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Evaluation of four prescriptions of traditional Chinese medicine: Syh-Mo-Yiin, Guizhi-Fuling-Wan, Shieh-Qing-Wan and Syh-Nih-Sann on experimental acute liver damage in rats

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Abstract

Syh-Mo-Yiin (SMY), Guizhi-Fuling-Wan (GFW), Shieh-Qing-Wan (SQW) and Syh-Nih-Sann (SNS) are four prescriptions of Traditional Chinese Medicine (TCM) used in the remedy of liver trouble in various types. The hepatoprotective effects of water extracts of these four recipes against D-galactosamine (D-GalN) and carbon tetrachloride (CCl_4)-induced acute hepatic damage were determined in rats. The results indicated that the serum glutamate-oxalate-transaminase (sGOT) and the serum glutamate-pyruvate-transaminase (sGPT) levels provoked by D-GalN and CCl_4 decreased after treatment with these prescriptions of TCM. Histological changes around portal area (D-GalN-induced hepatotoxicity) and central vein (CCl_4-induced hepatotoxicity) were simultaneously improved by the treatment with TCM mentioned above. © 1997 Elsevier Science Ireland Ltd.

Keywords: Traditional Chinese Medicine (TCM); Hepatoprotective effect; Carbon tetrachloride; D-Galactosamine; sGOT; sGPT

1. Introduction

Traditional Chinese Medicine (TCM) is characterized by the concept of wholism which views the various parts of the human body as an organic whole emphasizing the harmony and coordination of the internal organs with other parts or structures and the unity of the human body with the external environment, as well as by the theory for diagnosis and treatment based on overall analysis of symptoms and signs, the cause, nature and location of the illness and the physical condition

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of the patient (Yang et al., 1990). TCM, which dates back to ancient times, has a unique and profound theoretical system and proved to be useful in the remedy of liver trouble. The efficacy of prescribed medicine was based on personal experience accumulated over thousands of years in China. If more scientific information on the mechanisms of natural products became available, more effective prescriptions of TCM would be possible. Along these lines, studies have been done in this laboratory. In this present work acute liver damages induced by carbon tetrachloride (CCl_4) and D-galactosamine (D-GalN) in rats were conducted to elucidate the hepatoprotective effects of TCM and study the essence of it. Water extracts of four prescriptions of TCM: Syh-Mo-Yiin (SMY, prescription for regulation the flow of Qi), Guizhi-Fuling-Wan (GFW, prescription for treating blood disorders), Shieh-Qing-Wan (SQW, prescription for heat-clearing) and Syh-Nih-Sann (SNS, prescription for mediating), concerning the original references of the well-known Chinese medical works, medicinal herbs and prepared according to different factions of TCM, were used in this study. Silymarin, active constituents of the fruit from milk thistle (*Silybum marianum*, Compositae), which had been used for over 20 years in clinical practice for the treatment of toxic liver diseases (Koch and Loffler, 1985; Messner and Brissot, 1990) was tested simultaneously for comparison.

2. Materials and methods

2.1. Animals

Male Wistar albino rats, 4–6 weeks old were obtained from the National Laboratory Animal Breeding and Research Center, National Science Council, and fed with a standard laboratory diet and tap water ad libitum. The experimental animals were housed in air-conditioned room of 22 ± 3°C, 55 ± 5% humidity, and 12 h of light.

2.2. Prescriptions of Traditional Chinese Medicine and its composition

Crude ingredients of Syh-Mo-Yiin (SMY), Guizhi-Fuling-Wan (GFW), Shieh-Qing-Wan (SQW) and Syh-Nih-Sann (SNS) were purchased from a local herb grocery in Taichung. The herbal

Table 1
Components of Syh-Mo-Yiin (SMY), Guizhi-Fuling-Wan (GFW), Shieh-Qing-Wan (SQW) and Syh-Nih-Sann (SNS)

