Tibetan Medicine Shi-Wei-Gan-Ning-San Alleviates Carbon Tetrachloride-Induced Chronic Liver Injury by Inhibiting TGF-β1 in Wistar Rats

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Background. Shi-Wei-Gan-Ning-San (SWGNS) is a classic Tibetan prescription, which has obvious clinical effects in the treatment of viral hepatitis, fatty liver, liver fibrosis, liver cirrhosis, liver cancer, and other liver injuries. However, animal studies and mechanism studies are still lacking. This study aimed to investigate its hepatoprotective efficacy and pharmacological mechanism in animal experiments.

Methods. Chronic liver injury was induced by oral administration of carbon tetrachloride (CCl4) in Wistar rats for 13 weeks. SWGNS was administered orally to rats at doses of 235, 705, and 1410 mg/kg for 13 weeks. Blood samples were collected for biochemical, ELISA, and radioimmunoassay. Livers were harvested for H&E and immunohistochemical staining. The major constituents of SWGNS were analyzed by HPLC. In vitro experiments were used to explore the protective effect of Crocin on BRL-3A in the environment of H2O2.

Results. SWGNS reversed weight loss induced by CCl4. Serum assays showed that SWGNS reduced CCl4-induced alanine aminotransferase, aspartate aminotransferase, total bilirubin, and γ-glutamyltransferase levels and increased the total protein and albumin levels. Histopathological evaluation showed that SWGNS alleviated hepatic steatosis, fibrosis, and inflammation. Furthermore, SWNGS reduced CCl4-induced elevations of TGF-β1, hyaluronic acid, laminin, and collagen IV in serum and reduced the high expression of α-SMA in tissues. Moreover, Crocin I and II are the main components of SWGNS. Crocin attenuated the damaging effects of H2O2 on BRL-3A.

Conclusions. In conclusion, SWGNS alleviated CCl4-induced chronic liver injury by inhibiting the TGF-β1 pathway. This plays an important role in promoting traditional Tibetan medicine in clinical practice.

1. Introduction

The liver is an important metabolic organ in the body. It performs an important role in the metabolism, transformation, and detoxification of various endogenous and exogenous substances. Liver injury and liver diseases are one of the most dangerous diseases that are a threat to human life [1–3]. Traditional Chinese medicine (TCM) has shown a good hepatoprotective effect on various chronic liver diseases clinically [4]. Although, TCM has been used in Eastern medicine for thousands of years; however, there is a lack of scientific evidence data on its therapeutic benefits.

Liver injury can be classified as acute or chronic and is caused by a variety of different pathogens, such as chemicals, drugs, viruses, and toxins. Liver fibrosis, the final common stage of most chronic liver diseases, was once considered incurable. However, this view is changing in recent years, and some early-stage liver fibrosis can be reversed. Liver fibrosis is closely related to chronic liver injury and subsequent inflammatory response [5]. Inflammatory cytokines in the liver drive the activation of hepatic stellate cells (HSCs), the primary cells responsible for liver fibrosis. Transforming growth factor-β1 (TGF-β1) is an important profibrotic mediator that activates HSCs to upregulate the...
expression of hyaluronic acid (HA), laminin (LN), collagen IV (CIV), and α-smooth muscle actin (α-SMA) [6, 7]. In addition, the expression level of extracellular matrix (ECM) has also been considered a specific biomarker of liver fibrosis. Therefore, these factors are considered sensitive indicators of liver injury.

Traditional Tibetan medicine is one of the oldest well-known medical systems with a long history of more than 2000 years [8]. Shi-Wei-Gan-Ning-San (SWGNS) is a traditional Tibetan medicine formulation that has been used for the treatment of liver fibrosis for many years and has shown hepatoprotective effects in clinical applications [9–11]. The formula consists of seven herbs (Carthamus tinctorius, Crocus sativus, Herpetospermum pedunculosum, Meconopsis integrifolia, Draccephalum tangiticum, Saxifraga stolonifera, and Corydalis impatiens) and five mineral medicines (bear gallbladder, Calculus Bovis, Brag-zhun, Calciosinti, and Turquoisis). Although it has been used clinically for many years as an empirical formulation, however, little experimental data are available on its efficacy and underlying mechanisms. The lack of scientific data for SWGNS limits its broad clinical application.

