The effect of Jigan Xiaozhi Formula on oxidative stress and inflammatory factors in patients with non-alcoholic fatty liver disease

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Author contributions
Si Wang and Jia-Bao Liao contributed to study concept; Si Wang, Jia Zhu and Ning Wang contributed to study design and performance; Si Wang and Jia Zhu contributed to analysis of data; Si Wang contributed to drafting of the paper; Jia-Bao Liao contributed to study supervision.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
JGZ, Jigan Xiaozhi Formula; NAFLD, Non-alcoholic Fatty Liver Disease; TCM, Traditional Chinese Medicine; MDA, Malondialdehyde; GSH-Px, Glutathione peroxidase; SOD, Superoxide dismutase; IL-6, Interleukin-6; IL-8, Interleukin-8; TNF-α, Tumor necrosis factor-α; AST, Aspartate aminotransferase; TG, Triglycerides; TC, Cholesterol; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; ALT, Alanine aminotransferase; LDL, Low-density lipoprotein; PPC, Polyene phosphatidylcholine capsule; SOCS3, Signaling transfer suppressor 3; FFA, Free fatty acids; ROS, Reactive oxygen species; NASH, Non-alcoholic steatohepatitis.

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Abstract
Objective: To investigate the effects of Jigan Xiaozhi Formula (JGZX) on oxidative stress and inflammatory factors in patients with non-alcoholic fatty liver disease (NAFLD).

Methods: Between September 2022 and December 2023, our hospital admitted a total of 58 patients with NAFLD. These patients were split into two groups at random: one for experimentation and the other for control. There were 27 patients in the experimental group at the end, compared to 26 in the control group, reasonable exercise, weight management, lipid regulation, and oral polyene phosphatidylcholine capsules (PPC). The experimental group received JGZX in addition to the above treatments for 12 consecutive weeks. Changes in Traditional Chinese Medicine (TCM) syndrome scores, blood lipids, liver function indicators, the levels of oxidative stress markers, such as malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), as well as serum inflammatory factors, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α), were measured both before and after treatment. Results: After treatment, both groups showed significant reductions in TCM syndrome scores (P < 0.05) and improvements in blood lipids, liver function indicators, inflammatory factors, and oxidative stress markers (P < 0.05). Improvements were noticeably better in the experimental group than in the control group. (P < 0.05). Conclusion: JGZX can significantly improve clinical symptoms, regulate blood lipids, and protect liver function in patients with NAFLD. Its mechanism may be related to inhibiting inflammatory responses and regulating the balance of the oxidation-antioxidation system.

Keywords: Jigan Xiaozhi Formula; non-alcoholic fatty liver disease; oxidative stress response; inflammatory factors
**Introduction**

With the advancement of the social economy, shifts in dietary patterns and lifestyles have led to a steady rise in the prevalence of non-alcoholic fatty liver disease (NAFLD) [1]. Ten percent or so of patients with NAFLD may go on to develop nonalcoholic steatohepatitis (NASH). Within ten years, about 20% of NASH patients may develop hepatic cirrhosis. Moreover, NAFLD raises the chance of developing a number of chronic conditions, including atherosclerosis, type 2 diabetes, and hypertension [2]. Multiple clinical and basic studies have demonstrated that JGXZ can improve the ratio of liver-spleen CT values in patients with NAFLD [3], alleviate inflammation and oxidative stress in NAFLD rats, reduce liver injury, improve liver function, and activate the AMPK/JNK signaling pathway to reduce hepatocyte steatosis [4, 5]. Therefore, it is crucial to deeply explore the etiology and related mechanisms of NAFLD, and find effective early intervention measures to curb disease progression.

