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Compound *Cordyceps* TCM-700C exhibits potent hepatoprotective capability in animal model

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ABSTRACT

A herbal preparation "Compound Codyceps-TCM-700C (CC-700C)" was tested for hepatoprotective effect against the carbon tetrachloride induced liver damages in Sprague-Dawley rat model for a period of 6-weeks. Two dosage levels of CC-700C, respectively 286.2 mg/kg-bw (L-TCM) and 2862 mg/kg-bw (H-TCM), and a positive control Silymarin (Sigma) were used to compare their therapeutic effect. Both CC-700C's and Silymarin showed nontoxic in nature, as evidenced by body weight gain, organ weights and appearance including liver, spleen, and kidney. The activities of aspartate aminotransferase (AST) and alanine-aminotransferase (ALT) were more effectively suppressed by CC-700C than Silymarin. In addition, all levels of serum bilirubin, serum albumin, triglyceride (TG), total cholesterol (TC), platelet count (PLT), and prothombin time (PT) except TG were shown effectively restored to normal values by CC-700C and Silymarin. Moreover, although levels of glutathione (GSH), glutathione reductase (GSH-Rd), and superoxide dismutase (SOD) were equally maintained by these three preparations, glutathione peroxidase (GSH-Px) was suppressed only by H-TCM, and SOD only by Silymarin. In contrast, the activity of catalase efficiently recovered to control level on administration of CC-700C, being far better than Silymarin. Finally the liver collagen content, an indication of fibrosis, was also significantly suppressed by CC-700C, better effect was by L-TCM, but both levels were superior to Silymarin. Conclusively, the herbal preparation "Compound *Cordyceps* TCM-700C" is a potent hepatoprotective preparation. For therapeutic use, a dosage of 286.2 mg/kg-bw would be sufficiently effective.

1. Introduction

Liver is the largest and the most functionally operating organ. Pathologically, chronic hepatitis or long term intoxification can severely injure hepatic cells. Initially, the damaged cells are denatured, but subsequently transformed to hypertrophic fibrosis [1] and necrosis [2], and eventually may progress to hepatoma. Clinically, the hepatofibrotic stage is a very crucial pathogenetic point closely related to its prognosis. To terminate the serial consequences at the fibrotic stage or to retrograde such an event to the normal status currently has become the target strategy in treatment of liver diseases. *Codyceps sinensis* (a herb in Chinese it means "The Winter worm-Summer grass") has been used as a Chinese traditional medicine for over fifteen hundred years. The well known commercialized Chinese herbal medicine "Compound Cordyceps TCM-700" (CC-TCM-700), consisting of pulverized mycelial *Cordyceps sinensis*, radix Huan-Chi powder and zinc glucuronate as its main ingredients, has been frequently reported to be effective in activating

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monocytes and macrophages [3] and inhibiting proliferation of leukemia U937 cells [4]. In pancreas and blood, it effectively increased the ratio of T-cells $CD4^+/CD8^+$ [5]. Radix Huan-Chi was demonstrated to be a strong immuno-activating, anti-cancer and anti-ageing herbal medicine [6,7], and zinc ions are essential for *de novo* DNA synthesis. Moreover, it is also an important prothetic factor of enzymes participating in many biochemical functions. Literature often demonstrated that low zinc level tends to trigger a number of microbial infections. And replacement with an appropriate amount of zinc ions greatly reduced the prevalence of many infections. Pharmacologically, zinc insufficiency reduced secretion of thymulin and damaged the cell-mediated immune system. Alternatively, an appropriate zinc supplement to children was shown to have actively improved the production of CD3, CD4 and the ratio of $CD4^+/CD8^+$. Furthermore, zinc insufficiency was found to suppress the secretory function of β cells [8–12]. In this present thesis, the Sprague-Dawley rats were used to study the hepatoprotective effect of CC-TCM-700C against the carbon tetrachloride induced liver damages. Amazingly, its therapeutic effect was found to be more prominent comparing to the popular Silymarin preparation.

2. Materials and methods

2.1. Chemicals

Silymarin, a product of Sigma (Sigma Co., S0292, MO, USA) and carbon tetrachloride were provided by the local Leo-Ho Pharmaceutical Trading Company. Olive oil was purchased from the local pharmacy. The herbal medicine Compound *Codyceps* TCM-700C (CC-700C) was provided by the local TCM Biotech International Corp. (Taipei, Taiwan). Each gram of CC-700C consisted of *Codyceps sinesis* mycelial powder 77.0 g, Radix Huan-Chi powder [pulverized Radix Astragali *Astragali membranaceus* (Fisch) Bge.] 15.4 g, and zinc glucuronate 7.6 g.

