Review Article

Therapeutic Effect of Tanshinone IIA on Liver Fibrosis and the Possible Mechanism: A Preclinical Meta-Analysis

Qingji Ying, Yangyang Teng, Jing Zhang, Zhenzhai Cai, and Zhanxiong Xue

Department of Gastroenterology, The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang Province, China

Correspondence should be addressed to Zhenzhai Cai; czz77@sina.com and Zhanxiong Xue; xuezhanxiong2016@163.com

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Background. Liver fibrosis is a serious human health problem, and there is a need for specific antifibrosis drugs in the clinic. Tanshinone IIA has recently been reported to have a role in the treatment of liver fibrosis. However, the evidence supporting its antifibrotic effect is not sufficient, and the underlying mechanism is not clear. We thus performed this meta-analysis of animal research to assess the therapeutic effect of tanshinone IIA on liver fibrosis and analyzed the possible associated mechanism to provide a reference for further clinical drug preparation and clinical research.

Methods. We collect related articles from the databases PubMed, Web of Science, Embase, Wanfang, VIP, and CNKI. The quality of the included studies was evaluated according to the SYRCLE risk of bias tool for animal studies. Data were analyzed using RevMan 5.3 and Stata 12.0 software.

Results. A total of 404 articles were retrieved from the databases. After screening, 11 articles were included in the analysis. The included studies’ methodological quality was generally low, and an obvious publication bias was found. The results showed that tanshinone IIA significantly improved liver function in experimental animals and reduced the level of liver fibrosis by reducing inflammation and inhibiting immunity, antiapoptotic processes, and HSC activation.

Conclusion. Tanshinone IIA can effectively improve liver fibrosis and liver function in animal models and is worthy of future higher quality animal studies and clinical drug trials.

1. Introduction

Liver fibrosis is an important pathological process in the gradual development of various chronic liver diseases, such as viral hepatitis and alcoholic or nonalcoholic fatty liver disease, and it mainly manifests as necrosis in liver cells, excessive deposition of extracellular matrix components, and scar formation by liver fibers. Liver diseases gradually destroy the fibrous mesh scaffolds of the liver, which prevents the liver from reestablishing its normal structure and can cause the liver to lose its normal physiological function, eventually leading to end-stage cirrhosis. Cirrhosis causes approximately 1 million deaths per year [1]. The treatment costs associated with gastrointestinal bleeding, ascites, and other complications are more than three times higher for patients with cirrhosis than for those without cirrhosis [2]. The global burden of liver fibrosis is increasing [3], and drugs to improve liver fibrosis will bring huge benefits.

Liver fibrosis is the early stage of cirrhosis. Pathological biopsy is the gold standard for diagnosing liver fibrosis. The liver fibrosis score is based on liver pathology, and the hydroxyproline (Hyp) content in liver tissue can reflect the degree of liver fibrosis. However, clinically, for noninvasive examinations, serum liver fibrosis markers, such as hyaluronic acid (HA), laminin (LN), fibrinogen, alanine aminotransferase (ALT), albumin (ALB), and total bilirubin, which are included in this study, are increasingly used to diagnose liver fibrosis [1]. More importantly, their changes in these levels can be used to evaluate fibrosis regression after treatment [4].

Currently, it is believed that effective etiological treatment for liver fibrosis can block the development of fibrosis and even reverse this process [5, 6]. However, over the past two decades, the biology and pathophysiology of hepatic fibrosis have been increasingly understood, and more potential therapeutic targets have been found, providing us with increasingly specific antifibrotic methods. Many
antifibrotic drugs have begun clinical trials. However, these antifibrotic agents, such as IL-10, adenosine methionine, ursodeoxycholic acid, silymarin, and colchicine, have not been routinely used in the clinic, and notably, TNF-α treatment has been shown to increase infection and mortality rates in patients. Therefore, a product with strong efficacy, high safety, and a low cost is needed for clinical practice [7].

The traditional Chinese medicine Salvia miltiorrhiza is made from the root of the lip-shaped plant Salvia miltiorrhiza. It is a commonly used in traditional Chinese medicine practices in China. In Chinese medicine theory, Salvia miltiorrhiza belongs to the heart and liver channels and various prescriptions are used to promote blood circulation, calm nerves, regulate menstruation, and relieve pain [8]. Tanshinone IIA is a fat-soluble extract of Salvia miltiorrhiza, and its structure is shown in Figure 1. Recent studies have suggested that tanshinone IIA has anti-inflammatory, antioxidative, antitumor, neuroprotective, and cardioprotective effects in the treatment of atherosclerosis, lung cancer, cervical cancer, Alzheimer’s disease, and heart failure [8–12]. Sodium tanshinone IIA sulfonate is a water-soluble sulfonate made from tanshinone IIA, that shows greatly improved water solubility compared with tanshinone IIA, and is more suitable for intravenous infusion. This injectable has been used clinically for the treatment of coronary heart disease for nearly 30 years in China [13]. In recent years, it has been found that tanshinone IIA has a significant effect on organ fibrosis [14–16]. Recently, in vitro studies [17, 18] have suggested that tanshinone IIA can induce activation of hepatic stellate cell (HSC) apoptosis through a variety of pathways, thereby significantly reducing the level of liver fibrosis [19], and may have dose-dependent and time-dependent characteristics [18]. However, the evidence supporting the antifibrotic effect of tanshinone IIA is not sufficient [20], and the underlying mechanism is not clear.