This study aimed to investigate the efficacy of SWGNS on carbon tetrachloride (CCL4)-induced chronic liver injury in Wistar rats and to explore the underlying mechanisms. We hypothesized that SWGNS alleviated CCL4-induced chronic liver injury by reducing TGF-β1 expression. To this end, we collected the livers of treated rats and evaluated the therapeutic effect of SWGNS by serological and histological assays. Furthermore, we also analyzed the composition of SWGNS by HPLC. In vitro experiments were conducted to study the protective effect of Crocin, the main component of SWGNS, on BRL-3A cells under peroxide conditions. This is of great significance for understanding the hepatoprotective effect and pharmacological mechanism of SWGNS, which can provide a data basis for its further research and development.

2. Materials and Methods

2.1. Ethical. This study was performed in the laboratory of the Hubei Provincial Academy of Preventive Medicine. All procedures involving animals were performed according to Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) (Permit No. 2015021). Animals were allowed to acclimate at least for one week before being subjected to experimental procedures. Wistar rats were purchased from the Hubei Experimental Animal Research Center (license number: SCXK (Hubei) 2015–0018). Animals were allowed to acclimate at least for one week before being subjected to experimental procedures. The animals were housed in an IVcage with 40 air exchanges/h. All the cages were placed in a controlled room with temperature (20–25°C), humidity (40%–70%), and light (a 12 h light/dark cycle).

2.2. Reagents. CCL4 was purchased from Aladdin (C131583, Shanghai, China). Peanut oil was obtained from Beijing Bailinway Technology Co., Ltd. (C805618, Beijing, China). Enzyme-linked immunosorbent assay (ELISA) kits for TGF-β1 (EK0514), rabbit anti-α-SMA polyclonal antibody (BM3902), and SABC (rabbit IgG)-AP Kit (SK1052) were purchased from Boster Biological Technology Co., Ltd. (Wuhan, China). HA, LN, and collagen type IV (CIV) radiation immunoassay kit was obtained from Beijing North Biotechnology Research Institute Co., Ltd. (Beijing, China).

2.3. Animals. 78 male Wistar rats aged 4-5 weeks were used for chronic liver injury experiments. Models of CCL4-induced chronic liver injury refer to previous studies [12,13]. The animals were randomly divided into 6 groups of 13 rats each. Group 1 served as a blank control and was administrated with vehicle (0.5% SCC) only. Group 2 served as a CCL4 model group and was also gavaged with 0.5% SCC. Group 3 served as a positive control and was gavaged with bifendate (100 mg/kg/day). Groups 4, 5, and 6 were treated with SWGNS at three different dose levels (235 mg/kg/day, 705 mg/kg/day, and 1410 mg/kg/day, respectively). SCC, bifendate, and SWGNS were treated by oral gavage once daily for 13 weeks. Meanwhile, animals in groups 2–6 were orally gavaged with 1 mL/kg bodyweight of 50% CCL4 peanut oil twice a week for the first 10 weeks and once a week for the last 3 weeks. During week 8, a blood sample was collected from the jugular vein of each animal to monitor biochemistry. After 13 weeks of treatment, the rats were euthanized under phenobarbital, and blood samples and livers were collected for the following tests.

