

Effects of Zaozhu Yincheng Decoction on a Rat Model of Nonalcoholic Steatohepatitis via Regulation of Myeloid-Derived Suppressor Cells

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Objective: To observe the therapeutic effect of Zaozhu Yincheng Decoction (ZZYC) on rats with non-alcoholic steatohepatitis (NASH) and explore its mechanism of action related to myeloid-derived suppressor cells (MDSCs).

Methods: A NASH rat model was established by feeding a high-fat diet for 16 weeks, and drug intervention was initiated from the 9th week of modeling, lasting for 8 weeks. The general status of rats was observed. Biochemical methods were used to detect the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as the triglyceride (TG) content in liver tissue. Hematoxylin-eosin (HE) staining and Oil Red O staining were performed to observe the pathological changes of liver tissue. Flow cytometry was used to detect the expression level of MDSCs in peripheral blood. Enzyme-linked immunosorbent assay (ELISA) was employed to determine the protein expression levels of arginase 1 (ARG-1) and the pro-inflammatory factor S100 calcium-binding protein A9 (S100A9) in liver tissue.

Results: Compared with the normal group, rats in the model group exhibited typical histological features of NASH. After treatment with ZZYC decoction, hepatocellular steatosis and inflammatory infiltration were significantly alleviated. Compared with the normal group, the model group showed a significant increase in liver weight, serum ALT and AST activities, liver TG and free fatty acid (FFA) content, peripheral blood MDSC level, and the expression of ARG-1 and S100A9 in liver tissue (all $P < 0.01$). These indicators were significantly reduced after ZZYC decoction treatment (all $P < 0.05$).

Conclusion: ZZYC decoction exerts a significant pharmacological effect on improving hepatocellular steatosis, ballooning degeneration, and inflammation in NASH rats. Meanwhile, it can markedly reduce the level of peripheral blood MDSCs and the expression of ARG-1 and S100A9 in liver tissue. These findings suggest that the therapeutic mechanism of ZZYC decoction for NASH is closely associated with the regulation of MDSCs.

Keywords: Zaozhu Yincheng decoction, non-alcoholic steatohepatitis, myeloid-derived suppressor cells

Non-alcoholic Fatty Liver Disease (NAFLD) is a spectrum of progressive chronic liver diseases, including simple hepatic steatosis, non-alcoholic steatohepatitis (NASH), and associated cirrhosis and hepatocellular carcinoma.¹ Recently, the nomenclature of NAFLD has been updated to metabolic dysfunction-associated steatotic liver disease (MASLD), and non-alcoholic steatohepatitis is now referred to as metabolic dysfunction-associated steatohepatitis (MASH). About 20–30% of MASLD patients progress to MASH, and 2–5% of those develop cirrhosis.² With the changes in lifestyle, the incidence of NAFLD in China has increased significantly, and up to 33% of NASH cases may progress to liver fibrosis and even various end-stage liver diseases.³ Simple steatosis occurs during the early stage of MASLD/NAFLD, where fat accumulates in hepatocytes without significant inflammation.⁴ At this stage, the condition is generally considered

reversible through lifestyle interventions such as dietary modification and increased physical activity. However, persistent metabolic dysfunction, such as obesity, insulin resistance, and dyslipidemia, can promote oxidative stress, lipotoxicity, and inflammatory signaling.⁵ The progression to an inflammatory stage leads to hepatocyte injury, which may further drive fibrosis and eventually cirrhosis through activation of hepatic stellate cells, resulting in the excessive production of extracellular matrix and scar tissue.⁶ Therefore, active prevention and treatment of NASH to halt its progression to cirrhosis and hepatocellular carcinoma are of great social and economic significance.⁷

Although several pharmacological treatments such as pioglitazone, vitamin E, and GLP-1 receptor agonists have shown therapeutic potential for NASH,⁸ their clinical application remains limited due to side effects and inconsistent efficacy. Therefore, the development of alternative therapeutic strategies, including traditional Chinese medicine formulations, has attracted increasing attention.

Previous studies have demonstrated that ZZYC decoction exerts a significant therapeutic effect on NASH,⁹ but its underlying mechanism remains unclear. Recent studies have confirmed that the hepatic immune microenvironment plays a crucial role in maintaining liver functional homeostasis, and MDSCs are characterized by their ability to regulate the hepatic immune microenvironment and participate in the pathogenesis of NASH.¹⁰ Thus, this study focused on MDSCs to investigate the therapeutic mechanism of ZZYC decoction in NASH.

