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Research article

Integrative proteomics and metabolomics explore the effect and mechanism of Qiyin granules on improving nonalcoholic fatty liver disease

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) has emerged as a prominent global health concern, representing a substantial burden within the spectrum of chronic liver diseases. Despite its escalating prevalence, a definitive therapeutic strategy or efficacious pharmacological intervention for NAFLD has yet to receive official approval to date. While Fu Fang Qiyin granules have exhibited efficacy in addressing NAFLD, the intricacies of their underlying mechanism of action remain inadequately elucidated. In this study, we substantiated the ameliorative impact of Qiyin on highfat diet (HFD)induced NAFLD in rat models. The results of metabonomics showed that 108 potential biomarkers in serum and urine related to amino acid metabolism, energy metabolism, and pyrimidine metabolism, have returned to normal levels compared to the model group. Hepatic transcriptomics further indicated that Qiyin potentially confers protective effects against NAFLD by mediating liver inflammation and fibrosis through lumican (LUM) and decorin (DCN). In summation, our investigation provides compelling evidence affirming the therapeutic promise of Qiyin for NAFLD. It elucidates the underlying mechanistic pathways, furnishing a compelling rationale for its prospective clinical application.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), referring to a series of liver diseases caused by nonexcessive drinking, was defined by the presence of steatosis in more than 5 % of hepatocytes [1]. It was reported that the global prevalence of NAFLD rose from 25.26% in the period from 1990 to 2006 to 38% in 2016–2019 [2,3]. and can be reaching up to 88% in the obese people [4]. Surprisingly, individuals are increasingly being affected by NAFLD at a younger age, which could lead to more time for the development of severe complications [5]. NAFLD has become the most prevalent chronic liver disorder globally [6]. NAFLD consists of nonalcoholic fatty liver (NAFL), which is steatosis, without hepatocyte damage, and the more severe nonalcoholic steatohepatitis (NASH), which characterized by

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histological evidence of injury and inflammation, with or without fibrosis [7]. The incidence of NAFLD is intricately associated with several metabolic disorders, notably obesity, insulin resistance, and diabetes mellitus, thereby posing a significant risk to human health [8,9].

NAFLD is a multisystem disease [10], with the cardinal histopathological manifestation being hepatic fibrosis [11]. Additionally, NAFLD serves as a primary etiological factor for the development of hepatocellular carcinoma (HCC) [12]. The pathogenesis of NAFLD involves all aspects of the body, such as insulin resistance, endoplasmic reticulum (ER) stress, and disorders of fatty acid metabolism [13], while the dysregulation of inflammatory mediators constitutes a pivotal component in its etiopathogenesis. Clinical interventions commonly utilize techniques aimed at hepatoprotection and lipid reduction. In treating NAFLD, therapeutic lifestyle modifications are always regarded as a cornerstone of therapy, accompanied by adjunctive administration of antiinflammatory agents, hepatoprotective agents, and insulin sensitizers [14]. Despite the fact that it poses a growing threat to world health, no ideal way and the agent was approved yet to treat NAFLD to date [15].

Fu Fang Qiyin granules constitute a traditional prescription that has been utilized to therapeutically address fatty liver in Xinjiang Medical University Affiliated Traditional Chinese Medicine Hospital. This prescription comprises of a unique blend of nine distinct medicinal elements, including Astragalus membranaceus and Artemisia capillaris Thunb [16]. Previous results from our laboratory ascertained that Qiyin demonstrates a substantial potential to alleviate the hepatic steatosis condition in rats with NAFLD, which was induced by the intervention of HFD. There is sufficient evidence that Qiyin possesses the capability to decrease CYP2E1, SREBP1 and SREBP2 protein expression levels in hepatocytes, thereby mitigating oxidative stress, ameliorating hepatocyte steatosis, and diminishing both inflammation and injury to the hepatocytes [17,18]. Previous clinical investigations have shown that the administration of this prescription confers not only a marked relief in clinical symptoms but also significantly regulates serum glutamic pyruvic transaminase (GPT), triglyceride (TG), cholesterol (TC), body mass index (BMI), and other related parameters. Moreover, no significant adverse reactions were observed [19].

The primary objective of the present investigation was to establish the material foundation of Qiyin and further assess its therapeutic efficacy in rats afflicted with NAFLD. To accomplish this, ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) was employed for the analysis of Qiyin's chemical constituents. Additionally, the application of metabonomics to serum and urine was utilized to gauge the effectiveness of Qiyin as a treatment for NAFLD and to explore its mechanism in combination with proteomics. The results of this study hold significant potential in fostering an understanding of the therapeutic mechanism of Qiyin for the treatment of NAFLD.

2. Materia and methods

2.1. Reagents

Fu Fang Qiyin granules were purchased from Tianjiang Pharmaceutical Co. Ltd. 2% pentobarbital sodium was provided by Tianjiang Pharmaceutical Co. Ltd (Jiangyin, China). Tiopronin Entericcoated Tablets was from Shanghai Kaibao Xinyi (Xinxiang) Pharmaceutical Co. Ltd (Xinxiang, China). Acetonitrile was from Merck & Co Inc. (Kenilworth, USA), Ammonium acetate was from Sigma (Aldrich, USA).

