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Hepatoprotective and Antioxidative Properties of Chinese Herbal Medicine Xiao-Chai-Hu-Tang Formulated with *Bupleurum Kaoi* Liu on Carbon Tetrachloride-Induced Acute Hepatotoxicity in Rats

CHING-YI WU[1], YIH-MING WENG[1], CHU-TING LIU[2], PEI-TING CHUANG[2], SIN-YIE LIU[3] AND CHIN-YIN TSENG[3,*]

[1.] *Department of Food Science, National Chiayi University, No. 300, Syuefu Rd., Chiayi City 60004, Taiwan, R.O.C.*
[2.] *Institute of Food Science and Technology, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., Taipei City 10617, Taiwan, R.O.C.*
[3.] *Department of Nutrition and Health Science, Chung Chou Institute of Technology, No. 6, Lane 2, Sec. 3, Shanjiao Rd., Yuanlin Township, Changhua County 51003, Taiwan, R.O.C.*

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ABSTRACT

The Chinese herbal medicine, Xiao-Chai-Hu-Tang (XCHT), with *Bupleurum* radix as the main ingredient, is used for treating hepatitis. Although there are many varieties of *Bupleurum* in the herbal market, *Bupleurum kaoi* is the only indigenous species belonging to the *Bupleurum* genus plant in Taiwan which is commercially cultivated for making health-promoting products. The present study examined the hepatoprotective and antioxidative properties of XCHT formulated with commercially cultivated *B. kaoi*. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GRd) and glutathione-S-transferase (GST) as well as lipid peroxidation in rats treated with CCl_4 were analyzed to evaluate the hepatoprotective property of XCHT. The results showed that the enzyme levels in the serum of rats treated with XCHT extract prior to receiving CCl_4 were significantly lower ($p < 0.05$) than those in the rats that received CCl_4 only. Malondialdehyde formation was also reduced when XCHT extract was given. In comparison with the extract of XCHT, the extract of *B. kaoi* showed similar hepatoprotective abilities. Since the levels of SOD, catalase, GPx, GRd and GST were compensation increased in CCl_4 treatment, it was suggested that XCHT or *B. kaoi* markedly suppressed lipid peroxidation and reversed the activities of the antioxidant enzymes to the normal levels.

Key words: *Bupleurum kaoi* Liu, carbon tetrachloride, Xiao-Chai-Hu-Tang, antioxidative enzymes, hepatoprotective functionality

INTRODUCTION

Xiao-Chai-Hu-Tang (XCHT) is a traditional Chinese herbal medicine which is used mainly for treating chronic hepatitis, and also for distress and fullness in the chest and ribs in China and Japan[(1)]. Although XCHT is composed of seven medicinal herbs, it is generally recognized that *Bupleurum* radix contributes the major protective functionality because it is the principal component of XCHT[(2)]. While many varieties of *Bupleurum* species (belonging to the family *Umbelliferae*) are available in the herbal market, *Bupleurum kaoi* Liu (Chao et Chuang) is the only indigenous *Bupleurum* plant in Taiwan. It is reported that *B. kaoi* Liu possesses free radical scavenging activities and inhibits the formation of superoxide radicals[(3-5)]. To broaden the utilization of traditional herbal prescriptions, XCHT has been targeted for commercial production as health food and large-scale cultivation of *B. kaoi* is performed to meet the raw material requirement. Wu *et al.* demonstrated that XCHT that contained *B. kaoi* exhibited the same levels of DPPH radical scavenging effect, reducing power, and total antioxidant capacity as XCHT that contained *B. Chinense*[(6)]. Although the water extracts of *B. kaoi* roots alone had been shown to protect the liver against carbon tetrachloride (CCl_4)-, D-galactosamine- or dimethylnitrosamine-induced hepatotoxicity[(7-9)], the hepatoprotective functionality of XCHT formulated with commercially cultivated *B. kaoi* needs to be verified.

As the mechanism of CCl_4-induced hepatic necrosis

* Author for correspondence. Tel: +886-4-9311498 ext. 1108; Fax: +886-4-8395316; E-mail: cytseng@dragon.ccut.edu.tw

has been well-documented, CCl_4 is widely used to investigate hepatotoxic effects in various animal studies[10,11]. Superoxides and hydroxyl radicals derived from CCl_4 are the principal toxic substances interacting with the liver tissue. Detoxifying reactions catalyzed by superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GRd), and glutathione-S-transferase (GST) transform toxic free radicals to less toxic or non-toxic species and serve as defense mechanisms against CCl_4 in biological systems. SOD and catalase inactivate free radical precursors and prevent the formation of active oxygen species[12]. While GPx decomposes hydrogen peroxide and other peroxides, GRd serves as a reductase of GSSG (oxidized glutathione). In addition to the protective role of the enzymes described above, compounds such as vitamin E and glutathione that are known for their antioxidant properties, and other compounds that act as inhibitors of cytochrome P-450 have also been reported to reduce the extent of peroxidation and decrease the toxicity induced by CCl_4 in animal models[13]. In this study, CCl_4 was used as a liver damage-inducing agent and the activities of protective enzymes were analyzed to demonstrate the hepatoprotective properties of XCHT formulated with commercially cultivated *B. kaoi*.