SMY		
1.	Root of *Panax ginseng* C.A. Meyer (Araliaceae)	3.0 g
2.	Seed of *Areca catechu* L. (Palmae)	3.0 g
3.	Lignum of *Aquilaria agallocha* Roxb. (Euphorbiaceae)	3.0 g
4.	Root of *Lindera strychnifolia* Vill. (Lauraceae)	3.0 g
GFW		
1.	Bark of *Cinnamomum cassia* Presl (Lauraceae)	3.0 g
2.	Carpophores of *Poria cocos* Wolf (Polyporaceae)	3.0 g
3.	Seed of *Prunus persica* Batsch (Rosaceae)	3.0 g
4.	Root of *Paeonia lactiflora* Pall. (Paeoniaceae)	3.0 g
5.	Root bark of *Paeonia suffruticosa* Andr. (Paeoniaceae)	3.0 g
SQW		
1.	Root of *Angelica sinensis* Diels (Umbelliferae)	3.0 g
2.	Root and rhizome of *Gentiana scabra* Bunge (Gentianaceae)	3.0 g
3.	Fruit of *Gardenia jasminoides* Ellis (Rubiaceae)	3.0 g
4.	Root and rhizome of *Rheum palmatum* L. (Polygonaceae)	3.0 g
5.	Rhizome of *Ligusticum chuanxiong* Hort. (Umbelliferae)	3.0 g
6.	Root and rhizome of *Notopterygium incisum* Ting. (Umibelliferae)	3.0 g
7.	Root of *Saposhnikovia divaricata* Schischk. (Umbelliferae)	3.0 g
SNS		
1.	Root of *Bupleurum chinense* DC. (Umbelliferae)	2.8 g
2.	Root of *Paeonia lactiflora* Pall. (Paeoniaceae)	2.8 g
3.	Immature fruit of *Citrus aurantium* L. (Rutaceae)	2.8 g
4.	Root of *Glycyrrhiza uralensis* Fisch. (Leguminosae)	2.8 g

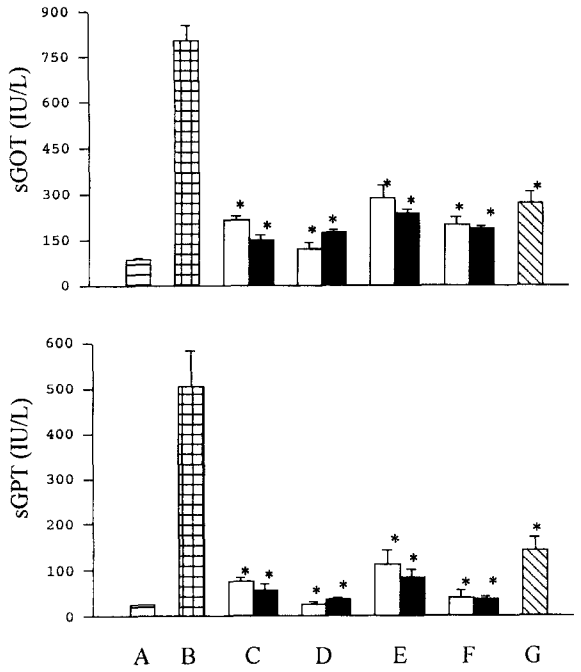

Fig. 1. The hepatoprotective effects of four prescriptions of TCM (SMY, GFW, SQW and SNS) and silymarin on D-GalN-induced hepatitis in rats. (A) Control (saline injection only). (B) D-GalN (400 mg/kg) injection only. (C) SMY + D-GalN. (D) GFW + D-GalN. (E) SQW + D-GalN. (F) SNS + D-GalN. (G) Silymarin (25 mg/kg) + D-GalN. Dose: □, 300 mg/kg; ■, 500 mg/kg for each extract of TCM. Values represent the mean ± S.E.M. of six rats. Statistically significant from bar B $*P < 0.01$ (Student's t-test).

ingredients were authenticated by Dr. Y.S. Chang, Department of Pharmacy, China Medical College Hospital. They are composed of nos. 4, 5, 7 and 4 medical plants as shown in Table 1, respectively.

2.3. Preparation of TCM extract

Four mixtures consisting of milled components in the ratio as stated in Table 1 were prepared. Each recipe was decocted with adequate boiling d-H_2O two times for 1 h. The decoction was filtered, mixed, concentrated and lyophilized. Yields were 12.3%, 15.7%, 11.9% and 12.6% for SMY, GFW, SQW and SNS, respectively, in terms of dried starting materials.

