

## Prophylactic effect of four prescriptions of traditional Chinese medicine on $\alpha$ -naphthylisothiocyanate and carbon tetrachloride induced toxicity in rats<sup>1</sup>

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**KEY WORDS** traditional Chinese medicine; 1-naphthylisothiocyanate; cholestasis; carbon tetrachloride; free radicals

### ABSTRACT

**AIM:** To study the prophylactic effects of four Chinese traditional prescriptions against experimental liver injury.

**METHODS:** Liver toxins,  $\alpha$ -naphthylisothiocyanate (ANIT), and carbon tetrachloride (CCl<sub>4</sub>) were used to induce acute liver injury. Simo Yin (SMY), Guizhi Fuling Wan (GFW), Xieqing Wan (XQW), and Sini San (SNS) were fed (500 mg/kg, in saline, *po*) to the rats before toxin administration. All the animals were killed 48 h after toxin insulted. Serum index of liver function and hepatic lipid peroxidation (LPO) were estimated. Histopathological observation was conducted simultaneously. **RESULTS:** The rats treated with ANIT exhibited elevations of serum total bilirubin (TBI), alkaline phosphatase (ALP), glutamate-oxalate-transaminase (GOT), glutamate-pyruvate-transaminase (GPT), as well as cholestasis and parenchyma necrosis. In rats, challenged with ANIT, receiving the pre-treatment of prescriptions of SMY, XQW, and SNS, the biochemical and morphological parameters of liver injury were significantly reduced. The increased LPO level in liver tissue, associated with the provoked serum GOT and GPT levels were the salient features observed in CCl<sub>4</sub>-insulting rats. Pre-treatment of four prescriptions showed

a remarkable protective effect, and also was effective in counteracting the free radical toxicity by bringing about a significant decrease in peroxidative level. **CONCLUSION:** These recipes ameliorate liver damage induced by both ANIT and CCl<sub>4</sub> despite the differences in their mechanisms of injury. Therefore they may be able to exert hepatoprotective effects through more than one mechanism of action because they contained a mixture of anti-hepatotoxic ingredients with mutual reinforcement and assistance.

### INTRODUCTION

Traditional Chinese medicine (TCM) is characterized by the concept of holism 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 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 of the patient<sup>(1)</sup>. TCM, which dates back to ancient times, has a unique and profound theoretical system and is 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.

Simo Yin, Guizhi Fuling Wan, Xieqing Wan, and Sini San are four prescriptions of TCM used in the remedy of liver trouble in various types. We previously reported that intraperitoneal injection of aqua extracts of these four prescriptions, prevented acute liver damages

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induced by CCl<sub>4</sub> and *D*-galactosamine (*D*-GalN) in rats<sup>[2]</sup>. Hence, interest has centered on the hepatoprotective effect of TCM recipes by oral administration. The present study was undertaken to determine whether these four prescriptions could protect liver from ANIT insult. In order to further clarify the hepatoprotective effects of TCM on CCl<sub>4</sub>-induced liver injury, we examined the effect of pre-oral TCM administration on the change of hepatic lipid peroxidative metabolism in rats intoxicated once with CCl<sub>4</sub>. 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, was tested simultaneously for comparison<sup>[3,5]</sup>.

## MATERIALS AND METHODS

**Chemicals and reagents** Thiobarbituric acid (TBA) and TMP (1, 1, 3, 3-tetramethoxypropane) were obtained from the Sigma Chemical Co, St Louis, MO, USA. Kits code B 8120 for glutamate-pyruvate-transaminase (GPT), B 8110 for glutamate-oxalate-transaminase (GOT), B 7917 for alkaline phosphatase (ALP), and B 8055 for total bilirubin (TBI) activity respectively were purchased from Menarini Industrie Farmaceutiche Riunite s r l Divisione Diagnostici (Italy). Silymarin were obtained from Aldrich Chemical

Co (Milwaukee, WI, USA). All other chemicals were of reagent grade and were used without further purification.

**Test 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 °C ± 3 °C, 55 % ± 5 % humidity, and 12 h of light.

**Prescriptions of traditional Chinese medicine and its composition** Crude ingredients of Simo Yin (SMY), Guizhi Fuling Wan (GFW), Xieqing Wan (XQW), and Sini San (SNS) were purchased from a local herb grocery in Taichung. The herbal ingredients were authenticated by Dr CHANG YS, Department of Pharmacy, China Medical College Hospital. They are composed of 4, 5, 7, and 4 medical plants as shown in Tab 1, respectively.

