Prevention of hepatic oxidative injury by *Xiao-Chen-Chi-Tang* in mice

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**Abstract**

The three purgative *Cheng-Chi-Tang* decoctions (CCTDs) including *Ta-Cheng-Chi-Tang* (TCCT), *Xiao-Chen-Chi-Tang* (XCCT), and *Tiao-Wei-Chen-Chi-Tang* (TWCCT) are used for treating gastrointestinal disorders, including liver diseases in traditional Chinese medicine. However, the underlying mechanisms as liver disease remedies are far from fully clarified. The objective of the study is to investigate and compare the antioxidant activity of the three purgative CCTDs in order to delineate their hepatic protective potential and mechanism. Antioxidant activity measured with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test indicated XCCT as the most potent preparation (IC50 8.94 μg/ml). In tert-butylhydroperoxide (TBH, 50 mM)-induced lipid peroxidation in ICR mice liver homogenates, XCCT also showed stronger and dose-dependent inhibitory activity against TBH-induced malondialdehyde (MDA, a marker of lipid peroxidation) production (IC50 53.66 μg/ml). In addition, XCCT showed dose-dependent protective effect against TBH-induced cytotoxicity in normal human Chung liver cells Furthermore, in carbon tetrachloride (CCl4)-induced acute liver injury model, mice pretreated with 0.2 g/kg and 0.4 g/kg of XCCT extracts showed a decrease of 59.8 and 43.1% in serum glutamic oxaloactetic transaminase (GOT) level, 51.4 and 52% in glutamic pyruvate transaminase (GPT) level, along with a reduction of 31 and 15% in MDA level, respectively, similar to the effects exerted by silymarin. XCCT pretreated mice also showed milder necrotic changes in the microscopic picture of the liver. The results suggest that XCCT has significant antioxidant activity and hepatic protection potential.

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**Keywords:** Oxidative injury; Liver disease; Purgative decoctions; *Xiao-Chen-Chi-Tang*; *Rheum palmatum*; Polygonaceae

**1. Introduction**

Oxidative stress has been implicated in the pathogenesis of various liver diseases including alcoholic liver disease, non-alcoholic fatty liver disease, and chronic hepatitis C (Roskams et al., 2003; Seki et al., 2003, 2005; Kitase et al., 2005). But the exact etiopathogenetic mechanisms of these hepatic lesions are not fully delineated yet. In many patients, hepatitis such as non-alcoholic fatty liver disease becomes chronic and eventually progresses to more-serious liver pathologies, such as fibrosis, cirrhosis, or even carcinogenesis, causing devastating economic losses and mortality (Mulhall et al., 2002; Albano et al., 2005). At present, treatment for hepatic lesions remains unsatisfactory. As a consequence, patients with liver disease constitute a significant portion of the population using complementary and alternative medicine (CAM) to improve their health (Strader et al., 2002). Among the CAM used by patients with liver disease, antioxidant agents have been postulated to be efficacious (Krueger et al., 2004). Milk thistle, extract from *Silybum marianum*, is one of the most popular liver tonics, and its hepatoprotective effect is at least contributed by the antioxidant effect exerted by its active compound, silymarin (Campos et al., 1989; Farghali et al., 2000; Comar and Kirby, 2005). The classical antioxidants, Vitamins E and C, have also been shown to improve fibrosis in patients with non-alcoholic steatohepatitis (Harrison et al., 2003). However, while definite treatment strategies for various liver diseases remain undefined, potential hepatoprotective agents from natural sources may be found from the time-honored traditional Chinese medicine, as many Chinese herbs have been found to possess potent antioxidant effects (Cai et al., 2004a).

Most Chinese herbs are taken in the form of formulation composed according to the philosophies of Chinese medicine. Although most of the preparations have been used clinically for a long time, many of them are not supported with adequate.