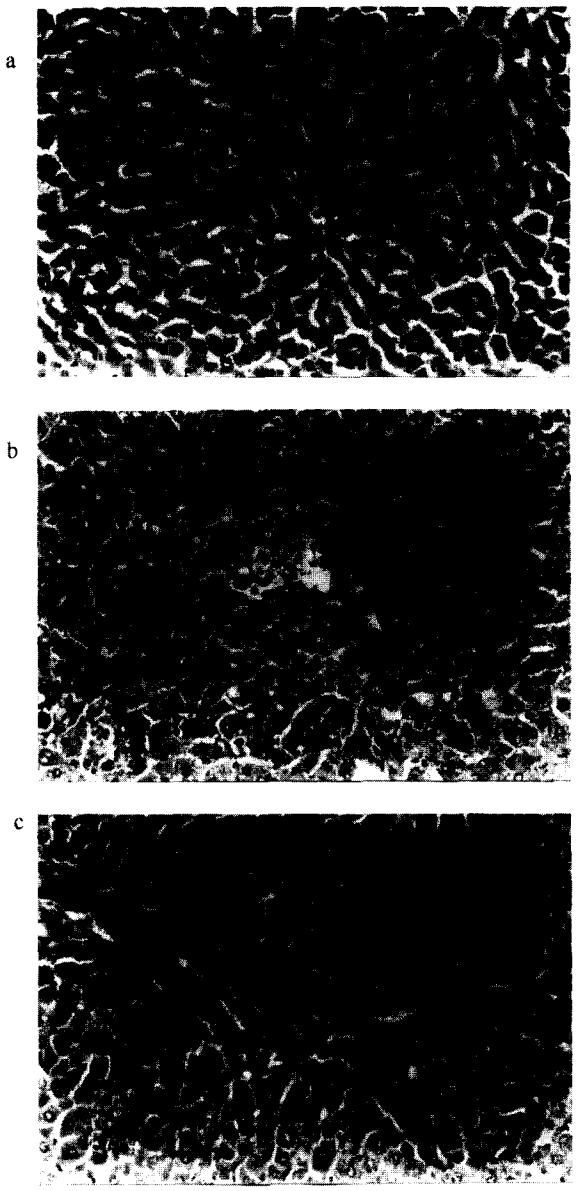

Fig. 2. The photomicrographs of liver section taken from rats who, (a) received saline as a control group; (b) received D-GalN (400 mg/kg). Note that D-GalN induced diffuse focal necrosis, inflammatory infiltration of the periportal area, mitosis and cell proliferation, as well as an increased number of Kupffer cells; (c) D-GalN + silymarin (25 mg/kg) (H-E stain, 176 ×).

2.4. D-GalN-induced hepatotoxicity in rats

The method of acute hepatotoxicity inducement

followed that of our previous reports(Lin et al., 1994b, 1995). Rats were divided into 11 groups of eight animals each. Group A was injected with saline (10 ml/kg, i.p.) and was used as a normal control. Group B was given D-GalN (Sigma Chemical Co., USA) (400 mg/kg in saline, i.p.) as a treated control. The other nine groups were treated with aqueous extracts of one of the four prescriptions of TCM (300 mg/kg and 500 mg/kg in saline, i.p.) or with the reference drug silymarin (G & F Hanse Biopharma) (25 mg/kg in 2% carboxymethyl cellulose, i.p.) 2 h before D-GalN administration.

2.5. CCl_4-induced acute hepatotoxicity in rats

The method was as described previously (Lin et al., 1994a,b, 1995, 1996). Rats were divided into 11 groups of eight animals in each group. Group A was administered with saline (10 ml/kg, i.p.) as a normal control. Group B was given CCl_4/olive oil (1:1, 3 ml/kg, s.c.) (CCl_4, Merk, Darmstadt, Germany). The other nine groups were treated with aqueous extracts of one of the four prescriptions of TCM (300 mg/kg and 500 mg/kg in saline, i.p.) or with the reference drug silymarin (G & F Hanse Biopharma) (25 mg/kg in 2% carboxymethyl cellulose, i.p.) concomitantly with the CCl_4/olive oil administration, two additional injections of herbal extracts and silymarin were given 24 and 48 h later.

2.6. Estimation of serum glutamate-oxalate-transaminase (sGOT) and serum glutamate-pyruvate-transaminase (sGPT) levels

For D-GalN-induced hepatotoxicity, the animals were killed and blood was withdrawn from the carotid artery 24 h after D-GalN intoxication. The blood was centrifuged at 3000 revs./min (Kubota 8800 centrifuge, Japan) at 4°C for 10 min to separate the sera. The sGOT and sGPT values were measured according to the method described by Ritman and Frankel (1957). For CCl_4-induced hepatotoxicity, the rats were killed and blood was withdrawn from the carotid artery 72 h after CCl_4 administration. The activities of sGOT and sGPT were measured by the method previously described (Ritman and Frankel, 1957).

2.7. Histopathological observation

After blood draining, liver sections were taken from each lobe of the liver. The tissue was fixed in 10% neutral formalin, dehydrated with different ethanol solutions from 50–100% and embedded in paraffin, then cut into 4–5 μm thick sections, stained with haematoxylin-eosin and observed under a photomicroscope. Morphological changes such as cell necrosis, fatty change, infiltration of lymphocytes and Kupffer cells were observed.

2.8. Statistical analysis

Data were expressed as mean $\pm$ S.E.M. ($n = 6$) and statistically assessed by one-way analysis of variance (ANOVA). The difference between drug treated animal and control groups was evaluated by Student's t-test using the Sigma plot software program. Further analysis, among the drug treated groups, was statistically evaluated by Newman-Keuls' test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Effect on D-GalN-induced hepatotoxicity

Administration of D-GalN (400 mg/kg, i.p.) resulted in a marked increase in liver transaminase activities which was significantly different from those of the control group (Fig. 1: bar B vs. bar A). The sGOT and sGPT activities of the drug treatment groups are summarized in Fig. 1. Treatments by all the extracts of TCM (300 mg/kg, 500 mg/kg) and silymarin (25 mg/kg) significantly reduced the enzyme activities promotion caused by D-GalN-intoxication ($P < 0.01$). By means of Newman-Keuls' test analysis, the result indicated that the effect of GFW was the most potent.