**Data and methods**

**General information**

The study's subjects were 58 NAFLD patients hospitalized at Jiaxing Hospital of Traditional Chinese Medicine between September 2022 and December 2023. Diagnostic criteria that met the guidelines for the diagnosis and treatment of NAFLD were the inclusion criteria [6]. Additionally, fasting 8-hour ultrasonography satisfied the diagnostic requirements for diffuse fatty liver imaging. No history of alcohol consumption or alcohol consumption equivalent to less than 140 g/week for men and less than 70 g/week for women. Exclusion of viral liver disease, drug-induced liver disease, fatty liver brought on either abrupt weight loss or extreme malnourishment, autoimmune disease, hepatotumoral degeneration, clinically diagnosed liver fibrosis, or cirrhosis patients. Randomized numbers were used to divide the two groups; There were 29 instances in the control group, 15 males and 11 females; 3 cases dropped out, 26 actual cases, with an age range from 44 to 77 years old, average (58.4 ± 7.2) years old; 29 cases in the experimental group, 2 cases dropped out, 27 actual cases, 14 males and 13 females, age range from 45 to 77 years old, average (56.5 ± 8.2) years old. Between the two groups, there was no discernible difference in general information. (P > 0.05), which was comparable. This study obtained informed consent from the patients. All the patients signed informed consent form. The collection of these clinical samples was in accordance with medical ethics guidelines and was approved by the Medical Ethics Committee of the Jiaxing Hospital of Traditional Chinese Medicine (SL-2023-0081).

**Method**

Both groups were given basic treatment, including diet control, rational exercise, body mass control, blood lipid regulation, and oral administration of polyene phosphatidylcholine capsules (PPC) [Sanofi (Beijing) Pharmaceutical Co., Ltd., national drug approval number H20059010], 228 mg/capsule, 2 capsules/time, 3 times/d. The experimental group was given JGXZ treatment on the basis of the above basic treatment. The formula was: 15 g of Poria, 10 g of Ailimu, 10 g of Atractylodes, 20 g of Astragali, 10 g of Bupleurum, 10 g of Curcuma, 9 g of lotus leaf, 10 g of Coixaegus pinnatifida, 10 g of Cassia uniflora seeds, 9 g of Salvia miltiorrhiza, 6 g of Rhizoma Curcuma, and 6 g of Glycyrrhiza uralensis Risch. Add water to 800 mL and decoct it to 200 mL. Take one dose per day in two doses, each time taking 100 mL. Treat for 12 weeks.

**Observation indicators**

**TCM Syndrome Ratings both before and after therapy.** Reference of the "Consensus Opinions on the Diagnosis and Treatment of NAFLD with Integrated Traditional Chinese and Western Medicine" were the "TCM syndrome scores for the syndrome of phlegm and blood stasis interweaving" [7]. Main symptoms included: 1) abdominal fullness; 2) sticky stools; 3) emotional depression. Secondary symptoms included: 1) bowel sounds; 2) flatulence; 3) poor appetite and fatigue; 4) belching. Tongue and pulse indicators: pale red tongue, white and greasy tongue coating, wiry and moderate or soft pulse. Each syndrome was scored as absent (0 points), mild (2 points), moderate (4 points), or severe (6 points).

**Blood lipids and liver function.** Two groups of patients were drawn for fasting venous blood before and after treatment, as well as the levels of aspartate aminotransferase (AST), triglycerides (TG), cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and alanine aminotransferase (ALT) were detected.

**Indicators of oxidative stress and inflammatory factors.** Compare the two patient groups' inflammatory and oxidative stress indicators before and after treatment. ELISA was used to quantify the levels of interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD).

**Statistical methods**

Software called SPSS 21.0 was used to do the statistical analysis. T-tests were used to examine the measurement data, which were represented as mean ± standard deviation. Chi-square tests were used to assess the count data, which were reported as rates. It was deemed statistically significant when P < 0.05.

**Results**

**The TCM syndrome scores of the two groups were compared before and after treatment**

After treatment, the TCM syndrome score of both groups decreased (P < 0.05). Compared with the two groups, the improvement was more significant in the treatment group (P < 0.05) (Table 1).