MATERIALS AND METHODS

I. *Chemicals*

Baicalin, baicalein, glycyrrhizic acid, saikosaponin a, saikosaponin c and saikosaponin d were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Folin-Ciocalteu's reagent, gallic acid, potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), quercetin, sodium carbonate (Na_2CO_3), sodium dodecylsulphate (SDS), 1,1,3,3-tetraethoxy propane (TEP) and Triton X-100 were purchased from Sigma Chemical Co. (St Louis, MO). Acetonitrile, ethanol, hydrochloric acid (HCl) and methanol were purchased from Merck Co. (Darmstadt, Germany). Aluminum nitrate ($Al(NO_3)_3$), formaldehyde solution (CH_2O) and potassium acetate (CH_3COOK) were purchased from J. T. Baker Co. (Phillipsburg, NJ). Hydrogen peroxide (H_2O_2) was purchased from Sigma-Aldrich Inc. (St Louis, MO). Tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl) and thiobarbituric acid (TBA) were purchased from Acros Chemical (Morris Plains, NJ). N-butanol was purchased from Fisher Co. (Fair Lawn, NJ). Carbon tetrachloride (CCl_4) was obtained from Hayashi Pure Chemical Ind. (Osaka, Japan). Silymarin was obtained from Tecmed Medico GmbH (Hamburg, Germany).

II. *Preparation of B. kaoi and Xiao-Chai-Hu-Tang (XCHT) Extracts*

Dried roots of 8 month-old *B. kaoi* were obtained from the plantation of the Green Health Biotechnology Corporation (Yunlin, Taiwan). The roots were ground with a blender, mixed with 10 volumes of distilled water (w/v), and boiled under reflux for 2 h. The aqueous extract was filtered through a Whatman filter paper (No. 1) and the filtrate was freeze-dried (The 12ES, Freeze Dryer, Virtes Company, Inc., Gardiner, NY) into dry powder for further analysis. The yield of freeze-dried *Bupleurum kaoi* powder was 17.1%.

The tuber of *Pinellia ternate* (*Pinelliae* tuber), fruits of *Zizyphus jujube* (*Zizyphi* fructus), rhizome of *Zingiber officinale* (*Zingiberis* rhizome) and roots of *Panax ginseng* (*Ginseng* radix), *Scutellaria baicalensis* (*Scutellariae* radix) and *Glycyrrhiza uralensis* (*Glycyrrhizae* radix) were purchased from a local herb pharmacy and authenticated. The formulation of XCHT was prepared according to Sakaguchi *et al.*[2] and the ratio (by weight) of *Bupleurum kaoi*, *Pinelliae* tuber, *Ginseng* radix, *Zizyphi* fructus, *Scutellariae* radix, *Glycyrrhizae* radix and *Zingiberis* rhizome was 7: 5: 3: 3: 3: 2: 1. The herbal ingredients were pooled together and ground with a blender. The pulverized herbs were mixed with 10 volumes of distilled water (w/v) and then boiled under reflux for 2 h. The freeze-dried powder of XCHT was prepared in the same way as *B. kaoi* powder described above. The yield of freeze-dried XCHT powder was 22.0%.

III. *Determination of Flavonoids and Total Phenols*

Freeze-dried *B. kaoi* or XCHT powder was dissolved in distilled water and the flavonoid concentration was determined using the method described by Jia *et al.*[14]. The sample solution (0.5 mL), ethanol (1.5 mL), $Al(NO_3)_3$ (0.1 mL, 10%), CH_3COOK (0.1 mL, 1 M) and H_2O (2.8 mL) were thoroughly mixed and kept at room temperature for 40 min. The absorbance of the reaction mixture at 415 nm was measured with a spectrophotometer (U-2000 Spectrophotometer, Hitachi Ltd., Tokyo, Japan). The flavonoid content was calculated according to a standard curve established with quercetin. The content of total phenols was determined using Folin-Ciocalteu's reagent as described by Taga *et al.*[15]. The sample solution (0.1 mL), Folin-Ciocalteu's reagent (0.5 mL), sodium carbonate (0.4 mL, 7.5%) and distilled H_2O (5 mL) were thoroughly mixed and kept at room temperature for 30 min before its absorbance at 760 nm was measured. The content of total phenols was calculated according to a standard curve established with gallic acid.