Materials and Methods

Animals

Specific pathogen-free (SPF) grade male Sprague-Dawley (SD) rats, aged 8 weeks and weighing 180–200 g, were provided by Shanghai SIPPR-BK Laboratory Animal Co., Ltd. (License No.: SCXK (Hu) 2013–2016) and housed in the Animal Experiment Center of Medical College of Xiamen University. The experimental protocol was approved by the Animal Ethics Committee of Xiamen University (Approval No.: XMULAC20120020). All animal procedures were performed in accordance with institutional guidelines for the care and use of laboratory animals.

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) followed by cervical dislocation, in accordance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

Drugs

ZZYC decoction, composed of Semen Gleditsiae Sinensis (15 g), Rhizoma Atractylodis Macrocephalae Praeparatum (15 g), Herba Artemisiae Scopariae (15 g), Fructus Gardeniae (9 g), and Radix et Rhizoma Rhei Praeparatum cum Vino (6 g), was prepared into a traditional Chinese medicine suspension with a concentration of 1 g/mL by the Traditional Chinese Medicine Laboratory of Medical College of Xiamen University. The high-fat diet (formulation: 77.5% regular chow, 0.5% sodium cholate, 2% cholesterol, 5% soybean powder, 5% sucrose, and 10% lard) was purchased from Suzhou Shuangshi Experimental Animal Feed Technology Co., Ltd. (License No.: (2017) 05005). Pioglitazone hydrochloride tablets (15 mg per tablet) were obtained from Chongqing Kerui Pharmaceutical (Group) Co., Ltd. (National Medicine Standard No.: H20080271).

Reagents

Triglyceride (TG) assay kit (Cat. No.: R21738-96T), alanine aminotransferase (ALT) assay kit (Cat. No.: BC1555), aspartate aminotransferase (AST) assay kit (Cat. No.: BC1565), fasting blood glucose (FBG) assay kit (Cat. No.: BC2496), arginase 1 (ARG-1) ELISA kit (Cat. No.: MB-6667B), and S100 calcium-binding protein A9 (S100A9) ELISA kit (Cat. No.: MB-7354A) were all purchased from Xiamen Minyan Biotechnology Co., Ltd. Rat flow cytometry antibodies CD11b-FITC (Cat. No.: 130–113-796) and His48-PE (Cat. No.: sc-19613 PE) were purchased from Hangzhou Bioss Antibodies Co., Ltd. and Xiamen Taijing Biotechnology Co., Ltd., respectively.

Instruments

The instruments used in this study included M200 Pro microplate reader (TECAN), R134a high-speed refrigerated centrifuge (Eppendorf), DW-AL240V ultra-low temperature freezer (Zhongke Meiling), RM2245 microtome (Leica),

BX53 intelligent biological microscope (Olympus), Excelsior AS tissue processor (Thermo Fisher Scientific), and IKA T18 homogenizer (ULTRA-TURRAX).

Methods

Animal Grouping and NASH Model Establishment

The sample size ($n = 10$ per group) was selected based on previous experimental studies using similar NASH rat models and was considered sufficient to detect significant biochemical and histological changes. After 1 week of adaptive feeding, the rats were randomly divided into 4 groups using a random number table ($n=10$ per group): normal group, model group, ZZYC decoction group, and pioglitazone group (the latter three groups were further randomized at week 9). The model group, ZZYC decoction group, and pioglitazone group were fed a high-fat diet to induce NASH, while the normal group was fed a regular chow diet for 16 consecutive weeks. All rats were housed under controlled conditions: temperature (23 ± 2) °C, relative humidity (60 ± 10)%, and a 12-hour light/dark cycle.

Drug Administration

Gavage administration was initiated from week 9 and continued for 8 weeks. The dosage of ZZYC decoction was converted based on clinical human dosage (assuming an adult weight of 60 kg). The ZZYC decoction group was given 1 g/mL suspension at a dose of 10 mL/kg per day by gavage, equivalent to 10 g/kg of crude drug. Pioglitazone hydrochloride was dissolved in distilled water to prepare a 1 mg/mL solution, and the pioglitazone group was gavaged with 10 mL/kg per day, equivalent to 10 mg/kg of pioglitazone. The normal group and model group were gavaged with 10 mL/kg of double-distilled water once daily for 8 consecutive weeks.