Animal trials are usually performed before clinical trials to evaluate the effectiveness of interventions, which mean that animal studies can be used as the preliminary evidence for the clinical use of drugs. Systematic reviews of animal studies play critical roles in the clarification of physiological and pathological mechanisms in clinical research [21].

Therefore, we conducted this meta-analysis focusing on liver fibrosis animal trials to evaluate tanshinone IIA compared to a placebo in the treatment of liver fibrosis, by assessing levels of change in liver fibrosis markers, particularly with regard to efficacy and the possible mechanism, and to determine whether the results of animal trials of tanshinone IIA can be applied in the clinic.

2. Methods

2.1. Eligibility Criteria

2.1.1. Studies. Only preclinical animal studies investigating the treatment of liver fibrosis with tanshinone IIA were included in this meta-analysis, regardless of binding and publication status. Article languages included English and Chinese.

2.1.2. Participants. Animals that successfully model liver fibrosis were included, regardless of the modeling method, age, gender, or species. The diagnosis of liver fibrosis is based on the pathological manifestations of liver tissue.

2.1.3. Intervention and Comparison. Studies which used tanshinone IIA or sodium tanshinone IIA sulfonate as a monotherapy were included. There is no restriction on dosage including frequency, dose, or intensity. Comparator interventions were placebo (inert fluid) or no treatment.

2.1.4. Outcomes. The primary outcome measurements were liver fibrosis index (liver fibrosis score or the level of Hyp, HA, LN, collagen type I, or procollagen type II) and a liver function index (the level of ALT, ALB, or total bilirubin), which can be used to evaluate fibrosis regression after treatment. The secondary outcome measure was a possible mechanism in which tanshinone IIA improves liver fibrosis.

2.1.5. Exclusion Criteria. The publication included previously published results. The full text was not found.

2.2. Search Strategies. This meta-analysis follows the PRISMA statement [22]. We searched all the articles on animal experiments evaluating the effects of tanshinone IIA on liver fibrosis in the databases PubMed, Web of Science, Embase, Wanfang, Chinese Scientific Journals Full-Text Database (VIP), and Chinese National Knowledge Infrastructure (CNKI). We searched the databases between inception and 2019.3.20. The article languages included English and Chinese. The search terms included all the keywords such as “Tanshinone” and “Liver Cirrhosis” and free words such as “Tanshinone IIA,” “Tanshinone IIA,” “Cirrhosis, Liver,” “Cirrhoses, Liver,” “Liver Cirrhoses,” “Hepatic Cirrhosis,” “Cirrhoses, Hepatic,” “Cirrhosis, Hepatic,” “Hepatic Cirrhoses,” “Fibrosis, Liver,” “Fibroses, Liver,” “Liver Fibroses,” and “Liver Fibrosis.” The specific search strategies are shown in the supplemental materials.

2.3. Study Selection and Data Extraction. According to the eligibility criteria, two authors independently read the titles and abstracts to select potential articles. Then, they independently read the full text of selected articles and made a
3. Results

3.1. Study Inclusion and Characteristics. We screened a total of 404 articles and were left with 215 articles after removing 189 duplicate or irrelevant documents. Through reading the title and abstract, 173 articles were eliminated. By reading the full text, 5 of the remaining 16 articles were eliminated. Therefore, a total of 11 articles comprising 13 groups of experiments were included [24–34] (Figure 2).