IV. *Saikosaponins, Baicalin, Baicalein and Glycyrrhizic Acid Analyses*

The freeze-dried *B. kaoi* or XCHT powder was dissolved in distilled water and the concentrations of saikosaponins (saikosaponin a, c and d) were analyzed with a high performance liquid chromatograph (HPLC L-7000, Hitachi Ltd., Tokyo, Japan) equipped with an

Inertsil ODS-3 C_{18} column (250 × 4.6 mm, GL Sciences, Tokyo, Japan). A programmed gradient elution using acetonitrile-water from 40 : 60 (v/v) to 50 : 50 (v/v) in 10 min at a flow rate of 1.0 mL/min and detection wavelength of 203 nm was used[16]. In the analysis of baicalin, baicalein and glycyrrhizic acid, the mobile phase consisted of (A) 25 mM NaH_2PO_4 (pH 2.5) and (B) acetonitrile. The eluent composition was initially set at 30% of B, increased to 100% B in 25 min, and then held for 10 min before returning to the initial composition. The detection wavelength was 277 nm for baicalin, 280 nm for baicalein and 252 nm for glycyrrhizic acid. Standard curves established with saikosaponins a, c and d, baicalin, baicalein, and glycyrrhizic acid were used for the calculation.

V. *Animal Treatment*

Eight-week old male Sprague-Dawley rats, weighing 280-310 g, were purchased from the National Laboratory Animal Center (Taipei City, Taiwan). They were processed according to the suggested international ethical guidelines for the care of laboratory animals. The experimental protocol was approved by the Laboratory Animal Management Committee of National Chiayi University (Chiayi, Taiwan). The animals were housed individually and maintained under standard laboratory conditions of a 12-h light/dark cycle and fixed temperature (25 ± 2°C). The rats were provided with tap water and chow diets (Lab Diet; PMI Nutrition International Inc., Brentwood, MO) *ad libitum* during the entire duration of the experiment. The rats were randomly assigned into seven groups, with six animals in each group. For four of the groups, freeze-dried powder of *B. kaoi* or XCHT dissolved in distilled water was given at the level of 100 or 500 mg/kg body weight (bw) daily by gavage to the animals on three consecutive days. Immediately after the third gavage, CCl_4 (CCl_4 : olive oil = 1 : 1, 3 mL/kg bw) was injected (*Intraperitoneal injection,* i.p.) into the animals to induce acute liver damage. The rats were anesthetized with ethyl ether and scarified 24 h after CCl_4 injection. The experiment procedures in the control groups (normal control, CCl_4-treated, and silymarin group) were the same as in the experiment groups, except for the following: Rats in the normal control group were given distilled water to replace the herbal extracts and injected with olive oil without CCl_4 (3 mL/kg bw, i.p.). rats in CCl_4-treated group were given distilled water to replace the herbal extracts and injected with CCl_4, while the rats in the silymarin control group did not receive herbal extract or distilled water but were given silymarin (25 mg/kg bw) by gavage before CCl_4 injection. Silymarin was used a medicine that protects the liver against CCl_4[11,17].

VI. *Analysis of Serum Transaminases and Cholesterol*

Blood was collected through the hepatic vein of animals 24 h after treatment with CCl_4 or olive oil. After centrifuging the blood at 3000 ×*g* and 4°C for 10 min, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined as the biochemical markers for hepatic cellular damage using commercial kits (Raichem, Division of Hemagen Diagnostics, Inc., San Diego, CA). They were analyzed by spectroscopy (Chiron/Diagnostics Express Plus, East Walpole, MA) and the results were expressed in U/L. The levels of serum cholesterol were assayed with a commercial kit (Raichem, Division of Hemagen Diagnostics, Inc., San Diego, CA) and the results were expressed in mg/dL.

VII. *Analysis of Rat Liver Lipid Peroxidation*

Lipid peroxidation of the liver homogenate was measured according to the method of Sano *et al.*[18] and Fraga *et al.*[19]. Immediately after the animals were sacrificed, their livers were removed, weighed and rinsed with ice-cold 0.1 M Tris-HCl buffer. The liver samples were homogenized in ice-cold buffer (8 mM Na_2HPO_4, 12 mM NaH_2PO_4, 1.5% KCl, pH 7.4) in a homogenizer (Glas-Col, Terre Haute, IN) to make a 10% (w/v) liver homogenate. The protein content was determined with the method by Lowry *et al.*[20] using a commercial kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). After centrifuging at 105,000 ×*g*, 4°C for 20 min (CF15D2, Hitachi Ltd., Tokyo, Japan), lipid peroxidation was determined with the thiobarbituric acid fluorometric method with emission at 553 nm and excitation at 515 nm using TEP as the standard[21,22]. The results were expressed as MDA nmol/mg.

VIII. *Activities of Rat Liver Antioxidant Enzymes*

Liver homogenate was used for the assay of antioxidant enzyme activities. The activity of SOD in the liver was evaluated using commercial kits (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK). The specific enzyme activity of SOD was expressed as U/mg protein. Catalase activity in the liver was measured using the method described by Aebi[23]. The reaction mixture (2 mL), which consisted of 10 μL of liver homogenate, 90 μL of 1% Triton X-100 and 1.9 mL of 20 mM of phosphate buffer, was added to a photometric quartz cuvette containing 1 mL of H_2O_2 (0.03 M). The initial absorbance (A1) and final absorbance (A2) of a 1-min time interval at 240 nm were measured and used to calculate the reaction rate constant (K) using the equation $K = (2.3/\Delta t) \times \log(A1/A2)$. The specific activity of the enzyme was expressed as K/mg protein.