2.2. Composition analysis

The composition of Qiyin was analyzed by ACQUITY UPLC with a Xevo G2S QTOF (Waters Co., USA) using an ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm). The 60 min was extracted by ultrasonic extraction with 50% methanol, and standby after filtering. The gradient elution program is shown in Table 1. A was acetonitrile while B was water containing 0.1% formic acid.

2.3. Animals and ethics statement

Seventy healthy male Sprague–Dawley rats with initial weights of 160–200 g, were provided by the Medical Experimental Animal Center of Xinjiang Medical University. The rats were given free access to food and water under standard temperature and humidity conditions. The rats were randomly divided into 6 groups including blank control group, model group, tiopronin group and QYtreatment group. The normal control group was fed with a normal diet while the NAFLD group was fed with high fat emulsion (ingredients: 30 g lard, 10 mL tween, 10 g cholesterol, 10 mL propanediol, 3 g porcine cholic acid plus distilled water to 100 mL,

Table 1
Chromatographic gradient elution program.

Time (min)	A (%)	B (%)
0	5	95
4	15	85
18	35	65
30	80	20
30.1	5	95
33	5	95

Beijing Keao Xieli Feed Co.,Ltd.) 10 mL/kg body weight once daily over a period of 30d. After confirming that the NAFLD model is established successfully, Qiyin was dissolved in warm water and prepared to the required concentration. Then it was infused into the stomach once per day 0.84 g/kg·d^1, 1.67 g/kg·d^1 and3.34 g/kg·d^1, while the blank control group and model group were administered distilled water 10 mL/kg for 30 days. After the final administration, the rats were fasted for 12 h and then anesthetized using an intraperitoneal injection of 2% pentobarbital sodium at a dose of 20 mL/kg. Subsequently, serum, urine and liver tissue were collected for further analysis. This study was approved by the Experimental Animal Ethics Committee of Xinjiang Medical University, Urumqi, P. R China (Ethical approval number: IACUC2017060111).

2.4. Serum biochemical indicators

The levels of TC (total cholesterol), TG (triglycerides), HDLC (highdensity lipoprotein cholesterol), LDLC (lowdensity lipoprotein cholesterol), ALT (alanine transaminase), and AST (aspartate transaminase) in serum were determined by commercially available kits (Mindray, China) using a 7600–010 automatic biochemical analyzer (Hitachi HighTech Corporation, Japan).

2.5. Histological analysis

In each experimental group, a standardized specimen of liver tissue was collected from a consistent anatomical region. Following fixation and dehydration, the sample was embedded in paraffin and subjected to histological examination using hematoxylin and eosin (HE) staining. Another frozen specimen from the identical anatomical location was staining with oil red O for observation.

2.6. Metabonomic analysis

The serum and urine samples of 100 μL from each rat were respectively added to 400 μL of precooled methanol/acetonitrile/water solution (4:4:2, v/v), vortexed and stored at 20 °C for 60 min. Following centrifugation at 14,000 g and 4 °C for 20 min, the supernatant was dried under vacuum. Then 100 μL of acetonitrile/water solution (1:1, v/v) was added, vortexed, centrifuged at 14,000 g and 4 °C for 15 min, and 2 μL of the supernatant was injected for analysis.

The samples were separated using an Agilent 1290 Infinity LC ultrahigh performance liquid chromatography (UPLC) system with a hydrophilic interaction liquid chromatography (HILIC) column. The samples separated by UPLC were subjected to mass spectrometry analysis using the Triple TOF 6600 mass spectrometer (AB SCIEX). Quality control (QC) samples were incorporated into the sample queue to assess both the stability of the system and the reliability of the experimental data.

Peak alignment, retention time correction and peak area extraction were performed by XCMS program after the raw data was converted into mzXML by ProteoWizard. The identification of metabolite structure was performed by utilizing precise mass matching (<25 ppm) and tandem mass spectrometry matching, with retrieval of data from a laboratory-developed database.Pareto scaling was used for data scaling by SIMCAP 14.1 (Umetrics, Umea, Sweden), which was also used for the data analysis, including unsupervised Principal component Analysis (PCA) and Partial Least Squares Discrimination Analysis (PLSDA). Metabolites with VIP >1, fold change >1.5 or fold change <0.67 were considered to be differential metabolites [20]. KEGG enrichment analysis was used to analyze pathways that the differential metabolites are involved in.

2.7. Proteomics analysis

The liver tissues from each group were randomly divided into 3 samples, each of which contained liver samples of three rats. Protein extraction was carried out by ultrasonic pulverization, and then the concentration was subsequently quantified using the BCA assay. The protein was subjected to hydrolysis by trypsin using the Filteraided proteome preparation (FASP) method. The resulting hydrolyzed peptides were then desalted using a C18 cartridge, lyophilized, reconstituted with 40 μL of dissolution buffer, and quantified. Subsequently, 100 μL peptides of each sample was marked with iTRAQ (AB SCIEX) following to the manufacturer's instructions for the reagent.