Animal species: eight studies used SD rats [24–26, 29–33]; 1 study used Wistar rats [27]; 1 study used Kunming mice [28]; and 1 study used ICR mice [34]. Three studies used female SD rats [29, 30, 33]; 1 study used a 50:50 split of male and female SD rats [25]; and the remaining 7 studies used male animals [24, 26–28, 31, 32, 34]. Anesthesia: three studies used pentobarbital [24, 29, 32]; 2 studies used ether [25, 28]; 1 study used chloral hydrate [24], and 1 study used xylazine and ketamine hydrochloride [33]; none of the other 4 studies clearly named the anesthetics used [26, 30, 31, 34]. Modeling method: six studies used carbon tetrachloride (CCL₄) modeling [24, 25, 27, 29, 31, 32]; 3 studies used thioacetamide (TAA) modeling [28, 33, 34]; 1 study used pig serum modeling [30]; and 1 study used dimethyl nitrosamine (DMN) modeling [26]. Modeling time: one study ran for 3 weeks [26]; 1 study performed prevention group modeling for 4 weeks, and treatment group modeling for 6 weeks [28]; 3 studies took 6 weeks [27, 31, 32]; 3 studies took for 8 weeks [25, 30, 34]; 2 studies took 12 weeks [24, 29]; and 1 study took 14 weeks [33]. Intervention initiation time: five experiments initiated the intervention before or concurrent with modeling [26–28, 30, 32], while 8 experiments initiated the intervention after modeling [24, 25, 28–31, 33, 34]. Dose: one study treated animals with 2 mg/kg tanshinone IIA [34]; 1 study treated animals with 20 mg/kg tanshinone IIA [33]; 3 studies treated animals with 21.3 mg/kg tanshinone IIA [24, 25, 27]; 1 study treated animals with 100 mg/kg tanshinone IIA [26]; 1 study treated animals with 200 mg/kg tanshinone IIA [29]; 2 studies treated animals with 15 mg/kg sodium tanshinone IIA sulfonate [30, 32]; and 2 studies treated animals with 20 mg/kg sodium tanshinone IIA sulfonate [28, 31]. Administration: six studies utilized intragastric administration [24–27, 29, 32]; 3 studies utilized intraperitoneal injection [28, 30, 31]; 1 study utilized tail vein injection [34]; and 1 study did not explicitly state the mode of administration [33]. The characteristics of the 11 included studies are summarized in detail in Table 1.
while analyzing the results, and it was not possible to determine whether there were other biases.

3.3. Ameliorative Effects of Tanshinone IIA on Liver Fibrosis

3.3.1. Liver Fibrosis Scores. A total of 3 studies evaluated the degree of fibrosis in the liver by examining pathological staining of liver sections [25, 32, 34]. The scores ranged from 0 to 4 according to the extent of liver structural damage. The three studies used different criteria, but the criteria were similar. The scoring criteria have been uploaded as a supplement. The tanshinone IIA-treated group showed significantly reduced liver fibrosis scores (n = 55, SMD −1.52, 95% CI [−2.15 to −0.89], P < 0.01; heterogeneity: χ² = 1.28, df = 2 (P = 0.53); I² = 0%) (Figure 3(a)).

3.3.2. Hydroxyproline (Hyp). Four studies examined the level of Hyp in liver tissue [24, 26, 27, 29], and the level of Hyp in the tanshinone IIA-treated group was significantly lower than that in the model group (n = 61, SMD −3.55, 95% CI [−4.52 to −2.58], P < 0.01; heterogeneity: χ² = 16.06, df = 3 (P = 0.53); I² = 81%) (Figure 3(b)).

3.3.3. Hyaluronic Acid (HA). Five studies [26, 27, 29–31] showed a significant decrease in the hyaluronic acid level, but the heterogeneity was significant (n = 89, SMD −6.72, 95% CI [−9.63 to −3.81], P < 0.01; heterogeneity: χ² = 31.52, df = 5 (P < 0.01); I² = 84%) (Figure 3(c)).

3.3.4. Laminin (LN). In the 5 studies that evaluated hyaluronic acid levels mentioned above [26, 27, 29–31], the laminin level showed a significant decrease in the tanshinone IIA-treated group (n = 89, SMD −3.22, 95% CI [−4.72 to −1.73], P < 0.01; heterogeneity: χ² = 21.15, df = 5 (P < 0.01); I² = 76%) (Figure 3(d)).

3.3.5. Collagen Type I (Col I) and Procollagen Type III (PCIII). The levels of serum type I collagen [24, 28] (n = 36, SMD −4.54, 95% CI [−6.00 to −3.08], P < 0.01; heterogeneity: χ² = 1.59, df = 2 (P = 0.45); I² = 0%) (Figure 3(e)) and type III procollagen [30, 31] (n = 40, SMD −4.18, 95% CI [−5.84 to −2.53], P < 0.01; heterogeneity: χ² = 3.13, df = 2 (P = 0.21); I² = 36%) (Figure 3(f)) were significantly lower in the tanshinone IIA-treated group than in the model group.

3.4. Ameliorative Effects of Tanshinone IIA on Liver Function

3.4.1. Alanine Aminotransferase (ALT). Eight studies [24, 26, 28, 29, 31–34] evaluated serum ALT. The tanshinone IIA-treated group showed a decrease in the ALT level (n = 132, SMD −7.12, 95% CI [−9.97 to −4.27], P < 0.01; heterogeneity: χ² = 93.52, df = 8 (P < 0.01); I² = 91%) (Figure 4(a)).