IX. *Determination of Rat Liver Glutathione Metabolic Enzyme Activities*

The glutathione peroxidase (GPx) and glutathione reductase (GRd) activities of rat liver were spectroscopically analyzed using commercial kits (Randox Laboratories Ltd., Crumlin, Co. Antrim, UK) and a spectrophotometer

Table 1. The contents of total phenols, flavonoids, saikosaponins, baicalin, baicalein and glycyrrhizic acid in the extracts of *B. kaoi* and Xiao-Chai-Hu-Tang (XCHT)

Sample	Compounds[1] (mg/g dry weight)					
	Total phenols[2]	Flavonoids[3]	Saikosaponins	Baicalin	Baicalein	Glycyrrhizic acid
B. kaoi	13.5 ± 0.7	4.0 ± 0.1	1.0 ± 0.0	ND[4]	ND	ND
XCHT	12.0 ± 0.4	3.6 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.4 ± 0.0	0.3 ± 0.0

[1] Each value represents means ± SD (n = 3).
[2] The content of total phenols was calculated against gallic acid standard.
[3] The content of flavonoids was calculated against quercetin standard.
[4] ND = Not detected.

as specified. The GPx and GRd activities were expressed as U/mg protein. Hepatic glutathione-S-transferase (GST) activity was analyzed according to the method described by Habig *et al*[24]. Liver homogenate (5 μL) was added with phosphate buffer solution (95 μL, pH 7.0, 20 mM), GSH (1 mM in 100 mM phosphate buffer), and 1-chloro-2,4-dinitrobenzene (CDNB) (20 μL, 50 mM). The CDNB-GSH conjugate formed in 5 min was measured at 340 nm with a spectrophotometer (U-2000, Hitachi Ltd., Tokyo, Japan). The GST activity was expressed as nM CDNB-GSH conjugate/min/mg protein. For the determination of blank samples, liver homogenate was replaced with the same buffer of the homogenate.

X. *Histopathological Examination*

Liver tissues of rats were collected from the central lobe of the liver for comparison purpose. The liver tissue was fixed in 10% neutral formalin, dehydrated with a graded series of ethanol solutions and embedded in paraffin. Thin sections of liver tissues were stained with haematoxylin and eosin (H&E) for microscopic examination. Histopathological examination was performed by Professor Ming-Huang Chang (Department of Veterinary Medicine, National Chiayi University, Chiayi, Taiwan).

XI. *Statistical Analyses*

Data were presented as means ± SD. One-way analysis of variance was used to analyze the variance among the samples. Duncan's multiple range tests, at $p < 0.05$, was used to determine significant differences among sample means.

RESULTS

I. *Bioactive Compounds in B. Kaoi and XCHT*

The contents of total phenols, flavonoids, saikosaponons, baicalin, baicalein and glycyrrhizic acid in the extracts of *B. kaoi* and XCHT are listed in Table 1. No significant differences ($p > 0.05$) in the contents of total phenols, flavonoids and saikosaponins were found between *B. kaoi* and XCHT. However, baicalin, baicalein, and glycyrrhizic acid were detected only in XCHT because baicalin and baicalein are found in *Scutellariae* radix only and glycyrrhizic acid is a specific compound found in *Glycyrrhizae* radix.

Table 2. The effects of *B. kaoi* and XCHT extracts on the serum transaminase activities of Sprague-Dawley rats treated with CCl_4

Animal treatment group[1]	Serum Transaminases (U/L)[2]	
	AST	ALT
Normal control	293 ± 70^{d}	82 ± 21^{e}
CCl_4	2058 ± 443^{a}	1249 ± 218^{a}
Silymarin (25) + CCl_4	651 ± 434^{cd}	205 ± 185de
B. kaoi (100) + CCl_4	1948 ± 177^{a}	1016 ± 160^{b}
B. kaoi (500) + CCl_4	1038 ± 492^{bc}	418 ± 219^{c}
XCHT (100) + CCl_4	483 ± 373^{d}	360 ± 160^{cd}
XCHT (500) + CCl_4	1213 ± 403^{b}	428 ± 162^{c}

[1] Normal control: The rats were given distilled water and injected with olive oil; CCl_4: The rats were given distilled water and injected with CCl_4; Silymarin (25) + CCl_4: The rats were given silymarin (25 mg/kg bw) before CCl_4 injection; *B. kaoi* (100) + CCl_4: The rats were given *B. kaoi* extract (100 mg/kg bw) before CCl_4 injection; *B. kaoi* (500) + CCl_4: The rats were given *B. kaoi* extract (500 mg/kg bw) before CCl_4 injection; XCHT (100) + CCl_4: The rats were given XCHT extract (100 mg/kg bw) before CCl_4 injection; XCHT (500) + CCl_4: The rats were given XCHT extract (500 mg/kg bw) before CCl_4 injection.
[2] Each value represents means ± SD (n = 6). Means with different letters within the same column are significantly different ($p < 0.05$).