**Preparation of TCM extract** Four mixtures consisting of milled components in the ratio as stated in Tab 1 were prepared. Each recipe was decocted with adequate boiling dH<sub>2</sub>O 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, XQW, and SNS, respectively, in terms of dried starting materials.

Tab 1. Components of Simo Yin (SMY), Guizhi Fuling Wan (GFW), Xieqing Wan (XQW), and Sini San (SNS).

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

### $\alpha$ -Naphthylisothiocyanate (ANIT)-induced hyperbilirubinemia and cholangitis in rats

The method of cholestasis inducement followed that of our previous reports<sup>[3]</sup>. Rats (average weight 200 g) were stratified by weight and allotted into 7 groups randomly. Initially ANIT (100 mg/kg, in olive oil) was administered to animals of the first 6 groups by gastric intubation. In the 7th group was received olive oil (10 mL/kg, *po*) and was used as a vehicle control. Aqueous extracts of TCM (500 mg/kg, in saline, *po*) and the reference drug silymarin (25 mg/kg in 2 % carboxymethyl cellulose, *po*) were fed to 5 groups 6 h, 24 h, and 42 h before ANIT administration, while the remaining two groups, ie, one with ANIT and other vehicle treated, were given no treatment. All the animals were killed and the blood was withdrawn from the carotid artery 48 h after ANIT insulting and serum separated for different estimations.

**CCl<sub>4</sub>-induced hepatotoxicity in rats** The protocol adopted in the case of pre-treatment studies was the same as in ANIT-induced hyperbilirubinemia and cholangitis mentioned above. CCl<sub>4</sub>, replaced as a toxicant, was injected (*ip*) in a dose of 1.5 mL/kg body weight as a 20 % olive oil solution.

**Assessment of liver functions** At the end of the experimental period, the animals were killed and blood was withdrawn from the carotid artery. The blood was centrifuged using the centrifuge (KUBOTA 8800, Japan), at 4 °C for 10 min to separate the sera. For ANIT-induced hyperbilirubinemia and cholangitis, the activities of TBI, ALP, GOT, and GPT were measured, using a clinical test kit, spectrophotometrically on a "Home Screen" chemistry analyzer system (ARTAX<sup>+</sup>). For CCl<sub>4</sub>-induced acute hepatotoxicity, the values of GOT and GPT were measured on the same apparatus.

**Measurement of hepatic lipid peroxidation (LPO) in CCl<sub>4</sub>-induced liver injury** At the end of the experimental period, the liver tissue was surgically removed after the animals were killed and blood was collected for biochemical assay. The isolated livers were washed in ice-cold KCl 0.15 mol/L, blotted on a filter, then were weighed and frozen in liquid nitrogen as soon as possible. After above, the livers were stored at -80 °C until used for the following assay.

The liver samples were homogenized in Tris-HCl buffer (0.01 mol/L, pH 7.4) using a homogenizer to give a 10 % homogenate. Lipid peroxidation was quantified by measuring thiobarbituric acid reactive

substance by the spectrophotometric assay (on a Beckman model DU<sup>+</sup> 650 spectrophotometer) described by Ohkawa *et al.*<sup>[6]</sup>. The level of lipid peroxides was expressed in terms of nanomoles of malondialdehyde (MDA) per milligram of protein, which was calculated from the absorbance at 532 nm using TMP as an external standard. The protein was also simultaneously assayed by the method of Lowry *et al.*<sup>[7]</sup>, which correlated with the A<sub>230</sub> curve.

**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  $\mu$ m thick sections, stained with haematoxylin-eosin and observed under a photomicroscope.

**Statistical analysis** Data were express as  $\bar{x} \pm s_x$  ( $n=6$ ) 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.

## RESULTS

**Effect on ANIT-induced hyperbilirubinemia and cholangitis in rats** Administration of ANIT (100 mg/kg) resulted in a marked increase in serum bilirubin, alkaline phosphatase, and transaminase activities which were significantly different from those of the vehicle control group (bar B vs bar A in Fig 1 and 2). The levels of TBI and ALP as well as GOT and GPT activities of the drug treatment groups were summarized in Fig 1 and 2. Treatments by the extracts of TCM (500 mg/kg) and silymarin (25 mg/kg) significantly reduced the enzyme activities promotion caused by ANIT-intoxication, except the XQW extract (bar E).