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pharmacological explanations. In order to promote the safety and efficacy of traditional Chinese herbal medicines for people in the 21st century, it is important to support these traditional theories and practices with sound scientific evidence, so that old wisdom can be integrated with modern language. Three Chen-Chi-Tang decoctions (CCTDs) originally mentioned in Shan han lun (about 200 a.d.) consist of three famous purgative decoctions, i.e. Ta-Chen-Chi-Tang (TCCT), Xiao-Chen-Chi-Tang (XCCT), and Tiao-Wei-Chen-Chi-Tang (TWCCCT), traditionally used for treating gastrointestinal disorders, including liver diseases (Liu, 1988; Hsu and Hsu, 1980). Recently, we have shown that TCCT has anti-inflammatory effects in addition to its traditionally known purgative activity (Tseng et al., 2006). The constituent herbs or the active compound of the herbs in the three CCTDs have been shown to possess potent antioxidant properties (Shen et al., 1998; Cai et al., 2004b; Wilmsen et al., 2005). Based on these studies and traditional experience, we hypothesized that these natural prescriptions would exert a beneficial effect on biological systems under oxidative stress.

Several methods have been established to assay antioxidant activity in herbal medicines, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test and measurement of malondialdehyde (MDA) level by thiobarbituric acid (TBA) method (Cai et al., 2004a; Sohn et al., 2005). Lipid peroxidation in the cell membrane occurred when free radicals attack the unsaturated fatty acids in the cell membranes. Malondialdehyde (MDA) is one of the end products of oxidation of polyunsaturated fatty acids (PUFAs). tert-Butylhydroperoxide (TBH) is an organic prooxidant frequently used to induce lipid peroxidation in vivo or in vitro assay. CCl₄ is another frequently used chemical agent to create acute oxidative stress of oxidation of polyunsaturated fatty acids (PUFAs). tert-Butylhydroperoxide (TBH) is an organic prooxidant frequently used to induce lipid peroxidation in vivo or in vitro assay. Malondialdehyde (MDA) is one of the end products of oxidation of polyunsaturated fatty acids (PUFAs). tert-Butylhydroperoxide (TBH) is an organic prooxidant frequently used to induce lipid peroxidation in vivo or in vitro assay. CCl₄ is another frequently used chemical agent to create acute oxidative stress.

2. Materials and methods

2.1. Cell culture

The normal human Chang liver cell line (ATCC CCL-13) was obtained from American Type Culture Collection (Rockville, MD, USA). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Sigma) and 10% heat-inactivated fetal bovine serum (FBS, Gibco BRL, Grand Island, NY, USA), and then incubated at 37°C in a humidified incubator containing 5% CO₂.

2.2. Animals

ICR male mice weighing 20±2 g were obtained from the National Science Council, Taipei, Taiwan, and maintained in plastic cages at 21±2°C with free access to pellet food and water. They were kept on a 12-h light/12-h dark cycle. All mice used in this experiment were cared for according to the ethical regulations on animal research of our university.

2.3. Preparations of aqueous extracts of CCTDs and their constituents

The medicinal plants and materials used in the experiment included the root of Rheum palmatum L. (Polygonaceae), the bark of Magnolia officinalis Rehd. et Wils. (Magnoliaceae), the immature fruit of Citrus aurantium L. (Rutaceae), the root of Glycyrrhiza uralensis F. (Leguminosae), and Mirabilium (mirabilite, crystals of sodium sulfate, Na₂SO₄). The herbs were purchased from a traditional Chinese medicinal store in Taipei, Taiwan, and these medicinal materials were authenticated by Associate Prof. H.C. Chang, National Laboratories of Food and Drugs, Department of Health, Executive Yuan, Taipei, Taiwan. Voucher specimens (nos. RP-0001, MO-0001, CA-0001, GU-0001 and M-0001) were deposited at the Herbarium of the College of Pharmacy, Taipei Medical University. TCCT, XCCT, and TWCCCT were prepared according to the Unified Formula published by the Committee on Chinese Medicine and Pharmacy of the Department of Health in Taiwan (Table 1). Each herb or preparation was immersed in distilled water, and boiled in 20 times the weight of the material until half of the original amount of water was left. The extract was then filtered and freeze-dried.