**The blood lipids and liver function indexes of the two groups were compared before and after treatment**

After treatment, the levels of TC, TG, LDL-C, AST and ALT decreased and the levels of HDL-C increased in the two groups (P < 0.05). Compared with the two groups, the improvement was more significant in the treatment group (P < 0.05) (Table 2).

**Comparison of inflammatory factors and oxidative stress indexes between the two groups before and after treatment**

After treatment, the levels of IL-6, IL-8, TNF-α, and MDA decreased and SOD, GSH-Px levels increased in the two groups (P < 0.05). Compared with the two groups, the improvement was more significant in the treatment group (P < 0.05) (Table 3).

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*Table 1 The TCM syndrome scores of the two groups were compared before and after treatment (score, T ± S)*

<table>
<thead>
<tr>
<th></th>
<th>Abdominal fullness</th>
<th>Sticky stools</th>
<th>Emotional depression</th>
<th>Bowel sounds</th>
<th>Flatulence</th>
<th>Poor appetite and fatigue</th>
<th>Belching</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G</strong></td>
<td><strong>T</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GT</strong></td>
<td>Pre-therapy</td>
<td>4.70 ± 0.36</td>
<td>4.22 ± 0.31</td>
<td>4.21 ± 0.29</td>
<td>3.86 ± 0.23</td>
<td>3.37 ± 0.89</td>
<td>2.58 ± 0.81</td>
</tr>
<tr>
<td>(n = 26)</td>
<td>Post-treatment</td>
<td>2.12 ± 0.34</td>
<td>2.31 ± 0.18</td>
<td>2.13 ± 0.24</td>
<td>2.12 ± 0.15</td>
<td>1.34 ± 0.59</td>
<td>1.23 ± 0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.75 ± 0.54</td>
<td>4.16 ± 0.27</td>
<td>4.36 ± 0.35</td>
<td>3.87 ± 0.31</td>
<td>3.47 ± 0.93</td>
<td>2.65 ± 0.74</td>
</tr>
<tr>
<td><strong>GT + JGXZ</strong></td>
<td>Post-treatment</td>
<td>1.78 ± 0.29**</td>
<td>2.01 ± 0.16**</td>
<td>1.67 ± 0.15**</td>
<td>1.58 ± 0.17**</td>
<td>1.08 ± 0.39**</td>
<td>0.99 ± 0.24**</td>
</tr>
</tbody>
</table>

Note: Before and after treatment, P < 0.05; *P < 0.01; **P < 0.05; ***P < 0.01; #P < 0.05 and ##P < 0.01 between groups after treatment.
Table 2 The blood lipids and liver function indexes of the two groups were compared before and after treatment (*P < 0.05)

<table>
<thead>
<tr>
<th>G</th>
<th>T</th>
<th>Blood lipids (mmol/L)</th>
<th>Liver function (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TC</td>
<td>TG</td>
</tr>
<tr>
<td>GT</td>
<td>(n = 26)</td>
<td>Pre-therapy</td>
<td>6.50 ± 0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>4.12 ± 0.34</td>
</tr>
<tr>
<td>GT + JGXZ</td>
<td>(n = 27)</td>
<td>Pre-therapy</td>
<td>6.55 ± 0.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>3.78 ± 0.29'</td>
</tr>
</tbody>
</table>

Note: Before and after treatment, *P < 0.05; **P < 0.01; ***P < 0.001; 0.05 < P < 0.1; +P < 0.05 and +P < 0.01 between groups after treatment.

Table 3 Inflammatory factors and oxidative stress indexes were compared between the two groups before and after treatment (*P < 0.05)

<table>
<thead>
<tr>
<th>G</th>
<th>T</th>
<th>Inflammatory factors</th>
<th>Oxidative stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IL-6 (μg/L)</td>
<td>IL-8 (μg/L)</td>
</tr>
<tr>
<td>GT</td>
<td>(n = 26)</td>
<td>Pre-therapy</td>
<td>6.50 ± 0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>4.12 ± 0.34'</td>
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<tr>
<td>GT + JGXZ</td>
<td>(n = 27)</td>
<td>Pre-therapy</td>
<td>6.55 ± 0.54</td>
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<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>3.78 ± 0.29'</td>
</tr>
</tbody>
</table>

Note: Before and after treatment, *P < 0.05; **P < 0.01; ***P < 0.001; 0.05 < P < 0.1; +P < 0.05 and +P < 0.01 between groups after treatment.

