a similar dose served as the control group. Food intake and body weight were routinely recorded. All animal use protocols were approved and conducted under the guidelines of the Institutional Animal Care and Use Committee of Taipei Medical University.

Histopathological examination

After 8 weeks, all rats were killed under ether anesthesia. The liver was weighed. An excised liver fragment (1 cm × 1 cm) was fixed in 10% paraformaldehyde, embedded in paraffin wax, and stained with hematoxylin and eosin, Masson's trichrome, or silver. Coded specimens were scored under a light microscope by a pathologist in a blinded fashion.

Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities

Blood samples after overnight starvation were collected into heparin-containing tubes from the tail vein at weeks 0, 1, 3, 5, 7, and 8. Blood samples were centrifuged at 1,400 *g* and 4°C for 15 minutes. Plasma ALT and AST activities were measured spectrophotometrically at 570 nm (Iatron Laboratories, Tokyo, Japan).

Plasma and hepatic lipid concentrations

The liver was homogenized in a chloroform/methanol (2:1 vol/vol) solution and extracted with chloroform/methanol/water (3:48:47 by volume). Plasma and hepatic cholesterol and triglyceride levels were determined spectrophotometrically at 500 nm by cholesterol esterase/peroxidase and lipase glycerol kinase enzymatic methods (Randox Laboratories, Antrim, UK), respectively.

Hepatic antioxidant enzyme activities and antioxidant status

The liver was homogenized in buffer containing 0.25 *M* sucrose, 10 m*M* Tris-HCl, and 1 m*M* EDTA (pH 7.4). Hepatic catalase activity was determined spectrophotometrically at 240 nm. One unit of catalase activity was defined as the consumption of 1 μmol of H_2O_2/minute. Hepatic superoxide dismutase (SOD) activity was measured colorimetrically at 525 nm.[7] One unit is the activity that doubles the autooxidation background in the absence of SOD. The protein content was quantitated by a method modified from that of Lowry *et al*.[8] The glutathione level was measured spectrophotometrically at 412 nm (EMD Biosciences, San Diego, CA).[9] The liver was homogenized in 5% metaphosphoric acid with or without the reactant (1-methyl-2-vinylpyridium trifluoromethane sulfonate in HCl) at 4°C. After centrifugation, the supernatant was mixed with the chromogenic regent (5,5′-dithio-bis-2-nitrobenzoic acid), glutathione reductase, and NADPH. The oxidized (GSSG) and total glutathione levels were determined at 412 nm. The reduced glutathione (GSH) concentration was calculated by subtracting 2 GSSG from total glutathione.

The hepatic total antioxidant status was determined spectrophotometrically by the ability of antioxidants to inhibit the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline sulfonate) (ABTS) to $ABTS^{\bullet+}$ by metmyoglobin (Randox Laboratories).[10] The liver homogenate (20 μL) was mixed with 1 mL of chromogen (5 μM metmyoglobin and 500 μM ABTS in 600 m*M* phosphate-buffered saline, pH 7.4), and the antioxidant status was measured at 600 nm and 37°C.

The liver homogenate (200 μL in 50 m*M* Tris-HCl, pH 7.4) was mixed with 650 μL of reagent 1 (7.7 m*M N*-methyl-2-phenylindole in 75% acetonitrile and 25% methanol) (EMD Biosciences, Inc.) and 150 μL of reagent 2 (15.4 *M* methanesulfonic acid) at 45°C for 60 minutes. The hepatic malondialdehyde level was assessed colorimetrically at 586 nm.[11]

Statistical analysis

All data are expressed as mean ± SEM values. Data were analyzed by one-way analysis of variance and Fisher's least significant difference test using SAS version 8.2 (SAS Institute, Cary, NC). A level of $P < .05$ was considered as a significant difference.