2.4. Chromatographic analysis of the CCTDs

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-10ATvp liquid chromatograph equipped with a DGU-14A degasser, an FCV-10ALvp low-pressure gradient flow control valve, a SIL-10Advp auto injector, an SPD-M10Avp diode array detector, and an SCL-10Avp system controller. Peak areas were calculated using Shimadzu Class-VP software (Version 6.12 sp5).

The mobile phase was composed of 0.05% H₃PO₄ in water–acetonitrile (v/v) with gradient elution (0–5 min, 95:5; Table 1). Preparation of extracts of the Chen-Chi-Tang decoctions (CCTDs) and their constituents

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Part used</th>
<th>Weight (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheum palmatum L.</td>
<td>Rhizome</td>
<td>10</td>
<td>27.3</td>
</tr>
<tr>
<td>Magnolia officinalis R. et W.</td>
<td>Bark</td>
<td>10</td>
<td>9.0</td>
</tr>
<tr>
<td>Citrus aurantium L.</td>
<td>Immature fruit</td>
<td>10</td>
<td>21.0</td>
</tr>
<tr>
<td>Glycyrrhiza uralensis F.</td>
<td>Root</td>
<td>10</td>
<td>28.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formula name</th>
<th>Constituent</th>
<th>Weight (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xiao-Chen-Chi-Tang (XCCT)</td>
<td>Rheum palmatum</td>
<td>14</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>Magnolia officinalis</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Citrus aurantium</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Ta-Chen-Chi-Tang (TCCT)</td>
<td>Rheum palmatum</td>
<td>8</td>
<td>27.3</td>
</tr>
<tr>
<td></td>
<td>Magnolia officinalis</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Citrus aurantium</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mirabilium</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Tiao-Wei-Chen-Chi-Tang (TWCCCT)</td>
<td>Rheum palmatum</td>
<td>12</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>Mirabilium</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glycyrrhiza uralensis</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
5.01–15 min, 90:10; 40 min, 80:20; 60 min, 20:80; 65 min, 0:100). Solvents were filtered through a 0.45 μm FP Vericel (PVDF) membrane filter from Pall Corporation (Ann Arbor, MI, USA). A Purospher STAR RP-18e reverse-phase column (250 mm × 4 mm i.d.) and a Purospher STAR RP-18e guard column (4 mm × 4 mm i.d.) (Merck, Darmstadt, Germany) were used. The flow-rate was 1.0 ml/min with UV absorbance detection at 280 nm. The analysis involved 20 μl of sample solution. The operation was carried out at room temperature (25 °C).

The following compounds were identified for each decoction: (+)-catechin (with a retention time (Rt) of 14.63 min), sennoside B (with a Rt of 38.77 min), sennoside A (with a Rt of 44.81 min), rhein (with a Rt of 60.01 min), and emodin (with a Rt of 60.42 min) (Fig. 1). Authentic standards of (+)-catechin, sennoside A, sennoside B, emodin, and rhein were obtained from Sigma (St. Louis, MO, USA).

2.5. DPPH scavenging effect of extracts

The scavenging effect of the prepared samples on the DPPH radical was evaluated as previously described (Yokozawa et al., 1998). An aqueous solution of each sample was serially diluted in triplicate wells of a 96-well microtiter plate, with a final volume of 100 μl aliquots (concentration ranged from 1 mg/ml to 1 μg/ml for the extracts and 1 mM to 1 μM for the compounds) in each well. Then, 100 μl of an ethanolic solution of DPPH (50 μM) was added to the well containing 100 μl of an aqueous solution of each sample at room temperature for 30 min. The DPPH level in each well was evaluated by measuring the optical density of each well at 530 nm, using an MRX microplate reader (Dynex Technologies, Guernsey, Channel Islands, UK). The equation was DPPH radical scavenging rate (%) = [1 − (ST/EC)] × 100, where ST is the optical density value of the sample and EC is the optical density value of the control.