Observation Indicators and Detection Methods

Sample Collection

At the end of week 16, after weighing the rats, blood samples were collected from the abdominal aorta. The liver and epididymal adipose tissue were harvested and weighed. A portion of liver tissue was fixed in 10% paraformaldehyde for histopathological analysis, and the remaining liver tissue was stored at -80 °C for biochemical detection.

Liver Histopathological Observation

Liver tissue fixed in 10% paraformaldehyde was embedded in paraffin, sectioned, and stained with HE and Oil Red O. The stained sections were observed and photographed under a light microscope to evaluate pathological changes.

Detection of Serum ALT, AST, and FBG Levels

The levels of serum ALT, AST, and FBG were detected using an automated biochemical analyzer according to the kit instructions.

Detection of TG and FFA Content in Liver Tissue

Approximately 100 mg of liver tissue from the same location was excised and accurately weighed, then placed in a centrifuge tube with 0.9 mL of anhydrous ethanol. The liver tissue was homogenized using an electric homogenizer in an ice-water bath and centrifuged at 2,500 r/min for 10 min at 4 °C. A 2.5 μ L aliquot of the supernatant was collected, and the contents of TG and free fatty acid (FFA) were determined using commercial assay kits.

Detection of MDSC Expression in Peripheral Blood

The expression level of MDSCs in peripheral blood was detected by flow cytometry following the standard protocol of the antibodies used.

Detection of ARG-I and S100A9 Protein Expression in Liver Tissue

The protein expression levels of ARG-I and S100A9 in rat liver tissue were measured using corresponding ELISA kits according to the manufacturer's instructions.

Statistical Analysis

All data were analyzed using SPSS 22.0 statistical software. Data conforming to normal distribution were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Multiple group comparisons were performed using one-way analysis of variance (ANOVA), and pairwise comparisons were conducted using *t*-test. A *P* value of <0.05 was considered statistically significant.

Results

Comparison of Body Weight, Liver Weight Index, and Liver TG/FFA Content (TABLE 1)

Compared with the normal group, liver weight in the model group increased significantly (19.51 ± 2.41 g vs. 12.31 ± 2.22 g). ZZYC decoction reduced this to 15.44 ± 2.58 g ($P < 0.05$), representing a 20.8% reduction in liver weight compared to the model group. Hepatic TG levels in the model group increased markedly to 11.83 ± 2.35 μmol/g. ZZYC treatment significantly lowered TG to 8.22 ± 2.58 μmol/g ($P < 0.01$), achieving a 30.5% reduction. Pioglitazone only reduced TG to 9.81 ± 2.23 μmol/g. ZZYC decoction showed a robust effect on FFA, decreasing levels from 96.52 ± 19.43 μmol/g in the model group to 68.94 ± 16.24 μmol/g ($P < 0.01$), a 28.6% reduction. Compared with the model group, both the ZZYC decoction group and pioglitazone group exhibited a significant decrease in these indicators ($P < 0.01$ or $P < 0.05$), and the therapeutic effect of ZZYC decoction was more pronounced. The body weight of the model group was higher than that of the normal group, but the difference was not statistically significant ($P > 0.05$) (Table 1).

Comparison of Serum ALT, AST, and FBG Levels

Compared with the normal group, the model group exhibited significantly elevated serum ALT, AST, and FBG levels. Treatment with ZZYC decoction significantly reduced these parameters compared with the model group. Serum ALT and AST activities in the model group were 69.23 ± 8.72 IU/L and 72.07 ± 7.27 IU/L, respectively. ZZYC treatment led to a 34.6% reduction in ALT (45.27 ± 4.57 IU/L) and a 33.1% reduction in AST (48.25 ± 5.17 IU/L), both with high statistical significance ($P < 0.01$) (Table 2).

Liver Histopathological Changes

HE staining showed that hepatocytes in the normal group had a normal structure without steatosis, with clear lobular architecture and no significant inflammatory cell infiltration in the lobules. In the model group, liver tissue showed obvious hepatocellular ballooning degeneration, cell swelling, steatosis, diffuse inflammatory cell infiltration in the lobules, and fusion of some necrotic foci into patches. In the ZZYC decoction group, hepatocellular ballooning degeneration was significantly alleviated, with only a small number of swollen cells, centrally located nuclei, and localized mild inflammatory cell infiltration. In the pioglitazone group, hepatocellular ballooning degeneration, cell swelling, and localized inflammatory cell infiltration were still observed (Figure 1).