2.4. Serum Biochemical Indicators. To detect liver health, serum samples were collected at 3000 RPM at room temperature. Details are as described in previous studies [14,15]. Biomarkers of hepatic injury, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), total bilirubin (TBIL), and gamma-glutamyl transferase (γ-GGT) were analyzed by an automatic biochemical analyzer (AU-680, Beckmann, USA). Serum TGF-β1 levels were measured using an ELISA kit according to the manufacturer’s protocol. The content of HA, LN, and CIV in serum was measured by GC-1200 gamma radioimmunoassay instrument in Wuhan Zhongnan Hospital.
2.5. Histopathology. Livers were embedded in paraffin and sectioned to 5 μm thickness. Then, slides were stained with hematoxylin–eosin (H&E). Details are as described in the previous studies [16,17]. Histological changes were observed under a microscope (DM2500, Leica, Germany). Hepatocellular degeneration/steatosis, inflammatory infiltration, necrosis, and fibrosis were evaluated as previously described [18–21]. Lesion scores for each type are as follows: steatosis: 0 (−), no steatosis; 1 (+), steatosis ≤25%; 2 (++), steatosis in 25%–50%; 3 (+++), steatosis ≥50%; inflammatory infiltration: 0 (−), no inflammatory cells infiltration; 1 (+), a small amount of inflammatory cells scattered in the hepatic sines, no inflammatory cells in other parts; 2 (++), more inflammatory cells infiltrated in the hepatic lobules and portal areas; 3 (+++), most of the inflammatory cells infiltrated in the hepatic lobules and portal areas; necrosis: 0 (−), no necrotic cells; 1 (+), necrotic cells ≤25%; 2 (++), necrotic cells in 25%–50%; 3 (+++), necrotic cells ≥50%; fibrosis: 0 (−), no fibrosis; 1 (+), slight fibrosis; 2 (++), medium fibrosis, but no pseudolobular formation; 3 (+++), severe fibrosis, visible pseudolobular formation. Total histopathology scores were calculated by summing all scores obtained for each parameter. Liver slides were prepared for immunohistochemical staining with the streptavidin-biotinidase complex (SABC) method according to the manufacturer’s protocol.

2.6. Composition Analysis. The active components of SWGNS were analyzed by high-performance liquid chromatography (HPLC). 1.0 g of SWGNS powder was dissolved in 50 mL of 50% methanol in an ultrasonic waterbath for 20 min, and the filtrate was collected for analysis. All the samples were analyzed on octadecyl chemically bonded phase silica gel using methanol as mobile phase A and 0.1% trifluoroacetic acid in water as mobile phase B. The separation was achieved using the following gradient program: 0–25 min, 55% B; 25–45 min, 55–30% B; 45–50 min, 30–55% B; 50–60 min, 55% B. The flow rate was set at 1.0 mL/min, and the injection volume was 10 μL. The detection wavelength was kept at 440 nm. Standard control stocksolutions containing 30 μg/mL of Crocin I and Crocin II were purchased from China National Institute for Food and Drug Control. Prepare standard control stock solutions containing 30 μg/mL of Crocin I and 12 μg/mL of Crocin II in 50% methanol. The HPLC method for quantitative and qualitative analyses was according to previous literature (Chinese Pharmacopoeia).

2.7. Cell Culture. Rat hepatocyte cell line BRL-3A (CL0376, Fenghui, Changsha, China) was cultured in H-DMEM (C11995500BT, Gibco, USA)+10% FBS (B21001, QuaCell, Zhongshan, China)+1% penicillin-streptomycin (G4003, Servicebio, Wuhan, China) medium at 37°C with 5% CO2. The experiments were divided into four groups: control group, H2O2 group (200 nmol/L), low-dose (1 μg/mL), medium-dose (2 μg/mL), and high-dose (5 μg/mL) treatment groups of Crocin. The control group was cultured normally with DMEM; the H2O2 group was treated with H2O2 for 1 hour and then replaced with DMEM for 24 hours. The Crocin-treated groups were changed to DMEM medium containing Crocin after H2O2 pretreatment. Drug concentrations were used as per previous studies [22,23]. Cell viability was detected with a 96-well plate and CCK-8 kit. ALT, AST, MDA, and SOD were measured with ELISA kits. An instruction manual was followed for the detection method.

2.8. Statistics. All measurement data are expressed as mean ± SEM. Statistics were performed using SPSS 17 (SPSS Science Inc., Chicago, Illinois, USA). Analysis of variance

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### Table 1: The composition of Tibetan medicine SWGNS formula.