2.2. Animals

According to the Helsinki Declaration (1972), this animal experiment has been approved by The Ethic Committee of Animal Experiment of The Taipei Veteran General Hospital, Taichung Branch (TVGH, Taichung, Taiwan). Five to six week aged mature male Sprague-Dawley (SD) rats, body weight averaged 246 g ± 11.2 g, were purchased from the National Laboratory Animal Breeding and Research Centre (LABRC, Hsin-Chu City, Taiwan). Rats were caged in plastic cages, six in each, in which saw wood dusts (Beta-Chip, Hardwood Lab Bedding, Northeastern) were evenly spread to serve the substrate pad. The animal room was maintained at 24 ± 1 °C and RH 60 ± 10%. The light and dark cycle was changed in a 12 h cycles. These SD rats were allowed free access to diets (Fu-Sou Brand, specific for rats) and drinking water (the reverse osmotic water, ROW). All other regulations were followed according to the Guidance for Animal Experimenters instructed by the animal rooms of TVGH.

2.3. Animal grouping and experimentation

The animals were first adapted in the first week in animal room and then grouped into 5 groups, six in each. Group 1 served the control healthy group. Group 2 was treated with carbon tetrachloride (CCl_4) to induce liver damages. Group 3 was per os treated with CCl_4 + Silymarin; group 4 received a per os dose of CC-700C (286.2 mg/kg bw/day) and designated as the low dosage group L-TCM. And group 5 administered per os a high dose of CC-700C (2862 mg/kg bw/day) and designated as the high dosage group H-TCM. These dosages given were calculated according to the regulation issued by the Taiwan Institute of Health (The NIH of Taiwan). The method for feeding CCl_4 to the experimental animals was according to Lin el al. [13]. Briefly, CCl_4 was previously dissolved in olive oils to make a final concentration of 40%. Feeding was carried out at 9:00 am every Monday, Wednesday, and Friday, the dosages given were 1 mL per os per kg body weight with the ROW. The whole experimental protocol is summarized in Table 1. This treatment successively continued for 6 weeks.

2.4. Sample collection and analysis

The blood samples were successively collected from the rat tail vein at week 1, 3 and 6 respectively post the experimentation. After left to stand at room temperature for 1 h, the sample blood were frozen at 4 °C and ultracentrifuged at 5000 rpm for 20 min. The sera were separated and collected to perform biochemical analyses. The rats were euthanized by CO_2 anesthesia. Abdominal surgery was performed and blood was bled from the caudal vena cava. The blood was untra-centrifuged at 5000 rpm and were used for determination of aspartate aminotransferase (AST; GOT), alanine aminotransferase (ALT; GPT), bilirubin, albumin, triglyceride (TG), total cholesterol (TC), prothrombin time (PT), and platelet counts (PLT) by routine medical laboratory assay methods used in TVGH. In addition, the livers, kidneys and spleen of rats were dissected and rinsed with saline several times. The moisture adhered onto the rinsed organs were soaked off with tissues and the weights of organs were taken. The larger lobes of livers were divided into three parts. From the middle part of each sample two sample pieces (1 cm × 1 cm) were sliced out. The slices were fixed with formalin (10%), coated with paraffin and sliced for further H&E and Sirus Red staining. The finished specimens were subjected to further pathological examinations. The remainder of organs were placed into plastic bags, clip-sealed, and kept at −70 °C for enzymatic analysis. Alternatively, the

Table 1
Experimental protocol of treatment.*

Group	Administration	Dosage
Control	Olive oil	Olive oil 1 mL/kg, with ROW
CCl_4	CCl_4	CCl_4 1 mL/kg, with ROW
CCl_4 + Silymarin	CCl_4 + Silymarin 200 mg/kg	CCl_4 1 mL/kg, with ROW
CCl_4 + L-CC-700	286.2 mg/kg	CCl_4 1 mL/kg + CC-700, with ROW
CCl_4 + H-CC-700	2862 mg/kg	CCl_4 1 mL/kg + CC-700, with ROW*

*CC-700: compound *Cordyceps* TCM-700 (CC-700C). L-TCM: group fed low dose of CC-700 (286.2 mg/kg bw/day). And H-TCM: group fed high dose of CC-700C (2862 mg/kg bw/day). ROW: reverse osmotic water. Administration time for Silymarin and CC-700C was regularly fixed at 14:00 pm every day.

