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Original Article

Modified Buyang Huanwu Decoction alleviates diabetic liver injury via inhibiting oxidative stress in db/db mice

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Abstract: Objectives: In diabetes, chronic hyperglycemia increases the overactivation of oxidative phosphorylation of mitochondria in the liver, resulting in oxidative stress (OS) damage. The Nrf2 signaling pathway plays a key role in preventing hepatic oxidative injury and inflammation. This study aims to investigate the therapeutic effect and mechanism of Modified Buyang Huanwu Decoction (mBYHWD) on diabetic liver injury (DLI) by regulating oxidative stress mediated by Nrf2 signaling pathway. Methods: The experiment was divided into three groups: a control group (db/m mice, Con), a diabetes model group (db/db mice, Mod), and a traditional Chinese medicine group (db/m mice, mBYHWD). Post-treatment, serum from each group was analyzed to assess changes of blood glucose, blood lipid, and liver function. These results were combined with data mining to explore the possible pathogenesis of DLI. Liver tissues were collected to observe the pathological morphology and detect related proteins. Results: The results demonstrated that mBYHWD significantly reduced blood lipids and improved liver function following diabetic liver injury. The histopathological results demonstrated that mBYHWD could significantly ameliorate damage of diabetic hepatocytes. Protein analysis revealed that mBYHWD treatment significantly increased the expression of antioxidant proteins in diabetic liver tissue and inhibited inflammation. Conclusions: The therapeutic mechanism of mBYHWD on DLI may involve activating the Nrf2 signaling pathway to improve oxidative stress, inhibit inflammation, and reduce liver tissue fibrosis.

Keywords: Diabetic liver injury, Modified Buyang Huanwu Decoction, oxidative stress, inflammation, hepatic fibrosis

Introduction

Diabetic liver injury (DLI) is a common complication of diabetes mellitus (DM), characterized by histopathological changes and enzymatic function impairment in the liver [1]. Approximately 78% of type 2 diabetes mellitus (T2DM) patients suffered liver injury, which became one of the most prevalent causes of death in diabetic patients [2-4]. The pathogenesis of DLI is very complex, involving fat droplet accumulation and balloon-like transformation of liver cells under chronic hyperglycemia. This lipid accumulation leads to liver steatosis, lipid peroxidation, pro-inflammatory cytokines release, and eventually induces irreversible damage such as hepatocyte steatosis, inflammatory cell infiltration, fibrosis, etc. [5]. Among them, oxidative stress (OS) induces hepatocyte apoptosis and inflammatory factor release, leading to liver dysfunction, which is central to the development of DLI [6, 7]. Nrf2, a redox-sensitive transcription factor encoded by the NFE2L2 gene, protects against reactive oxygen species-induced oxidative damage by regulating OS and antioxidant mechanisms [8]. Recent evidence suggests that Nrf2 activation can significantly enhance glucose homeostasis and reduce inflammatory responses in diabetic mice [9]. In addition, the changes in oxidative status in DLI can induce the activation of the nuclear factor κB (NF-κB) inflammatory signal transduction pathway [10-12]. These findings suggest that an imbalance in the Nrf2 signaling pathway

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leads to OS and inflammatory injury, which may contribute to the onset of DLI.

In traditional Chinese medicine (TCM) theory, DLI is classified under the "Expelling Thirst" syndrome, commonly presenting with symptoms such as fatigue, dizziness, distention, waist and knee tenderness, and exhaustion. This categorization implies that the TCM syndromes of DM patients with liver complications are primarily liver-kidney co-diseases, with Yin deficiency playing a significant role in the pathogenesis. Modern research supports this view, demonstrating that DM complicated by liver and kidney injury influences each other, with OS playing a crucial role in the pathogenesis [13, 14]. The Buyang Huanwu Decoction (BYHWD) is known for its anti-inflammatory [15], antioxidant [16, 17], and vascular and nerve regeneration properties [18]. It has therefore been extensively researched for treating conditions like atherosclerosis, apoplexy, and diabetic lower limb ischemia, among others [19-21]. The Modified Buyang Huanwu Decoction (mBYHWD), which combines BYHWD and Shenqi Dihuang Decoction with certain modifications, shows enhanced efficacy in benefiting qi and nourishing Yin. Our previous research results showed that mBYHWD can protect glomerular podocytes, reduce albuminuria, inhibit kidney fibrosis, and improve diabetic nephropathy [18]. Moreover, it was also found to significantly improve the liver function of mice. However, the therapeutic effects and mechanisms of mBYHWD on DLI have not been fully elucidated.

In this study, spontaneous diabetes db/db mice were selected to establish an in vivo animal model, with mBYHWD administered as a drug intervention. The results demonstrated that mBYHWD could regulate the Nrf2 and NF-κB signaling pathways, improving DLI by mitigating oxidative stress injury, inhibiting intrahepatic inflammation, and reducing liver fibrosis. These results indicate that mBYHWD has a significant therapeutic effect on hepatic fibrosis mediated by oxidative stress and inflammation, providing a valuable theoretical basis for further research into the clinical treatment of DLI.

Materials and methods

Preparation and grouping of animal models

Control group mice (db/m, Con group) and spontaneous diabetic (db/db) mice were purchased to establish the diabetic mouse model. The db/db mice were randomly divided into model group (Mod group) and traditional Chinese medicine group (mBYHWD group), with each group comprising 6 mice. The dose for the mice was calculated based on the equivalent dose converted from the body surface area ratio of humans to mice, resulting in a dose 9.1 times of that given to adults. According to the calculation of 188 g of Chinese medicine for a 70 kg adult daily, the mouse dosage was set at 24.44 g/kg. 24.44 g of TCM granules were respectively weighed and dissolved in 10 mL of 0.9% sodium chloride solution. The volume of intragastric drugs in the mBYHWD group was 10 mL/kg. The Con and Mod groups were given intragastric 0.9% sodium chloride solution at 10 mL/kg. Mice were given intragastric administration once a day for 12 weeks. After the intervention, serum and liver were collected for follow-up experiments. All animal experiments were approved by the Ethical Committee of Hebei University of Chinese Medicine and were conducted in accordance with all ethical standards (No. DWLL202203117).