<table>
<thead>
<tr>
<th>English name</th>
<th>Chinese name</th>
<th>Source</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carthamus tinctorius</td>
<td>Honghua</td>
<td>Dry flowers of Carthamus tinctorius L</td>
<td>70 g (13%)</td>
</tr>
<tr>
<td>Crocus sativus</td>
<td>Fanhonghua</td>
<td>The dry stigma of Crocus sativus L</td>
<td>15 g (2.8%)</td>
</tr>
<tr>
<td>Herpeotuspermum pedunculosum</td>
<td>Bolengguazi</td>
<td>Dried seeds of Herpeotuspermum pedunculosum (Ser.) Baill</td>
<td>50 g (9.3%)</td>
</tr>
<tr>
<td>Meconopsis integrifolia</td>
<td>Lyronghao</td>
<td>The dried whole grass of Meconopsis integrifolia (Maxim.) Franch, Meconopsis quintupinervia Regel, and Meconopsis lancifolia (Franch.) Franch.</td>
<td>70 g (13%)</td>
</tr>
<tr>
<td>Dracocephalum tanguticum</td>
<td>Ganqingqinglan</td>
<td>The dry aboveground part of Dracocephalum tanguticum Maxim</td>
<td>70 g (13%)</td>
</tr>
<tr>
<td>Saxifraga stolonifera</td>
<td>Lianzuohuercao</td>
<td>Saxifraga pasumensis, a plant of the genus snaill Marq.et Dry whole grass of Saxifraga confertifolia Engl. and Saxifraga candelabrum Franch.</td>
<td>50 g (9.3%)</td>
</tr>
<tr>
<td>Corydalis impatiens</td>
<td>Zijn</td>
<td>The dried whole plant of Corydalis henderonii Hems. (c. nepalesis Kitamura) and c. mucionafera Maxim.</td>
<td>50 g (9.3%)</td>
</tr>
<tr>
<td>Bear gallbladder</td>
<td>Xiongdanfen</td>
<td>The dried product of Selenarctos thibetanus Cuvier, a bear in the family Ursidae, which is drained bile by gallbladder operation</td>
<td>0.5 g (0.093%)</td>
</tr>
<tr>
<td>Calculus Bovis</td>
<td>Niuhuang</td>
<td>Gallstones in the gallbladder of Bos taurus domesticus Gmelin</td>
<td>1.0 g (0.186%)</td>
</tr>
<tr>
<td>Brag-Zhun</td>
<td>Shuhuhua</td>
<td>The cream is made from the dry feces of Trogopterus xanthipes Milne Edwards</td>
<td>50 g (9.3%)</td>
</tr>
<tr>
<td>Calciosinti</td>
<td></td>
<td>Carbonate minerals, mainly containing calcium carbonate (CaCO3)</td>
<td>50 g (9.3%)</td>
</tr>
<tr>
<td>Turquoisis</td>
<td>Songshi</td>
<td>A mineral formed by the precipitation of copper-bearing aqueous solution with alumina-bearing minerals and phosphorus-bearing minerals in fractures under supraege conditions. The main water-bearing phosphate containing copper and aluminum is CuAl6 (PO4)4 (OH)8·4H2O.</td>
<td>60 g (11%)</td>
</tr>
</tbody>
</table>
(ANOVA) was used to compare differences in multiple groups with a post-hoc LSD test. Histopathological scores were analyzed with Kruskal–Wallis and Mann–Whitney tests. Student’s unpaired t-test was used to compare differences between the two groups. \( P < 0.05 \) was considered significant. More significant differences are described in the legends.