3.4.2. Albumin (ALB). Serum ALB was assessed in 2 studies [31, 34], and the serum albumin level was significantly higher in the tanshinone IIA-treated group than in the model group (n = 28, SMD 3.49, 95% CI [2.15 to 4.83], P < 0.01; heterogeneity: χ² = 1.01, df = 1 (P = 0.31); I² = 1%) (Figure 4(b)).

3.4.3. Total Bilirubin. Two studies [32, 34] reported data for total bilirubin, and there was a significant decrease in the serum total bilirubin level in the tanshinone IIA-treated group (n = 35, SMD −2.65, 95% CI [−3.63 to −1.68], P < 0.01; heterogeneity: χ² = 0.61, df = 1 (P = 0.43); I² = 0%) (Figure 4(c)).
<table>
<thead>
<tr>
<th>First author</th>
<th>Animal species</th>
<th>Number</th>
<th>Modeling methods</th>
<th>Anesthesia</th>
<th>Interventions</th>
<th>Outcome</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhang [24]</td>
<td>Male SD rats</td>
<td>6/6</td>
<td>40% CCl₄ (2.5 ml/kg) twice a week for 12 weeks subcutaneously</td>
<td>Pentobarbital sodium</td>
<td>Tanshinone IIA (21.3 mg/(kg-d)) for 10 weeks (3–12) intragastrically</td>
<td>(1) Hyp (2) ALT (3) AST (4) Col I (5) ANG II (6) AT1R (7) TGF-β1 (1) Fibrosis score (2) TGF-β1 (3) Smad6, 7 (4) BMP7</td>
<td>(1) P &lt; 0.05 (2) P = 0.22 (3) P = 0.08 (4) P &lt; 0.05 (5) P &lt; 0.01 (6) P &lt; 0.01 (7) P &lt; 0.05</td>
</tr>
<tr>
<td>Zhang [25]</td>
<td>Male and female SD rats</td>
<td>10/10</td>
<td>10% CCl₄ (5 ml/kg) for 8 weeks subcutaneously</td>
<td>Ether</td>
<td>Tanshinone IIA (21.3 mg/(kg-d)) for 4 weeks (5–8) intragastrically</td>
<td>(1) Hyp (2) HA (3) LN (4) ALT (5) MDA (6) SOD (7) GSH-Px</td>
<td>(1) P &lt; 0.01 (2) P &lt; 0.01 (3) P &lt; 0.01 (4) P &lt; 0.01</td>
</tr>
<tr>
<td>Yang and Cheng [26]</td>
<td>Male SD rats</td>
<td>7/7</td>
<td>DMN (10 mg/kg) for 3 weeks (3 consecutive days/week)</td>
<td>Not mentioned</td>
<td>Tanshinone IIA (100 mg/kg) for 3 weeks (same time) intraperitoneally</td>
<td>(1) Hyp (2) HA (3) LN</td>
<td>(1) P &lt; 0.01 (2) P &lt; 0.01 (3) P &lt; 0.01</td>
</tr>
<tr>
<td>Qin and Yan [27]</td>
<td>Male Wistar rats</td>
<td>10/10</td>
<td>40% CCl₄ twice a week for 6 weeks (3–8) (first time 3 ml/kg and then 1 ml/kg) intragastrically</td>
<td>Chloral hydrate</td>
<td>Tanshinone IIA (21.3 mg/(kg-d)) for 8 weeks intragastrically</td>
<td>Prevention group: sodium tanshinone IIA sulfonate (20 mg/kg) for 4 weeks, intraperitoneally (1) Hyp (2) HA (3) LN</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Sun et al. [28]</td>
<td>Male Kunming mice</td>
<td>Prevention: 6/6 treatment: 6/6</td>
<td>TAA (200 mg/kg) three times a week for 4 weeks (treatment group)/6 weeks (treatment group) intraperitoneally</td>
<td>Ether</td>
<td>Tanshinone IIA sulfonate (20 mg/kg) for 3 weeks (4–6) intraperitoneally</td>
<td>Prevention and treatment group: (1) ALT (2) Col I (3) TGF-β1 (4) Smad3 (5) IGFBP7</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Liu et al. [29]</td>
<td>Female SD rats</td>
<td>8/7</td>
<td>CCl₄ twice a week for 12 weeks (first time pure CCl₄ (5 ml/kg) and then 20% CCl₄ (3 ml/kg)), subcutaneously</td>
<td>Pentobarbital</td>
<td>Tanshinone IIA (200 mg/(kg-d)) for 6 weeks (7–12), intragastrically</td>
<td>Prevention group: sodium tanshinone IIA sulfonate (15 mg/(kg-d)) for 6 weeks (9–16) intraperitoneally</td>
<td>(1) Hyp (2) HA (3) ALT (4) PCIII</td>
</tr>
<tr>
<td>Guo [30]</td>
<td>Female SD rats</td>
<td>Prevention: 8/6 treatment: 8/6</td>
<td>Pig serum (0.5 ml) twice a week for 8 weeks intraperitoneally</td>
<td>Not mentioned</td>
<td>Tanshinone IIA sulfonate (15 mg/(kg-d)) for 8 weeks intraperitoneally</td>
<td>Prevention and treatment group: sodium tanshinone IIA sulfonate (15 mg/(kg-d)) for 8 weeks (9–16) intraperitoneally</td>
<td>(1) ALB (2) HA (3) ALT (4) PCIII</td>
</tr>
<tr>
<td>Bai [31]</td>
<td>SD rats</td>
<td>6/6</td>
<td>15% CCl₄ (0.75/kg) three times a week for 6 weeks intraperitoneally</td>
<td>Not mentioned</td>
<td>Sodium tanshinone IIA sulfonate (20 mg/(kg-d)) for 3 days after successful modeling intraperitoneally</td>
<td>(1) ALB (2) HA (3) ALT (4) PCIII</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