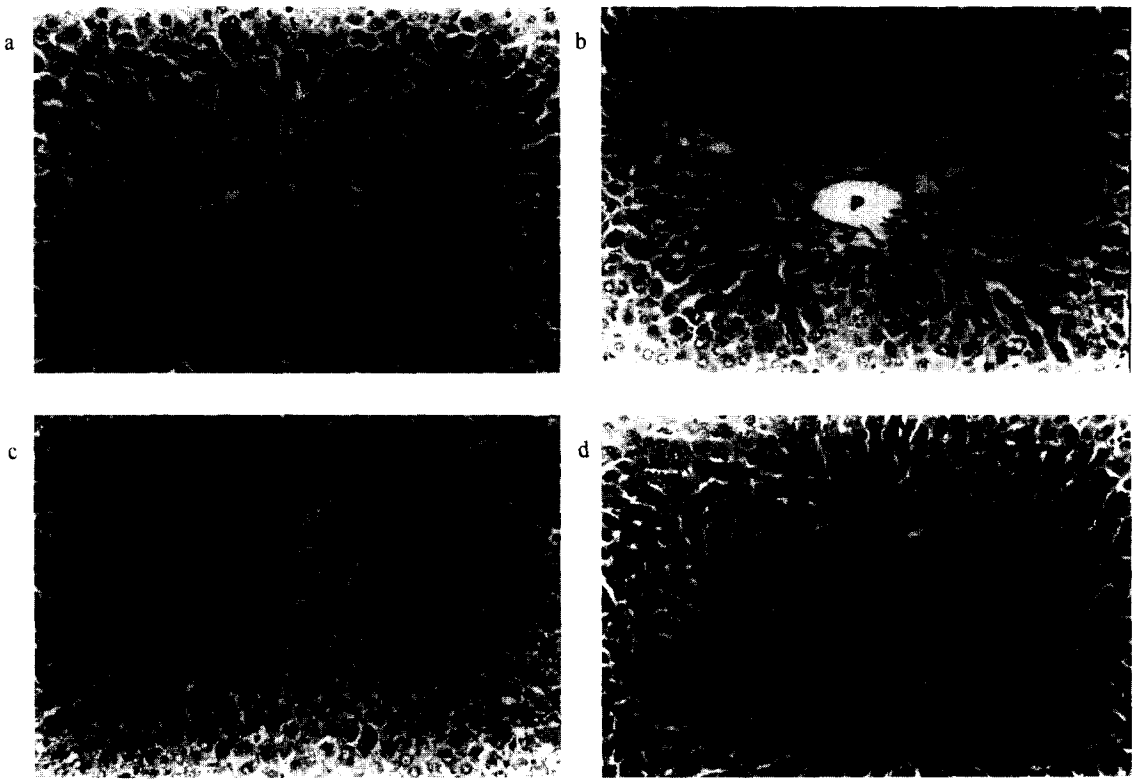

Fig. 3. The photomicrographs of liver section taken from D-GalN + drug treated groups (300 mg/kg). (a) D-GalN + SMY; (b) D-GalN + GFW; (c) D-GalN + SQW; (d) D-GalN + SNS (H-E stain, 176 ×).

The histological observations basically supported the results obtained from serum enzyme assays. Diffuse areas of hepatitis, especially in the periportal areas, focal necrosis and inflammatory infiltration, mitoses and cell proliferation, fatty change, sinusoidal distension and an increased number of Kupffer cells of liver damage were observed after intoxication of D-GalN at a dose of 400 mg/kg i.p. (Fig. 2b). In the reference group, i.e. treatment with silymarin (25 mg/kg), the hepatic architectural pattern, with mild hepatitis, was the same as in the normal control group (Fig. 2c).

Treatment of animals with herbal extracts of TCM reversed, to a large extent, the hepatic lesions produced by D-GalN, as is obvious from the absence of cellular necrosis, fatty accumulation, Kupffer cells and lymphocytes infiltration around the portal area (Figs. 3 and 4).

3.2. Effect on CCl_4-induced hepatotoxicity

Table 2 shows the serum enzyme levels of rats in the TCM-treated and other groups. Administration of CCl_4 (50%, 3 ml/kg) resulted in a marked increase of sGOT and sGPT, and which were significantly different from those of the control group. Treatment of rats with all the extracts of TCM and silymarin exhibited a significant reduction of the biochemical parameters, viz. sGOT and sGPT, induced by CCl_4-intoxication ($P < 0.01$).