The histological observations basically supported the results obtained from serum enzyme assays. Histologic findings of vehicle control rats was normal (Fig 3A). In Fig 3B, liver slice of ANIT-treated rats, the lesions were characterized by multifocal, moderate portal edema and the infiltration of numerous inflammatory cells into portal triads. Bile ductule epithelial cells were swollen, vacuolated, and had foci of cell necrosis as well as

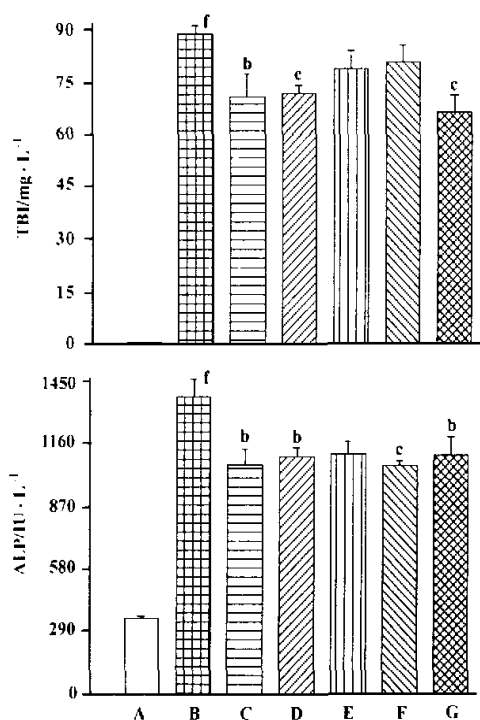


Fig 1. The prophylactic effect of TCM prescriptions (SMY, GFW, XQW, and SNS) and silymarin on TBI and ALP in ANIT-insult rats. Bar A: vehicle control; Bar B: ANIT/olive oil; Bar C: SMY + ANIT; Bar D: GFW + ANIT; Bar E: XQW + ANIT; Bar F: SNS + ANIT; Bar G: silymarin + ANIT.  $n = 6$ .  $\bar{x} \pm s_x$ . <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs bar B. <sup>f</sup> $P < 0.01$  vs bar A.

increased mitotic activity. In the surrounding liver parenchyma, occasional scattered necrotic hepatocytes and macrophages adjacent to portal tract were seen. Some increase in number of sinusoid lining cells was also noticed. TCM administration in ANIT-insulted animals resulted in a significant antagonism of all morphological alterations due to ANIT (Fig 3C, D, E and F). In the reference group, ie, treatment with silymarin (25 mg/kg), the liver lesions were qualitatively similar but significantly less severe (Fig 3G).

**Effect on CCl<sub>4</sub>-induced hepatotoxicity** Tab 2 shows the serum enzyme levels of rats in the prescriptions of TCM-treated and other groups. Administration of CCl<sub>4</sub> resulted in a marked increase of serum GOT, GPT, and hepatic LPO content, which were significantly different from those of the vehicle control group. Treatment of rats with all the extracts of TCM and silymarin exhibited a significant reduction of the bio-