The samples were separated by nanoliter flow rate HPLC liquid phase system EasynLC (Thermo Fisher Scientific, USA), then mass spectrometry analysis was carried out by QExactive mass spectrometer (Thermo Fisher Scientific, USA). The detection method is a positive ion, and the scanning range of the parent ion is 3001800 *m/z*.

The raw data obtained from Q Exactive was processed using Proteome Discoverer 1.4 (Thermo Scientific) to convert it into a compatible format, and screened the database (uniprot_rat_35837_20161104.fasta, accessed on November 4, 2016) using MASCOT2.2. Proteome Discoverer 1.4 was utilized for the extraction and normalization of peak intensities. Proteins with p value > 0.05, fold change >1.2 or fold change <0.83 were considered to be differential proteins. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were utilized the function of differential proteins.

2.8. Detection of differential proteins by western blotting

The concentration of protein extracted from rat liver using RIPA buffer was then determined using the BCA assay. SDSPAGE was employed to separate the proteins, followed by transfer onto a PVDF membranes. The membrane was then incubated with a blocking buffer containing 5% skimmed milk for 1 h, washed with TBST three times, and subsequently incubated overnight with primary antibodies including βactin (Sino Biological), Desmin (Boster), Tagln (Boster), Tpm2 (Sangon Biotech), Csrp1 (Sangon Biotech), and

Myh11(Cusabio) at dilutions of 1:1000, 1:400, 1:500, 1:300, 1:500, and 1:300, respectively, at 4 °C. After washing three times with TBST, the membrane was incubated with secondary antibodies for 1 h, followed by staining. Results were visualized using a Chemiscope 3000 (Clinx Science Instruments Co., Ltd).

2.9. Statistical analysis

The data were reported as means ± SD and analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). For the comparison of means among multiple groups of samples, oneway analysis of variance (ANOVA) was used when the assumptions of normality and homogeneity of variance were met. Pairwise comparisons between groups were conducted using the LSDt test. In cases where there was heterogeneity of variance, pairwise comparisons among groups were performed using the DunnettT3 test. A value of $P \leq 0.05$ indicated statistical significance of the observed differences.

3. Result

3.1. Composition analysis

The sample solution was subjected to positive and negative ion mode scans using an electrospray ionization source, and the total ion chromatogram (TIC) of Qiyin solution was obtained, as shown in Fig. 1A and B. By integrating information from the extant literature and database, a total of 67 components were identified and shown in Table S1.

3.2. Qiyin improved NAFLD in HFD rats

Following a 60-day period of high-fat diet (HFD) feeding, a notable loss in both body weight and liver weight was observed when comparing the model group to the blank group. In the model group, conspicuous hepatic fat particles were observed in the liver tissue of rats, displaying a rough texture, and exhibiting a yellowish ischemic appearance while it was bright red and smooth in the blank group. Meanwhile, the swelling and color of liver tissue in the administration group were improved in varying degrees compared to the model group (Fig. S1). HE staining showed diffuse hepatocyte steatosis and hepatocyte balloon degeneration in the model group (Fig. 2B), while Qiyin could improve the number of hepatocytes in the cytoplasm of steatosis decreased significantly. Moreover, there was a significant decrease in the number of lipid droplets between the administration group and the model group based on oil red O staining (Fig. 2C), which suggested that Qiyin have inhibitory effect on hepatic steatosis in rats. Additionally, the level of serum TC, TG, HDLC, LDLC and AST/ALT, which were influenced by HFD feeding, were improved by Qiyin (Fig. 2 A). Furthermore, the Qiyin intervention exhibited a positive effect on the levels of serum TC, TG, HDLC, LDLC, and the AST/ALT ratio, all of which demonstrated significant alterations due to HFD consumption. In brief, our results indicate that Qiyin improved NAFLD in HFD rats.

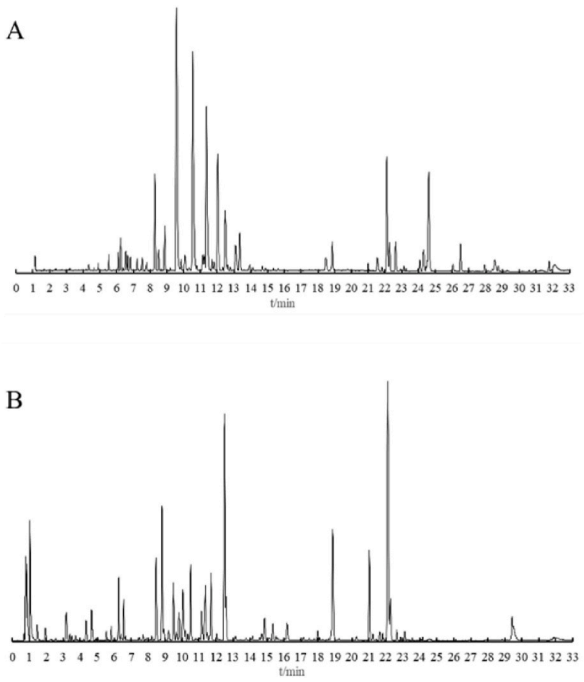

Fig. 1. TIC of Qiyin. (A) positive. (B) negative.