### 3. Results

3.1. SWGNS Restored CCl₄-Induced Weight Loss. Bodyweight is a sensitive indicator of animal growth. As shown in Figure 1, compared with the control group, the CCl₄-treated rats showed a significant decrease in bodyweight right after day 12 of dosing (\( P < 0.01 \) or \( P < 0.05 \)). However, SWGNS significantly reduced bodyweight loss in rats. In particular, in the SWGNS-fed group at a dose of 705 mg/kg, bodyweight almost returned to normal levels from day 74. The positive control bifendate had little effect on bodyweight improvement.

#### 3.2. SWGNS Ameliorated CCl₄-Induced Decline in Liver Function. To understand whether SWGNS can improve CCl₄-induced liver injury, the biomarkers of liver injury ALT, AST, TP, ALB, TBIL, and γ-GGT were measured in serum. As shown in Figure 2, compared with the control group, the serum levels of ALT, AST, TBIL, and γ-GGT of CCl₄-treated rats were significantly increased, and the levels of TP and ALB were significantly decreased (\( P < 0.01 \)). However, compared with the CCl₄-treated model group, TBIL and γ-GGT levels were significantly decreased in SWGNS-treated groups in a dose-dependent manner (\( P < 0.05 \) or \( P < 0.01 \)). In addition, ALB levels at weeks 8 and 13 and TP levels at week 13 were significantly recovered (\( P < 0.05 \) or \( P < 0.01 \)). Serum biochemical results indicated that SWGNS had significant hepatoprotective ability against CCl₄-induced liver injury.

#### 3.3. SWGNS Alleviated CCl₄-Induced Liver Pathological Changes. To understand the role of SWGNS in alleviating CCl₄-induced hepatic pathological changes, hepatic pathological changes were assessed. As shown in Figure 3, the histological evaluation results showed that SWGNS reduced CCl₄-induced liver fibrosis. Histopathological examination of CCl₄-treated rats showed that the histological changes of chronic liver injury were mainly characterized by severe hepatic steatosis and fibrosis, as well as mild inflammatory infiltration and necrosis. In contrast, CCl₄-induced liver injury was significantly improved in all rats pretreated with SWGNS. These histopathological data were consistent with serum biochemical results. It suggests that SWGNS can alleviate CCl₄-induced liver fibrosis.

#### 3.4. SWGNS Ameliorated CCl₄-Induced Liver Injury by Inhibiting TGF-β1 Expression. To understand whether the liver fibrosis alleviated by SWGNS is related to the TGF-β1 pathway, the expression of TGF-β1, hyaluronic acid, laminin, and collagen IV in serum was measured. As shown in Figures 4(a) and 4(b), TGF-β1 and hyaluronic acid were significantly elevated in the CCl₄-induced chronic liver injury model; and the SWGNS decreased the level of TGF-β1 and hyaluronic acid. However, as for laminin and collagen IV, there were no significant differences between the groups (Figures 4(c) and 4(d)). Furthermore, the expression of α-SMA was also examined by immunohistochemistry. As shown in Figure 4(e), the expression of α-SMA was increased in the CCl₄ model group, and the increased expression level was inhibited by SWGNS.

#### 3.5. Analysis of SWGNS Composition. SWGNS mainly consists of 12 herbs and minerals. We successfully characterized them, especially Crocin I and Crocin II, by HPLC. HPLC results showed that the linear ranges of Crocin I and Crocin II were 3.33–66.67 μg/mL (0.9998) and 1.21–24.24 μg/mL (0.9998), respectively (Figure 5(a)). The mean recovery of Crocin I was 96.50% with an RSD of 1.62%, and the mean recovery of Crocin II was 90.41% with an RSD of 1.85% (Figure 5(b)). It shows that the established HPLC method has high sensitivity, accuracy, and good repeatability and is suitable for the determination of SWGNS phytochemical characteristics. The contents of Crocin I and Crocin II in the samples were 0.23% and 0.07%, respectively.
3.6. Crocin Attenuated H₂O₂-Induced BRL-3A Cell Injury.