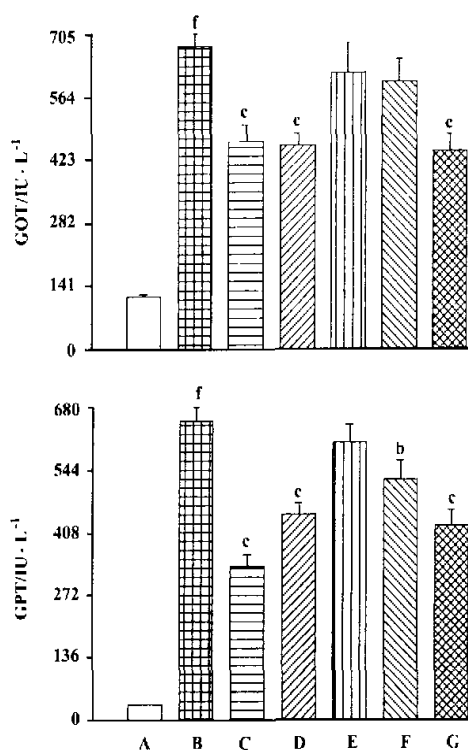


Fig 2. The prophylactic effect of TCM prescriptions (SMY, GFW, XQW, and SNS) and silymarin on GOT and GPT in ANIT-insult rats. Bar A: vehicle control; Bar B: ANIT/olive oil; Bar C: SMY + ANIT; Bar D: GFW + ANIT; Bar E: XQW + ANIT; Bar F: SNS + ANIT; Bar G: silymarin + ANIT.  $n = 6$ .  $\bar{x} \pm s_x$ . <sup>b</sup> $P < 0.05$ , <sup>c</sup> $P < 0.01$  vs bar. <sup>f</sup> $P < 0.01$  vs bar A.

chemical parameters viz. GOT and GPT, as well as the level of LPO, induced by CCl<sub>4</sub>-intoxication.

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<sub>4</sub>-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 4B. The histological pattern of the livers of the rats treated with extracts of TCM showed a mild degree of fatty change, necrosis and lymphocyte infiltration (Fig 4C, D, E, and F).

## DISCUSSION

SMY (prescription for regulation the flow of Qi) was compiled originally by CHEN Yan, a physician in

Tab 2. Effect of pre-oral TCM (SMY, GFW, XQW, and SNS) and silymarin administration on serum enzymes activity and hepatic LPO level in CCl<sub>4</sub> intoxication rats. n = 6.  $\bar{x} \pm s_x$ . \*P < 0.01 vs control group. †P < 0.01 vs CCl<sub>4</sub>-intoxicated group.

Group	Dose/mg·kg <sup>-1</sup>	GOT/IU·L <sup>-1</sup>	GPT/IU·L <sup>-1</sup>	LPO <sup>1)</sup>
Control	—	89.8 ± 2.2	28.1 ± 0.3	2.6 ± 0.6
CCl <sub>4</sub> <sup>2)</sup>	1.5 ml/kg (ip)	422 ± 24 <sup>c</sup>	152 ± 16 <sup>c</sup>	7.1 ± 1.0 <sup>c</sup>
SMY + CCl <sub>4</sub>	500 mg/kg (po)	269 ± 16 <sup>e</sup>	119 ± 16	3.0 ± 0.8 <sup>e</sup>
GFW + CCl <sub>4</sub>	500 mg/kg (po)	329 ± 15 <sup>f</sup>	112 ± 12	4.0 ± 1.4 <sup>f</sup>
XQW + CCl <sub>4</sub>	500 mg/kg (po)	294 ± 17 <sup>f</sup>	91 ± 8 <sup>f</sup>	2.9 ± 1.3 <sup>f</sup>
SNS + CCl <sub>4</sub>	500 mg/kg (po)	228 ± 21 <sup>f</sup>	71 ± 11 <sup>f</sup>	3.1 ± 0.6 <sup>f</sup>
Silymarin + CCl <sub>4</sub>	25 mg/kg (po)	292 ± 17 <sup>f</sup>	93 ± 7 <sup>f</sup>	2.8 ± 1.5 <sup>f</sup>

1) n moles of MDA/mg protein. 2) CCl<sub>4</sub> in olive oil (1/4, v/v).

the 12th century of the Southern Song Dynasty (1131-1189 AD). GFW (prescription for treating blood disorders) and SNS (prescription for mediating) were compiled by ZHANG Zhong-Jing, an outstanding physician in the Eastern Han Dynasty (150 – 219 AD). XQW (prescription for heat-clearing) was compiled by QIAN Yi, a distinguished pediatrician of the Northern Song Dynasty (1035 – 1117 AD). SMY, GFW, XQW, and SNS, historically, are considered by traditional medical practitioners to be beneficial in the treatment of various liver disorders<sup>(8,9)</sup>.

The characteristics of the clinical practice in TCM are 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, living habit of the patient, as well as the weather and the environment.