RESULTS

Food intake, body weight, and liver weight

Food intake (20 ± 2–23 ± 2 g/day) did not differ among the five groups (data not shown). The initial weight, final weight, or weight gain did not differ (Table 1). The absolute and relative liver weights in the CCl_4 group significantly increased compared with those in the control group ($P < .05$). The relative liver weight did not differ among the CU, CU+SS, and control groups. The absolute and relative liver

Table 1. Body Weight and Liver Weight in Rats Fed Experimental Diets for 8 Weeks

	Group				
	Control	*CCl_4*	*CU*	*SS*	*CU + SS*
Initial weight (g)	362 ± 7	363 ± 11	363 ± 6	362 ± 11	363 ± 5
Final weight (g)	409 ± 7	388 ± 38	398 ± 4	397 ± 4	394 ± 5
Weight gain (g)	47 ± 5	26 ± 13	35 ± 7	35 ± 11	32 ± 9
Liver weight (g)	13.8 ± 0.7[b]	15.4 ± 0.5[a]	15.1 ± 0.2[ab]	14.6 ± 0.4[ab]	14.2 ± 0.4[ab]
Relative liver weight (g/kg)	33.9 ± 1.9[c]	40.2 ± 1.4[a]	36.9 ± 1.0[abc]	37.9 ± 1.0[ab]	36.1 ± 1.3[bc]

Data are mean ± SEM values ($n = 10$). CU, 0.005% curcumin in feed; SS, 0.004% saikosaponin a in feed; CU + SS, 0.005% curcumin + 0.004% saikosaponin a in feed.

Values not sharing a common superscript letter within a row significantly differ ($P < .05$) as determined by one-way analysis of variance and Fisher's least significant difference test.

weights did not differ among the curcumin and/or saikosaponin a-supplemented groups.

Histopathological evaluation

Rat liver sections were stained with hematoxylin and eosin, Masson's trichrome, or silver to evaluate the general morphology and fibrosis. No fat vacuoles were found in the liver biopsy of the control group (Table 2). However, fat vacuoles occurred in all CCl_4-treated groups, and numbers of large and small vacuoles increased compared with those in the control group ($P < .05$). Only the CU+SS group showed a decreased number of small vacuoles compared with the CCl_4 group ($P < .05$). Hematoxylin and eosin staining showed that all CCl_4-treated groups developed severe inflammation, necrosis, and fibrosis compared with the control group ($P < .05$). Necrosis was significantly inhibited in the CU+SS group, and the SS and CU+SS groups exhibited improvements in fibrosis caused by CCl_4 compared with the CCl_4 group according to both hematoxylin and eosin and Masson's trichrome stains ($P < .05$). The silver stain showed that supplementation with curcumin or saikosaponin a inhibited the formation of reticular fibers ($P < .05$).

Liver function index

Supplementation with curcumin and/or saikosaponin a significantly reversed the elevated plasma ALT and AST activities due to CCl_4 treatment in week 8 ($P < .05$) (Fig. 1). However, there were no significant differences among curcumin and/or saikosaponin a-supplemented groups. Plasma ALT and AST activities in the CCl_4 group increased rapidly in week 1 but decreased significantly with supplementation of curcumin and/or saikosaponin a after week 7 ($P < .05$) (data not shown).

Plasma and hepatic lipids

Supplementation with curcumin and/or saikosaponin a significantly reduced plasma and hepatic cholesterol and

Table 2. Scores for Rat Liver Biopsy Specimens Stained with Hematoxylin and Eosin, Masson's Trichrome, or Silver

	Group				
	Control	*CCl_4*	*CU*	*SS*	*CU + SS*
Hematoxylin and eosin stain					
Fatty change in central veins					
Large vacuoles	0.0 ± 0.0[b]	1.4 ± 0.2[a]	1.5 ± 0.1[a]	1.3 ± 0.1[a]	1.5 ± 0.2[a]
Small vacuoles	0.0 ± 0.0[c]	1.0 ± 0.1[a]	0.9 ± 0.2[ab]	0.6 ± 0.2[ab]	0.6 ± 0.2[b]
Inflammation	0.0 ± 0.0[b]	1.0 ± 0.3[a]	0.9 ± 0.1[a]	1.1 ± 0.1[a]	1.0 ± 0.2[a]
Necrosis	0.0 ± 0.0[c]	2.3 ± 0.2[a]	0.9 ± 0.5[a]	1.5 ± 0.3[a]	0.7 ± 0.2[b]
Fibrosis	0.0 ± 0.0[d]	1.5 ± 0.1[a]	1.4 ± 0.4[ab]	1.2 ± 0.2[bc]	1.0 ± 0.2[c]
Masson stain	0.0 ± 0.0[c]	1.4 ± 0.1[a]	1.1 ± 0.5[ab]	0.9 ± 0.2[b]	1.0 ± 0.2[b]
Silver stain	0.0 ± 0.0[d]	2.2 ± 0.1[a]	1.9 ± 0.3[bc]	1.6 ± 0.1[c]	1.9 ± 0.1[ab]