Table 2
Body weight and organ weight affected by different treatment during 6 weeks of experimental period.*

Experimental period	Group				
	Control	CCl_4	CCl_4 + L-TCM	CCl_4 + H-TCM	CCl_4 + Silymarin
Body weight variation (g)					
Week 0	248 ± 16	249 ± 4	253 ± 7	247 ± 7	246 ± 11
Week 3	318 ± 9^{b}	266 ± 13^{a}	335 ± 21^{b}	345 ± 14^{c}	313 ± 15^{b}
Week 6	368 ± 19^{a}	316 ± 24^{b}	384 ± 14^{a}	403 ± 20^{c}	$367 \pm 20^{a,b}$
Liver weight (g)					
Week 6	14 ± 1^{a}	19 ± 3^{b}	15 ± 1^{a}	14 ± 2^{a}	16 ± 2^{a}
Spleen weight (g)					
Week 6	$0.8 \pm 0.3^{a,b}$	1.4 ± 0.3^{b}	0.7 ± 0.1^{a}	0.9 ± 0.1^{a}	$1.1 \pm 0.4^{a,b}$
Kidney weight (g)					
Week 6	1.3 ± 0.2^{a}	1.9 ± 0.2^{b}	1.4 ± 0.3^{a}	1.3 ± 0.2^{a}	1.5 ± 0.3^{a}

1. *TCM: compound TCM-700C (CC-700C).
2. Results expressed as means ± SD ($n = 6$).
3. Values in the same row with different superscripts are significantly different ($p < 0.05$).
4. Rat group CCl_4 + L-TCM was fed low dose of CC-700C (286.2 mg/kg bw/day). And group CCl_4 + H-TCM was fed high dose of CC-700C (2862 mg/kg bw/day).

remaining part of the larger liver lobes was further chopped into masses, added ten-fold (W/V) buffer solution containing 8 mM of KH_2PO_4, 12 mM of K_2HPO_4, and 1.5% of KCl (pH 7.40) and homogenized. The level of glutathione (GSH), and the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), superoxide dismutase (SOD) and catalase (CAT) were assayed by following the manufacturer's instructions with the experimental kits supplied by Calbiochem-Novbiochem Corp.

2.5. Statistical method

The data obtained were processed with the statistical software SPSS (Version 10.0) to evaluate the significance in difference. A confidence level of $p < 0.05$ was considered to be significantly different between two data pairs to be compared.

3. Results

3.1. Effect of CC-TCM-700C on body- and organ weights

As can be seen from Table 2, the body weight gain was normal in all groups except the CCl_4-damaged group ($p < 0.05$). The increase in body weight of H-TCM was more prominent than both the L-TCM and Silymarin groups ($p < 0.05$). Apparent swellings of liver, pancreas, and kidney were observed in the liver intoxicated CCl_4 group (Table 2). All the organs concerned remained normal when compared with those of CCl_4-treated. Amazingly, CC-700C exhibited better efficiency than the conventionally reputed Silymarin ($p < 0.05$) (Table 2).

3.2. Effect of CC-TCM-700C on levels of AST and ALT

In CCl_4-damaged rat livers, the activities of AST (GOT) and ALT (GPT) were elevated to over 700 U/L after a 6-week of intoxification ($p < 0.05$). However, both H-TCM and L-TCM showed promising protective effect on such a hepatic injury (Table 3).

3.3. Effect of CC-TCM 700C on some important biochemical parameters

The CCl_4-intoxicated rats all showed significantly elevated levels of serum bilirubin ($p < 0.05$). As often cited, apparently decreased PLT, prolonged PT, and elevated levels of serum albumin, triglyceride and total cholesterol were typical symptoms of CCl_4 intoxification (Table 4). Levels of serum bilirubin, serum albumin, serum TG and TC, PLT and PT all restored to normal levels after treated with CC-700C. Again, Silymarin revealed to be much less effective when taking these parameters into consideration ($p < 0.05$) (Table 4).