ANIT is metabolized in the liver, which produced cholestasis and cytotoxicity<sup>(10)</sup>. The rise of serum bilirubin indicates obstruction to the bile flow. Another useful biochemical index of bile duct damage is leakage of enzymes localized to bile duct, particularly alkaline phosphatase<sup>(11)</sup>. It has been shown that clinical and experimental conditions affecting the biliary passages are frequently accompanied by elevated serum GOT and GPT levels. Another factor of high GOT and GPT levels may be due to their entrance into the blood stream in large amounts from stores in the damaged liver cells<sup>(12)</sup>. In

this study, the liver of rats exposed to ANIT reveals diffuse alteration of intrahepatic bile ducts, edema of bile ductules, periductular inflammation, as well as occasional mild hepatic necrosis. These morphological changes were accompanied by elevation in bilirubin, GOT and GPT, as described by Goldfarb *et al*<sup>(13)</sup>. In rats receiving TCM before ANIT-intoxication, biochemical and morphological parameters of liver injury were reduced to some extent significantly. Further analysis by Newman-Keuls test and histological examination showed that GFW was the most potent of the four recipes. These results demonstrated that TCM therapy showed a protective effect on ANIT-induced liver damage in rats.

CCl<sub>4</sub> 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<sub>3</sub>·), which are highly unstable and immediately react with membrane components<sup>(14)</sup>. 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<sup>(15)</sup>. Microsomal oxidation of chloroform was found to involve the formation of phosgene. It is thought that a secondary metabolite caused cell death<sup>(16)</sup>. CCl<sub>4</sub>-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<sub>4</sub>-induced hepatotoxicity as judged from the