Previous studies have reported that Crocin have anti-inflammatory and antioxidant effects [23,24]. Inflammation is a major source of oxidative stress. We investigated the antioxidant protective effect of Crocin through in vitro experiments. The results showed that Crocin increased the cell viability of BRL-3A after H₂O₂ pretreatment in a concentration-dependent manner. Further studies showed that Crocin reduced the expression of ALT, AST, MDA, and SOD. It is suggested that Crocin may protect liver cells through their antioxidant effect (Figure 6).

4. Discussion

As a traditional Tibetan medicine, SWGNS has been mainly used to treat liver diseases for many years. Many Tibetan medicines have shown obvious advantages in the treatment of liver diseases; and the commonly used natural products include Carthamus tinctorius, Crocus sativus, Meconopsis integrifolia, Brag-zhun, Herpetospermum pedunculosum, and Phyllanthus emblica [8], which are all included in our SWGNS formula. This study found that SWGNS can alleviate the process of CCl₄-induced chronic liver injury, such as preventing weight loss, reducing liver injury biomarkers, and improving liver tissue lesions. Additionally, SWGNS may prevent liver pathology by reducing TGF-β1 secretion, inflammation, and oxidative stress, which indicates that it has a hepatoprotective effect.

The dosage of herbal medicine is the key to the treatment. The clinical dose of SWGNS is 1.5 g, 2 times a day, so the dosage is 3.0 g/day. In this study, the calculated dose, such as low, medium, and high, was 1, 3, and 6 times the clinically equivalent dose, respectively. According to the conversion formula of body surface area and bodyweight \[ \text{IgS (surface)} = 0.8762 + 0.698 \text{lgW (weight)} \], the three-dose levels were calculated as 235, 705, and 1410 mg/kg in rats, respectively. All of these SWGNS doses demonstrated their protective effect in the treatment of chronic liver injury models, especially in liver fibrosis. Furthermore, the therapeutic effect was dose-dependent.

Serum ALT and AST have been the commonly used biomarkers of liver injury, and their release from hepatocytes into the circulation is proportional to the degree of

![Figure 2: SWGNS improved enzymatic activity of ALT, AST, and γ-GGT and the levels of TP, ALB, and TBIL in the CCl₄-induced hepatic fibrosis. #P < 0.05 and ##P < 0.01 versus the normal control group; *P < 0.05 and **P < 0.01 versus the CCl₄-induced model group (n = 10–13 each group).]
hepatocyte injury [25]. An interesting finding in this study is that the serum ALT and AST levels were significantly increased in the CCl₄ model group at the early stage (week 8), but no significant changes were observed at the end stage (week 13). Asymptomatic liver fibrosis with normal ALT and AST has been reported clinically [26]. Regardless, prophylactic treatment of SWGNS restored AST and ALT levels in animal models. In addition, SWGNS improved other serum liver function biomarkers, including \( \gamma \)-GGT, TBIL, and ALB, which further demonstrated its hepatoprotective activity.

Histopathological evaluation is an important method for the treatment and prognosis of chronic liver disease [27, 28]. Our findings suggest that CCl₄-induced chronic liver injury is characterized by liver fibrosis, whereas SWGNS reduces the incidence and extent of steatosis and fibrosis. In contrast, although bifendate significantly reduced ALT levels, it did not attenuate histopathological changes. This suggests that SWGNS may have more therapeutic potential than other traditional liver injury drugs.