Table 3
Variation of serum AST and ALT levels in rats affected by different treatment method and experimental periods.*

Group	Week 0 (U/L)		Week 3 (U/L)		Week 6 (U/L)	
	AST	ALT	AST	ALT	AST	ALT
Control	118 ± 16	67 ± 6	109 ± 27^{a}	51 ± 26^{a}	90 ± 26^{a}	55 ± 10^{a}
CCl_4	129 ± 9	67 ± 5	938 ± 147^{b}	660 ± 92^{b}	725 ± 327^{b}	711 ± 174^{b}
CCl_4 + L-TCM	115 ± 12	69 ± 6	74 ± 15^{a}	40 ± 5^{a}	91 ± 23^{a}	47 ± 16^{a}
CCl_4 + H-TCM	132 ± 22	59 ± 11	78 ± 16^{a}	52 ± 10^{a}	84 ± 13^{a}	51 ± 4^{a}
CCl_4 + Silymarin	130 ± 7	62 ± 11	432 ± 207^{c}	133 ± 20^{a}	93 ± 17^{a}	62 ± 12^{a}

1. *AST: aspartate aminotransferase. ALT: alanine aminotransferase.
2. TCM: compound TCM-700C (CC-700C).
3. Results expressed as means ± SD ($n = 6$).
4. Values in the same column with different superscripts are significantly different ($p < 0.05$).
5. Rat group CCl_4 + L-TCM was fed low dose of CC-700C (286.2 mg/kg bw/day). And group CCl_4 + H-TCM was fed high dose of CC-700C (2862 mg/kg bw/day).

Table 4
Levels of serum biochemical parameters affected by treatment method and period.*

Group	Week 0	Week 3	Week 6
Control	–	–	–
Bilirubin, μmol/L	0.1	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Albumin, g%	4.9 ± 0.0	4.9 ± 0.1^{a}	4.9 ± 0.0^{a}
TG, mg/dL	188 ± 32.3	$77 \pm 14^{a,b}$	71 ± 24
TC, mg/dL	153 ± 20	78 ± 17	80 ± 19
PLT, 10^3/μL	–	–	993.5 ± 39.4^{b}
PT, s	–	–	13.4 ± 0.9^{a}
Liver collagen content, %	–	–	0.8 ± 0.2^{a}
CCl_4	–	–	–
Bilirubin, μmol/L	0.1	0.3 ± 0.0^{b}	0.6 ± 0.3^{b}
Albumin, g%	4.9 ± 0.0	4.4 ± 0.3^{b}	4.3 ± 0.3^{b}
TG, mg/dL	183 ± 22	67 ± 17^{b}	65 ± 16^{a}
TC, mg/dL	161 ± 20	73 ± 11	95 ± 30
PLT, 10^3/μL	–	–	677.8 ± 77.6^{a}
PT, s	–	–	16.6 ± 1.5^{b}
Liver collagen content, %	–	–	9.7 ± 5.0^{c}
CCl_4 + L-TCM	–	–	–
Bilirubin, μmol/L	0.1	0.1 ± 0.0^{a}	0.1 ± 0.1^{a}
Albumin, g%	4.9 ± 0.0	4.4 ± 0.2^{b}	4.8 ± 0.2^{a}
TG, mg/dL	194 ± 40	108 ± 20^{c}	$98 \pm 22^{a,b}$
TC, mg/dL	166 ± 36	80 ± 12	72 ± 8
PLT, 10^3/μL	–	–	1184.0 ± 146.0^{b}
PT, s	–	–	$14.5 \pm 0.9^{a,c}$
Liver collagen content, %	–	–	1.7 ± 0.4^{b}
CCl_4 + H-TCM	–	–	–
Bilirubin, μmol/L	0.1	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Albumin, g%	4.9 ± 0.0	4.3 ± 0.1^{b}	4.8 ± 0.1^{a}
TG, mg/dL	201 ± 48	$102 \pm 14^{b,c}$	113 ± 34^{b}
TC, mg/dL	157 ± 25	80 ± 10	68 ± 10
PLT, 10^3/μL	–	–	1030.2 ± 220.6^{b}
PT, s	–	–	15.1 ± 0.8^{c}
Liver collagen content, %	–	–	2.5 ± 1.1^{b}
CCl_4 + Silymarin	–	–	–
Bilirubin, μmol/L	0.1	0.2 ± 0.0^{c}	0.2 ± 0.1^{a}
Albumin, g%	4.9 ± 0.0	4.3 ± 0.4^{b}	4.4 ± 0.2^{b}
TG, mg/dL	192 ± 50	55 ± 35^{a}	$83 \pm 22^{a,b}$
TC, mg/dL	152 ± 17	72 ± 17	81 ± 12
PLT, 10^3/μL	–	–	$999.8 \pm 305.4^{a,b}$
PT, s	–	–	15.0 ± 1.0^{c}
Liver collagen content, %	–	–	5.7 ± 4.0^{c}