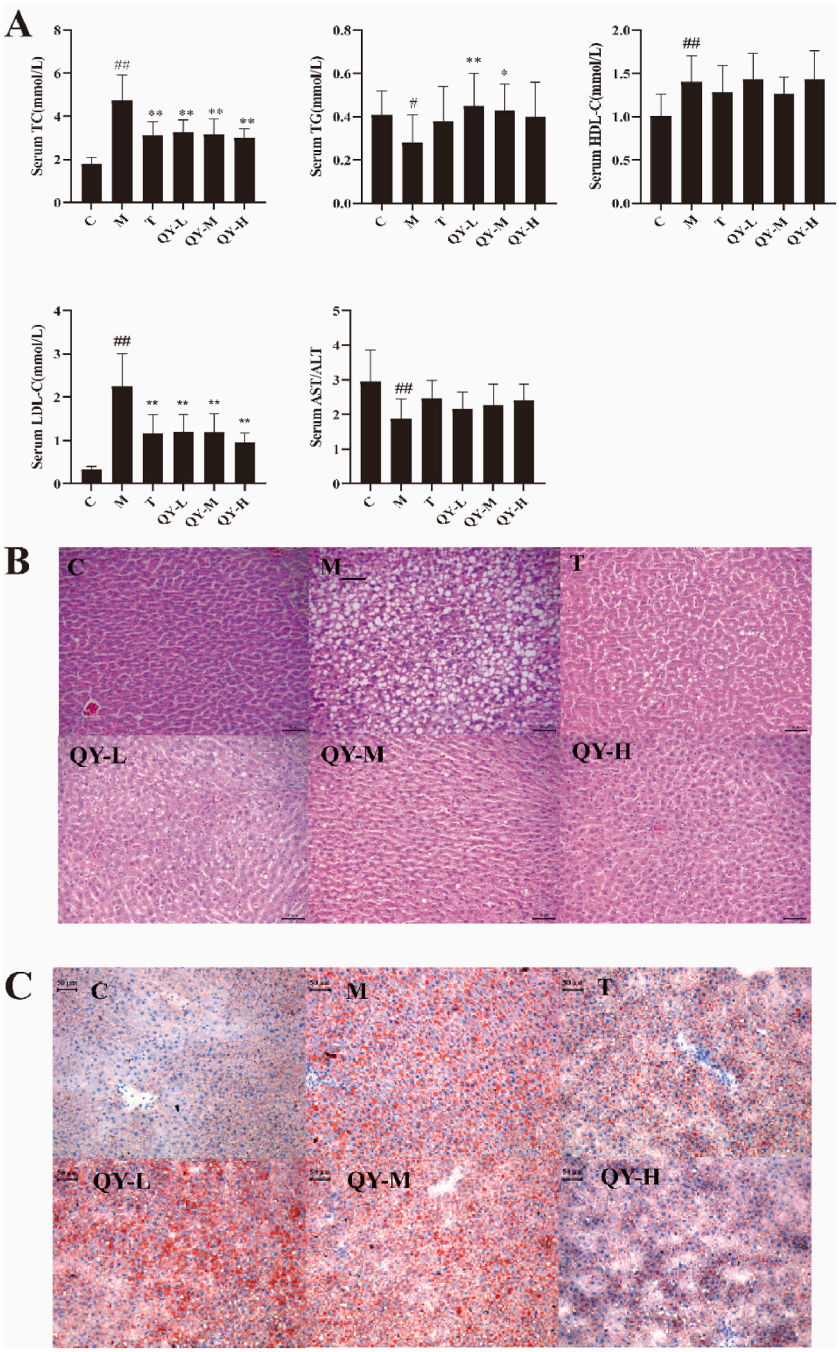

Fig. 2. Serum biochemistry and representative pathological photomicrographs. (A) Serum biochemistry analysis. (B) H&E staining of the liver in different groups. (C) Oil Red O staining (bottom) of the liver in different groups. C: control group; M: model group; T: tiopronin group; QYL, QYM and QYH are QY treatment with low, medium and high concentrations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Metabolomics analysis

To further reveal the therapeutic effect of Qiyin on NAFLD, we performed metabolic of the serum and urine from the three group of rats. The PCA analysis showed that serum (Fig. 3 A) and urine (Fig. 3 B) metabolites were significantly different in the three group. By conducting a crosscomparative analysis of the metabolites among the three groups, as shown in Fig. 3C and Fig. 3 D, it was observed a total of 52 shared differential metabolites in serum and 56 shared differential metabolites in urine (Table S2). Many differential

metabolites in serum, such as 1-Palmitoyllysophosphatidylcholine, 1-Oleoylsnglycero3phosphocholine, PC(16:0/16:0), and Taurocholate, are closely related to lipid metabolism and their variations are consistent with changes of the level of TC, HDLC, and LDLC. The KEGG enrichment analysis (Fig. 3 E) showed that linoleic acid metabolism, alanine, aspartate and glutamate metabolism, phenylalanine metabolism, and biosynthesis of unsaturated fatty acids has been strongly influenced in serum, while they were alanine, aspartate and glutamate metabolism, butanoate metabolism, pyrimidine metabolism and TCA cycle in urine. Those pathways demonstrated that Qiyin has effects on fatty acid, amino acid metabolism and energy cycle in rats, which underscore the potential of these pathways as therapeutic avenues for mitigating NAFLD through Qiyin intervention.