Preparation of mBYHWD

The mBYHWD drug composition and the adult clinical dosage include Huang Qi (Milkvetch Root) 30 g, Dang Gui (Angelicae Sinensis Radix) 10 g, Chi Shao (Paeoniae Radix Rubra) 10 g, Tao Ren (Persicae Semen) 10 g, Di Long (Pheretima) 12 g, Jiang Can (Bombyx Batryticatus) 10 g, Chan Tui (Cicadae Periostracum) 6 g, Tai Zishen (Pseudostellariae Radix) 15 g, Fu Ling (Poria) 15 g, Bai Zhu (Macrocephalae Rhizoma) 10 g, Ze Lan (Lycopi Herba) 10 g, Shu Di (Rehmanniae Radix Praeparata) 15 g, Shan Yao (Common Yam Rhizome) 15 g, Mu Danpi (Tree Peony Bark) 10 g, and Shan Zhuyu (Common Macrocarpium Fruit) 10 g. This formulation is composed of traditional Chinese medicine granules, sourced from the Traditional Chinese Medicine Hall of Hebei University of Traditional Chinese Medicine and produced by SHINEWAY Pharmaceutical.

Biochemical analyses

Collected whole blood samples were left at room temperature for 2 hours to allow for clotting, then centrifuged at 3000 RPM for 15 minutes to obtain serum. Serum levels of alanine aminotransferase (ALT), aspartate transaminase (AST), total cholesterol (TC), triglyceride

(TG), and high-density lipoprotein (HDL) were quantified using an Automatic Biochemical Analyzer.

Equal weights of liver tissue were mechanically homogenized in ice-cold water to prepare a 10% liver tissue homogenate. The homogenate was then centrifuged at 3000 RPM for 10 minutes. The supernatant was used with corresponding assay kits (Jiancheng Bioengineering Institute, Nanjing) for the detection of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and malondialdehyde (MDA).

Analysis of differential gene expression sequencing data in DLI

Gene sequencing data for mouse diabetic liver injury were obtained from the Gene Expression Omnibus database (GEO). The Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used for Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The TRING database was utilized for Protein-Protein Interaction Networks (PPI) analysis.

Hematoxylin and eosin (HE) staining

For H&E staining, dewaxed paraffin sections were stained with hematoxylin for 1-2 minutes, then rinsed with tap water. They were differentiated briefly in 1% hydrochloric acid in ethanol and washed, followed by immersion in eosin solution for 1-2 minutes before rinsing. The liver tissue sections were then dehydrated and mounted with neutral gum. Histopathology was observed and images were captured using an optical microscope.

Masson's trichrome staining

The dewaxed and hydrated sections were stained with prepared Weigert's hematoxylin for 4-8 minutes, then differentiated with acidic ethanol solution and washed with water. Sections were stained with Masson's bluing solution and Ponceau S acid fuchsin for 4-8 minutes, followed by washing with weak acid solution and phosphomolybdic acid solution. The sections were then stained with aniline blue for 1-2 minutes, washed with weak acid solution for 1 minute, dehydrated, cleared, and mounted with neutral gum. The stained sections were observed under an optical microscope.

Periodic acid-Schiff (PAS) staining

Paraffin sections were dewaxed and incubated with periodic acid solution for 4-5 minutes, rinsed with distilled water, and stained with Schiff reagent for 10-20 minutes. After washing with running water, sections were stained with hematoxylin for 1-2 minutes, differentiated in acidic ethanol solution, and soaked in Scott's tap water. The steps for dehydration, clearing, and mounting followed those in H&E staining. Collagen fibers in liver tissue appear blue under a light microscope.

Immunocytochemistry (IHC) and immunofluorescence (IF)

Paraffin sections were baked at 60°C for 1 h, dewaxed, and subjected to antigen retrieval. After blocking with 5% BSA, primary antibodies were applied to the tissue overnight at 4°C. Secondary antibodies were then added and incubated at 37°C for 15-30 minutes. After washing with PBS, SABC was applied to the tissue sections at 37°C for 30 minutes. Freshly mixed chromogen was applied to liver tissue, and the reaction was monitored under a microscope, then terminated by rinsing with tap water. Hematoxylin was used to stain nuclei. The sections were dehydrated, cleared, and mounted for observation.

For immunofluorescence, primary and secondary antibodies were replaced with a fluorescent conjugate, with all other procedures similar to those of immunohistochemistry.

Western blot

Total liver tissue protein was extracted following manufacturer's protocols. Equal amounts of protein extract were separated by agarose gel electrophoresis. After electrophoresis, proteins were transferred to a PVDF membrane. The PVDF membrane was blocked in 5% skim milk and incubated at room temperature for 1 hour. Corresponding primary antibodies were diluted and incubated with the PVDF membrane overnight on a shaker at 4°C. The dilution ratios for primary antibodies were as follows: rabbit anti-IL-1β (1:1000, Servicebio), rabbit anti-TNF-α (1:250, Servicebio), mouse anti-NFκB-P65