2.6. Induction of oxidative stress in mice liver homogenates and Chang liver cells

Oxidative stress was induced in liver homogenates from ICR mice and Chang liver cell cultures by the addition of TBH (Sigma). Liver homogenates of ICR mice was treated with TBH (50 mM) and with or without the three CCTD extracts (concentration ranged from 6 to 50 μg/ml). The extent of lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) with TBA method as previously described (Ohkawa et al., 1979). The liver samples were first homogenized in Tris–HCl buffer to provide a 10% homogenate. The protein level in the homogenized tissue was quantified with Bioquant (Merck) as previously described (Wang et al., 2002a). Results are expressed in terms of the percentage reduction of the MDA level by the extracts:

Percentage reduction of MDA level (%) = [1 − (MT/MC)] × 100; where MT is the MDA level of the sample and MC is the MDA level of the control.

TBH treatment reduced the viability of Chang liver cells (Sohn et al., 2005). The hepatoprotective effect of the sample (XCCT) was determined by the viabilities of TBH-stimulated Chang liver cells using the 3-(4,5-dimethlythiaol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described (Wang et al., 2002a). Chang liver cells were pretreated with different concentrations of XCCT for 30 min (6–100 μg/ml), and then 2 or 4 mM of a TBH solution was added to the cell culture. After 24 h of incubation, viabilities of the cells were determined with the MTT assay. Finally, the products were evaluated by measuring the optical density of each well at 600 nm, using an MRX microplate reader (Dynex Technologies).

2.7. Carbon tetrachloride (CCL4)-induced acute liver injury

ICR mice were divided into five groups. The XCCT-pretreated group received different concentrations of XCCT (0.2 or 0.4 g/kg) every day for 7 days, and the last dose was given 1 h orally before an intraperitoneal injection (i.p.) of 2% CCL4–corn oil solution in a dose of 1.0 ml/kg body weight. The control mice received the vehicle (0.1 ml/10 g) and same dose of CCL4–corn oil injection, i.p. Another group of mice received no treatment at all, and was designated the blank group. Another group of mice received 25 mg/kg silymarin (Sigma) as positive control, as sily-
marin is a well-known liver tonic (Comar and Kirby, 2005). Four hours after CCl₄ treatment, blood was first obtained from the animal under light anesthesia through the orbital vessels using capillary tubes for immediate serum GOT and GPT analysis on a FUGI DRI-CHEM 3500i analyzer with GOT and GPT slide using 10µl of serum according to the manufacturer’s instruction. Then, the animals were sacrificed under ether anesthesia to obtain the liver for histopathological investigations and lipid peroxidation determination. Liver sections were kept at −80 °C until use. MDA levels in liver homogenates were determined as previously described in Section 2.6. After being removed from the animals, livers for histopathological examination were immediately fixed in 10% formaldehyde, embedded in paraffin, cut into 4–5 µm-thick sections, stained with hematoxylin–eosin (H&E), and observed under a light microscope.

2.8. Statistical analysis

Data were first statistically assessed by one-way analysis of variance (ANOVA). Difference between drug-treated groups and control group was then evaluated by Bonferroni’s t-test. p < 0.05 was considered significant. All data are expressed as the mean ± S.D.

3. Results

3.1. HPLC profiles of the CCTDs

The major compounds in the three CCTDs were analyzed with HPLC. Major peaks identified included (+)-catechin, sennoside A, sennoside B, rhein, and emodin in each decoction (Fig. 1). The concentrations of (+)-catechin were highest in XCCT (11.28 ± 0.08 µg/g) followed by TCCT (7.51 ± 0.02 µg/g), and least in TWCCT (1.23 ± 0.04 µg/g).