Fibrosis is the final common histological change in chronic liver disease, characterized by excessive deposition and reorganization of the ECM [29]. Biomarkers of ECM synthesis include HA, LN, and CIV [30, 31]. Furthermore, upon HSC activation, they lose vitamin A-containing lipid droplets and transdifferentiate into myofibroblasts that express high \( \alpha \)-SMA [32]. Thus, these parameters reflect the stage of liver fibrosis. In our model, the levels of HA and \( \alpha \)-SMA increased significantly in the CCl₄ model group, whereas the levels of LN and CIV did not change. It has been reported that HA is a sensitive biomarker of early liver fibrosis, and LN and CIV are biomarkers of a later stage [33], which explains our results. We observed that SWGNS significantly decreased HA and \( \alpha \)-SMA in a dose-dependent manner, implying that SWGNS could inhibit ECM synthesis. TGF-\( \beta \) is a central mediator of the liver fibrosis process, activating transdifferentiation from HSCs to myofibroblasts and producing ECM. Previous animal studies demonstrated that hepatic TGF-\( \beta \) overexpression increased fibrosis, whereas TGF-\( \beta \) neutralization abolished this process [34–36]. Therefore, anti-TGF-\( \beta \) strategies have
been developed to treat chronic liver diseases [37]. Here, we further demonstrated that SWGNS may prevent liver fibrosis by inhibiting TGF-β1. Previous studies have demonstrated that excessive inflammation and ROS are important causes of liver injury, and inhibiting inflammation and ROS can effectively
Figure 5: The HPLC chromatogram of standard Crocin I and II sample (a) and SWGNS sample (b). 1, Crocin I; 2, Crocin II.

Figure 6: The role of Crocin in protecting BRL-3A cells from a peroxidative environment. (a) Cell viability. (b) ALT. (c) AST. (d) MDA. (e) Total SOD. *P < 0.05, compared to the control group. †P < 0.05, compared to the H$_2$O$_2$ group. n = 5.
alleviate liver injury [38–42]. Especially in the CCL4-induced liver injury model, several studies indicate that natural products can alleviate tissue injury by modulating immune responses and oxidative stress [43–46]. TGF-β1 is well known for regulating inflammation and oxidative stress [47,48]. In other words, TGF-β1 can be associated with the process of liver injury by mediating inflammation and oxidative stress, for example, downregulation of miR-10a can suppress sepsis-induced liver injury by downregulating the TGF-β1 pathway, as well as inflammation and oxidative stress [49]. Silymarin can alleviate CCL4-induced liver injury by inhibiting oxidative stress, inflammation, and TGF-β1 [50]. The study also indicates that oxidative stress is a key factor driving inflammation and TGF-β1. Our view/results are parallel to the previous literature. Since there are more studies on inflammation and oxidative stress, we paid more attention to the changes in TGF-β1. We hypothesized that SWGNS significantly downregulated the expression of TGB-1 in vivo and that Crocin significantly reduced oxidative stress in vitro. Therefore, we believe that SWGNS protects the liver by reducing TGF-β, inflammation, and oxidative stress.

This study has some limitations. First, toxicity testing has not been performed. The composition of herbal medicines is complex, and in addition to the effective components, there is the possibility of toxic components. Further testing of the effects of SWGNS on other organs is required. Second, there is a lack of dose testing: we tested the effect of SWGNS at three different doses and found that its liver-protective effect was dose-dependent. However, higher doses may have unpredictable side effects. Therefore, verification is required. Furthermore, more active ingredients for liver protection need to be identified. In the future, we are planning to isolate more active ingredients to further characterize their effects and toxicity because this will be more important for mass production and promotion. In addition, numerous studies have shown that herbal medicines generally have anti-inflammatory and antioxidant effects, which is one of the key mechanisms of treatment. SWGNS also has anti-inflammatory and antioxidant effects, but lack of in-depth mechanism research. This is also the focus of future research.

5. Conclusions
In conclusion, this study demonstrates that SWGNS reduces CCL4-induced chronic liver injury by inhibiting TGF-β1 expression for the first time. Also, it shows that SWGNS is a promising drug for the treatment of liver fibrosis, which is worthy of further research, development, and promotion.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that there are no conflicts of interest.

Authors’ Contributions
Ziming Jia and Yanhua Zhen contributed equally to this work. All authors participated in the implementation and design of this study and read and approved the final manuscript. WQ, YHZ, ZMJ, and ZNM designed the study, and drafted the manuscript. WQ, YHZ, SHF, JJQ, and JT conducted the experiments and analyzed the data. WQ, YHZ, ZMJ, and ZNM edited, corrected, and wrote the manuscript in its final form.

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References
Evidence-Based Complementary and Alternative Medicine