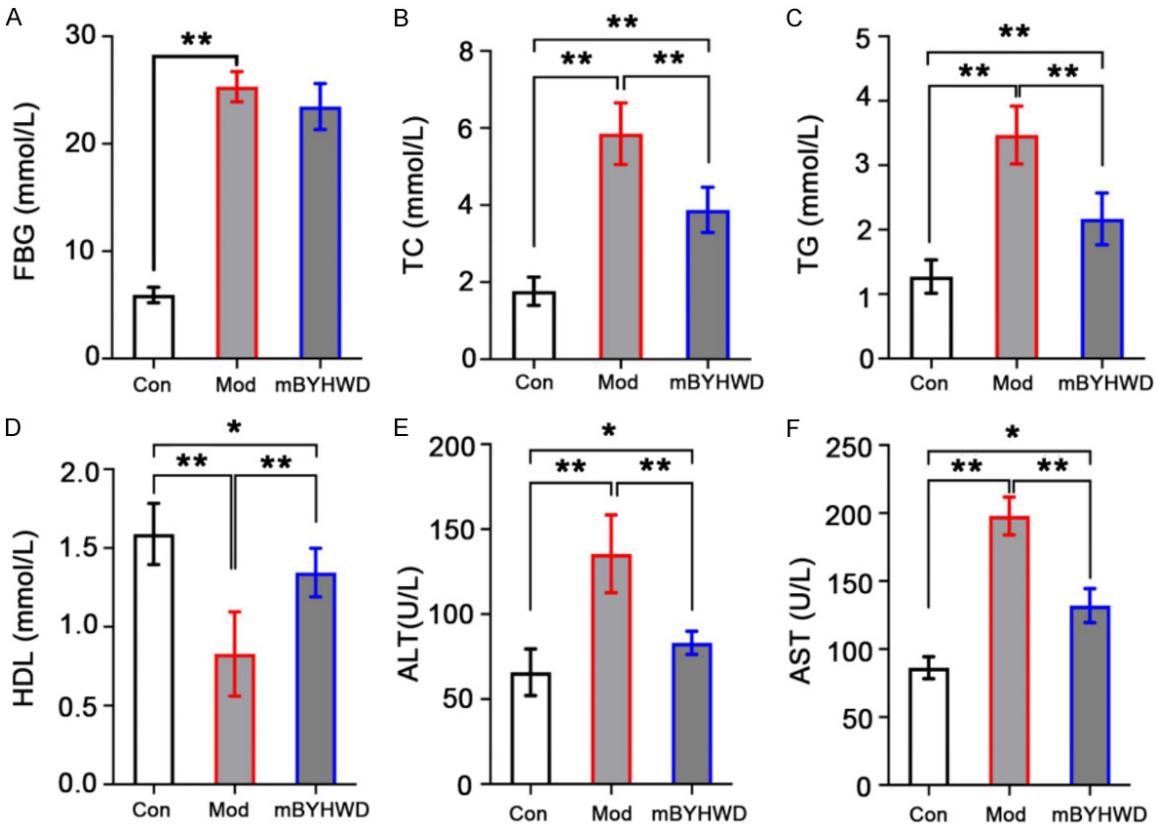

Figure 1. Effects of Modified Buyang Huanwu Decoction (mBYHWD) on biochemical indices of liver function. A. Fasting blood glucose (FBG). $^{**}P < 0.01$; B. Total cholesterol (TC). $^{**}P < 0.01$; C. Triglycerides (TG). $^{**}P < 0.01$; D. High density liptein cholesterol (HDL). $^{*}P < 0.05$, $^{**}P < 0.01$; E. Alanine aminotransferase (ALT). $^{*}P < 0.05$, $^{**}P < 0.01$; F. Aspartate aminotransferase (AST). $^{*}P < 0.05$, $^{**}P < 0.01$.

(1:500, Bioss), mouse anti-IκBα (1:1000, Proteintech), rabbit anti-p-IκBα (1:800, Bioss), rabbit anti-Nrf2 (1:800, Protientech), mouse anti-HO-1 (1:500, Servicebio), rabbit anti-NQO1 (1:800, Servicebio), or mouse anti-β-actin (1:1000, Abcam). After incubation, the PVDF membrane was washed and incubated with the secondary antibody at room temperature for 1 hour. Protein expression was detected using ECL, and band intensity was quantified using Image J software.

Statistical analysis

GraphPad Prism 9 was used for experimental data analysis. Data are presented as mean ± standard error. Comparisons between two or multiple groups were analyzed by t-test or one-way ANOVA, respectively. A *p*-value of less than 0.05 was considered statistically significant.

Results

Effects of mBYHWD on improving metabolic and liver biochemical parameters in diabetic mice

In comparison to the control (Con) group, fasting blood glucose (FBG) in the Mod group and mBYHWD group increased significantly (**Figure 1A**). However, mBYHWD exhibited a limited therapeutic effect on FBG. Type 2 diabetes is often associated with lipid metabolism disorders. Therefore, we assessed changes in serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) in each group. Our results indicated significant increases in TC (**Figure 1B**) and TG (**Figure 1C**) in the Mod group compared to the Con group, alongside a significant decrease in HDL (**Figure 1D**). The mBYHWD group showed a significant decrease in TC