3.2. DPPH radical-scavenging effect of the three CCTDs and their constituents

Evaluation of antioxidant activity of the three CCTDs and their constituent medicinal materials that composed the three preparations was first performed with a DPPH radical-producing system. As shown in Fig. 2, the scavenging effects of the three CCTDs on DPPH radicals were in the order of XCCT > TCCT > TWCCT, with IC₅₀ values of 8.94, 27.3 and 61.8 µg/ml, respectively. The scavenging effects of the five component medicinal materials on DPPH radicals were in the order of Rheum palmatum > Magnolia officinalis > Citrus aurantium > Mirabilium and Glycyrrhiza uralensis, with IC₅₀ values of 7.76 µg/ml for Rheum palmatum, 59.8 µg/ml for Magnolia officinalis, and 272.6 µg/ml for Citrus aurantium; while Mirabilium and Glycyrrhiza uralensis exhibited no activity on DPPH radicals scavenging (IC₅₀ values > 500 µg/ml). (+)-Catechin showed potent DPPH scavenging effect as the IC₅₀ value was 5.94 µM, while rhein and emodin exhibited no significant activity on DPPH radicals scavenging (IC₅₀ values > 500 µM) (Table 2).

3.3. Prevention of oxidative injury in liver homogenates and Chang liver cells by CCTDs

As oxidative stress is an important inducer of liver diseases, we evaluated the effect of the three CCTDs on TBH (50 mM)-induced lipid peroxidation in ICR mice liver homogenates. At 50 µg/ml, XCCT again showed stronger inhibitory activity against TBH-induced MDA production than did TCCT or TWCCT in mice liver homogenates (Fig. 3). Moreover, XCCT showed dose-dependent inhibitory activity against TBH-induced MDA production (IC₅₀ 53.66 µg/ml). These findings suggest that XCCT may be a potentially beneficial natural antioxidant agent when oxidative stress is present.

We also investigated the protective effect of XCCT against TBH-induced cytotoxicity by preincubating normal human Chang liver cells with or without XCCT for 30 min and then TBH was added to the well. The cells were harvested after 24 h of incubation. As shown in Fig. 4, treatment with 2 or 4 mM

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Table 2 IC₅₀ values of the extract of Chen-Chi-Tang decoctions (CCTDs), constituent medicinal material, and the main active compounds in Rheum palmatum on the DPPH radical formation system

<table>
<thead>
<tr>
<th>Name of extract</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decoctio</td>
<td></td>
</tr>
<tr>
<td>Ta-Chen-Chi-Tang (µg/ml)</td>
<td>27.3</td>
</tr>
<tr>
<td>Xiao-Chen-Chi-Tang (µg/ml)</td>
<td>8.94</td>
</tr>
<tr>
<td>Tuao-Wei-Chen-Tang (µg/ml)</td>
<td>61.8</td>
</tr>
<tr>
<td>Constituen</td>
<td></td>
</tr>
<tr>
<td>Rheum palmatum (µg/ml)</td>
<td>7.76</td>
</tr>
<tr>
<td>Magnolia officinalis (µg/ml)</td>
<td>59.8</td>
</tr>
<tr>
<td>Citrus aurantium (µg/ml)</td>
<td>272.6</td>
</tr>
<tr>
<td>Natural products of Rheum</td>
<td></td>
</tr>
<tr>
<td>Rhein (µM)</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Emodin (µM)</td>
<td>&gt;500</td>
</tr>
<tr>
<td>(+)-Catechin (µM)</td>
<td>5.94</td>
</tr>
</tbody>
</table>

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Fig. 2. Antioxidant activity of three Chen-Chi-Tang decoctions (CCTDs) on DPPH radical scavenging. The concentration of DPPH was 50 µM. Concentration of the formulas or herbal extract was 50 µg/ml. Results are presented as the mean ± S.D., n = 3. Numbers: 1, TCCT; 2, TWCCT; 3, XCCT; 4, Rheum palmatum; 5, Magnolia officinalis; 6, Citrus aurantium; 7, Glycyrrhiza uralensis; 8, Mirabilium.
TBH resulted in nearly 90% loss of viability of Chang liver cells. Pretreatment of cells with XCCT prevented TBH-induced Chang liver cell cytotoxicity in a dose-dependent and statistically significant manner. Thus, XCCT may protect the liver from oxidative damage.

3.4. Hepatoprotective effect of XCCT against CCl₄-induced acute liver injury in mice

In order to further evaluate XCCT as a potential hepatic protective antioxidant, we examined the hepatic protective effect of XCCT using CCl₄-induced acute liver injury model in mice.