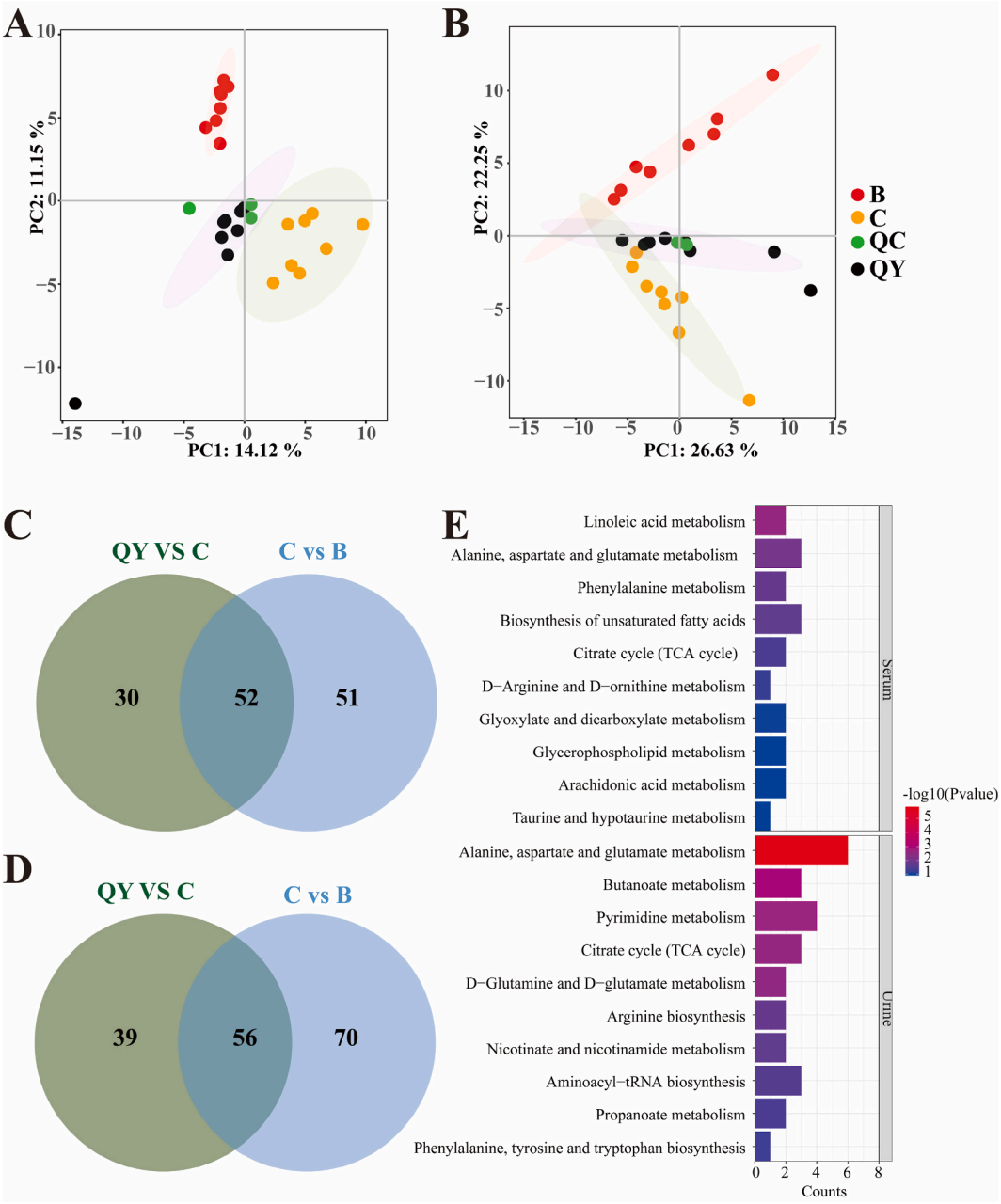

Fig. 3. Serum and urine metabolomics analysis. (A) Score plot of the PCA of serum. (B) Score plot of the PCA of urine. (C) and (D) Venn diagram showing the shared differential metabolites that commonly changed in the three groups in serum and urine. (E) KEGG enrichment analysis of shared differential metabolites in serum and urine.

3.4. Proteomic analysis

Proteomics was used to analyze the variations in protein expression levels extracted from liver tissues of the rats in normal control, NAFLD and Qiyintreatment groups, and a total of 4418 proteins were matched by the comparative analysis of the labeled peptides. Differential protein screening was conducted using a combination of criteria: |fold change| >1.2 and P < 0.05. As shown in Fig. 4 A,