II. *Effects of B. Kaoi and XCHT on AST and ALT Activities*

The levels of serum transaminase activities in the rats subjected to different treatment are shown in Table 2. AST and ALT of the CCl_4-treated group were significantly higher than the normal control group. *B. kaoi* extract at a dose of 500 mg/kg bw and XCHT extract at a dose of 100 or 500 mg/kg bw significantly lowered the

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AST and ALT activities when compared with the rats in the CCl_4-treated group.

III. *Effects of B. Kaoi and XCHT on Serum Cholesterol and Lipid Peroxidation*

Serum cholesterol level can be used as a diagnostic indicator for artherosclosis, high blood pressure, malabsorption, fatty liver and anemia[25]. Although the administration of CCl_4 slightly increased serum cholesterol level (Table 3), it is interesting to note that the serum cholesterol levels of animals treated with extracts of *B. kaoi* or XCHT were lower than those found in the animals of the normal control group. The formation of MDA was used to represent the peroxidation of liver tissue (Table 3). In comparison with the normal control group, animals treated with CCl_4 showed a 12-fold increase in MDA level. Furthermore, the extract of *B. kaoi* (500 mg/kg bw) and the extract of XCHT (100 and 500 mg/kg bw) significantly reduced lipid peroxidation when compared with the CCl_4-treated animals.

Table 3. The effects of *B. kaoi* and XCHT extracts on the serum cholesterol and hepatic malondialdehyde (MDA) concentrations of Sprague-Dawley rats treated with CCl_4

Animal treatment group[1]	Cholesterol	MDA
	mg/dL[2]	nmol/mg protein[2]
Normal control	55 ± 7^{a}	1 ± 0^{d}
CCl_4	57 ± 10^{a}	13 ± 4^{b}
Silymarin (25) + CCl_4	56 ± 15^{a}	16 ± 4^{a}
B. kaoi (100) + CCl_4	47 ± 9^{ab}	18 ± 3^{a}
B. kaoi (500) + CCl_4	42 ± 14^{b}	4 ± 1^{cd}
XCHT (100) + CCl_4	50 ± 8^{ab}	4 ± 0^{c}
XCHT (500) + CCl_4	42 ± 8^{b}	2 ± 0^{cd}

[1] Animal treatment is the same as in Table 2.
[2] Each value represents means ± SD (n = 6). Means with different letters within the same column are significantly different ($p < 0.05$).

Table 4. The effects of *B. kaoi* and XCHT extracts on the liver superoxide dismutase (SOD) and catalase activities of Sprague-Dawley rats treated with CCl_4

Animal treatment group[1]	SOD	Catalase
	mU/mg protein[2]	mK/mg protein[2]
Normal control	151 ± 18^{c}	169 ± 55^{b}
CCl_4	254 ± 49^{a}	246 ± 35^{a}
Silymarin (25) + CCl_4	198 ± 24^{b}	140 ± 30^{bc}
B. kaoi (100) + CCl_4	190 ± 13^{b}	131 ± 20^{c}
B. kaoi (500) + CCl_4	171 ± 30^{bc}	106 ± 12^{c}
XCHT (100) + CCl_4	166 ± 19^{bc}	124 ± 13^{c}
XCHT (500) + CCl_4	141 ± 8^{c}	102 ± 10^{c}

[1] Animal treatment is the same as in Table 2.
[2] Each value represents means ± SD (n = 6). Means with different letters within the same column are significantly different ($p < 0.05$).

IV. *Effects of B. Kaoi and XCHT on SOD and Catalase Activities*

The SOD and catalase activities of the rat liver homogenates are listed in Table 4. Acute liver damage resulting from CCl_4 administration was indicated by an increase in the activities of these two enzymes. Although silymarin reduced the activities of both enzymes, the extracts of *B. kaoi* and XCHT exerted even stronger protective effects in the case of SOD and catalase.

V. *Effects B. Kaoi and XCHT on Glutathione Metabolic Enzymes*

The activities of hepatic enzymes involving glutathione metabolism are listed in Table 5. Compensative increases of hepatic GPx, GRd and GST were observed after CCl_4 was injected into the rats. The extracts of *B. kaoi* and XCHT exerted similar protective effects when compared to silymarin. No significant differences in the activities of GPx, GRd and GST were found between animals given the extracts of *B. kaoi* and XCHT extracts and animals in the normal control group.