The histological changes associated with the hepatoprotective activity in four prescriptions of TCM basically support the estimation of the serum enzymes. The livers of CCl_4-intoxicated rats showed massive fatty change, gross necrosis, broad infiltration of the lymphocytes and Kupffer cells around the central vein, loss of cellular boundary in Fig. 5b. The histological pattern of

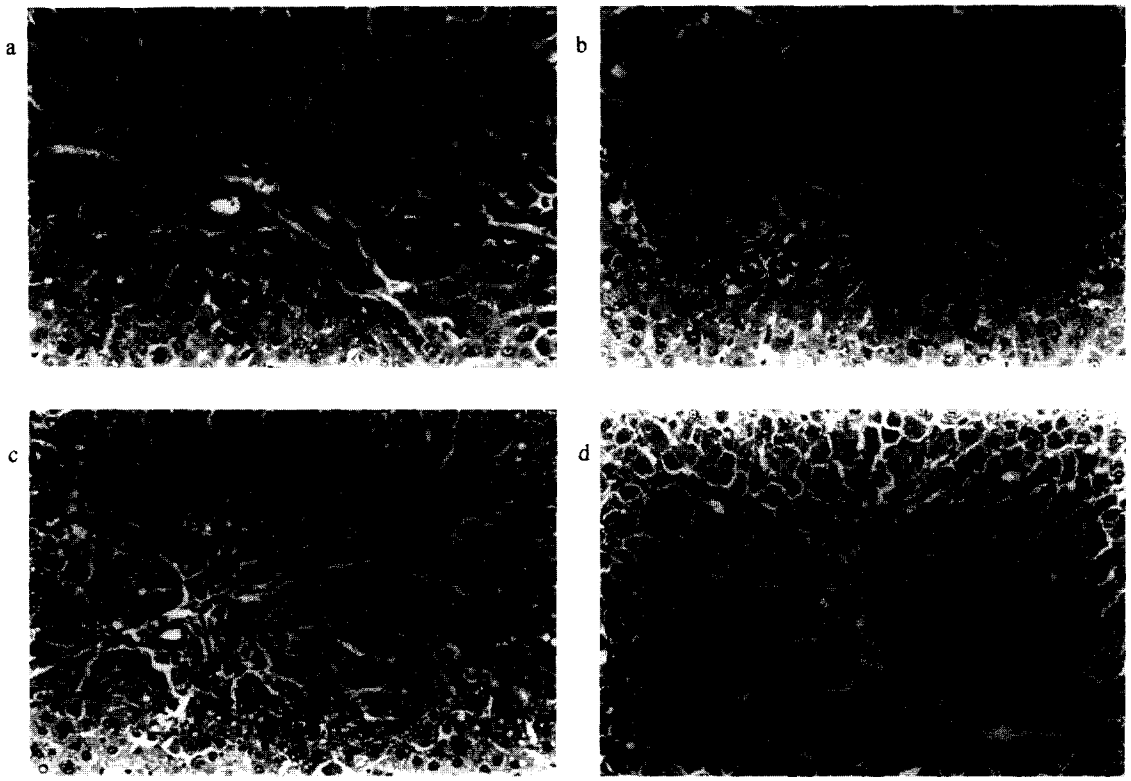

Fig. 4. The photomicrographs of liver section taken from D-GalN + drug treated groups (500 mg/kg). (a) D-GalN + SMY; (b) D-GalN + GFW; (c) D-GalN + SQW; (d) D-GalN + SNS (H-E stain, 176 ×).

the livers of the rats treated with extracts of TCM showed a normal lobular pattern with a mild degree of fatty change, necrosis and lymphocyte infiltration (Figs. 6 and 7).

4. Discussion and conclusion

The characteristics of the clinical practice in TCM is to treat a patient in accordance with an overall differentiation of symptoms and signs, which is accomplished by a sequence of determination of mechanism, application of therapeutic principle, selection of prescription and use of medicaments. The medicaments were developed through clinical practices and proved to be useful for thousands of years in the form of herbal mixtures. Although there are certain principles for the selection of drugs in a prescription, it should vary with the severity of the disease, the age, constitution and living habit of the patient as well as the weather and the environment.

Syh-Mo-Yiin (SMY, prescription for regulation the flow of Qi), was compiled originally by Chen Yan, a physician in the 12th century of the Southern Song Dynasty (1131–1189 AD), Guizhi-Fuling-Wan (GFW, prescription for treating blood disorders) and Syh-Nih-Sann (SNS, prescription for mediating) were compiled by Zhang Zhongjing, an outstanding physician in the Eastern Han Dynasty (150–219 AD). Shieh-Qing-Wan (SQW, prescription for heat-clearing), was compiled by Qian Yi, a distinguished pediatrician of the Northern Song Dynasty (1035–1117 AD). SMY, GFW, SQW and SNS, historically, are considered by traditional medical practitioners to be beneficial in the treatment of various liver disorders (Ou, 1988; Zhang, 1990).