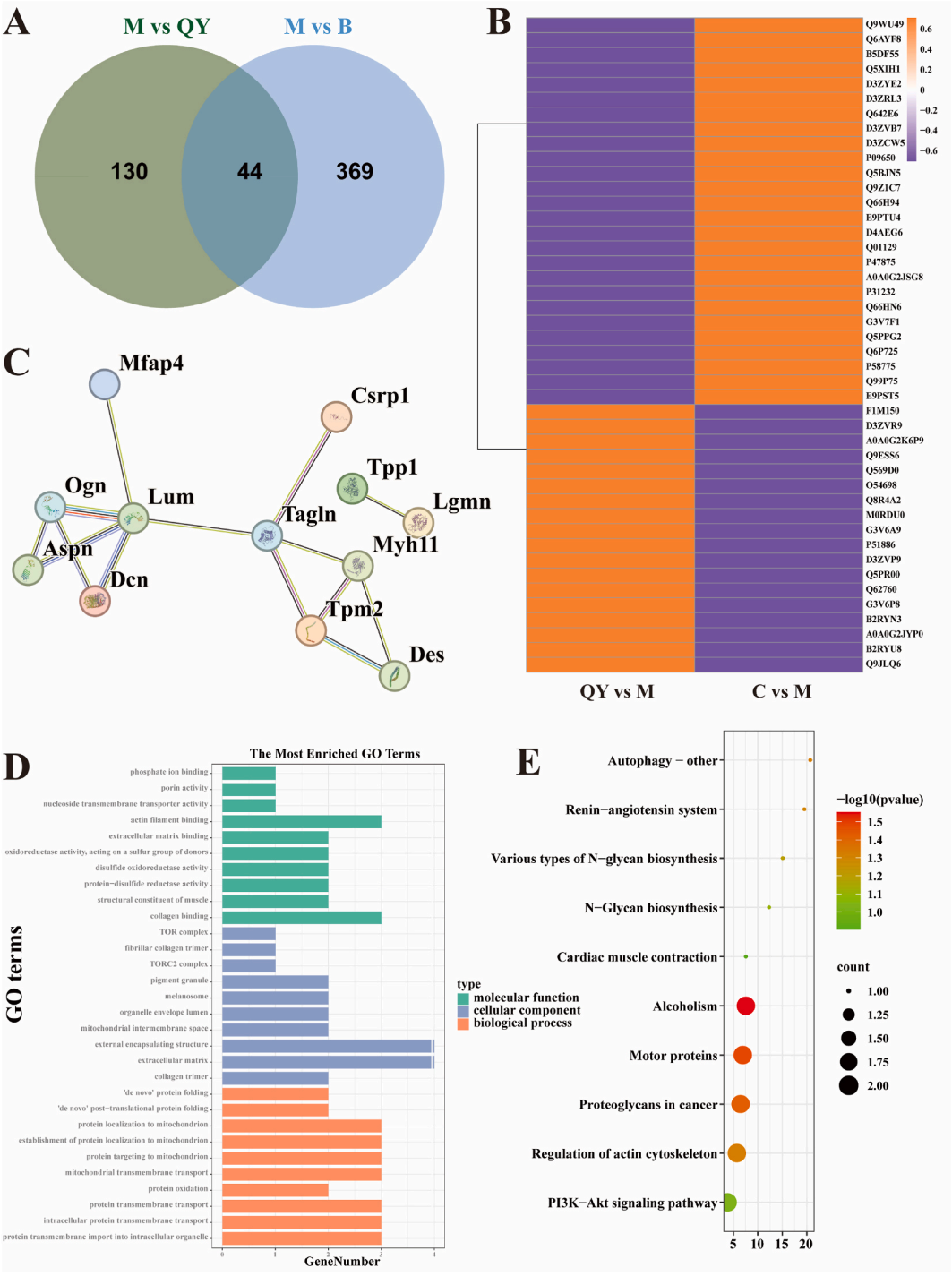

Fig. 4. Shared differential proteins analysis. (A) venn diagrams. (B) heatmap of shared differential proteins in the three groups. (C) PPI of shared differential proteins in the three groups. (D) GO enrichment analysis of shared differential proteins in the three groups. (E) KEGG enrichment analysis of shared differential proteins in the three groups.

there were 174 differential proteins between Qiyintreatment and NAFLD groups and 413 differential proteins between the NAFLD model group and control group. Through the implementation of a cross-comparative analysis of metabolites across the three groups, as delineated in Fig. 4A, a collective presence of 44 shared differential proteins within the liver was identified. The analysis of fold changes in shared differential proteins revealed that the administration of Qiyin effectively ameliorated the impact of the NAFLD progression in rats (Fig. 4 B). PPI network (Fig. 4C). which employed for the analysis of shared differential proteins' interactions, suggested that Desmin, Tagln, Tpm2, Myh11, and Crsp1 may represent the core targets through which Qiyin exerts its anti-NAFLD effects. GO an enrichment analysis was performed to elucidate the biological mechanisms that shared differential proteins are involved in. As detail in Fig. 4 D, the proteins displaying divergent expression patterns among the three experimental groups were found to be associated with processes such as protein transmembrane import into intracellular organelles, collagen binding, and collagen trimer formation. Subsequently, an in-depth investigation into these shared differential proteins was conducted through KEGG pathway enrichment analysis (Fig. 4 E), revealing their potential relevance to pathways involving alcoholism, motor proteins, proteoglycans in cancer, the regulation of actin cytoskeleton, and autophagy, among others, which underscore the potential of these pathways as targeted therapeutic avenues for mitigating NAFLD through Qiyin intervention.