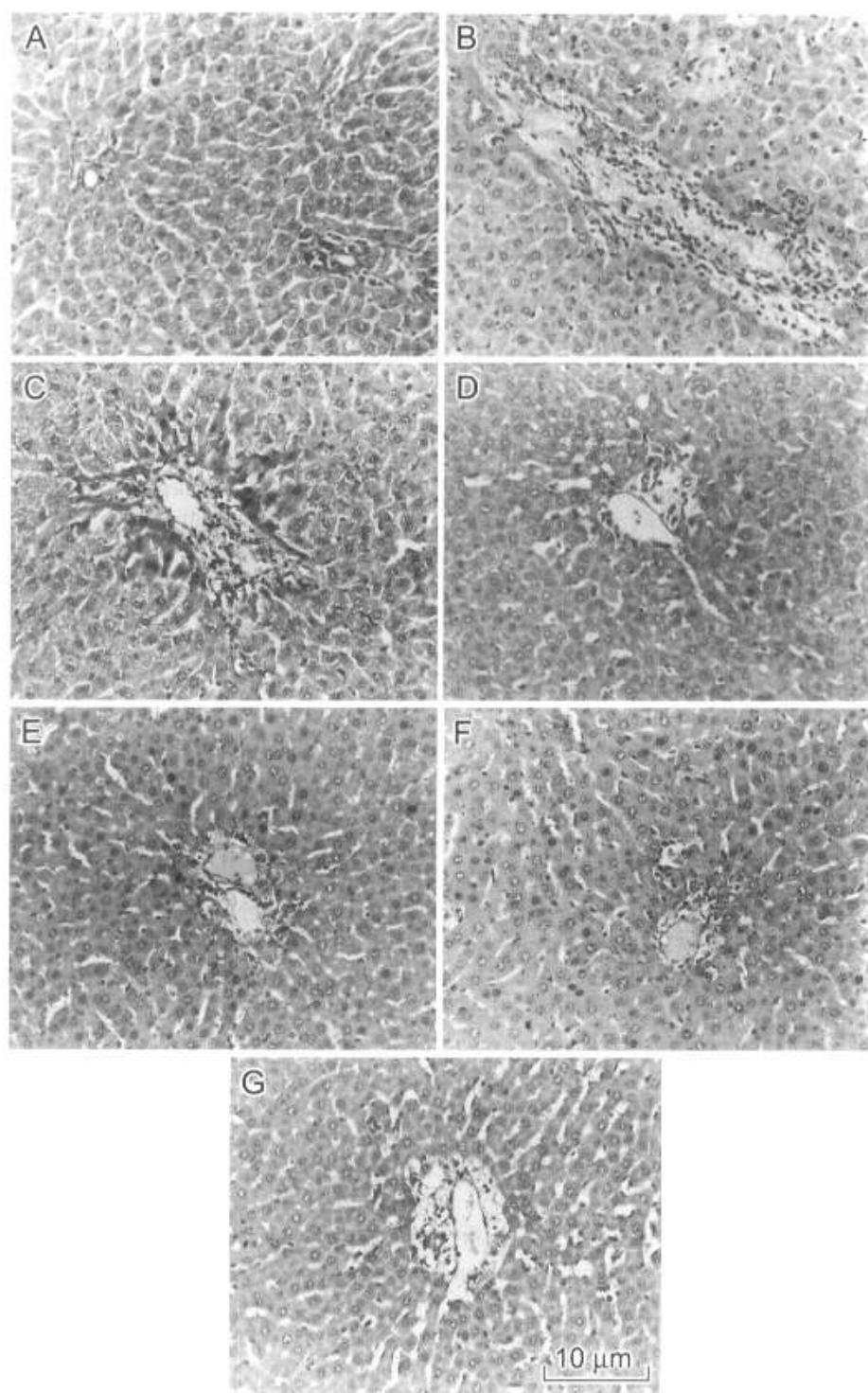


Fig 3. The photomicrographs of liver section taken from rats. A: vehicle control; B: received ANIT/olive oil. Note that edematous change in portal triads and periductular inflammation as well as hepatic necrosis are observed; C: SMY + ANIT; D: GFW + ANIT; E: XQW + ANIT; F: SNS + ANIT; G: silymarin + ANIT. HE stain,  $\times 1800$ .