D-GalN and CCl_4 are two well characterized hepatotoxins which mediate their toxic effects

through different modes of action. The toxicity of D-GalN results from inhibition of RNA and protein synthesis in the liver (Decker and Keppler, 1972; Konishi et al., 1974). After administration of D-GalN the main damage is the profound depletion in uridine diphosphate glucose (UDPG), which becomes trapped in the formation of uridindiphosphogalactosamine. Intense galactosamination of the membrane structures is thought to be responsible for loss in the ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium, is considered to be responsible for cell death (Dianzani, 1991). The hepatoprotective effect of medicaments from different aspects were studied in rats with liver injury provoked by D-GalN (Tyutulko et al., 1983; Kato et al., 1984; Yamaura et al., 1985; Ota et al., 1986; Czaja et al., 1994; Lin et al., 1994b, 1995; Thabew et al., 1995). This study demonstrated that the aqueous extract of each TCM prescription (dose: 300 mg/kg and 500 mg/kg) exhibited the best activity in declining sGOT and sGPT levels induced by D-GalN ($P < 0.01$). This result was also confirmed by histological observation (Figs. 2–4). As evident from the results, GFW seems to be a better hepatoprotective agent than the other recipes when compared in a dose-to-dose manner (Fig. 1). The protective action of

Table 2
The hepatoprotective effect of four prescriptions of traditional Chinese medicine (SMY, GFW, SQW and SNS) and silymarin on carbon tetrachloride (CCl_4)-induced hepatitis in rats

Groups	Dose (mg/kg)	sGOT (IU/l)	sGPT (IU/l)
Control		89.0 ± 4.2	31.2 ± 0.6
CCl_4	3 ml/kg	622.7 ± 71.3	259.5 ± 19.4
SMY	300	120.3 ± 14.0*	36.3 ± 4.3*
	500	112.0 ± 4.7*	34.7 ± 2.2*
GFW	300	141.2 ± 9.3*	49.0 ± 4.2*
	500	155.0 ± 12.0*	47.0 ± 6.1*
SQW	300	181.7 ± 25.0*	53.3 ± 9.4*
	500	137.5 ± 9.4*	38.2 ± 1.1*
SNS	300	146.7 ± 15.0*	48.3 ± 4.7*
	500	139.8 ± 16.0*	44.0 ± 6.9*
Silymarin	25	232.5 ± 14.9*	71.2 ± 9.0*

Each value represents mean ± S.E.M. ($n = 6$).
Student's t-test was performed.
*$P < 0.01$ significantly different from CCl_4-intoxicated group.

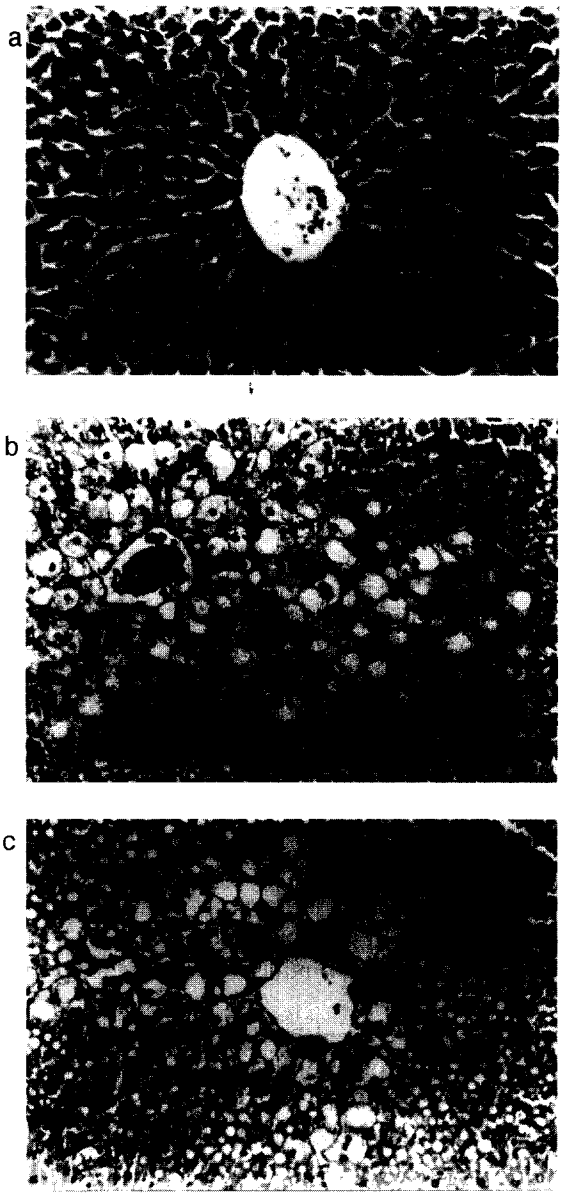

Fig. 5. The photomicrographs of liver section taken from rats who. (a) received saline as a normal control group; (b) received CCl_4/olive oil (1:1, 3 ml/kg). Note that massive fatty change, centrilobular necrosis, ballooning degeneration, infiltrating lymphocytes and loss of cell boundaries are observed. (c) CCl_4 + silymarin (25 mg/kg) (H-E stain, 176 ×).