3.5. Western blot analysis

Western blot was carried out to verify the expression changes of five differential proteins with high degrees which are consistent with PPI network (Fig. 5 A and Figure S2~S7). The findings of this investigation reveal that the expression levels of Desmin, Tagln, Tpm2, and Myh11 were elevated in the NAFLD model group ($p < 0.05$) and were subsequently ameliorated by intervention with Qiyin ($p < 0.05$) (Fig. 5 B, C, D and E). However, crsp1 has not changed (Fig. 5 F). Compared to the model group, the middle and high-dose groups of Qiyin exhibited significant alterations in the expressions of Tagln, Tpm2, and Myh11 ($p < 0.05$). In contrast, a markedly decreased expression of Desmin was noted exclusively in the high-dose group of Qiyin ($p < 0.05$).

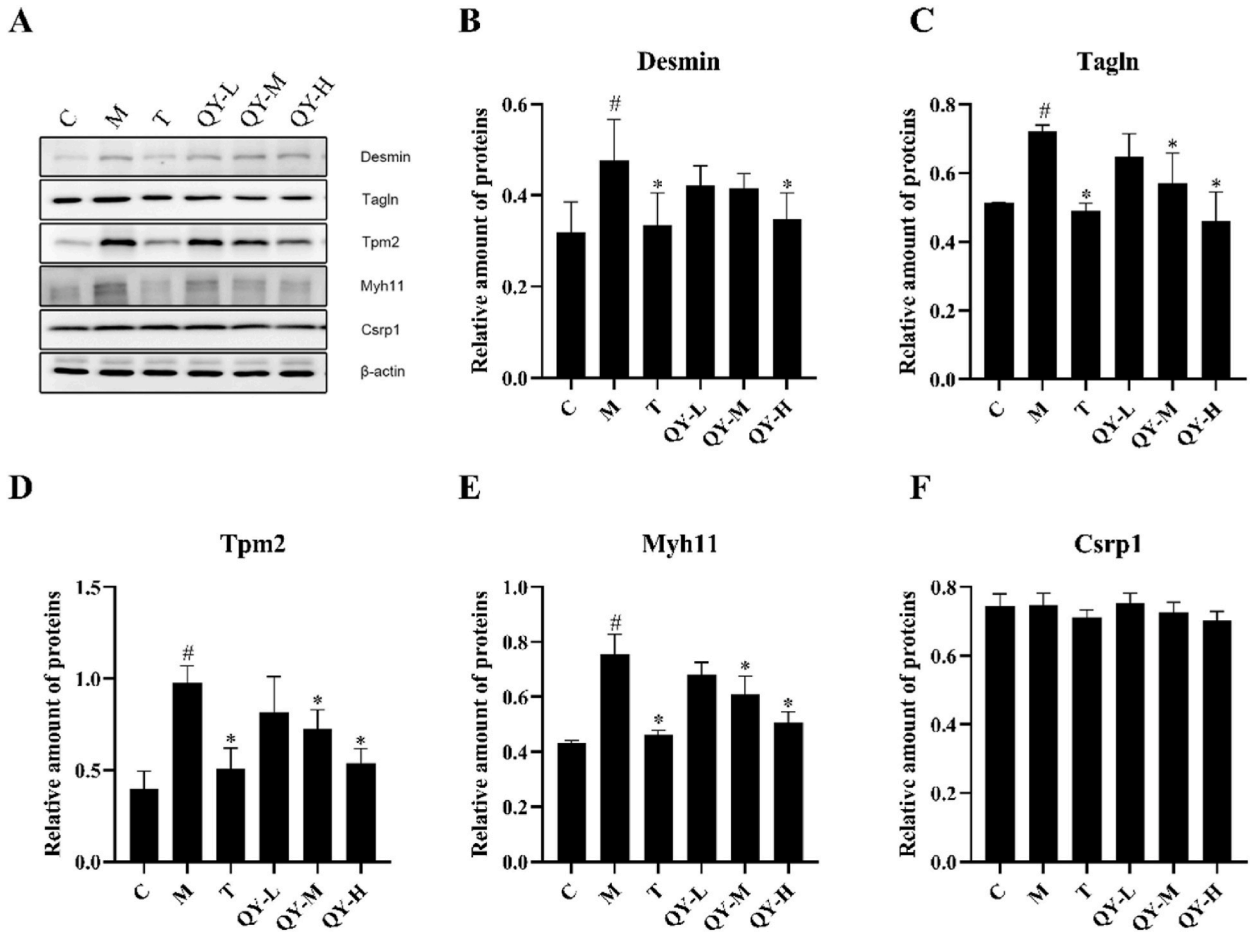

Fig. 5. Expression of differential proteins in lumbar samples from the three groups. (A) Western blotting analyses of protein levels in lumbar. (B) to (F) Protein levels in the lumbar of different rat groups. #$p < 0.5$ represent M vs. C. *$p < 0.5$ represent data vs. the M. C: control group; M: model group; T: tiopronin group; QYL, QYM and QYH are QY treatment with low, medium and high concentrations.