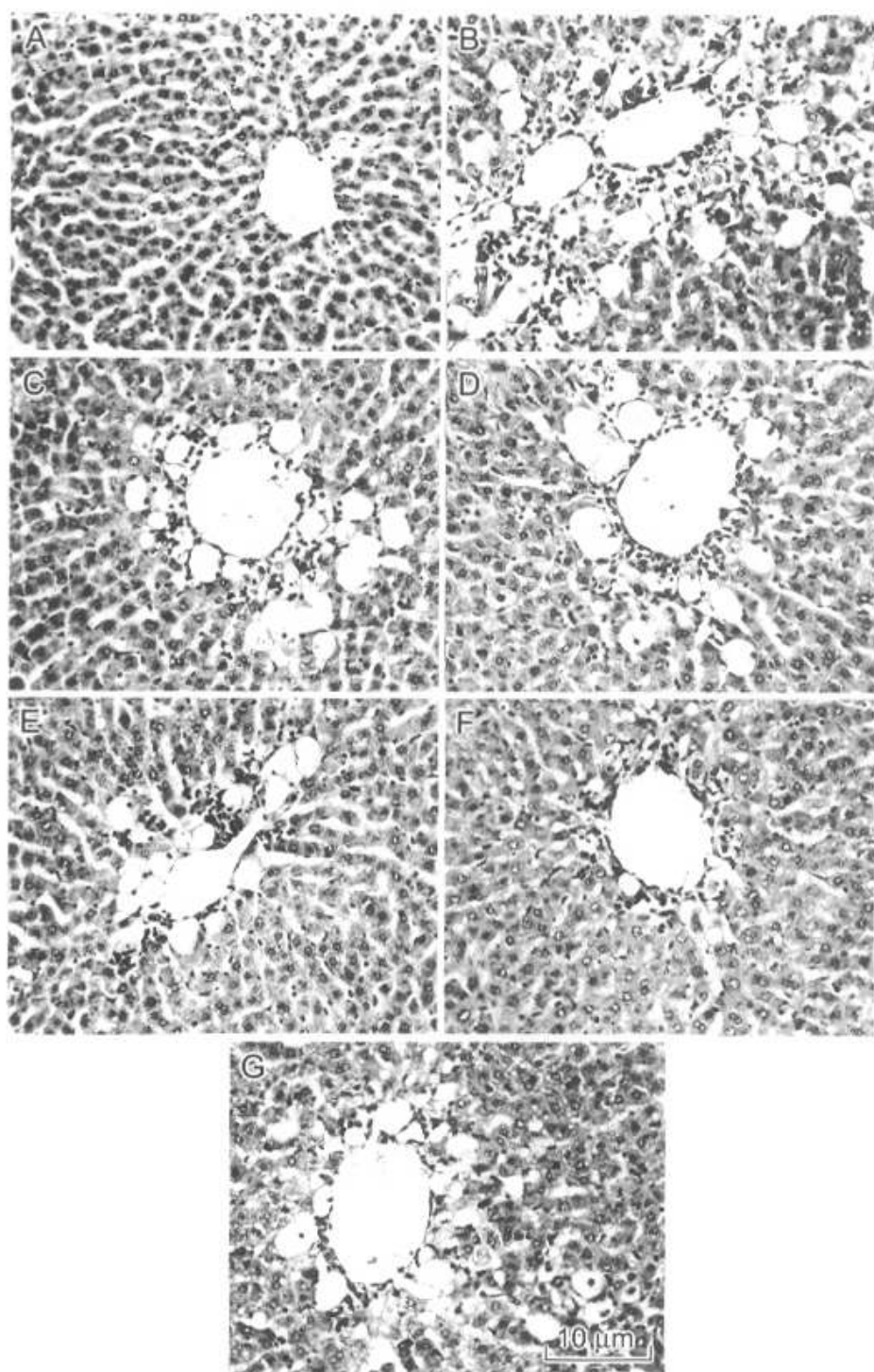


Fig 4. The photomicrographs of liver section taken from rats. A: vehicle control; B: received CCl<sub>4</sub>/olive oil. Note that massive fatty change, centrilobular necrosis, ballooning degeneration, infiltrating lymphocytes and loss of cell boundaries are observed; C: SMY + CCl<sub>4</sub>; D: GFW + CCl<sub>4</sub>; E: XQW + CCl<sub>4</sub>; F: SNS + CCl<sub>4</sub>; G: silymarin + CCl<sub>4</sub>. HE stain, × 1800.

recovery of GOT, GPT, and hepatic LPO content. This phenomenon was also confirmed by liver biopsy (Fig 4). Further analysis by Newman-Keuls test and histological examination showed that SNS was the most potent of the four recipes. There was positive correlation between liver peroxidation and the activities of serum transaminase in SMY, GFW, XQW and silymarin whereas negative for SNS.

Endemic liver disease became one of the ten leading cause of death in Taiwan area for many years<sup>[7]</sup>. Interest in TCM on hepatic medications has increased in recent years and more investigation to it is warranted.

The present study has demonstrated that aqueous extracts of these TCM recipes ameliorated liver damage induced by both ANIT and CCl<sub>4</sub> 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 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.

TCM has been through all the ages attaching great importance to prevention. As early as over two thousand years ago, the theory of "preventive treatment of a disease" was put forward in the "Yellow Emperor's Canon of Internal Medicine". It refers to taking various precautions against the possible occurrence of a disease. As mentioned above, the occurrence of a disease is due to both deficiency of the vital-qi and dysfunction of the human body and the pathological damages to the body caused by pathogenic factors<sup>[8]</sup>. This study rationalizes the native prophylactic use of herbal prescriptions in liver diseases.

Furthermore, protective mechanism not only specific to ANIT or CCl<sub>4</sub> 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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## 中医四方剂对异硫氰酸 $\alpha$ -萘酯及四氯化碳诱发的大鼠肝损伤的预防作用<sup>1</sup>

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**关键词** 中国传统医学; 异硫氰酸 1-萘酯; 胆汁郁积; 四氯化碳; 自由基

**目的:** 探讨四种中医方剂对实验性肝损伤的作用。

**方法:** 事先给予四磨饮(SMY)、桂枝茯苓丸(GFW)、泻青丸(XQW)及四逆散(SNS)的水提取物, 对异硫

氰酸  $\alpha$ -萘酯(ANIT)、四氯化碳( $\text{CCl}_4$ )诱发的肝损伤, 进行预防保肝评估, 包括生化指标、肝组织脂质过氧化及组织病理。结果: 大鼠给予 ANIT 后, 总胆红素(TBI)、碱性磷酸酶(ALP)、谷草转氨酶(GOT)、谷丙转氨酶(GPT)皆有明显升高, 组织病理显示胆道郁阻及局部坏死。四磨饮、泻青丸及四逆散在生化指标及病理观察上皆有显著的预防效果。在四氯化碳诱发的肝损伤实验中, 四方剂对肝功能生化指标与肝组织脂质过氧化及病理变化等, 皆显示出预防效果。结论: 虽然异硫氰酸  $\alpha$ -萘酯、四氯化碳的肝损伤机制不同, 但此四方都有不同程度的保肝作用, 且其作用是由多重机制来完成的。方剂处方均由君臣佐使的配合来设计, 显示对不同毒物的多重保护作用。

(责任编辑 韩向晖)