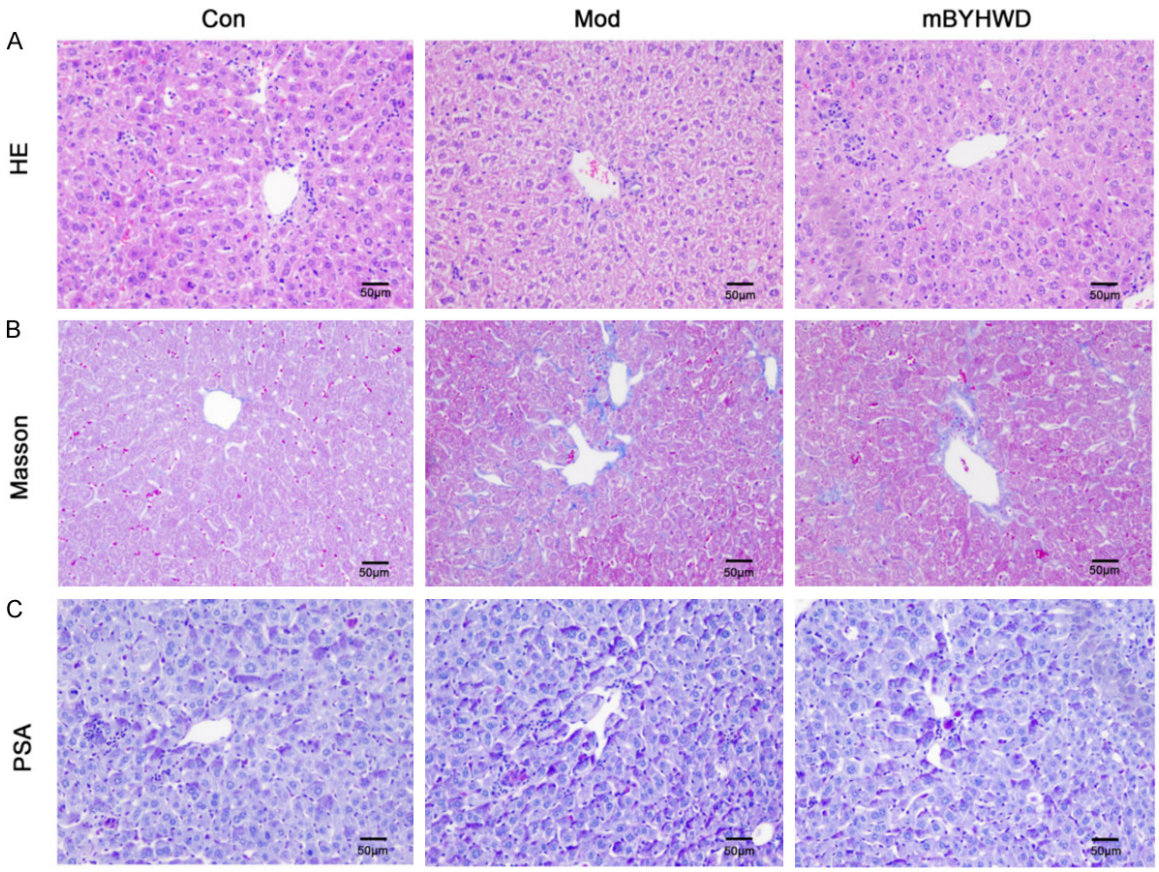

Figure 2. Effects of mBYHWD on hepatic histopathology. A. Hematoxylin-Eosin staining to observe the liver tissue pathological morphology. B. Masson staining to show the liver tissue fibers. C. Periodic Acid-Schiff stain to detect liver tissue glycogen accumulation. Bar = 50 μm.

and TG and an increase in HDL compared to the Mod group (**Figure 1B-D**). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, commonly used liver injury markers, were elevated in the Mod group (**Figure 1E** and **1F**), indicating notable liver damage. Treatment with mBYHWD significantly reduced ALT and AST levels (**Figure 1E** and **1F**), suggesting its efficacy in mitigating DLI.

Effects of mBYHWD on liver histopathology in diabetic mice

Hematoxylin and eosin (HE) staining (**Figure 2A**) revealed normal morphology of liver cells in the Con group, arranged radially around the central vein with minimal steatosis. In contrast, hepatocytes in the Mod group exhibited severe vacuolar degeneration and disarray. The mBYHWD group showed significantly reduced fatty degeneration, with liver cell morphology and arrangement largely normalized. Masson's trichrome staining, used to identify fibrous tissue, showed minimal blue collagen in the central vein area of the Con group (**Figure 2B**). In the Mod group, extensive blue collagen fibers were present around the central vein and between hepatocytes. This fibrosis was significantly reduced in the mBYHWD group. Periodic acid-Schiff (PAS) staining revealed pronounced glycogen accumulation in hepatocytes of the Mod group compared to the Con group, which was markedly ameliorated by mBYHWD treatment (**Figure 2C**).

Sequencing data analysis to explore the possible pathogenesis of DLI

To investigate the potential pathogenic mechanisms of DLI, we analyzed differential gene sequencing data from the Gene Expression Omnibus (GEO) database. We selected three datasets (GSE137923, GSE21984, GSE39752) and identified 238 intersecting genes (**Figure**