Data are mean ± SEM values ($n = 10$). CU, 0.005% curcumin in feed; SS, 0.004% saikosaponin a in feed; CU + SS, 0.005% curcumin + 0.004% saikosaponin a in feed. Scores were graded from 0 (normal) to 3 (severe). Fat vacuoles were graded on a scale of 0 (none), 1 (formation in central veins), 2 (formation in central veins and middle zone, but not reach to portal tract), or 3 (formation from central veins to portal tract). Fibrosis was graded on a scale of 0 (none), 1 (fibrosis in central veins), 2 (fibrous septa formation between central veins or between central veins and portal tract), or 3 (cirrhosis).

Values not sharing a common superscript letter within a row significantly differ ($P < .05$) as determined by one-way analysis of variance and Fisher's least significant difference test.

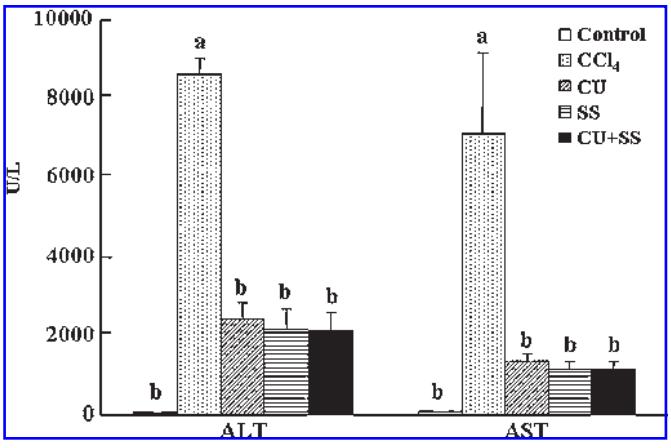

FIG. 1. Plasma ALT and AST activities in week 8. Data are mean ± SEM values ($n = 10$). CU, 0.005% curcumin in feed: SS, 0.004% saikosaponin a in feed; CU+SS, 0.005% curcumin + 0.004% saikosaponin a in feed. Values not sharing a common letter significantly differ ($P < .05$) as determined by one-way analysis of variance and Fisher's least significant difference test.

triglyceride levels in week 8 ($P < .05$) (Fig. 2). However, no additive effect was found between curcumin and saikosaponin a. Plasma cholesterol levels in the CCl_4 group were significantly increased after week 5 and decreased with supplementation with curcumin and/or saikosaponin a after week 7 ($P < .05$) (data not shown). Plasma triglyceride levels in the CCl_4 group were significantly elevated in week 1 ($P < .05$) but did not differ from those in the control group thereafter (data not shown).

Hepatic antioxidant enzyme activities and antioxidant status

Treatment with CCl_4 impaired hepatic SOD activity and the antioxidant status and elevated hepatic lipid peroxidation ($P < .05$) (Table 3). However, hepatic catalase activity and the glutathione redox status were not influenced by CCl_4. The SS group showed elevated hepatic catalase activity and decreased GSSG levels compared with those of the CCl_4 and control groups ($P < .05$). The CU+SS group showed increased hepatic SOD activity, GSH, and total glutathione levels compared with the CCl_4 group ($P < .05$). Supplementation with curcumin and/or saikosaponin a improved the hepatic antioxidant status and inhibited lipid peroxidation ($P < .05$). Additionally, combined curcumin and saikosaponin a enhanced the hepatic total glutathione level and antioxidant status compared with individual supplementation alone ($P < .05$).