TCM against D-GalN toxicity is of clinical importance because there is a close resemblance between the multifocal necrosis produced by D-GalN and the lesion of viral hepatitis in

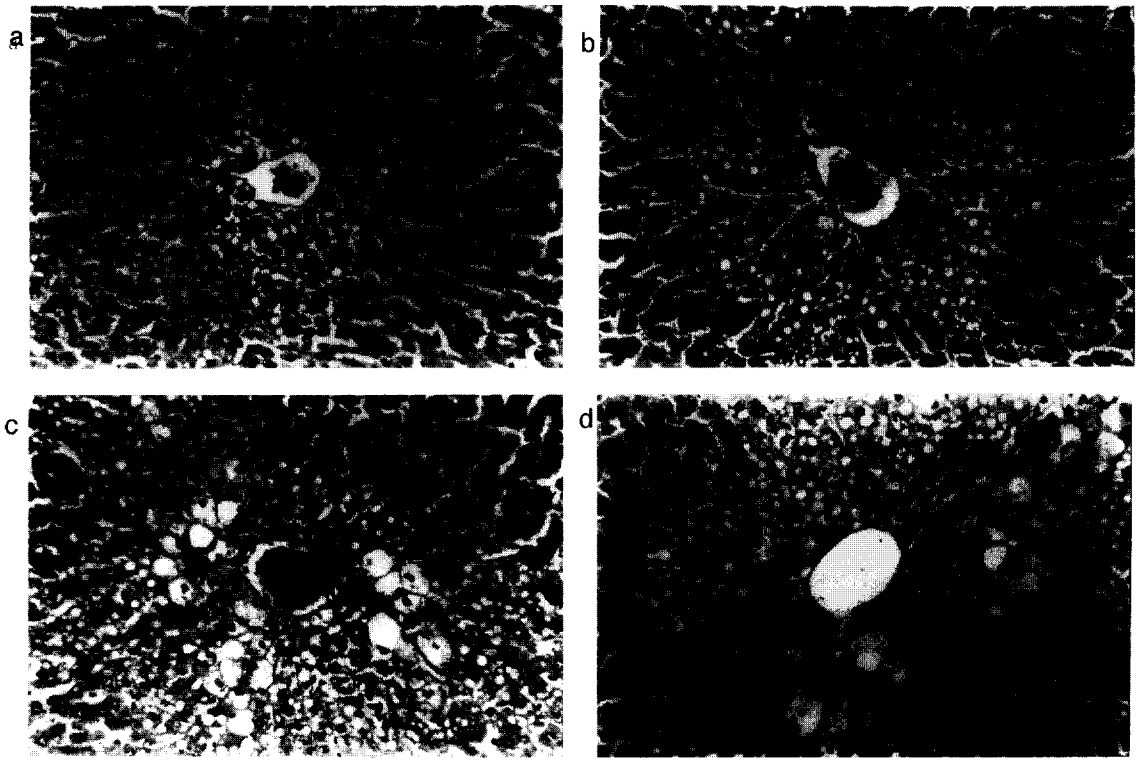

Fig. 6. The photomicrographs of liver section taken from CCl_4 + drug treated groups (300 mg/kg). (a) CCl_4 + SMY; (b) CCl_4 + GFW; (c) CCl_4 + SQW; (d) CCl_4 + SNS (H-E stain, 176 ×).

human (Decker and Keppler, 1972). With the high prevalence of viral hepatitis in Taiwan, elucidating the hepatoprotective activity of TCM by the model of D-GalN-induced liver damage is very meaningful.

CCl_4 is metabolized by the mixed-function oxidase system in the endoplasmic reticulum of the liver. Cleavage of the carbon-chloride bond results in the formation of free trichloromethyl radicals ($CCl_3\cdot$), which are highly unstable and immediately react with membrane components (Recknagel and Glende, 1973). They form covalent bonds with unsaturated fatty acids, or take a hydrogen atom from the unsaturated fatty acids of membrane lipids, resulting in the production of chloroform and lipid radicals. The lipid radicals react with molecular oxygen, which initiates peroxidative decomposition of phospholipids in the endoplasmic reticulum. The peroxidation process results in the release of soluble products that may affect other membranes, such as the cell membrane (Packer et al., 1978). Microsomal oxidation of chloroform was found to involve the formation of phosgene. It is thought that a secondary metabolite causes cell death (Shah et al., 1979). CCl_4-induced necrosis is most severe in the centrilobular hepatocytes (zone 3), as here the concentration of cytochrome P-450 is highest. This study indicated that treatment with herbal extracts of TCM and silymarin appeared to enhance the recovery from the CCl_4-induced hepatotoxicity as judged from the recovery of sGOT and sGPT ($P < 0.01$). This phenomenon was also confirmed by liver biopsy (Figs. 5–7). Further analysis by Newman-Keuls test and histological examination showed that SMY was the most potent of the four recipes (Table 2). Endemic liver disease became one of the ten leading cause of death in Taiwan area for many years (General Health Statistics, 1992). Interest in TCM on hepatic medications has increased in recent years and more investigation into it is warranted.