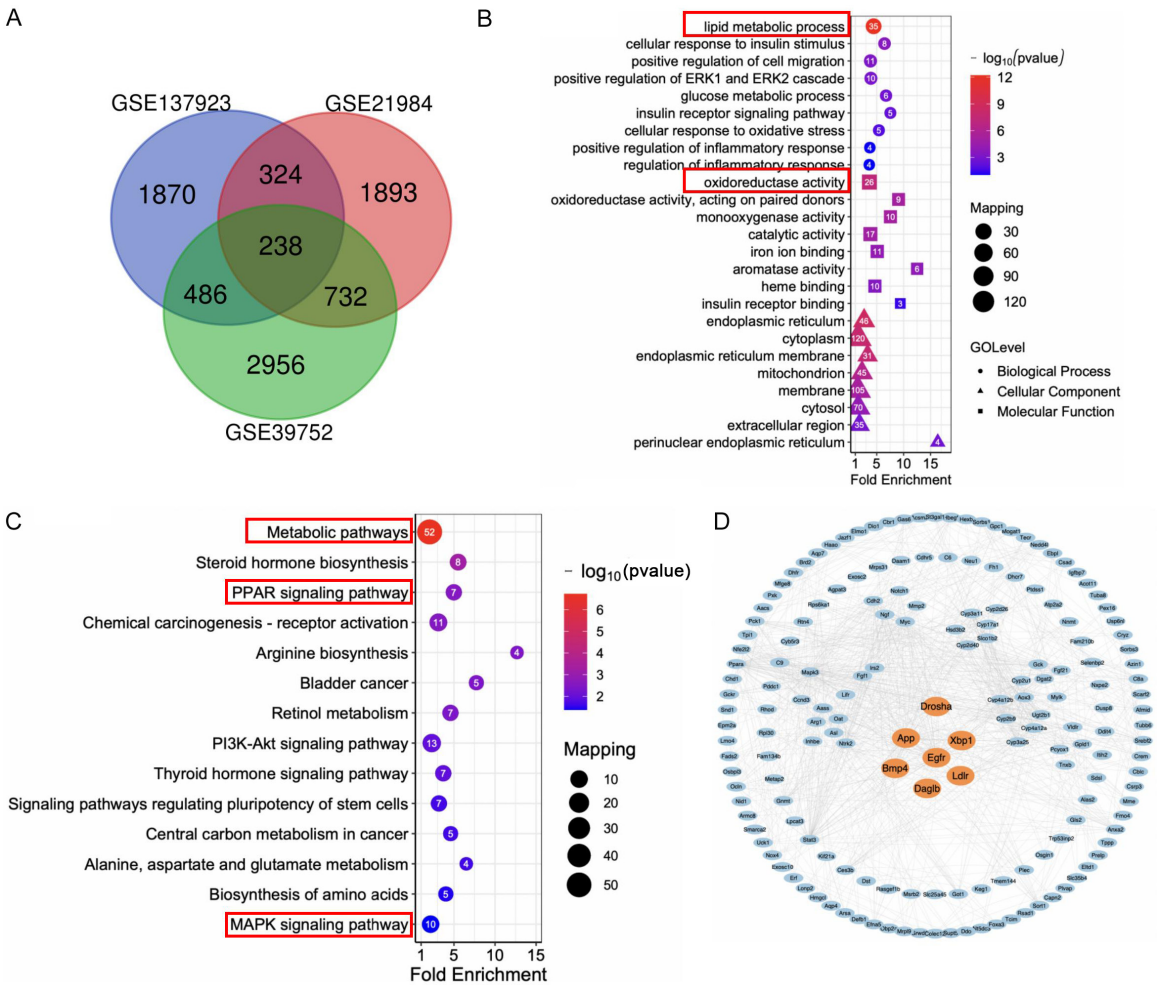

Figure 3. The related pathogenesis of diabetic liver injury. A. Three DLI-related gene sequencing data were screened in GEO database. B. GO analysis. DLI-related descriptive genes, gene products and biological pathways were analyzed. C. KEGG analysis. The distribution of metabolic pathways of gene products in DLI was systematically analyzed. D. PPI network. The protein interaction network was constructed to describe the interaction between DLI-related genes or proteins.

3A). Functional enrichment analysis of these genes was conducted using Gene Ontology (GO) analysis, focusing on molecular functions, biological processes, and cellular components. Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis and Protein-Protein Interaction Networks (PPI) were also utilized for gene function and disease-related protein-protein interaction assessment [22, 23]. KEGG Analysis is often used to study gene function and screen key genes in differential gene sets. PPI are composed of disease-related proteins with interactions involving biological signal transmission, gene expression regulation, energy and material metabolism, and cell cycle regulation. GO, KEGG, and PPI analyses are commonly used for bioinformatic analysis of disease genes.

Our analysis from GO revealed that these genes are mainly involved in cell metabolism and inflammatory responses, with significant implications for oxidative stress (OS) (**Figure 3B**). KEGG pathway analysis implicated these genes in metabolic pathways, PPAR signaling, MAPK signaling, among others (**Figure 3C**). PPAR signaling pathway and MAPK signaling pathway are also closely related to OS and inflammatory pathological processes [24, 25]. PPI analysis highlighted the top seven differentially expressed genes potentially associated with DLI, including EGFR, XBP1, APP, LDLR, BMP4, DAGLB, and DROSHA (**Figure 3D**). These results indicate a strong correlation between DLI and pathological processes such as OS and inflammation.

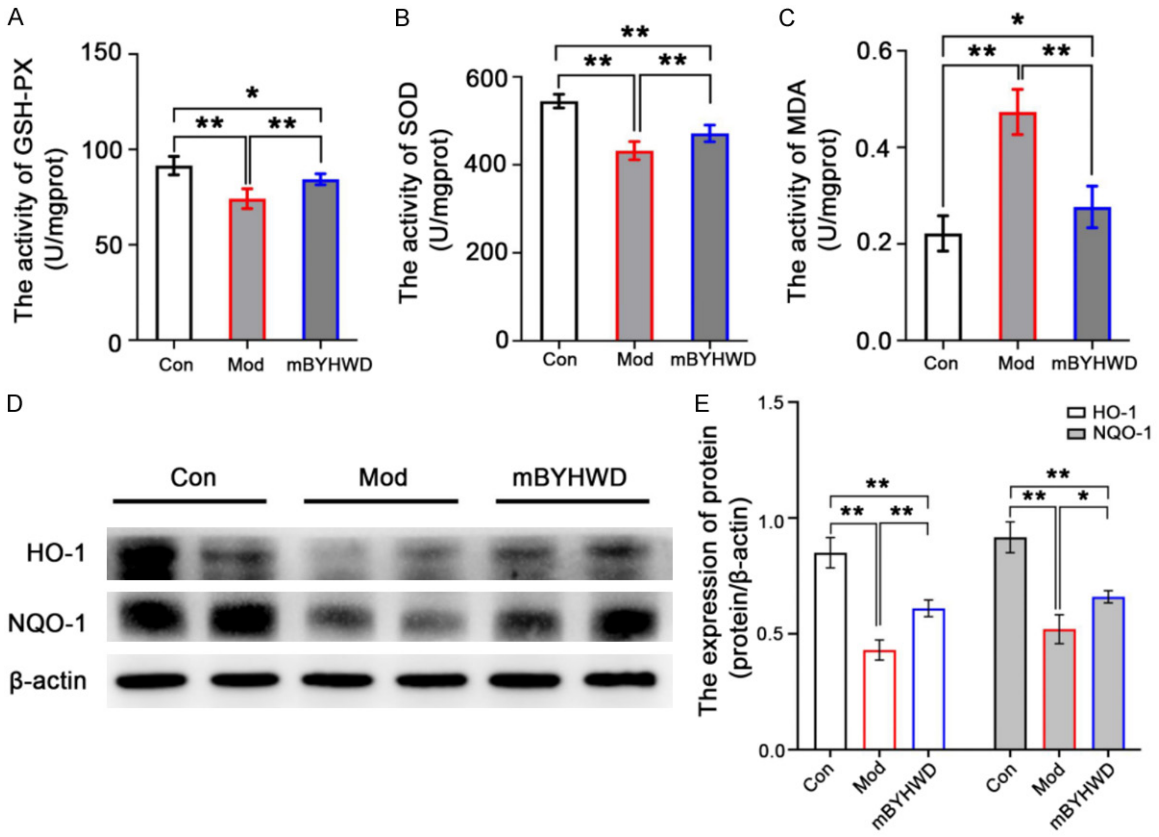

Figure 4. The ameliorating effect of mBYHWD on oxidative stress DLI. A. Glutathione peroxidase. *$P < 0.05$, **$P < 0.01$; B. Superoxide dismutase. **$P < 0.01$; C. Triglycerides. *$P < 0.05$, **$P < 0.01$; D. Western blot detected the expressions of HO-1 protein and NQO-1 protein in each group liver tissue; E. The expressions of HO-1 protein and NQO-1 protein were analyzed statistically with integrated density. *$P < 0.05$, **$P < 0.01$.