DISCUSSION

This study found that supplementation with curcumin or saikosaponin a reversed the increased levels of hepatic lipids and activities of plasma ALT and AST, as well as the impaired antioxidant defense, with CCl_4-induced liver injury. Combined curcumin and saikosaponin a exhibited no additive hepatoprotection in terms of the pathological evaluation, liver function index, fatty changes, or antioxidant enzyme activities, although the combined supplementation produced a higher total glutathione level and antioxidant status.

The orally administered dosages of curcumin and saikosaponin a were determined in an *in vitro* pilot study. Our *in vitro* study showed that curcumin or saikosaponin a at the respective doses of 18.4 (50 μM) and 10 μg/mL (12.8 μM) significantly increased catalase and SOD activities and inhibited lipid peroxidation after a 48-hour treatment in normal rat liver cells (clone 9). The *in vivo* dosage was calculated based on the *in vitro* effective dose as the *in vivo* concentration in the circulation, the conversion factor of 1/13 body weight for blood volume, and the respective absorption rates of 45%[12,13] and 30%[14] for curcumin and saikosaponin a, in a 330-g rat given 20 g of feed/day. The calculated weight percentages (wt/wt) of curcumin and saikosaponin a in the feed were 0.005% and 0.004%, re-

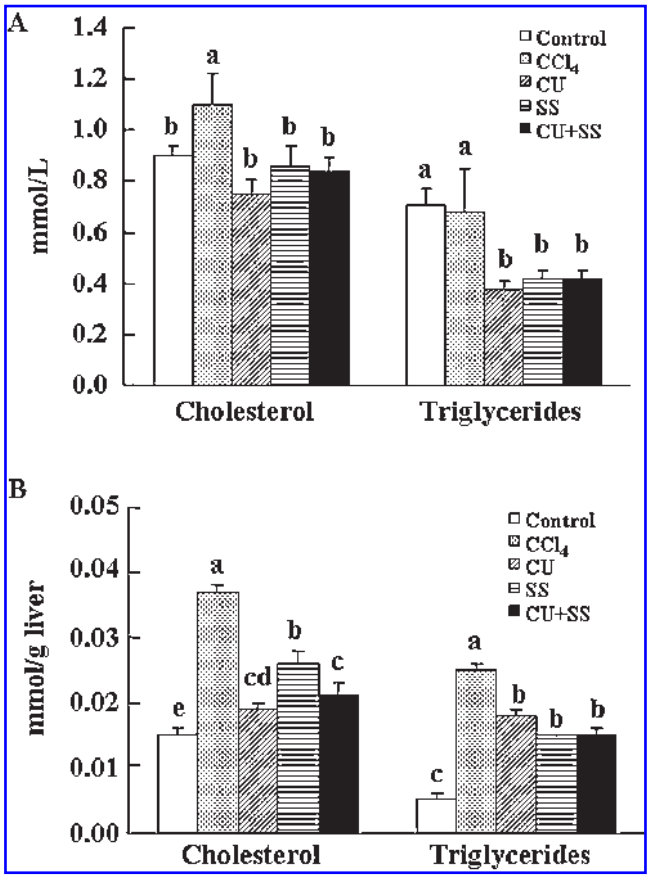

FIG. 2. (**A**) Plasma and (**B**) hepatic cholesterol and triglyceride levels in week 8. Data are mean ± SEM values ($n = 10$). CU, 0.005% curcumin in feed; SS, 0.004% saikosaponin a in feed; CU+SS, 0.005% curcumin + 0.004% saikosaponin a in feed. Values not sharing a common letter significantly differ ($P < .05$) as determined by one-way analysis of variance and Fisher's least significant difference test.