Table 1 Comparison of Body Weight, Liver Weight Index, and Liver TG/FFA Content

Group	n	Body Weight (g)	Liver Weight (g)	Liver Index (%)	TG (mg/g)	FFA (μmol/g)
Normal	10	423.2 $\pm$ 22.1	12.31 $\pm$ 2.22	2.83 $\pm$ 0.22	5.64 $\pm$ 1.26	65.21 $\pm$ 14.42
Model	10	454.1 $\pm$ 34.5	19.51 $\pm$ 2.41**	4.29 $\pm$ 0.44**	11.83 $\pm$ 2.35**	96.52 $\pm$ 19.43**
ZZYC decoction	10	435.8 $\pm$ 29.2	15.44 $\pm$ 2.58Δ	3.31 $\pm$ 0.31Δ	8.22 $\pm$ 2.58ΔΔ	68.94 $\pm$ 16.24ΔΔ
Pioglitazone	10	445.3 $\pm$ 28.4	17.67 $\pm$ 3.12	3.98 $\pm$ 0.53	9.81 $\pm$ 2.23Δ	73.45 $\pm$ 17.62Δ

Note: Compared with the normal group, ** $P < 0.01$; Compared with the model group, Δ $P < 0.05$, ΔΔ $P < 0.01$.

Abbreviations: FFA, Free Fatty Acid; TG, Triglyceride; ZZYC, Zaozhu Yinchen Decoction.

Table 2 Comparison of Serum ALT, AST, and FBG Levels

Group	n	ALT (IU/L)	AST (IU/L)	FBG (mmol/L)
Normal	10	26.25±4.53	27.88±5.23	5.26±0.18
Model	10	69.23±8.72**	72.07±7.27**	6.45±0.32*
ZZYC decoction	10	45.27±4.57ΔΔ	48.25±5.17ΔΔ	5.53±0.19Δ
Pioglitazone	10	56.22±4.36Δ	64.33±5.43Δ	5.64±0.21Δ

Note: Compared with the normal group, *P<0.05, **P<0.01; Compared with the model group, ΔP<0.05, ΔΔP<0.01. Data are expressed as mean ± SD (n = 10).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBG, fasting blood glucose.

Oil Red O staining showed significant steatosis in the model group, with numerous large lipid droplets, and the staining in the central lobular area was deeper than that in the peripheral area. The ZZYC decoction group showed a significant reduction in the degree of hepatic steatosis and the number of lipid droplets, while the pioglitazone group showed no obvious improvement (Figure 2).

Expression of MDSCs in Peripheral Blood

Compared with the normal group, the expression level of MDSCs in peripheral blood was significantly increased in the model group. Compared with the model group, ZZYC treatment significantly reduced the proportion of CD11b⁺His48⁺ cells (MDSCs) in peripheral blood, while no significant change was observed in the pioglitazone group (Figure 3).

Expression of ARG-1 and S100A9 in Liver Tissue

Levels of the pro-inflammatory factor S100A9 were reduced by 23.7% following ZZYC treatment (20.91 ± 4.26 pg/mg) compared to the model group (27.42 ± 6.62 pg/mg, $P < 0.01$). Compared with the normal group, the protein expression levels of ARG-1 and S100A9 in liver tissue were significantly increased in the model group (both $P < 0.01$). Compared