VI. *Pathologic Changes in Rat Liver*

Microscopic observation of histological samples

Table 5. The effects of *B. kaoi* and XCHT extracts on the liver glutathione metabolic enzymes activities of Sprague-Dawley rats treated with CCl_4

Animal treatment group[1]	GPx	GRd	GST
	mU/mg protein[2]	mU/mg protein[2]	nM CDNB-GSH conjugate/min/mg protein[2]
Normal control	149 ± 13^{bc}	595 ± 50^{b}	10 ± 1^{c}
CCl_4	263 ± 20^{a}	1051 ± 79^{a}	19 ± 6^{a}
Silymarin (25) + CCl_4	150 ± 25^{bc}	601 ± 100^{b}	9 ± 8^{c}
B. kaoi (100) + CCl_4	162 ± 16^{b}	669 ± 99^{b}	17 ± 5^{ab}
B. kaoi (500) + CCl_4	162 ± 34^{b}	648 ± 137^{b}	11 ± 5^{bc}
XCHT (100) + CCl_4	164 ± 15^{b}	655 ± 61^{b}	9 ± 2^{c}
XCHT (500) + CCl_4	131 ± 15^{c}	556 ± 61^{b}	12 ± 6^{bc}

[1] Animal treatment is the same as in Table 2.
[2] Each value represents means ± SD (n = 6). Means with different letters within the same column are significantly different ($p < 0.05$).

can provide direct evidence for pathogenic changes in animals. Typical histological samples of the liver tissue from the different treatment animal groups are shown in Figure 1. Hepatocyte inflammation, hepatocyte necrosis, fatty change, balloon degeneration and bile duct proliferation were scored and reported in Table 6. While hepatocyte inflammation was observed only in rats in the CCl_4 group, hepatocyte necrosis was observed in all the rats except the rats in the normal control group. The *B. kaoi* and XCHT extracts had little effect on fatty change of the liver. However, silymarin and extracts of *B. kaoi* and XCHT reduced the liver balloon degeneration induced by CCl_4. In the overall histological observation, *B. kaoi* and XCHT extracts demonstrated similar hepatoprotective effects as silymarin.

DISCUSSION

Herbs have been used in folk medicine around the world for thousands of years with little or no knowledge of their active components or modes of action. The isolation and purification of phytochemicals from some herbs and subsequent pharmacological studies have revealed a few of the long-sought mysteries of herbal medicine and their healing capabilities. In traditional Chinese philosophy, food and medicine are derived from the same origin and both serve to enhance human health. Although herbal medicine is traditionally used to treat diseases, herbal ingredients are currently being formulated into health food and drinks and play an important role in the prevention of diseases. The results of this study demonstrated that the administration of *B. kaoi* and XCHT formulated with commercially cultivated *B. kaoi* can reduce hepatic damage caused by CCl_4 in rats.

AST and ALT are two enzymes found in normal liver cells. When severe liver damage occurs, these enzymes are released into the blood stream. Increases of serum AST and ALT levels resulting from massive liver cell damage can be observed in acute hepatitis induced by CCl_4 in laboratory animals[26]. Central lobular necrosis, balloon degeneration and cellular infiltration were detected in intoxicated rat tissue samples. Ingestion of *B. kaoi* extract (500 mg/kg bw) and XCHT extract (100 and 500 mg/kg bw) prior to the administration of CCl_4 significantly decreased the AST and ALT levels in the serum. Reduced liver damage and lesser enzyme penetration through the cell membrane might be used to explain the