1. *TCM: compound TCM-700 (CC-700C). TG: triglyceride; TC: total cholesterol; PLT: platelet count; PT: prothrombin time.
2. Results expressed as means ± SD ($n = 6$).
3. Values in the same column with different superscripts are significantly different from each other ($p < 0.05$).
4. Rat group CCl_4 + L-TCM was fed low dose of CC-700C (286.2 mg/kg bw/day). And group CCl_4 + H-TCM was fed high dose of CC-700C (2862 mg/kg bw/day).

3.4. Effect of CC-700C on the in vivo antioxidative parameters

Usually some oxidative enzymes are related to many liver damages. In the CCl_4 damaged group, levels of glutathione (GSH) and catalase were significantly reduced at the end of week 6, while being comparatively normal in the two CC-700C groups (Table 5). In contrast, the levels of liver glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd) and the superoxide dismutase (SOD) were apparently lowered due to CCl_4 intoxification ($p < 0.05$). CC-700C showed excellent protecting effect as the Silymarin, although still incompletely recovered comparing to the control (Table 5).

Pathological examination revealed roughness on the liver superficial face with fibrosis and fibrotic septum in CCl_4 damaged group. The reticular fiber tissue extended outward from the central part of liver. Such phenomenon was greatly reduced by CC-700C as evidenced with slighter extent of fibrosis and less deformation occurred in liver lobules ($p < 0.05$) (Fig. 1). Besides, extracellular matrix accumulated in the fibrotic liver, which in turn, can induce more severe liver necrosis. As well known, the major component in extracellular matrix is collagen. Thus we performed the animal model to investigate whether the accumulation of collagen in CCl_4-damaged livers can be suppressed by treatment with CC-700C. Staining with Sirus Red is possible to detect the distribution of collagens in liver. As revealed in the stained specimens, the collagen in CCl_4 damaged liver specimens apparently increased and severely accumulated ($p < 0.05$). The liver lobules were hampered with huge amount of collagens leading to extensive liver fibrosis. In contrast, the phenomenon was far less severe when treated with CC-700C or Silymarin ($p < 0.05$) (Figs. 2 and 3). In addition, the Image-Pro Plus scanning also revealed rather consistent results (Table 4), implicating the effective hepatoprotective bioactivity of CC-700C.

4. Discussion

Results indicated that the CC-700C was able to suppress the hepatic toxicity of CCl_4. As can be seen it significantly enhanced the antioxidative constituent GSH and stimulated the antioxidant enzymes activities like catalase, GSH-Px, GSH-Rd and SOD.

Chang et al. [14] demonstrated that Cordyceps significantly retarded the body weight loss. And consistent with Liu and Shen [15], we confirmed that CC-700C retarded the swelling of liver, spleen, and kidney caused by CCl_4 intoxification.

In blood coagulating pathway, CCl_4 was reported to decrease platelet count and to relapse the coagulation time [16]. Literally, Sakamoto et al. [17] and Filimonov et al. [18] demonstrated CCl_4 intoxification decreased platelet count and simultaneously suppressed a huge number of immuno-functions. Regarding this, CC-700C showed a potential platelet count elevating and prothrombin time prolonging capability. Moreover, *Cordyceps* polysaccharides had been shown to accelerate the maturity and to activate human mononuclear cells (HMNC) in parallel to upregulation of IL-1, IFN-γ, and TNF-α in the Kupffer cells. Oral administration of *Cordyceps* aqueous extract activated Kupffer cell [4,19]. As seen, normal body weight was retained on administration of *Cordyceps*, indicating the nontoxic nature of *Cordyceps*. Clinically, it is often prescribed for enhancing the immunoactivity in order to retard tumor metastasis [20,21].

Apparently CCl_4 reduced the level of hepatic GSH. Szymonik-Lesiuk et al. [22] reported CCl_4-elevated oxidative stress damaged tissues and cells [23]. Such a mechanism has been also confirmed by us. The antioxidative enzymes in rats apparently were seen activated or induced by CC-700C. And the detrimental effects exerted by CCl_4 toxicity on catalase, SOD, GSH-PX, and GSH-Rd [22] and bilirubin synthesis [24,25] all were effectively recovered or improved. Moreover, liver fibrosis was found to be significantly suppressed by CC-700C due to the down-regulation of collagen synthesis.