**Table 1: Characteristics of the 11 included studies.**
3.5. Subgroup Analysis. We conducted a subgroup analysis to assess the source of heterogeneity in the included studies based on the intervention start time. Hyp, HA, LN, and ALT measured were used to divide the data into two groups: the treatment group (the intervention was performed after the model was induced) and the prevention group (the intervention was performed before the model was induced). The levels of all four markers, expect those of Hyp, showed significant decreases in both the treatment and the prevention groups compared with the
### Table 1: Forest plot for the comparison of treatment effects

#### (a) Treatment vs. control

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Experimental Mean</th>
<th>Experimental SD</th>
<th>Experimental Total</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>Control Total</th>
<th>Weight (%)</th>
<th>Std. mean difference (IV, random, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meng et al. 2015</td>
<td>2.15</td>
<td>0.53</td>
<td>8</td>
<td>3.14</td>
<td>0.55</td>
<td>8</td>
<td>27.2</td>
<td>-1.73 [-2.93, -0.53]</td>
</tr>
<tr>
<td>Zhang 2015</td>
<td>2</td>
<td>0.09</td>
<td>10</td>
<td>2.46</td>
<td>0.32</td>
<td>10</td>
<td>32.9</td>
<td>-1.87 [-2.96, -0.78]</td>
</tr>
<tr>
<td>Zhang 2013</td>
<td>2.18</td>
<td>0.98</td>
<td>11</td>
<td>3.25</td>
<td>0.89</td>
<td>8</td>
<td>39.9</td>
<td>-1.08 [-2.07, -0.09]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>29</td>
<td>26</td>
<td>100.0</td>
<td>-1.52</td>
<td>-2.15</td>
<td>-0.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 4.61$, $\chi^2 = 16.06$, $df = 2$ ($P = 0.53$); $I^2 = 0%$

Test for overall effect: $Z = 4.76$ ($P < 0.00001$)

#### (b) Prevention vs. control

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Experimental Mean</th>
<th>Experimental SD</th>
<th>Experimental Total</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>Control Total</th>
<th>Weight (%)</th>
<th>Std. mean difference (IV, random, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu et al. 2002</td>
<td>1.04</td>
<td>0.063</td>
<td>8</td>
<td>1.31</td>
<td>0.056</td>
<td>7</td>
<td>25.9</td>
<td>-4.24 [-6.28, -2.21]</td>
</tr>
<tr>
<td>Qin and Yan 2010</td>
<td>376.3</td>
<td>15.4</td>
<td>10</td>
<td>533.5</td>
<td>15.3</td>
<td>10</td>
<td>18.9</td>
<td>-9.81 [-13.31, -6.30]</td>
</tr>
<tr>
<td>Yang and Cheng 2004</td>
<td>0.388</td>
<td>0.05</td>
<td>7</td>
<td>0.621</td>
<td>0.079</td>
<td>7</td>
<td>27.1</td>
<td>-3.30 [-5.08, -1.52]</td>
</tr>
<tr>
<td>Zhang 2017</td>
<td>199.39</td>
<td>45.28</td>
<td>6</td>
<td>396.77</td>
<td>112.21</td>
<td>6</td>
<td>28.2</td>
<td>-2.13 [-3.67, -0.59]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>31</td>
<td>30</td>
<td>100.0</td>
<td>-4.44 [-6.82, -2.06]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 4.61$, $\chi^2 = 16.06$, $df = 3$ ($P = 0.001$); $I^2 = 81%$