Histological changes in liver tissue associated with CCl₄ toxicity include centrilobular necrosis, fatty changes, inflammatory cell infiltration, and elevation of serum markers of hepatic injury like GOT and GPT levels (Lu et al., 2002). The effect of XCCT pretreatment on CCl₄-induced histopathological changes of the liver was evaluated after the mice were sacrificed. After CCl₄ injection, diffuse small whitish-yellow macular changes were noted in the microscopic picture with severe coagulation necrosis around the central areas (Fig. 5B). The liver of mice pretreated with XCCT for 7 days showed a milder degree of necrosis and inflammation around the central areas (Fig. 5C). XCCT-pretreated mice also showed significantly lowered level of MDA production in liver tissue and serum GOT and GPT levels as compared to the vehicle-treated CCl₄-intoxicated mice (Table 3). Mice pretreated with silymarin at a dose of 25 mg/kg significantly decreased the CCl₄-induced GOT and GPT levels (53.5, 57.6%, respectively), and 24.8% in MDA level. Mice pretreated with 0.2 g/kg or 0.4 g/kg of XCCT extracts showed a decrease of 59.8% and 43.1% in GOT level, 51.4% and 52% in GPT level, along with a reduction of 31% and 15% in MDA level, respectively, similar to the effects exerted by silymarin.

4. Discussions

The three Chen-Chi-Tang decoction (CCTDs), Ta-Cheng-Chi-Tang (TCCT), Xiao-Chen-Chi-Tang (XCCT), and Tiao-Wei-Chen-Chi-Tang (TWCCCT) are traditionally used as purgative decoctions, prescribed to relieve abdominal fullness and distention, and constipation due to pathogenic heat through purgation (Liu et al., 2000; Hsu and Hsu, 1980). Today, these preparations are used to treat symptoms related to acute simple intestinal obstructions without complications, acute cholecystitis, acute appendicitis, constipation, and hepatitis (Qi et al., 2004; Kou et al., 2004). However, the underlying mechanisms as liver disease remedies are far from fully clarified. In this study, we have investigated and compared the antioxidant activity of the three purgative CCTDs using various in vitro and in vivo oxidative injury model in order to delineate their hepatic protective potential and mechanism. We have also compared the effect with a well-known liver tonic, silymarin. Antioxidant activity of the three CCTDs measured with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test indicated XCCT as the most
Fig. 5. Prophylactic effect of Xiao-Chen-Chi-Tang (XCCT) on the macroscopic and microscopic integrity of CCl₄-induced acute liver damage in mice. (A) A section of liver from a control animal treated with vehicle only; (B) a section of liver from a CCl₄-treated animal. Diffuse small whitish-yellow macular changes can be seen with severe coagulation necrosis (↓) around the central areas; (C) section of liver from an animal pretreated with XCCT and then with CCl₄. Focal macular change can be noted with mild to moderate necrosis and some ballooning hepatocytes (↑), with inflammation around the central areas.

potential preparation. We have also shown that XCCT could attenuate TBH-induced MDA production in liver homogenates and TBH-induced cytotoxicity in normal human Chang liver cells. Mice pretreated with XCCT also showed lower serum GOT, GPT levels and less MDA production. Elevated serum GOT and GPT level are biochemical markers of liver injury, and elevated MDA level detected with TBA method is used as an index of the extent of lipid peroxidation in tissue. Liver histopathological study also showed milder necrotic changes in the XCCT pretreated mice. The data suggested that XCCT is a potent antioxidant herbal preparation and has hepatic protection potential.

The results in our DPPH radical scavenging experiment are agreeable to previous report (Yokozawa et al., 1998). The roots of Glycyrrhiza showed no significant activity against DPPH radicals. In addition, the presence of Mirabilitum in a decocted preparation has been reported to qualitatively and quantitatively alter the constituents (Fan et al., 2001). XCCT is the only preparation, among the CCTDs, that contains no Mirabilitum or Glycyrrhiza uralensis. We have also simultaneously showed that (+)-catechin had a very low IC₅₀ (5.94 μM) for scavenging DPPH radicals and XCCT contained more (+)-catechin than did the other two decoctions. Statistically, together, these factors may explain higher antioxidant activity observed in XCCT than in either TCCT or TW CCT in our experiments.