Cohen [26] had proposed a treatment protocol for hepatitis C with a combined therapy of *Cordyceps sinensis*, a Chinese traditional herbal therapy. He pointed out such a combined

Table 5
GSH and some representative anti-oxidative enzyme levels in rat liver post CCl_4 damage in different treatment groups for a duration of 6 weeks.

Group	Parameters, (U/mg protein)				
	GSH	Catalase	GSH-Px	SOD	GSH-Rd
Control	4.7 ± 0.3^{c}	1286 ± 45^{a}	8.0 ± 0.2^{d}	12.7 ± 0.1^{a}	81.7 ± 1.8^{d}
CCL_4	3.6 ± 0.2^{a}	961 ± 48^{c}	5.3 ± 0.2^{a}	4.0 ± 0.4^{a}	69.5 ± 0.5^{a}
CCL_4 + L-TCM	4.6 ± 0.2^{c}	$1245 \pm 13^{a,b}$	7.5 ± 0.1^{c}	8.8 ± 0.2^{d}	$75.8 \pm 1.8^{b,c}$
CCL_4 + H-TCM	4.6 ± 0.1^{c}	$1242.6 \pm 18^{a,b}$	6.2 ± 0.2^{b}	8.3 ± 0.2^{c}	76.5 ± 0.5^{c}
CCL_4 + Silymarin	4.1 ± 0.1^{b}	120.3 ± 55^{b}	7.3 ± 0.1^{c}	7.2 ± 0.2^{b}	74.5 ± 1.4^{b}

1. *TCM: compound TCM-700 (CC-700C). GSH: glutathione; GSH-Px: glutathione peroxidase; SOD: superoxide dismutase; GSH-Rd: glutathione reductase.
2. Results expressed as means ± SD ($n = 6$).
3. Values in the same column with different superscripts are significantly different from each other ($p < 0.05$).
4. Rat group CCl_4 + L-TCM was fed low dose of CC-700C (286.2 mg/kg bw/day). And group CCl_4 + H-TCM was fed high dose of CC-700C (2862 mg/kg bw/day).

therapy could be more beneficial with better outcome. The macrophages and complement system were all activated by administration of *Cordyceps* [13,27]. In addition, much of the *in vitro* studies also demonstrated that cancer cell differentiation was significantly inhibited [28,29].

Alternatively, *Cordyceps* mycelia are effective activator for glucokinase and glucose-6-phosphate dehydrogenase [30]. It also possesses rather potent peroxide-eliminating [31] and free radical scavenging capabilities [32]. Zhou et al [27] reported liver function of hepatitis B patients was more improved by eliminating HBV, elevating complement levels and promoting protein metabolism.

As mentioned, CC-700C consisted of *Cordyceps* mycelia, Radix Huan-Chi powder [pulverized Radix Astragali *Astragali membranaceus* (Fisch) Bge.], and zinc gluconate. *Cordyceps* mycelia are able to activate monocytes and macrophages [33], to inhibit proliferation of leukemia U937 cells [4] and to elevate the proportionality of T cells including the ratio of $CD4^{+}/CD8^{+}$ [5]. Huan-Chi is effective in improving immuno-activity, anticancer and anti-aging [6,7]. Zinc ions are required for DNA synthesis, which participate in many physiological and metabolic functions acting as enzyme cofactors. Finally, besides the bioactivity exerted by its precious polysaccharide content, the potent hepatoprotective effect of *Cordyceps* preparations can be partly ascribed to its unique high content of selenium (6.22 ppm) in its mycelia (Appendix A).