Discussion

A major contributing factor to the development of NAFLD is lipid metabolic disease [8]. Under normal circumstances, fat intake (free fatty acids or "de novo fat synthesis" and esterification) and fat disposal (metabolism or β-oxidation, low-density lipoprotein (LDL) elimination) are in a dynamic balance. In NAFLD, this balance is broken, and the elimination capacity of LDL is lower than the rate of TG intake and hepatic synthesis [9]. This observation shows that the increase in TC and TG in NAFLD patients suggests that there is abnormal lipid metabolism in NAFLD patients. With the accumulation of lipids, the blood biochemistry of NAFLD patients also showed a significant increase in AST and ALT, indicating that there is abnormal liver function and injury in NAFLD patients; after intervention with JGXZ, the liver pathological injury in NAFLD patients was alleviated, and various indicators significantly improved, suggesting that JGXZ can significantly improve the abnormal lipid metabolism and liver pathological injury in NAFLD patients. At the same time, this study selected polyene phosphatidylcholine capsule as a positive control drug, which is widely used in clinical treatment of NAFLD. According to the study's findings, PCC can enhance lipid metabolism [10] and inflammatory response and oxidative-antioxidant imbalance in the pathogenesis of NAFLD "secondary attack" are of great significance [11]. Among these, cytokine signaling transfer suppressor 3 (SOCS3) can be induced to express by IL-6 and IL-8 promote systemic and local tissue (liver) insulin resistance, cause hepatic cell fat deposition and degeneration, and induce NAFLD [12]. TNF-α participates in various immune responses and inflammatory reactions, and can cause the release of other cytokines, which is considered to be the main cytokine that causes NAFLD [13]. Additionally, TNF-α can exacerbate the development and incidence of NAFLD by impairing the intestinal mucosa's mechanical barrier [14]. In addition, due to the large amount of free fatty acids (FFA) in the body, Through oxidation in the liver cells' endoplasmic reticulum, they can generate a significant quantity of reactive oxygen species (ROS), leading to an imbalance in the body's oxidative-antioxidant system, manifested as a decrease in antioxidant enzyme activities such as SOD and GSH-Px, while reflecting the body's unsaturated fatty acid oxidation and decomposition. The content of MDA, a final product of lipid peroxidation damage, increases significantly, eventually causing damage to liver cell biological membranes, cell swelling, degeneration necrosis or fibrosis, and severe cases of cirrhosis [15, 16]. MDA is commonly utilized as a crucial indicator for assessing oxidative stress, indicating the amount of oxidative damage and the amount of ROS present within cells [17]. Antioxidant enzymes that remove reactive oxygen species include SOD and GSH-Px, effectively mitigating intracellular peroxidative reactions [18]. The findings of this study indicate elevated levels of MDA, IL-8, IL-6, and TNF-α, in addition to lower SOD and GSH-Px levels in NAFLD patients. These alterations suggest disrupted SOD/GSH-Px homeostasis, significant oxidative stress injury, and inflammatory reactions in the body. Following intervention with JGXZ, these markers shifted in the opposite direction, resulting in the restoration of SOD/GSH-Px homeostasis. This indicates that JGXZ has a demonstrable therapeutic effect on NAFLD patients, offering a path for more research into JGXZ's mechanism of action in the treatment of NAFLD.

In conclusion, JGXZ can reduce lipids, protect the liver, and enhance the NAFLD patients' clinical symptoms, and may improve the clinical symptoms of NAFLD by inhibiting inflammatory reactions and regulating the balance of oxidative-antioxidant systems in the body.

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