Table 3. Hepatic Antioxidant Enzyme Activities, Glutathione Redox Status, Antioxidant Status, and Malondialdehyde Level

	Group				
	Control	*CCl_4*	*CU*	*SS*	*CU + SS*
Catalase (U/mg of protein)	5.8 ± 0.5[b]	5.3 ± 0.6[b]	6.9 ± 0.5[b]	14.1 ± 1.1[a]	6.5 ± 0.4[b]
SOD (U/mg of protein)	20.0 ± 3.9[a]	11.5 ± 1.2[c]	15.9 ± 0.7[bc]	15.3 ± 1.1[bc]	16.6 ± 1.2[ab]
GSSG (mmol/L)	141 ± 51[a]	185 ± 65[a]	113 ± 19[ab]	27 ± 4[b]	100 ± 20[ab]
GSH (mmol/L)	1,079 ± 9[ab]	508 ± 19[b]	1,110 ± 64[ab]	1,070 ± 84[ab]	1,697 ± 371[a]
Total glutathione (mmol/L)	1,220 ± 20[ab]	693 ± 130[b]	1,223 ± 55[b]	1,097 ± 93[b]	1,797 ± 368[a]
Antioxidant status (mmol/L)	289 ± 16[b]	207 ± 13[c]	264 ± 7[b]	293 ± 14[b]	332 ± 14[a]
Malondialdehyde (μmol/L)	106 ± 2[b]	277 ± 1[a]	107 ± 1[b]	107 ± 1[b]	107 ± 1[b]

Data are mean ± SEM values ($n = 10$). CU, 0.005% curcumin in feed; SS, 0.004% saikosaponin a in feed; CU + SS, 0.005% curcumin + 0.004% saikosaponin a in feed.

Values not sharing a common superscript letter within a row significantly differ ($P < .05$) as determined by one-way analysis of variance and Fisher's least significant difference test.

spectively. The daily equivalent intake levels of curcumin and saikosaponin a in humans would be 0.5 and 0.4 mg/kg of body weight, respectively, according to the rat-to-human conversion factor of 0.16.[15]

Food intake and weight gain of all rats did not significantly differ, indicating that CCl_4 *per se* did not affect growth. The liver weight significantly increased in the CCl_4 group probably because of increased accumulation of fat vacuoles shown by hematoxylin and eosin staining and increased hepatic cholesterol and triglyceride levels. Similarly, Nan *et al.*[16] demonstrated that relative liver weights significantly increased because of the accumulation of hepatic hydroxyproline content after surgery in rats with liver fibrosis induced by biliary obstruction. A previous study showed that the relative liver weight was a more sensitive indicator of hepatotoxicity than the absolute liver weight in a CCl_4-induced liver injury model.[17]

CCl_4 induces liver injury through its conversion into a trichloromethyl free radical ($^{\bullet}CCl_3$) by cytochrome P450 in the liver. The $^{\bullet}CCl_3$ free radical further causes polysome disaggregation, a distorted structure and dysfunction of endoplasmic reticula and plasma membranes,[18] and stimulation of lipid peroxidation.[19] Our study showed that plasma ALT and AST activities rapidly increased in parallel with CCl_4 injection, indicating the induction of acute hepatotoxicity by CCl_4. After week 7, plasma ALT and AST activities had significantly declined in all curcumin- and/or saikosaponin a-supplemented groups, suggesting that curcumin and saikosaponin a are beneficial for liver regeneration to reverse liver injury.

Our study showed that CCl_4 treatment led to fatty degeneration and increased liver cholesterol and triglyceride levels and plasma cholesterol level. Hepatic fat depots may have resulted from the transport of lipids from the peripheral stores and/or the interrupted transport of hepatic triglycerides by very-low-density lipoproteins because of the transformation of proteins and lipids induced by the free radicals derived from the metabolites of CCl_4.[20] Curcumin and saikosaponin a exerted an antihyperlipidemic effect. *In vitro* studies demonstrated that curcumin increased the expression of low-density lipoprotein receptor mRNA,[21,22] which resulted in a higher uptake of low-density lipoprotein-cholesterol from the plasma. Similarly, animal studies found that curcumin had a lipid-lowering effect on the modulation of lipid absorption and metabolism.[23,24] Saikosaponins also showed cholesterol-lowering activity through increasing fecal excretion of bile acids and neutral sterols.[25] A clinical study found that plasma cholesterol and triglyceride levels were significantly reduced and plasma high-density lipoprotein-cholesterol level markedly increased in 32 asymptomatic hyperlipidemic patients given daily Da Chai Hu Tang containing 12 g of Bupleuri radix dissolved in 300 mL of hot water for 8 weeks.[26] The stage of liver fibrosis was positively associated with blood ALT and AST activities and negatively related to blood triglycerides.[27] Our results revealed that supplementation with curcumin and/or saikosaponin a for 8 weeks not only decreased plasma ALT and AST activities, but also lowered plasma and hepatic cholesterol and triglyceride levels, suggesting that curcumin and saikosaponin a alleviate liver fatty degeneration and may further protect against the development of liver fibrosis.