5. Conclusion

Compound *Cordyceps* TCM-700C (CC-700C) is capable to retard the pathogenesis of CCl_4-induced hepatic injury and fibrosis in animal model. We attribute these effects to its

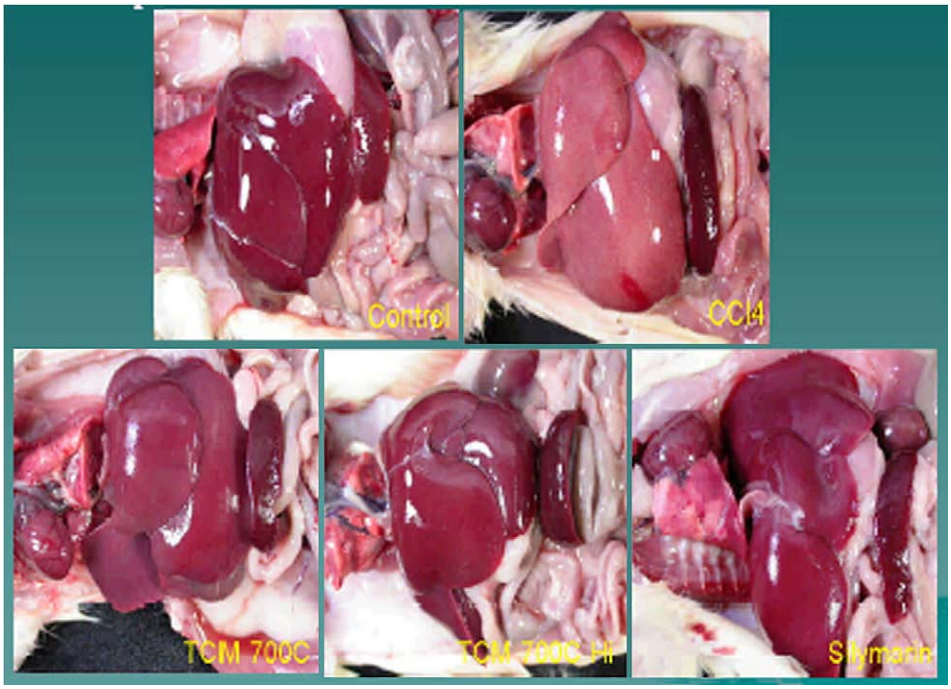

Fig. 1. Gross view of livers with different degree of damages. Upper left: control; upper right: CCl_4-treated; lower left: L-TCM; lower center: H-TCM; lower right: CCl_4 + Silymarin; TCM-700: CC-700C.

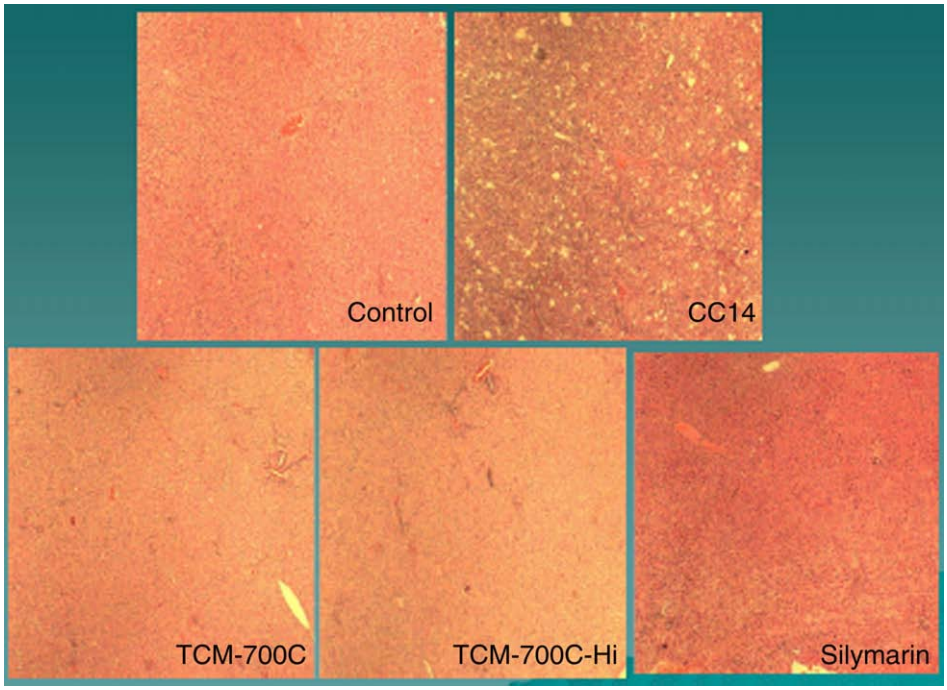

Fig. 2. H.E. stain of liver tissues. Upper left: control; upper right: CCl_4-treated; lower left: L-TCM-700C; lower center: H-TCM-700C; lower right: CCl_4 + Silymarin; TCM-700: CC-700C.