Effects of mBYHWD on oxidative stress in liver tissue of diabetic mice

Liver tissues were homogenized to measure the levels of glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), and malondialdehyde (MDA). In the Mod group, GSH-PX and SOD levels decreased significantly, while MDA levels increased compared to the Con group (**Figure 4A-C**). In the mBYHWD group, GSH-PX and SOD levels increased significantly, and MDA levels decreased compared to the Mod group (**Figure 4A-C**). The protein levels of heme oxygenase-1 (HO1) and NAD(P)H: quinone oxidoreductase 1 (NQO-1), important antioxidant enzymes, were significantly reduced in the Mod group but partially restored in the mBYHWD group (**Figure 4D** and **4E**).

Effects of mBYHWD on oxidative stress in liver tissue of diabetic mice

Nrf2 plays an important role in antioxidant stress signaling pathway. The expression of Nrf2 protein was analyzed via Western blot and immunofluorescence. Western blot results showed a significant downregulation of Nrf2 protein in the Mod group and an upregulation in the mBYHWD group (**Figure 5A** and **5B**). Immunofluorescence results for liver tissue Nrf2 protein expression aligned with the Western blot findings (**Figure 5C**).

Effects of mBYHWD on NF-κB signaling pathway in DLI

As shown in **Figure 6A**, expressions of inflammatory factors TNF-α (**Figure 6B**) and IL-1β (**Figure 6C**) were significantly higher in the Mod group compared to the Con group. Additionally, the ratio of phosphorylated IκB to IκB (**Figure 6D**) and the level of p65 (**Figure 6E**) were markedly elevated. In the mBYHWD group, TNF-α, IL-1β, and p65 levels were significantly reduced compared to the Mod group, and the p-IκB/IκB ratio was reduced.

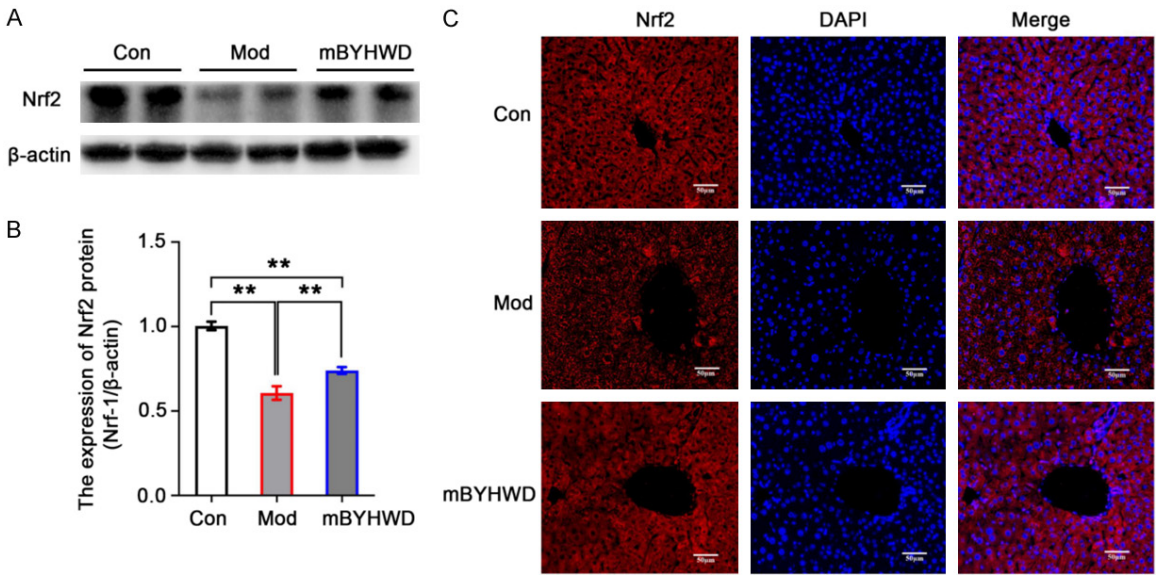

Figure 5. Effect of mBYHWD on the expression of Nrf2 protein. A. Western blot detected the expressions of Nrf2 protein in each group liver tissue. B. The expression of Nrf2 protein was analyzed statistically with integrated density. $^{**}P < 0.01$. C. Immunofluorescence detected the expressions of Nrf2 protein in each group liver tissue. Red fluorescence represents Nrf2 protein, while blue fluorescence staining by DAPI indicates the nucleus. Bar = 50 μm.