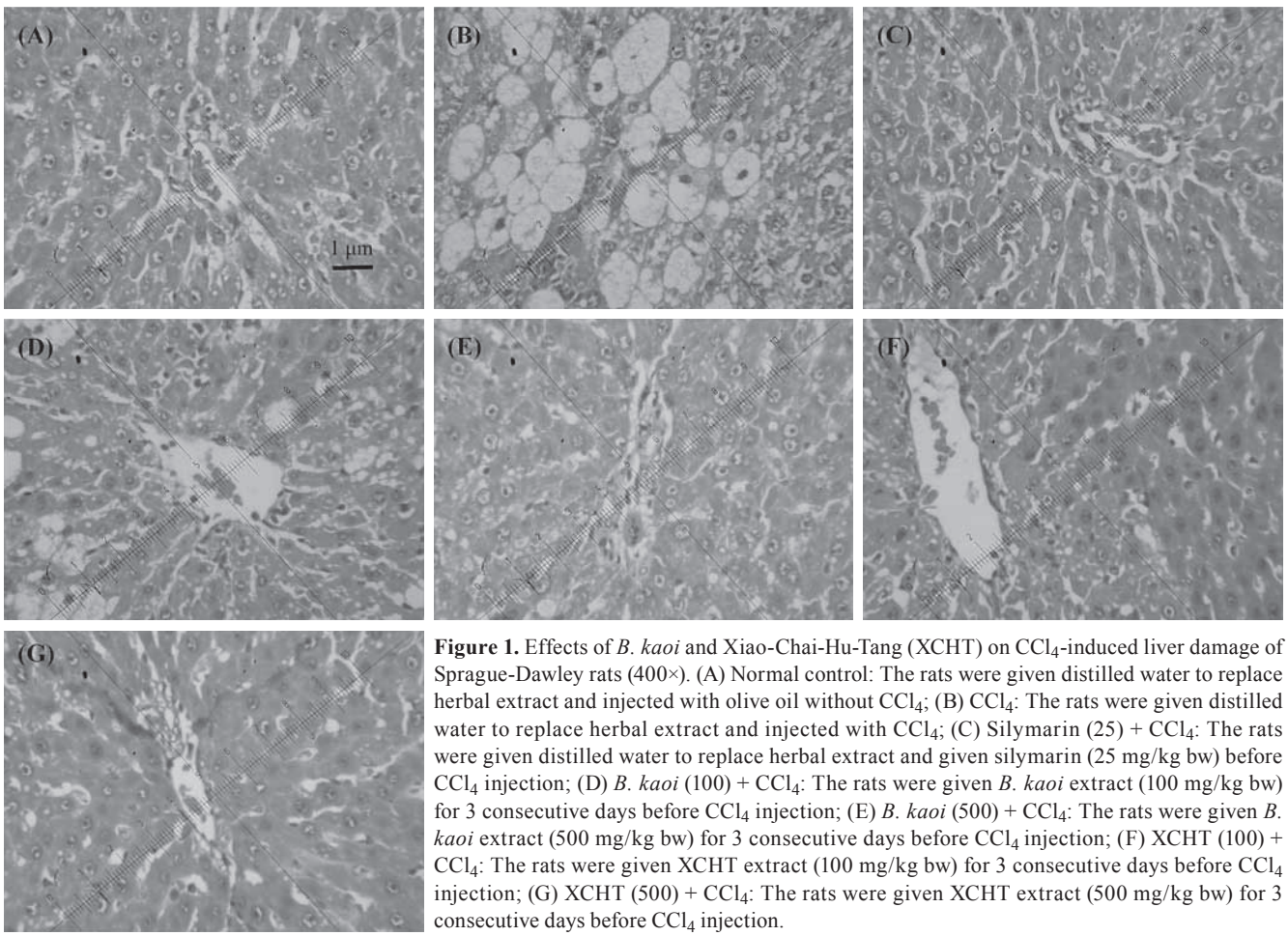

Figure 1. Effects of *B. kaoi* and Xiao-Chai-Hu-Tang (XCHT) on CCl_4-induced liver damage of Sprague-Dawley rats (400×). (A) Normal control: The rats were given distilled water to replace herbal extract and injected with olive oil without CCl_4; (B) CCl_4: The rats were given distilled water to replace herbal extract and injected with CCl_4; (C) Silymarin (25) + CCl_4: The rats were given distilled water to replace herbal extract and given silymarin (25 mg/kg bw) before CCl_4 injection; (D) *B. kaoi* (100) + CCl_4: The rats were given *B. kaoi* extract (100 mg/kg bw) for 3 consecutive days before CCl_4 injection; (E) *B. kaoi* (500) + CCl_4: The rats were given *B. kaoi* extract (500 mg/kg bw) for 3 consecutive days before CCl_4 injection; (F) XCHT (100) + CCl_4: The rats were given XCHT extract (100 mg/kg bw) for 3 consecutive days before CCl_4 injection; (G) XCHT (500) + CCl_4: The rats were given XCHT extract (500 mg/kg bw) for 3 consecutive days before CCl_4 injection.

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Table 6. Pathologic changes in the liver of Sprague-Dawley rat treated with *B. kaoi* or XCHT extracts and exposed to CCl_4-induced acute hepatic damage

Animal treatment group[a]	Injury Score				
	Inflammation[b]	Hepatocyte[c] necrosis	Fatty change[d]	Balloon[e] degeneration	Bile duct[f] proliferation
Normal control	0	0	0	0	0
CCl_4	1	1	2	2	0
Silymarin (25) + CCl_4	0	1	2	1	0
B. kaoi (100) + CCl_4	0	1	2	1	0
B. kaoi (500) + CCl_4	0	1	2	1	0
XCHT (100) + CCl_4	0	1	2	1	0
XCHT (500) + CCl_4	0	1	2	1	0

[a] Animal treatment is the same as in Table 2. The results are derived from the overall observation of the six animals in the group.
[b] Score 0 = no significant lesion; score 1 = mild inflammation; score 2 = limited in portal area; score 3 = piecemeal necrosis aggregation in parenchyma; score 4 = marked inflammation.
[c] Score 0 = no significant lesion; score 1 = spotty necrosis; score 2 = a lot of spotty necrosis; score 3 = confluent necrosis; score 4 = massive necrosis.
[d] Score 0 = no significant lesion; score 1 = micro fatty change; score 2 = sporadic or diffuse micro fatty change; score 3 = massive fatty change; score 4 = marked diffuse.
[e] Score 0 = no significant lesion; score 1 = slight; score 2 = mild; 3 = moderate; 4 = severe.
[f] Score 0 = no significant lesion; score 1 = 2-3 bile ducts in a big portal; score 2 = 2-3 bile ducts in each portal area; score 3 = over 3-5 bile ducts in each portal area; score 4 = adenoma.

hepatoprotective activity.