Exposure to CCl_4 caused decreases in hepatic SOD activity and the total antioxidant status, as well as an increase in the hepatic malondialdehyde level, indicating that CCl_4 induces hepatotoxicity and impairs the antioxidant status. The mitochondrial electron-transport chain is responsible for activating CCl_4. The formation of a conjugated diene was observed in the mitochondrial lipid extract, indicating that the stimulation of lipid peroxidation occurs as a result of the formation of free radical species.[28] Curcumin and saikosaponin a, acting as antioxidants, enhanced the hepatic total antioxidant status and inhibited lipid peroxidation to prevent CCl_4-induced oxidative damage in the liver. Consistent with previous studies, curcumin had the antioxidant capacity to inhibit lipid peroxidation and increase antioxidant enzyme activities.[29,30] Similarly, saikosaponin a, as a free radical scavenger, restored the decreased activities of SOD, catalase, and glutathione peroxidase in rats with nephritis.[31]

The evidence shows that supplementation with curcumin or saikosaponin a promotes potent hepatoprotective activi-

ties through the lipid-lowering effect, inhibition of lipid peroxidation (which decreases oxidative stress), and improvement of the antioxidant status (which enhances the antioxidant defense). However, combining curcumin and saikosaponin a had no additive effects on hepatoprotection even though there was greater improvement in the total glutathione level and antioxidant status.

ACKNOWLEDGMENTS

The authors thank the Department of Pathology at Taipei Medical University for assistance with histological staining.