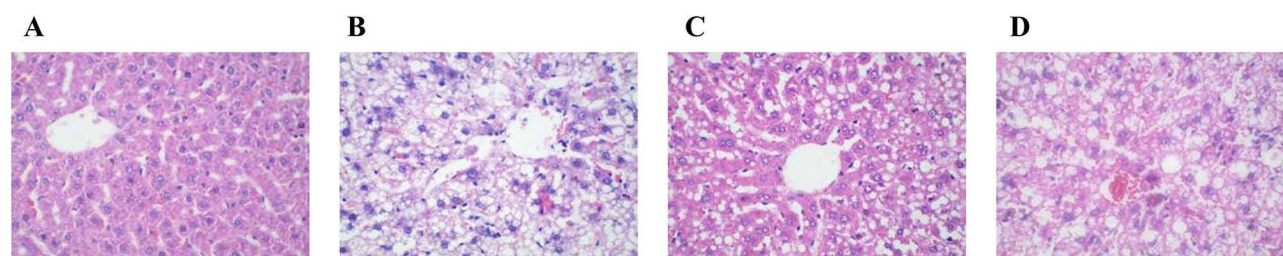


Figure 1 Pathological changes of rat liver tissue (HE staining, ×100). Representative liver sections from each group are shown: (A) Normal group, displaying intact hepatic architecture without steatosis or inflammation; (B) Model group, showing hepatocellular ballooning degeneration, steatosis, and diffuse inflammatory infiltration; (C) ZZYC decoction group with improved hepatocellular morphology and reduced inflammatory cell infiltration; (D) Pioglitazone group, showing partial improvement with residual ballooning and localized inflammation.

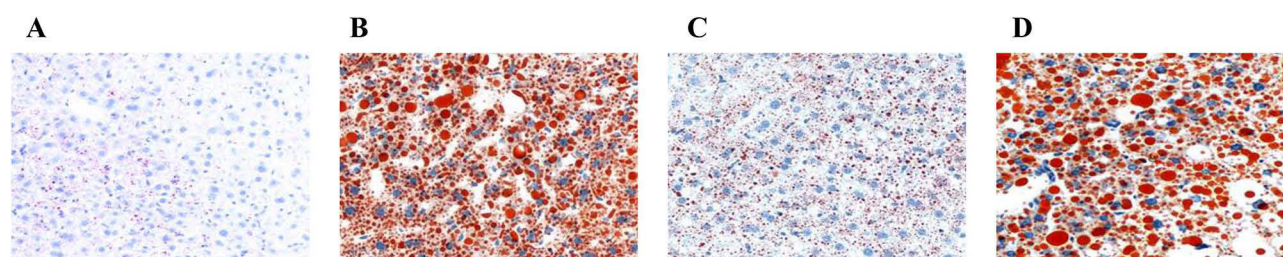


Figure 2 Oil Red O staining of rat liver tissue (×400). Representative sections demonstrate lipid accumulation in hepatocytes: (A) Normal group, minimal lipid droplets; (B) Model group, extensive steatosis with numerous large lipid droplets; (C) ZZYC decoction group, reduced lipid deposition and improved tissue morphology; (D) Pioglitazone group, moderate improvement with persistent lipid droplets.

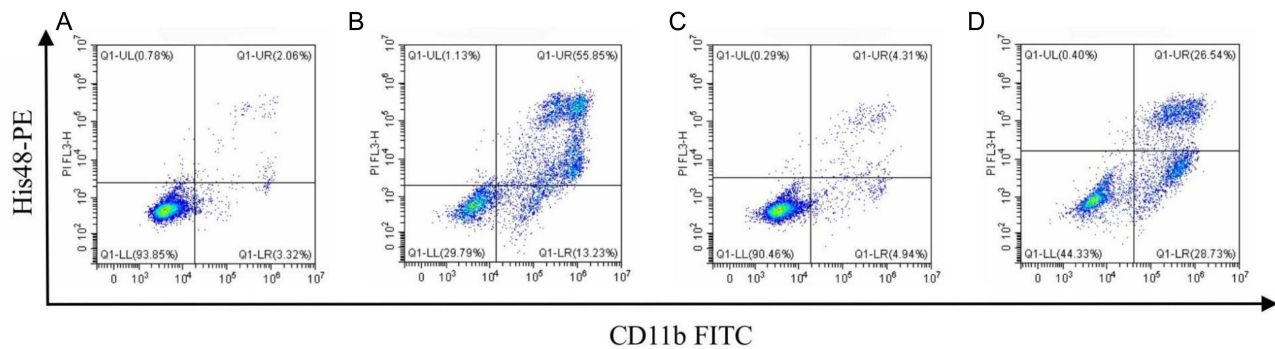


Figure 3 Changes in peripheral blood MDSC expression in rats (flow cytometry) Dot plots show CD11b-FITC versus His48-PE staining. The proportion of CD11b⁺His48⁺ cells (Q1-LR quadrant) represents MDSCs. Compared with the normal group (A), the model group (B) exhibited a significant increase in MDSCs. ZZYC decoction treatment (C) reduced MDSC levels, while the pioglitazone group (D) showed partial modulation.

with the model group, the ZZYC decoction group showed a significant decrease in the expression of ARG-1 and S100A9 (both $P<0.01$), while the pioglitazone group showed no significant change (both $P>0.05$) (Table 3).