Test for overall effect: $Z = 3.66$ ($P = 0.0003$)

#### (c) Treatment vs. prevention

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Experimental Mean</th>
<th>Experimental SD</th>
<th>Experimental Total</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>Control Total</th>
<th>Weight (%)</th>
<th>Std. mean difference (IV, random, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai 2015</td>
<td>253.33</td>
<td>18.62</td>
<td>6</td>
<td>425</td>
<td>47.54</td>
<td>6</td>
<td>19.2</td>
<td>-4.39 [-6.81, -1.97]</td>
</tr>
<tr>
<td>Guo (prevention) 2007</td>
<td>304.38</td>
<td>23.06</td>
<td>8</td>
<td>562</td>
<td>31.28</td>
<td>6</td>
<td>13.4</td>
<td>-9.00 [-13.07, -4.93]</td>
</tr>
<tr>
<td>Guo (treatment) 2007</td>
<td>309.75</td>
<td>21.81</td>
<td>8</td>
<td>562</td>
<td>31.28</td>
<td>6</td>
<td>13.4</td>
<td>-9.02 [-13.10, -4.94]</td>
</tr>
<tr>
<td>Liu et al. 2002</td>
<td>129.7</td>
<td>19.3</td>
<td>8</td>
<td>254.8</td>
<td>22.7</td>
<td>7</td>
<td>18.9</td>
<td>-5.60 [-8.14, -3.05]</td>
</tr>
<tr>
<td>Qin and Yan 2010</td>
<td>62.5</td>
<td>15.5</td>
<td>10</td>
<td>164.7</td>
<td>56.8</td>
<td>10</td>
<td>21.3</td>
<td>-2.35 [-3.55, -1.16]</td>
</tr>
<tr>
<td>Yang and Cheng 2004</td>
<td>246.075</td>
<td>24.303</td>
<td>7</td>
<td>564.422</td>
<td>10.798</td>
<td>7</td>
<td>9.7</td>
<td>-15.85 [-22.85, -8.84]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>47</td>
<td>42</td>
<td>100.0</td>
<td>-6.72 [-9.63, -3.81]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 9.96$, $\chi^2 = 31.52$, $df = 5$ ($P < 0.00001$); $I^2 = 84%$

Test for overall effect: $Z = 4.53$ ($P < 0.00001$)

#### (d) Prevention vs. treatment

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Experimental Mean</th>
<th>Experimental SD</th>
<th>Experimental Total</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>Control Total</th>
<th>Weight (%)</th>
<th>Std. mean difference (IV, random, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang and Cheng 2004</td>
<td>116.115</td>
<td>10.373</td>
<td>7</td>
<td>186.32</td>
<td>51.027</td>
<td>7</td>
<td>21.3</td>
<td>-1.79 [-3.09, -0.48]</td>
</tr>
<tr>
<td>Qin and Yan 2010</td>
<td>34.6</td>
<td>16.9</td>
<td>10</td>
<td>79.1</td>
<td>19.2</td>
<td>10</td>
<td>21.8</td>
<td>-2.36 [-3.55, -1.16]</td>
</tr>
<tr>
<td>Liu et al. 2002</td>
<td>79.4</td>
<td>19.1</td>
<td>8</td>
<td>310.7</td>
<td>34.5</td>
<td>7</td>
<td>10.7</td>
<td>-7.97 [-11.44, -4.50]</td>
</tr>
<tr>
<td>Guo (treatment) 2007</td>
<td>160.88</td>
<td>17.66</td>
<td>8</td>
<td>202.27</td>
<td>20.02</td>
<td>6</td>
<td>20.8</td>
<td>-2.07 [-3.47, -0.68]</td>
</tr>
<tr>
<td>Guo (prevention) 2007</td>
<td>161.88</td>
<td>14.91</td>
<td>8</td>
<td>202.27</td>
<td>20.02</td>
<td>6</td>
<td>20.6</td>
<td>-2.20 [-3.62, -0.77]</td>
</tr>
<tr>
<td>Bai 2015</td>
<td>99.94</td>
<td>2.49</td>
<td>6</td>
<td>277.55</td>
<td>18.54</td>
<td>6</td>
<td>4.8</td>
<td>-12.39 [-18.55, -6.24]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>47</td>
<td>42</td>
<td>100.0</td>
<td>-3.22 [-4.72, -1.73]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 2.29$, $\chi^2 = 21.15$, $df = 5$ ($P = 0.0008$); $I^2 = 76%$

Test for overall effect: $Z = 4.23$ ($P < 0.00001$)
Heterogeneity: $\chi^2 = 1.59$, $df = 2$ ($P = 0.45$); $I^2 = 0$