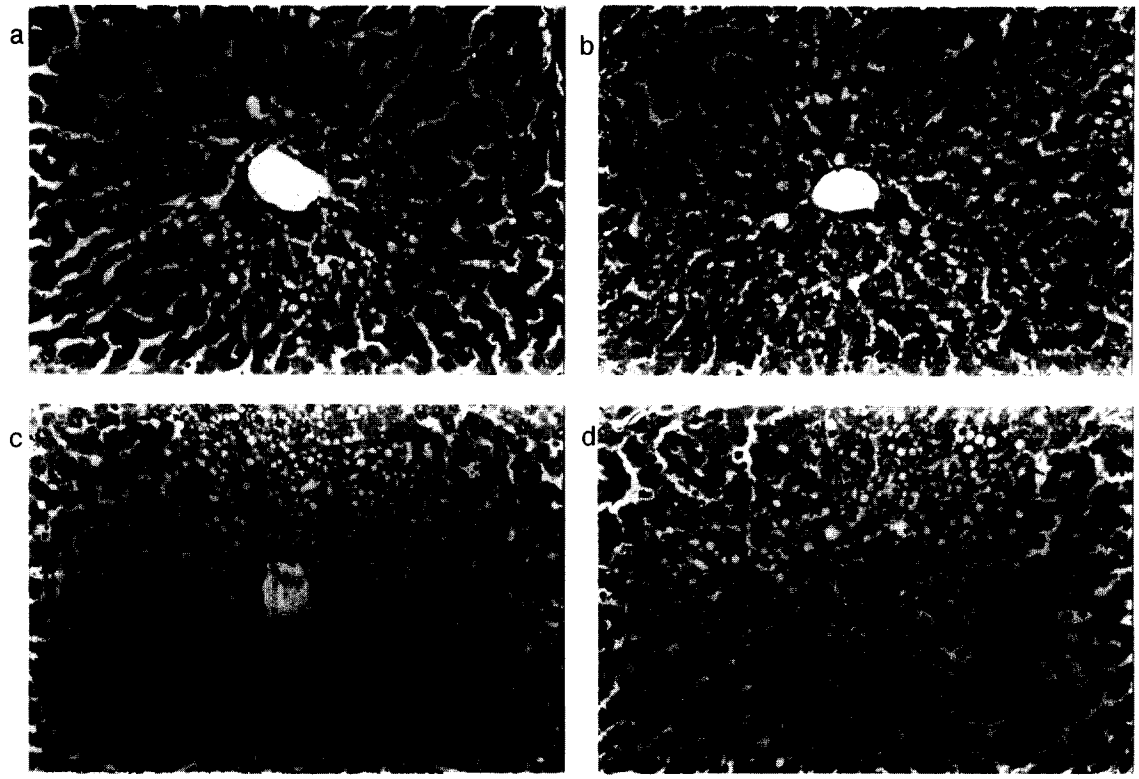

Fig. 7. The photomicrographs of liver section taken from CCl_4 + drug treated groups (500 mg/kg). (a) CCl_4 + SMY; (b) CCl_4 + GFW; (c) CCl_4 + SQW; (d) CCl_4 + SNS (H-E stain, 176 ×).

The present study has demonstrated that aqueous extracts of these TCM recipes protect liver against damage induced by both D-GalN and CCl_4 despite the differences in their mechanisms of injury. These prescriptions of TCM may therefore be able to exert their hepatoprotective effects through more than one mechanism of action. Four recipes shown might have contained a mixture of anti-hepatotoxic ingredients, some of which show preferential protection against certain toxins while others exert their protective activity against other toxins. In the concept of TCM, the healing effect on liver diseases is thought to be caused by the stagnation of pathogenic damp-heat and liver stasis or invasion of the stomach and spleen by hepatic Qi. Patient with liver disease may manifest different syndromes at different phases (Zhang et al., 1993). So the same disease can be treated in different ways and this study rationlizes the traditional use of herbal prescriptions in liver diseases.

Furthermore, protective mechanism not only specific to D-GalN or CCl_4 may be responsible for hepatoprotective activity of these prescriptions. Experiments to extract and identify the active components and mechanisms involved are now in progress.

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