Discussion

However, several limitations should be acknowledged. First, this study was conducted in a single animal model, which may not fully capture the complex pathophysiology of human NASH. Second, fibrosis scoring and long-term progression were not evaluated in this study. Third, the specific molecular signaling pathways involved in MDSC regulation were not investigated and require further mechanistic studies. Addressing these points in future work will help strengthen the evidence and improve the translational relevance of ZZYC decoction in NASH.

The pathogenesis of NASH is complex and multifactorial, and its underlying mechanisms have not been fully elucidated. The “multiple-hit” hypothesis, which involves insulin resistance, endoplasmic reticulum stress (ERS), cytokines, genetic polymorphisms, lipotoxicity, and gut microbiota, is currently the widely recognized pathogenic mechanism.¹¹ The “first hit” refers to the excessive accumulation of fat in hepatocytes, leading to hepatic steatosis and increased susceptibility of the liver to inflammatory damage. The “second hit” is based on the “first hit,” where the overproduction of reactive oxygen species (ROS) and their metabolites from oxidative metabolic products, adipokines, lipid peroxides, and inflammatory mediators induces oxidative stress and ERS, thereby activating hepatic immune cells and ultimately promoting the progression of simple steatosis to hepatocellular inflammation and necrosis.¹² The “third hit” involves the dysregulation of the hepatic immune microenvironment, which further drives the progression of NASH to liver fibrosis, cirrhosis, and hepatocellular carcinoma.¹³

As an important immune organ, the liver has a complex immune microenvironment composed of various immune cells and cytokines, which plays a critical role in maintaining liver functional homeostasis.¹⁴ MDSC-mediated regulation of the hepatic immune microenvironment is an important pathogenic mechanism of NASH. Studies have shown that MDSCs are characterized by their ability to regulate the hepatic immune microenvironment; they inhibit T cell proliferation and activation signaling by expressing high levels of ARG-1, while simultaneously promoting the release of inflammatory

Table 3 Expression of ARG-1 and S100A9 in Liver Tissue

Group	n	ARG-1 (pg/mg)	S100A9 (pg/mg)
Normal	10	12.33±3.25	14.63±4.21
Model	10	25.61±6.53**	27.42±6.62**
ZZYC decoction	10	17.48±5.78ΔΔ	20.91±4.26ΔΔ
Pioglitazone	10	24.22±6.25	25.12±4.27

Note: Compared with the normal group, ** $P<0.01$; Compared with the model group, ΔΔ $P<0.01$. Data are expressed as mean ± SD (n = 10).

Abbreviations: ARG-1, arginase-1; S100A9, S100 calcium-binding protein A9.

mediators.^{15,16} ARG-1 catalyzes the hydrolysis of L-arginine into ornithine and urea, leading to depletion of L-arginine in the local microenvironment. Since L-arginine is essential for T-cell receptor expression and T-cell proliferation, its depletion results in impaired T-cell activation and reduced immune surveillance.^{17,18} Meanwhile, the number of MDSCs in NASH mouse models increases significantly with lipid accumulation, and MDSCs promote intrahepatic lipid deposition by secreting the pro-inflammatory factor S100A9, which can amplify inflammatory signaling and enhance lipid accumulation in hepatocytes.^{19–21} Therefore, the combined effects of ARG-1-mediated T-cell suppression and S100A9-induced inflammatory signaling contribute to persistent hepatic inflammation and progression of NASH.

In this study, HE and Oil Red O staining results showed that the high-fat diet-induced model rats exhibited typical NASH histological features, including hepatocellular steatosis, diffuse intra-lobular inflammatory cell infiltration, and fusion of necrotic foci. The increased expression of peripheral blood MDSCs and the upregulated protein levels of ARG-1 and S100A9 in liver tissue further confirmed the presence of hepatic immune microenvironment disorder in NASH.