CCl_4 is a typical hepato-toxic chemical used in animal studies[11]. CCl_4 is converted to the trichloromethyl free radical ($CCl_3^{\bullet}$) and then to the trichloromethyl-peroxyl radical ($CCl_3O_2^{\bullet}$) in sequence by the cytochrome P-450 system. The resulting $CCl_3O_2^{\bullet}$ catalyzes lipid peroxidation, interferes with calcium homeostasis and causes cell death[27,28]. Reduction of the formation of $CCl_3^{\bullet}$ and $CCl_3O_2^{\bullet}$ free radicals can inhibit lipid peroxidation and subsequent cell damage. As our study showed that both *B. kaoi* and XCHT extracts lowered MDA formation as shown in Table 3, it can be concluded that lipid peroxidation induced by free radicals was reduced.

Membrane stabilization and inhibition of lipid peroxidation provided by the compounds in XCHT might play an important hepatoprotective role. Saikosaponins are triterpenoid compounds found in *B. kaoi* and have been reported to possess membrane stabilization capability[29]. Administration of saikosaponin-d to rats could effectively reduce hepatic microsomal lipid peroxidation induced by CCl_4[30]. The protective function was also attributed to the reduction of hepatic drug-metabolizing enzymes including NADPH-cytochrome C reductase and cytochrome P-450, which are responsible for the formation of highly toxic free radicals from CCl_4. Baicalin, a compound found in *Scutellariae* radix, has been reported to inhibit the formation of MDA in rat liver[31]. Baicalein, another bioactive compound found in *Scutellariae* radix, also showed antioxidant properties by reducing $FeCl_3$-induced lipid peroxidation[32]. Furthermore, glycyrrhizic acid from *Glycyrrhizae* radix exerted both superoxide scavenging activity and inhibited the $FeCl_2$/ascorbate-induced lipid peroxidation of rat liver homogenate[33].

Reactive oxygen species including superoxide free radical ($^{\bullet}O_2$), hydroxyl free radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2) can react with cellular macromolecules and result in membrane damage and tissue injury[34]. SOD, catalase and GPx are the principal antioxidant enzymes *in vivo*; and the activities of these enzymes represent the *in vivo* protection capability against oxidative stress[35]. SOD protects the biological system by converting $^{\bullet}O_2^-$ to hydroperoxide, thus reducing both direct and indirect cellular damage by free radicals[36]. The hydroperoxide is subsequently transformed to H_2O and O_2 by catalase and the toxicity from free radicals will be further reduced. While the activities of SOD and catalase in rat liver were stimulated by CCl_4, it is interesting to note that the activities of these two enzymes did not increase when the *B. kaoi* and XCHT extracts were administrated prior to CCl_4.

Although oxidative stress from ROS can stimulate the activities of antioxidant enzymes, the presence of endogenous or exogenous antioxidant compounds that are active against ROS would prevent the increase of such enzymes[37]. The increase in CAT and GPx may compensate the function of hepatocytes on acute peroxidation of CCl_4[4]. The results in Table 4 suggest that the

extracts of *B. kaoi* and XCHT exhibited a protective function as antioxidants by scavenging ROS.

Being low in catalase content, endothelium and muscle cells depend on glutathione metabolizing enzyme system to decompose H_2O_2. Coupling with the conversion of glutathione from its reduced form (GSH) to the oxidized form (GSSG), H_2O_2 is converted to H_2O and O_2 by GPx[38]. Cellular GSH level is restored by GRd through a NADPH-mediated oxidation-reduction reaction. On the other hand, endogenous and exogenous electrophilic compounds are linked to GSH by GST, a multiple function enzyme, to form less toxic glutathione-conjugates[39].

GPx, GRd and GST activities in rat liver were significantly increased by the administration of CCl_4 (Table 5). This increase in enzyme activities was probably a response towards increased $CCl_3O_2^{\bullet}$ generation in CCl_4 toxicity[4]. However, these enzyme activities did not show any significant increase when the rats were given the extracts of *B. kaoi* or XCHT before being given CCl_4. Since GPx, GRd and GST activities did not increase after the administration of the extracts of *B. kaoi* or XCHT, it is assumed that the hepatoprotective functionalities of *B. kaoi* or XCHT might not increase the activities of endogenous antioxidative enzymes systems.

The extracts of *B. kaoi* (500 mg/kg bw) or XCHT could reduce MDA formation in rat liver, which indicated that the formation of toxic free radicals and lipid peroxidation were inhibited by the extracts. Moreover, because the activities of SOD, catalase, GPx, GRd and GST were compensation ally increased by the administration of CCl_4, it is further confirmed that the hepatoprotective functionality of *B. kaoi* or XCHT extracts was achieved by direct interaction with toxic free radicals or peroxides and by reversing the activities of the antioxidant enzymes to the normal levels rather than by inducing detoxification antioxidant enzymes. Furthermore, the amount of *B. kaoi* or XCHT required by an adult to reach the dosage of 100 mg/kg bw will be in the range of 350 and 270 mL of *B. kaoi* or XCHT water extract, a reasonable quantity from a cup of health drink.

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