**Study or subgroup**

<table>
<thead>
<tr>
<th>Study or subgroup</th>
<th>Weight (%)</th>
<th>Weighted Mean difference</th>
<th>Std. mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sun et al. (prevention) 2009</td>
<td>33.4</td>
<td>0.87</td>
<td>38.3</td>
</tr>
<tr>
<td>Sun et al. (treatment) 2009</td>
<td>22.8</td>
<td>0.78</td>
<td>42.7</td>
</tr>
<tr>
<td>Zhang 2017</td>
<td>8.9</td>
<td>0.0147</td>
<td>19.0</td>
</tr>
</tbody>
</table>

**Total (95% CI)**

18

Heterogeneity: $\tau^2 = 0.00$, $\chi^2 = 1.59$, $df = 2$ ($P = 0.45$); $I^2 = 0$

Test for overall effect: $Z = 6.09$ ($P < 0.00001$)

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3.6. Sensitivity Analysis. The robustness of the integrated results, which showed heterogeneity $>70\%$, was assessed by sensitivity analysis. Two studies were removed. In one study [27], mice were given tanshinone IIA 3 weeks prior to the start of modeling, which was much earlier than in the other studies. Another study [30] performed modeling with pig serum, while the other studies used CCL4. After removing these two studies, only one study was left in the Hyp prevention group, indicating a significant decrease in the Hyp level ($P < 0.01$). The heterogeneity in the ALT level did not change, but the HA level in the prevention group and the LN level in the treatment group showed significant decreases in heterogeneity (HA: $I^2$ from 91% to 64%; ALT: $I^2$ from 89% to 34%). The results of sensitivity analysis are summarized in Table 3.

3.7. Mechanisms by Which Tanshinone IIA Improves Liver Fibrosis. The results of 3 studies [24, 28, 31] suggested a decrease in TGF-$\beta$1 protein expression ($n = 48$, SMD $-6.94$, 95% CI $[-9.14$ to $-4.74]$, $P < 0.01$; heterogeneity: $\chi^2 = 4.23$, $df = 3$ ($P = 0.24$); $I^2 = 29\%$) (Figure 5(a)). The results of 1 study [25] suggested a significant decrease in TGF-$\beta$1 mRNA expression ($P < 0.01$). Two studies [31, 33] suggested a decrease in TNF-α expression ($n = 32$, SMD $-109.98$, 95% CI $[-114.92$ to $-105.04]$, $P < 0.01$; heterogeneity: $\chi^2 = 0.71$, $df = 1$ ($P = 0.40$); $I^2 = 0\%$) (Figure 5(b)). After the sensitivity analysis, 1 study [26] was removed, and only 1 of the remaining studies [33] suggested that SOD and GSH-Px levels were significantly increased ($P < 0.01$) and the MDA level was significantly decreased ($P < 0.01$). One study [24] suggested that the levels of Ang II, AT1R, VEGF, and HIF-1α were significantly decreased ($P < 0.01$). One study [32] suggested that the protein expression of Bax was significantly decreased, while the protein expression of Bcl-2 was significantly increased ($P < 0.01$). One study [33] suggested that Akt activation and p38-MAPK were significantly inhibited, while HO-1 expression was significantly decreased ($P < 0.01$). Recent in vitro cytology studies have shown that tanshinone IIA can inhibit TIMP-1 expression and increase MMP-1 expression in HSCs [35], but whether tanshinone IIA can affect liver fibrosis by regulating MMPs and TIMPs in vivo in liver tissue has not been reported in relevant animal experiments. We summarized the mechanism of liver protection mediated by tanshinone IIA in liver fibrosis in Figure 6.
3.8. Publication Bias. Due to the small number of studies included, we only used the measurements of ALT to assess publication bias. Through Egger’s and Begg’s test, we found an obvious publication bias (Egger’s P value < 0.01 and Begg’s test P value = 0.016). There are many factors that influence the outcome of these tests, not only the nonpublication of negative results but also the heterogeneity among studies, low methodological quality, and having a limited number of small trials [36]. These factors all appeared in this study.

4. Discussion

4.1. Summary of Evidence. This is the first preclinical meta-analysis of the use of tanshinone IIA in the treatment of animal liver fibrosis. A total of 200 animals were included across 11 studies. According to the evidence, tanshinone IIA can reduce oxidative stress and the liver immune inflammatory response, inhibit liver cell apoptosis, improve the liver microcirculation, inhibit the TGF-β1 pathway, reduce the proliferation and activation of HSCs, and ultimately improve liver fibrosis and function. However, the quality of the included articles was generally low, so the results of this meta-analysis should be treated with caution.