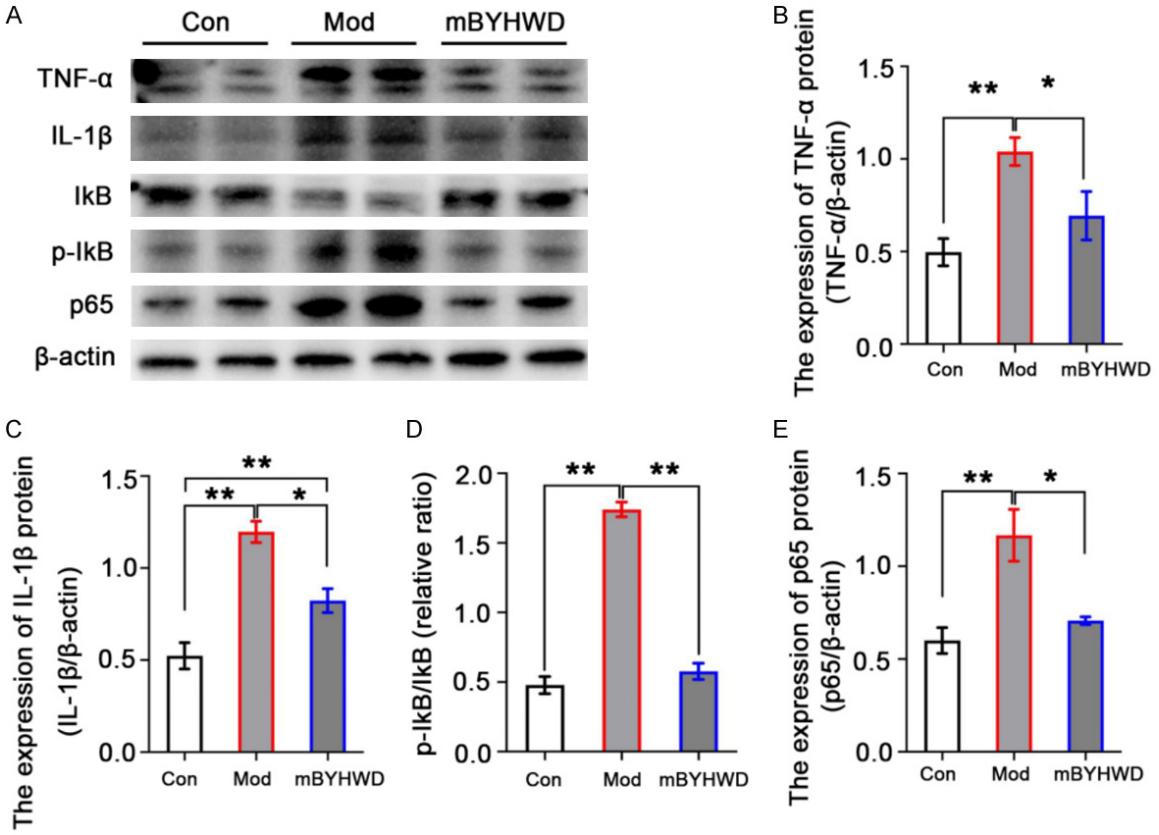

Figure 6. mBYHWD inhibits inflammation in DLI. (A) Western blot detected the expressions of TNF-α, IL-1β, IκB, p-IκB, and p65 in each group liver tissue. The expressions of TNF-α (B), IL-1β (C), p-IκB/IκB (D), and p65 (E) were analyzed statistically with integrated density. $^{*}P < 0.05$, $^{**}P < 0.01$.

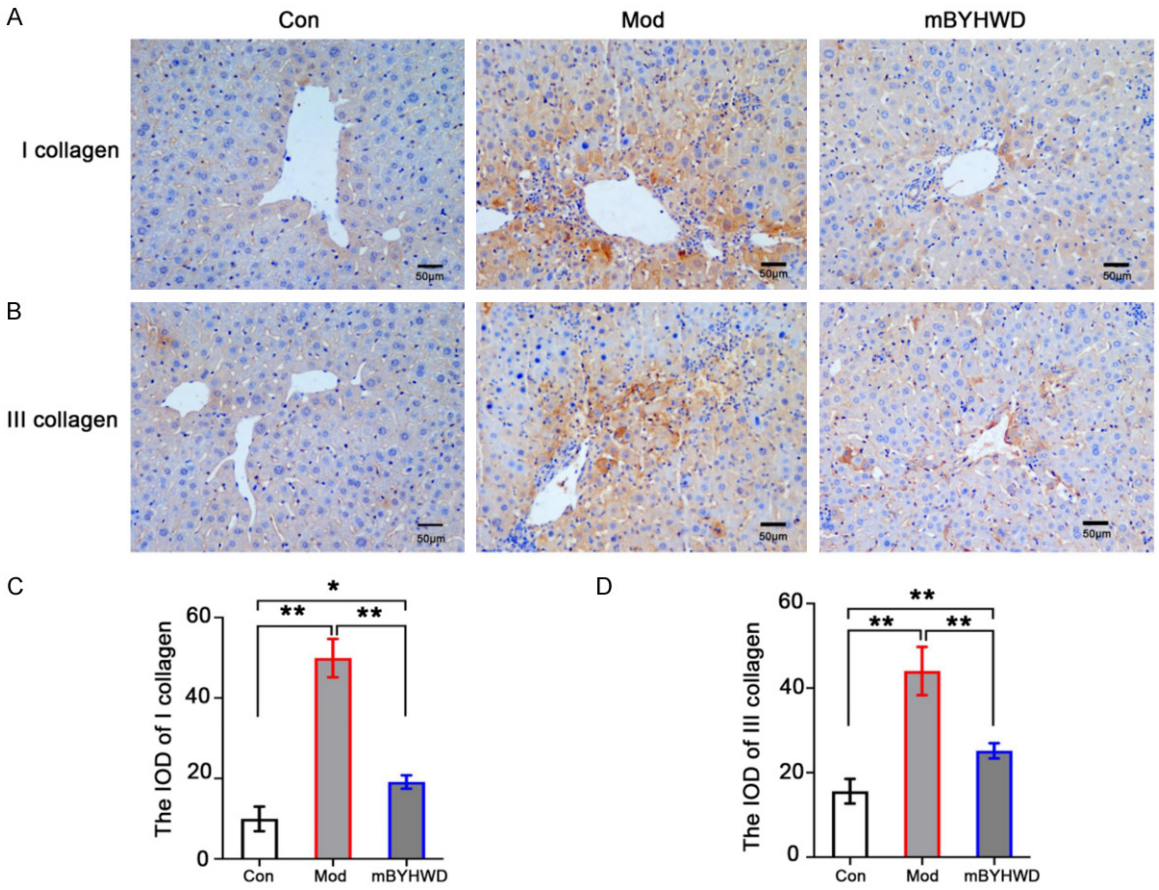

Figure 7. Effects of mBYHWD on collagen expression in liver tissue. A, B. Immunohistochemical staining detected the expressions of collagen I and collagen III. C. The expressions of collagen I were analyzed statistically with Integral Optical Density. *$P < 0.05$, **$P < 0.01$. D. The expressions of collagen III were analyzed statistically with Integral Optical Density. **$P < 0.01$. Bar = 50 μm.

Effects of mBYHWD on collagen expression in DLI

The expression of Type I and III Collagens in liver tissues was assessed (**Figure 7A** and **7B**). Immunohistochemical (IHC) staining showed a significant increase in Type I and III collagen types in the Mod group (**Figure 7C** and **7D**). However, these levels were significantly reduced in the mBYHWD group.