In traditional Chinese medicine, NASH is classified under the category of “Gan Pi” (liver obstruction). Its pathological factors are summarized as the accumulation of “phlegm, dampness, blood stasis, and heat,” which block the liver meridians. The pathogenesis is characterized by spleen dysfunction and liver damage as the root cause, and the mutual accumulation of phlegm, dampness, blood stasis, and heat as the superficial manifestation, belonging to a pattern of coexisting deficiency and excess. Based on the classic formula Yinchenhao Decoction, our research team developed ZZYC decoction by adding Semen Gleditsiae Sinensis and Rhizoma Atractylodis Macrocephalae Praeparatum. In this formula, pharmacological studies have shown that *Herba Artemisiae Scopariae* exhibits hepatoprotective and anti-inflammatory effects and can regulate bile acid metabolism,²² *Semen Gleditsiae Sinensis* demonstrates immunomodulatory and anti-fibrotic activity^{23,24} by suppressing inflammatory mediators and regulate cytokine expression. *Fructus Gardeniae* clears heat and purges fire, *Rhizoma Atractylodis Macrocephalae Praeparatum* strengthens the spleen and eliminates dampness, and *Radix et Rhizoma Rhei Praeparatum cum Vino* has been shown to alleviate hepatic lipid accumulation and inflammatory injury. The synergistic combination of these herbs may therefore contribute to the improvement of hepatic steatosis and inflammatory responses observed in this study. In particular, the observed reduction in MDSC levels and decreased expression of ARG-1 and S100A9 suggest that ZZYC decoction may exert its therapeutic effects partly through modulation of immune-mediated inflammatory pathways involved in NASH progression.²⁵ These results indicate that ZZYC decoction influences both metabolic and immune aspects of NASH pathogenesis, underscoring the value of multi-target herbal approaches in tackling complex metabolic liver diseases.

On the basis of previous studies, this study induced a fatty liver model by feeding a high-fat diet for 16 weeks and administered ZZYC decoction from week 9. The results confirmed that ZZYC decoction can improve liver histopathological changes, reduce liver weight index, liver tissue TG and FFA contents, and serum ALT, AST, and FBG levels in NASH rats. Although ZZYC significantly reduced liver weight and lipid content, the lack of a significant difference in total body weight suggests that the decoction's effects may be specifically targeted at hepatic metabolism rather than systemic obesity.

Conclusion

Overall, the present study demonstrates that ZZYC decoction reduces peripheral blood MDSCs and lowers hepatic ARG-1 and S100A9 protein levels, thereby alleviating hepatocellular inflammation and necrosis and inhibiting NASH progression. The therapeutic effects appear linked to modulation of the MDSC-mediated hepatic immune microenvironment. While pioglitazone, a PPAR- γ agonist primarily targeting insulin sensitivity, showed minimal impact on peripheral MDSC levels, ZZYC decoction seems to act through distinct immunological pathways that pioglitazone does not address. Pioglitazone, a PPAR- γ agonist, has been widely investigated for NASH because of its ability to improve insulin sensitivity and lipid metabolism. However, its therapeutic effects are primarily associated with metabolic regulation rather than direct modulation of immune cell populations.^{26,27} In contrast, our findings indicate that ZZYC decoction may also act on the immune axis, lowering MDSC levels and suppressing the expression of ARG-1 and S100A9, thereby modulate the hepatic immune microenvironment. These findings provide experimental evidence supporting the potential application of ZZYC decoction in the treatment of NASH. However, further studies are required to clarify the underlying molecular mechanisms and evaluate its translational potential in clinical settings.

Abbreviations

ALT, alanine aminotransferase; ARG-1, arginase 1; AST, aspartate aminotransferase; ELISA, enzyme-linked immunosorbent assay; FBG, fasting blood glucose; FFA, free fatty acid; HE, hematoxylin and eosin; MDSCs, myeloid-derived suppressor cells; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; S100A9, S100 calcium-binding protein A9; TG, triglyceride; ZZYC, Zaozhu Yincheng decoction.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article. Additional raw data supporting the findings of this study are available from the corresponding author, Shaodong Chen, upon reasonable request.

Ethics Approval and Consent to Participate

All animal experiments were conducted in accordance with the guidelines of the Animal Ethics Committee of Xiamen University (Approval No.: XMULAC20120020). No human participants were involved in this study.

Patient Consent for Publication

This study did not involve human participants or patient data.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

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