REFERENCES

1. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL: Unconventional medicine in the United States. Prevalence, costs and patterns of use. *N Engl J Med* 1993;328: 246–252.
2. Maellaro E, Casini AF, Del Bello B, Comporti M: Lipid peroxidation and antioxidant systems in the injury produced by glutathione depleting agents. *Biochem Pharmacol* 1990;39:1513–1521.
3. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK: Turmeric and curcumin: biological actions and medicinal applications. *Curr Sci* 2004;87:44–53.
4. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS: Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med* 1998;64:353–356.
5. Chiang LC, Ng LT, Liu LT, Shieh DE, Lin CC: Cytotoxicity and anti-hepatitis B virus activities of saikosaponins from *Bupleurum* species. *Planta Med* 2003;69:705–709.
6. Matsuda H, Murakami T, Ninomiya K, Inadzuki M, Yoshikawa M: New hepatoprotective saponins, bupleurosides III, VI, IX, and XIII, from Chinese Bupleuri Radix: structure-requirements for the cytoprotective activity in primary cultured rat hepatocytes. *Bioorg Med Chem Lett* 1997;7:2193–2198.
7. Nebot C, Moutet M, Huet P, Xu JZ, Yadan JC, Chaudiere J: Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. *Anal Biochem* 1993;214:442–451.
8. Lowry OH, Rosebrough NJ, Farr A, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951;193: 265–275.
9. Anderson ME: Enzymatic and chemical methods for the determination of glutathione. In: *Glutathione: Chemical, Biochemical and Medical Aspects, Vol. A* (Dolphin D, Poulson R, Avramovic O, eds.). John Wiley and Sons, New York, 1989, pp. 339–365.
10. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A: A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* 1993;84:407–412.
11. Esterbauer H, Cheeseman KH: Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hyderoxynonenal. *Methods Enzymol* 1990;186:407–421.
12. Wahlström B, Blennow G: A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol (Copenh)* 1978:43:86–92.
13. Ravindranath V, Chandrasekhara N: Absorption and tissue distribution of curcumin in rats. *Toxicology* 1980;16:259–265.
14. Fujiwara K, Ogihara Y: Pharmacological effects of oral saikosaponin a may differ depending on conditions of the gastrointestinal tract. *Life Sci* 1986;39:297–301.
15. Miao MS: Animal feed and nutrition. In: *Laboratory Animals and Animal Experimental Techniques*. China Press of Traditional Chinese Medicine, Beijing, 1997, pp. 127–132.
16. Nan JX, Park EJ, Kang HC, Park PH, Kim JY, Sohn DH: Antifibrotic effects of a hot-water extract from *Salvia miltiorrhiza* roots on liver fibrosis induced by biliary obstruction in rats. *J Pharm Pharmacol* 2000;53:197–204.
17. Uemitsu N, Nishimura C, Nakayoshi H: Evaluation of liver weight changes following repeated administration of carbon tetrachloride in rats and body-liver weight relationship. *Toxicology* 1986;40: 181–190.
18. Smuckler EA: Structural and functional changes in acute liver injury. *Environ Health Perspect* 1976;15:13–25.
19. Tomasi A, Albano E, Banni S, Botti B, Corongiu F, Dessi MA, Iannone A, Vannini V, Dianzani MU: Free-radical metabolism of carbon tetrachloride in rat liver mitochondria. A study of the mechanism of activation. *Biochem J* 1987;246:313–317.
20. Boll M, Weber LW, Becker E, Stampfl A: Pathogenesis of carbon tetrachloride-induced hepatocyte injury bioactivation of CCl_4 by cytochrome P450 and effects on lipid homeostasis. *Z Naturforsch [C]* 2001;56:111–121.
21. Fan C, Wo X, Qian Y, Yin J, Gao L: Effect of curcumin on the expression of LDL receptor in mouse macrophages. *J Ethnopharmacol* 2006;105:251–254.
22. Peschel D, Koerting R, Nass N: Curcumin induces changes in expression of genes involved in cholesterol homeostasis. *J Nutr Biochem* 2007;18:113–119.
23. Arafa HM: Curcumin attenuates diet-induced hypercholesterolemia in rats. *Med Sci Monit* 2005;11:BR228–BR234.
24. Kalpana C, Rajasekharan KN, Menon VP: Modulatory effects of curcumin and curcumin analog on circulatory lipid profiles during nicotine-induced toxicity in Wistar rats. *J Med Food* 2005;8: 246–250.
25. Yamamoto M, Kumagai A, Yamamura Y: Structure and action of saikosaponins isolated from *Bupleurum falcatum* L. II. Metabolic actions of saikosaponins, especially a plasma cholesterol-lowering action. *Arzneimittelforschung* 1975;25:1240–1243.
26. Qian X, Zhang M: Clinical research on asymptomatic hyperlipemia treated by Da Chai Hu Tang. *Forum Tradit Chin Med* 2001;16:11–12.
27. Zheng RD, Lu LG, Meng JR, Huang JD, Rao RC, Xu CR, Zeng MD: A clinical and pathological study of nonalcoholic fatty liver disease. *Zhonghua Gan Zang Bing Za Zhi* 2006;14:449–452.
28. Lee JY, Lee SH, Kim HJ, Ha JM, Lee SH, Lee JH, Ha BJ: The preventive inhibition of chondroitin sulfate against the CCl_4-induced oxidative stress of subcellular level. *Arch Pharm Res* 2004;27:340–345.
29. Durqaprasad S, Pai CG, Vasanthkumar, Alvres JF, Namitha S: A pilot study of the antioxidant effect of curcumin in tropical pancreatitis. *Indian J Med Res* 2005;122:315–318.
30. Kalpana C, Menon VP: Modulatory effect of curcumin on lipid peroxidation and antioxidant status during nicotine-induced toxicity. *Pol J Pharmacol* 2004;56:581–586.
31. Hattori T, Ito M, Suzuki Y: Studies on antinephritic effects of plant components in rats. (1). Effects of saikosaponins original-type anti-GBM nephritis in rats and its mechanisms. *Nippon Yakurigaku Zasshi* 1991;97:13–21.