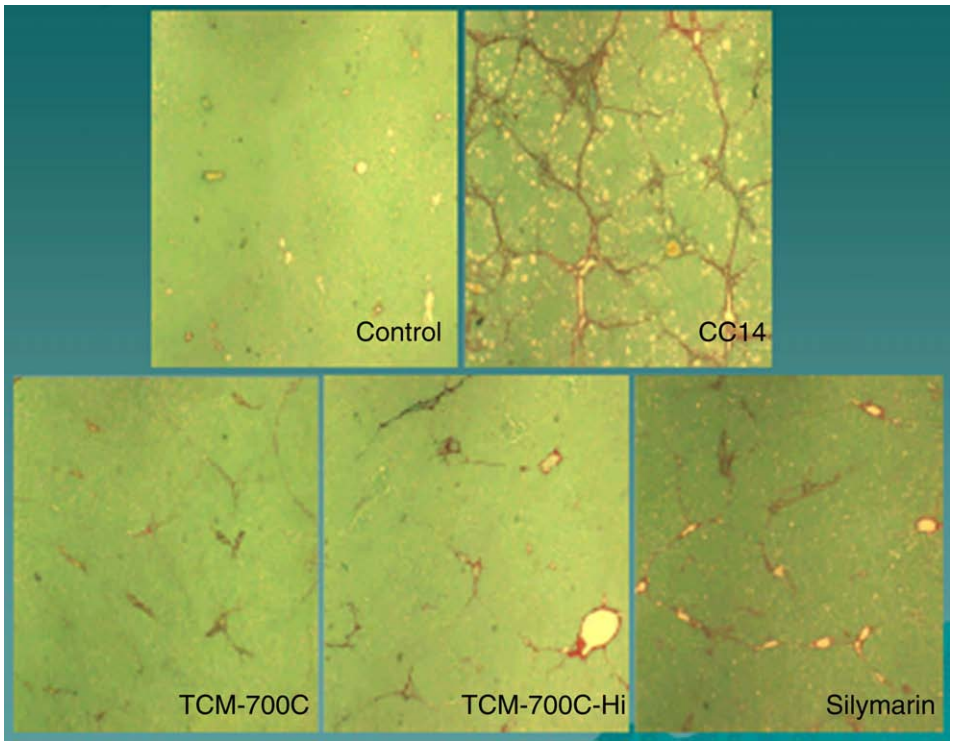

Fig. 3. Liver tissue with different degree of fibrosis. Upper left: control; upper right: CCl_4-treated; lower left: L-TCM-700C; lower center: H-TCM-700C; lower right: CCl_4 + Silymarin; TCM-700: CC-700C.

elevated antioxidative enzyme activity, increased albumin bioactivity, enhanced immuno-biofunctions and its lowered hepatic collagen content to favor fibrosis formation.

Appendix A

Content of heavy metallic ions and proximate compositional analysis of CC-700C.*

Composition	Content	Allowance
Polysaccharides, (%)	6.89 ± 0.35	>5.0%
Metallic ions, (ppm)	–	–
Lead	1.18 ± 0.552	<5.00
Mercury	N.D.	<0.50
Cadmium	0.14 ± 0.074	<0.60
Arsenic	N.D.	<2.00
Copper	5.90 ± 1.757	<150
Selenium	6.22 ± 0.835	Undefined
Zinc	4619 ± 358.6	Undefined
Nickel	5.50 ± 0.573	Undefined
Cobalt	7.70 ± 1.052	Undefined
Ferric + ferrous	1426 ± 453.6	Undefined
Manganese	26.63 ± 3.855	Undefined
Chromium	1.42 ± 0.457	Undefined
Magnesium	1834 ± 387.4	Undefined
Calcium	6369 ± 432.3	Undefined
Silver	N.D.	Undefined
Aluminum	189 ± 46.6	Undefined
Strontium	12.3 ± 2.24	Undefined
Sodium	577.1 ± 65.46	Undefined
Lithium	0.30 ± 0.078	Undefined
Potassium	11540 ± 465.7	Undefined

*Data by Curtsey of TCM Biotech International Corp. (F 7, No. 11, Lane 35, Keehu Rd., Neihu District, Taipei). Data are expressed in Mean ± S.D. ($n = 10$). N.D.: not detectable. Analysis was performed using an Induced Coupled Plasma Emission Spectrometer (ICP-OES). Detection limit: 0.0100 ppm.

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