Discussion

The progression of Type 2 Diabetes Mellitus (T2DM) often leads to sustained hyperglycemia and prolonged metabolic disorder, causing systemic tissue and organ damage. Diabetic Liver Injury (DLI) is a significant complication of diabetes [26]. DLI can lead to the occurrence of non-alcoholic fatty liver disease, potentially leading to Non-Alcoholic Fatty Liver Disease (NAFLD), Hepatocellular Carcinoma (HCC), bacterial liver abscess, liver infarction, and acute liver failure [27], with NAFLD being the most common one. The pathogenesis of DLI is multifaceted, with extended hyperglycemic states inducing triglyceride and free fatty acid accumulation in hepatocytes, resulting in steatosis. This lipid accumulation triggers oxidative stress (OS), mitochondrial dysfunction, lipid peroxidation, and pro-inflammatory cytokine release, culminating in fibrosis due to an imbalance in liver cell death and regeneration, ultimately leading to irreversible liver tissue damage [5, 28]. Furthermore, the inappropriate use of diabetes medications can exacerbate liver damage, making the exploration of safe and effective DLI treatments highly clinically significant.

Given the broad effects and minimal side effects of Traditional Chinese Medicine, this study investigated the therapeutic efficacy and

mechanism of mBYHWD in treating DLI. Initially, db/db mice were used to establish a diabetic mouse model. In the Mod group, significant increases in FBG, TC, and TG, alongside decreases in HDL and elevations in ALT and AST, were observed. ALT and AST serve as sensitive indicators of liver cell damage [29]. Serum biochemistry in the mBYHWD group revealed negligible effects on blood glucose reduction but significant decreases in TC and TG levels, indicating the potential therapeutic effect of mBYHWD on lowering blood lipid levels. Further examination of mouse liver function showed that ALT and AST were significantly decreased, suggesting that mBYHWD could significantly improve DLI. Histopathological observations revealed severe steatosis, irregular morphology and arrangement, significantly increased collagen fibers in the sink area, and increased glycogen accumulation in the liver of the Mod group, indicating serious liver cell damage and a degree of liver fibrosis. mBYHWD intervention significantly ameliorated these indications, indicating the potential therapeutic efficacy of mBYHWD for the treatment of DLI.

In order to further explore the pathogenesis of DLI, we analyzed gene sequencing data of liver injury in diabetic mice obtained from the GEO database. KEGG and GO analysis indicated that the occurrence of DLI was closely related to OS and inflammation. Previous studies have also shown that lipid metabolism disorder is closely related to OS. Lipid accumulation in hepatocytes can increase the generation of oxygen free radicals by increasing lipid peroxidation products, protein carbonylation, and reducing antioxidant status, thereby promoting OS in hepatocytes and aggravating liver injury. SOD and GSH-Px are important antioxidant enzymes in hepatocytes [30]. As one of the most important products of cytotoxic lipid peroxidation, MDA can diffuse into cells and exacerbate oxidative stress damage. The extent of lipid peroxidation damage can be evaluated by detecting MDA concentration [31, 32]. Therefore, we assessed the changes in oxidative stress related indexes of GSH-Px, SOD, and MDA in liver tissues of each group. The results showed that, compared with the Con group, the activities of GSH-Px and SOD in the liver homogenate of diabetic mice were significantly reduced, while the amount of MDA was significantly increased, indicating serious OS damage. After mBYHWD treatment, the activity of GSH-Px and SOD significantly increased, while MDA significantly decreased, suggesting that mBYHWD effectively mitigates oxidative stress damage in DLI.

The Nrf2/ARE signaling pathway is one of the important anti-oxidative stresses signaling pathways in vivo, which can significantly promote the expression of downstream proteins HO-1 and NQO-1 after activation [33]. We analyzed the protein expressions of HO-1, NQO-1 and Nrf2 in each group, and observed reduced protein expressions of HO-1, NQO-1 and Nrf2 in liver tissue during DLI treatment, partially recovering after mBYHWD treatment. This suggests that mBYHWD may improve the oxidative stress injury induced by DLI through activating the Nrf2/ARE signaling pathway.

Lipid peroxidation products produced from OS are mostly cytotoxic and can activate NF-κB, increasing production of cytokines such as TNF-α, TGF-β and IL-6, exacerbating liver inflammation, and promoting liver tissue inflammation, necrosis, and fibrosis [32, 34]. Based on the above theory, we examined the activation of NF-κB signaling pathway and found increased expressions of pro-inflammatory factors TNF-α, IL-1β, p-IκB/IκB, and p65 were significantly increased in the liver of diabetic mice, indicating NF-κB pathway activation. However, the expressions of TNF-α, IL-1β, p-IκB/IκB and p65 in liver of the mBYHWD group was significantly decreased, indicating that mBYHWD could induce inhibitory effects on the NF-κB pathway.

Studies have demonstrated that hepatic cell injury triggers an inflammatory response, which can activate hepatic stellate cells that migrate to the injury site, producing abundant extracellular matrix and inflammatory mediators. This results in the replacement of type IV collagen in the Disse cavity with fibrous type I and type III collagen, leading to liver fibrosis [35, 36]. Therefore, we further examined the contents of type I and type III collagen in the liver tissues of mice in each group. Compared with the control group, the contents of type I and type III collagen significantly increased in the livers of diabetic mice, suggesting DLI-induced liver fibrosis. However, the contents of type I and type III collagen in the livers were significantly reduced in the mBYHWD group, indicating an inhibitory effect on liver fibrosis caused by DLI.

In this study, we found that mBYHWD can improve liver oxidative stress injury by regulating the Nrf2 and NF-κB signaling pathways, subsequently inhibiting liver fibrosis induced by inflammation and alleviating DLI. Nevertheless, the study has limitations, including the absence of in vitro cell validation and the lack of clinical observation and application of mBYHWD in DLI treatment. Future research will focus on providing a more comprehensive theoretical basis for the clinical treatment of DLI with mBYHWD.

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Disclosure of conflict of interest

None.

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