Today, the separation and determination of the active chemical constituents is generally recommended for the standardization and quality control of herbal products and herb related investigations. Furthermore, the identification of major compounds in an herb or herbal preparation may be helpful in delineating the pharmacological activity and the underlying mechanisms. However, the compound in herbal medicine is so complex, that the relationship between the biological activity of a compound and the concentrations within medicinal herbs is still controversial. For instance, Iizuka et al. (2004) did not find significant correlation between the higher concentrations and stronger antioxidant activity in the compounds identified in several specimens of Rheum, while Cai et al. (2004a) reported that the high phenolic content in the crude extracts of Rheum officinale correlated with the high antioxidant activity of the extract. Besides (+)-catechin discussed above, magnolol, a major compound isolated from the cortex of M. officinalis, has been found to inhibit neutrophil adhesion by inhibition of the accumulation of reactive oxygen species, and treatment with magnolol significantly inhibited lipid peroxidation in primary hepatocytes (Shen et al., 1998; Park et al., 2003). Naringenin, a flavonoids found frequently in citrus fruits such as Citrus aurantium, also exhibited antioxidant activity (Li et al., 2002; Pari and Gnanasoundari, 2006). Anti-oxidative effects exhibited by XCCT may also have been contributed by these compounds. However, researches about the active compounds within the three CCTDs mostly focused on anthraquinones, the principle compounds for the laxative effect of the three CCTDs (Xi and Liu, 2001; Zeng et al., 2002). But these anthraquinoids had low antioxidant activity (Cai et al., 2004b), and therefore, are not suitable for the quality control when regarding the antioxidant activity of the three purgative preparations. Rheum palmatum was present in all three decoctions, and it is known as the principle constituent within these formulas. Rheum palmatum is a well-known laxative herb. Recently, some Rheum species or Rheum-containing preparations have been reported to inhibit liver fibrosis (Jin et al., 2005; Imanishi et al., 2004), and were found to be useful in the prevention and treatment of non-alcoholic steatohepatitis (NASH) in rats (Liu et al., 2000). Although the underlying mechanisms are not yet clearly defined, oxidative injury has been recognized as a pathogenetic factor in these hepatic lesions. In our DPPH experiment, the root of Rheum palmatum had the lowest IC₅₀ value among all the ingredients used to prepare the three preparations, suggesting the superior antioxidant property of the root of R. palmatum then other constituent herbs. According to Cai et al. (2004b), major
constituents in the root of Rheum species include hydroxyan-
thaquinone, tannins and phenolic acid, but strong antioxidative
activity in the root of Rheum was mainly due to the high concen-
tration of tannin and gallic acid. Hence, in our HPLC analysis of
the three CCTDs, we have identified the following compounds, i.e. (+)-catechin, sennoside A, sennoside B, rhein and emodin, all of which are the main constituents found in *Rheum palmatum* (Wang et al., 2002b; Liu et al., 2000). Among the compounds identified for each decoction in the present study, sennoside A and B are not antioxidative. (+)-Catechin is a potent natural antioxidative compound (Iizuka et al., 2004; Maatta-Riihinen et al., 2005). Rhein and emodin are also not active DPPH scav-
enger (Cai et al., 2004a,b). Results from our DPPH assay are
in agreement with the previous reports. Therefore, we proposed that (+)-catechin may be used for the quality control of future antioxidative experiments with CCTDs or XCCT in particular.

In conclusion, based on the results from this study, we may suggest that apart from the traditional uses of XCCT, this quite-
popular TCM recipe may also act as a prophylactic agent to prevent liver diseases related to oxidative injury, and that (+)-catechin may be used for the quality control of future antioxidative experiments with CCTDs or XCCT in particular.

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