4. Discussion

In recent times, there has been a notable rise in the incidence of obesity and metabolic syndrome (MetS), due to the overconsumption of highcalorie diets that are rich sugar and fat. However, there are currently no approved treatments for NAFLD [11,16]. Qiyin, as a traditional empirical formula for the treatment of NAFLD, has been used for many years in Xin Jiang, China. In this study, a rat model of NAFLD induced by a high-fat diet was successfully established, and the findings of biochemical and histologic support that the significant potential of Qiyin as a therapeutic intervention for mitigating dyslipidemia and hepatic tissue injury in NAFLD-afflicted rats.

Through the implementation of a cross-comparative analysis of metabolites across the three experimental groups a collective presence of 52 shared differential metabolites in serum and 56 shared differential metabolites in urine was discerned, including glycocholic acid, taurocholic acid, phenylalanine, aromatic amino acid, and branched-chain amino acids (BCAAs), which were reported as biomarkers for NAFLD [21–24]. They are related to the biosynthesis of unsaturated fatty acids, amino acid metabolism, and the TCA cycle, which are closely related to the occurrence and development of NAFLD [25,26]. The biosynthesis of unsaturated fatty acids, encompassing monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs), is intimately interconnected with lipid metabolism, which may lead to an altered hepatic lipid profile, characterized by increased saturated fatty acids (SFAs) and reduced unsaturated fatty acids [27]. This lipid imbalance contributes to hepatic lipid accumulation and inflammation in NAFLD [28]. Amino acids, essential for protein synthesis, also contribute significantly to energy metabolism and oxidative stress regulation. Perturbations in amino acid metabolism, particularly elevated levels of BCAAs, are associated with NAFLD. Increased BCAA concentrations are linked to insulin resistance and NAFLD severity, potentially contributing to hepatic steatosis and inflammation [29–31].

A parallel finding was made within the urinary metabolomic wherein the shared differential metabolites exhibited associations with disorders in amino acid metabolism, energy metabolism, and pyrimidine metabolism, which is aligned with prior research findings underscores the consistency of these metabolic patterns [32]. Pyrimidines are essential components of nucleotides, which are not only vital for nucleic acid biosynthesis but also play crucial roles in various cellular processes, including energy transfer, cofactor generation, and lipid metabolism. Emerging evidence suggests that disruptions in pyrimidine nucleotide biosynthesis and homeostasis may play a role in the molecular alterations associated with NAFLD [33,34]. In summary, the outcomes of the metabonomic analysis conducted on serum and urine collectively suggest that the therapeutic efficacy of Qiyin intervention in the context of NAFLD is realized through the modulation of amino acid metabolism, energy metabolism, and pyrimidine metabolism.

Lumican (LUM) and decorin (DCN), both small leucinerich proteoglycans residing within the extracellular matrix (ECM), including hepatic ECM, play pivotal roles in the orchestration of tissue architecture and function [35,36]. The pathogenesis of NAFLD involves dynamic alterations in liver ECM composition and structure, often coinciding with fibrotic processes [37]. LUM and DCN exert influence on ECM composition, thereby modulating the fibrotic progression. It was found that elevated LUM levels observed in the hepatic tissue and serum of individuals afflicted with advanced fibrosis suggest the potential utility of LUM as a diagnostic biomarker for assessing the severity of NAFLD liver fibrosis [38]. DCN operates by sequestering transforming growth factor β (TGFβ), a pivotal profibrogenic cytokine, thereby impeding its activity. This antifibrotic effect positions DCN as a potential target for mitigating liver fibrosis in NAFLD conditions [39,40]. Within our investigative inquiry, we observed that Qiyin intervention ameliorated NAFLD induced perturbations in LUM, DCN, and their associated proteins, culminating in the attenuation of liver inflammation and fibrosis within the NAFLD context.

5. Conclusions

In conclusion, our results that include serum biochemistry and liver histology demonstrated that Qiyin could improve hepatic steatosis, inflammation and fibrosis in rats with HFD. The results of metabonomic based on serum and urine showed that there were 52 shared differential metabolites in serum and 56 shared differential metabolites in urine, which indicates the effect of Qiyin on amino acid metabolism, energy metabolism, and pyrimidine metabolism. The liver proteomic analyses have illuminated the potential of Qiyin to mitigate liver inflammation and fibrosis, with particular regard to its impact on LUM, DCN, and their associated proteins. Collectively, these results furnish compelling evidence in support of the therapeutic promise of Qiyin for NAFLD and provide mechanistic insights, thereby rationalizing its prospective clinical application. Nevertheless, further investigation is needed to obtain a better understanding of the diverse effect of Qiyin on NAFLD and its mechanism in the clinic.

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Ethics statement

This study was approved by the Experimental Animal Ethics Committee of Xinjiang Medical University, Urumqi, P.R China (Ethical approval number: IACUC2017060111).

Data availability statement

The data has not been deposited in a publicly accessible repository, but will be made available upon reasonable request.

CRediT authorship contribution statement

Xuehua Yan: Writing – review & editing, Writing – original draft, Methodology. **Hongbing Liu:** Funding acquisition. **Meng Huang:** Funding acquisition. **Yujie Zhang:** Funding acquisition. **Binfang Zeng:** Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Thanks to the drawing service provided by the figure-draw (https://www.figdraw.com/#/).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27075.

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