4.2. Limitations. (1) This meta-analysis only included 11 articles including 9 articles in Chinese and 2 articles in English. The lack of articles in other languages may result in selection bias. None of the included articles mentioned the way in which random allocations were performed. There was no mention of allocation concealment or blinding, so there was a risk of other biases. (2) Many negative results may not be published, and positive results may cause publication bias, resulting in an overestimation of the effect of tanshinone IIA. (3) The results of this meta-analysis show a high degree of heterogeneity. Although some heterogeneity was reduced by sensitivity and subgroup analyses, the heterogeneity within some groups was still high. It is likely that this heterogeneity was related to the insufficient sample size in...
the included article, which may affect our judgment of the effect of tanshinone IIA. (4) There are articles suggesting that tanshinone IIA does not damage liver cells [17, 37], but no adverse reactions were reported in the included studies. (5) This meta-analysis was not registered so there may be some bias during the research process.

4.3. Implications. Liver fibrosis has long plagued clinical practices. Continued progression of any chronic liver disease can lead to liver fibrosis. By reading a number of guidelines for liver disease, it has been found that we can treat liver fibrosis using a variety of traditional Chinese medicine preparations. However, there are currently no large, randomized, multicenter clinical studies being performed to confirm the antifibrotic effects of traditional Chinese medicine preparations. Tanshinone IIA is an extract of Salvia miltiorrhiza, which has antioxidative and anti-inflammatory effects, but its application in liver fibrosis is still lacking. This meta-analysis comprehensively analyzed data from several animal experiments. According to the results, tanshinone IIA reduces liver fiber scores, collagen content in liver tissue, multiple serum fibrosis indexes, and serum liver enzyme levels and restores the serum albumin levels. This analysis also described possible mechanisms related to improving liver fibrosis. The results will provide an important reference for subsequent clinical trials [38].

We utilized the SYRCLE assessment tool for quality assessment. Unlike clinical trials, which are strictly randomized and blinded, most animal experiments do not

Figure 5: Forest plot: (a) ability of tanshinone IIA to decrease TGF-β1 protein expression compared with that of control treatment; (b) ability of tanshinone IIA to decrease TNF-α level compared with that of control treatment.
mention specific methods of distribution [39]. Like the studies we included, all studies do not report the methodology clearly; this deficiency makes us more likely to obtain positive results [40]. The number of samples in animal studies is usually small, and because there is no standard protocol, animal age and sex and experiment duration vary greatly. These shortcomings seriously affect our direct application of animal trial results and meta-analysis of these data [41]. Moreover, for animal experiments, the randomized allocation of animals is relatively important. Lighting and temperature differences during housing have impact on animal behavior, the metabolic rate, and drug toxicity [23]. Therefore, we recommend that subsequent animal trials follow the items in the SYRCLE assessment tool [23] and the ARRIVE Animal Experiment Report [42].

Preclinical animal models are indispensable for identifying novel drug targets for the development of future therapies. The variability among individual models sometimes complicates the comparability of studies and can hamper the translation of results to human diseases [43]. It is important to identify and develop clinically relevant and reliable animal models. The four modeling methods in the studies evaluated here have the disadvantages of high model animal mortality and differences in pathophysiological processes between the model animals and human liver fibrosis. There is currently no ideal animal model for all types of liver fibrosis [44], and different modeling methods must be used for different research purposes. Considering the large number of people infected with hepatitis B virus [3], the incidence of cirrhosis caused by alcoholic or non-alcoholic fatty liver disease has risen sharply [45, 46]; therefore, we recommend that, for HBV-induced liver fibrosis, we can use a primate HBV model or tree scorpion HBV model [47, 48]; for alcohol-induced liver fibrosis, we can use an alcohol-fed mouse model [49]; and for non-alcoholic fatty liver disease-induced liver fibrosis, we can use the iron load supplement diet-fed diabetic mouse model [50]. However, the abovementioned modeling methods are not completely in line with the pathophysiological processes of human liver fibrosis, and there are also ethical and cost-related problems. More ideal models still require subsequent research.

5. Conclusion

This meta-analysis suggests that tanshinone IIA may have a therapeutic effect on animal liver fibrosis through its antioxidative, anti-inflammatory, and antiapoptotic properties and its abilities to improve the microcirculation and inhibition of HSC proliferation and activation. Tanshinone IIA is worthy of study in subsequent higher quality animal studies and clinical drug trials.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Supplementary Materials

PRISMA 2009 Checklist. (Supplementary Materials)

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Evidence-Based Complementary and Alternative Medicine


[31] Y. F. Bai, Research on Celastrus Orbiculatus Extraction Treatment of Liver Fibrosis in Rats, Yangzhou University, Yangzhou, China, 2015.


[34] Z. Meng, L. Meng, K. Wang et al., "Enhanced hepatic tar-geting, biodistribution and antifibrotic efficacy of tanshinone


