**Supplementary methods**

***Biochemical analysis***

Blood samples were kept at 4°C for 2 h. Then serum was collected by centrifugation at 6000 rpm for 10 m. Liver tissue (0.5 g) was homogenized with 1% Triton 100 and lysed on ice for 1 h. The supernatant was obtained by centrifugation at 12000 rpm for 10 min. Serum content of aspartate aminotransferase (AST) was detected by a Hitachi Automatic Biochemical Analyzer 7180 (Hitachi, Yokohama, Japan). Serum low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were assayed with commercial kits (A112-1-1 and A113-1-1; Nanjing Jiancheng Bio, Nanjing, China) following the manufacturer's instructions.

***Extraction of*** ***Quzhi formula and*** ***Quzhi formula granules***

*Polygonum cuspidatum, Cassiae semen,* and *Crataegi fructus* were purchased from Shanghai Tong Ren Hospital. Herbs decoction was performed twice, the first added 10 times the amount of water with decoction for 2 h, the second added 8 times the amount of water with decoction for 1 h. The filtrates were combined and concentrated to a relative density of 1.10 ± 0.02. Ethanol was added while stirring to a concentration of 60%. The supernatant was collected after 12 h and the 2.08 g/ml crude herbal dose was concentrated and dried at 70-80ºC, crushed, mixed well with dextrin, and the soft material was mixed with the right amount of ethanol and granulated.

***Selection of active ingredients***

The TCMSP and BATMAN-TCM databases were search for the active ingredients in the herbal medicines of Quzhi formula. The oral bioavailability (OB) ≥30% and drug-like properties (DL) ≥0.18 identified from the TCMSP were used as the reference for screening the candidate active ingredients of the formula. In addition, several widely recognized chemical components were added by reviewing the literature.

***Screening for*** ***bioactive compounds and NASH-related targets***

The bioactive compound targets of the three herbs were searched in the TCMSP database platform. Based on the UniProt database, the full names of the target genes were converted into gene symbols, and the targets without corresponding gene names were deleted. The keywords "nonalcoholic steatohepatitis," "nonalcoholic fatty liver disease," and "hepatic steatosis" were used to search for NASH-related disease targets in the GeneCards and OMIM databases. Finally, the UniProt platform was used to restrict the target species to human and to obtain Quzhi formula-associated and NASH-related targets.

***Construction of a herb-compound-target network***

The potential targets of Quzhi formula against NASH were obtained by intersecting the targets of the active ingredients with the disease targets to create a drug-compound-target network file based on the above potential targets. The targets were imported by Cytoscape 3.8.2 software to build a drug-compound-target visualization network. The nodes of the network diagram were herbs, components, and targets. The interrelationships between the nodes were represented by edges. The CytoNCA Cytoscape plug-in was used to analyze the network diagram, and the results were exported to obtain the main active ingredients and core targets for Quzhi Formula against NASH.

***Construction of a PPI network***

PPI network analysis was performed with the STRING database and Cytoscape 3.8.2 software. The targets of the active chemical components were imported into the STRING platform to obtain PPI networks of drug targets, and the obtained interaction.tsv files were imported into Cytoscape 3.8.2 software. The CytoHubba plug-in is used to analyze the network, and the top 100 targets are ranked by MCC for graphical analysis to further filter the core targets.

***GO and KEGG pathway enrichment analysis***

GO and KEGG pathway enrichment analysis enrichment analysis of PPI core targets were performed using the Metascape platform. The 100 core targets in the PPI network were imported into the Metascape platform for GO and KEGG enrichment analysis, and the genus "homo sapiens" was selected with *p* ≤0.01. Three categories, biological processes, cellular components, molecular functions, and KEGG enrichment analysis were performed.

***Molecular docking***

Using the PubChem database and AutoDock software, the docking energy between the overlapped proteins and chemical ingredients of Quzhi formula were calculated. If the binding force is less than 0, then the ligand and the receptor can bind spontaneously. The docking score value of the binding activity between the component and the target met two standards: binding tighter than 5-ASA and a binding force below −5.5 to further filter the targets related to the treatment of NASH by Quzhi formula.

***Oil Red O staining***

Intracellular lipid accumulation was determined by Oil Red O staining. Mouse hepatocytes were stimulated by 0.75 mM FFA, with or without Quzhi formula extract (1.25 μg/ml) for 24 h. Then the hepatocytes were fixed in 4% paraformaldehyde for 0.5 h and stained with Oil ed O solution for 0.5 h. The stained hepatocytes were rinsed with 60% isopropanol and counterstained with hematoxylin. After washing with distilled water, the stained lipid droplets within cells were observed with an inverted microscope.

***Cell viability assay***

Mouse hepatocytes were seeded in 96-well plates at a density of 5×103 cells/well. After 24 h, cells were incubated in DEME (#SH30243.01, HyClone, Logan, UT, United States) containing 0.75 mM of FFA, with or without 60% ethanol extract of Quzhi formula treatment at 0.625, 1.25, 2.5, or 5 μg/mL for another 24 h. Cell viability was measured with CCK-8 assay kits (#G021-1-1, Nanjing Jiancheng Bio, Nanjing, China) following the manufacturer’s instructions.

***Quantitative real-time PCR***

The total RNA was extracted from liver or hepatocytes with Trizol reagent (#B511311-0100, Sangon Biotech, Shanghai, China), following the manufacturer’s instructions. cDNA synthesis was performed with reverse transcriptase kits (#RR047, Takara, Shiga, Japan). The mRNA levels of genes involved in inflammation and lipid metabolism were assayed by qRT-PCR using SYBR Green qPCR Master Mix kit (#RR820B, Takara, Shiga, Japan). The primer sequences are shown in Table 1. Relative mRNA expression was calculated using GAPDH as an internal control.

**Table 1. qRT-PCR primers**

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| --- | --- | --- |
| **Gene** | **Sequence 5′-3′** | |
| *FABP1* | F | TGGTCCGCAATGAGTTCACCCT |
| R | CCAGCTTGACGACTGCCTTGACTT |
| *CD36* | F | GACTGGGACCATTGGTGATGA |
| R | AAGGCCATCTCTACCATGCC |
| *DGAT1* | F | TCCGTCCAGGGTGGTAGTG |
| R | TGAACAAAGAATCTTGCAGACGA |
| *HMGCR* | F | ATCATGTGCTGCTTCGGCTGCAT |
| R | AAATTGGACGACCCTCACGGCT |
| *IL6* | F | TAGTCCTTCCTACCCCAATTTCC |
| R | TTGGTCCTTAGCCACTCCTTC |
| *IL10* | F | TGAATTCCCTGGGTGAGAAG |
| R | CTCTTCACCTGCTCCACTGC |
| *TNFα* | F | CATCTTCTCAAAATTCGAGTGACAA |
| R | TGGGAGTAGACAAGGTACAACCC |
| *GAPDH* | F | AGGAGTAAGAAACCCTGGAC |
| R | CTGGGATGGAATTGTGAG |

***Western blot assays***

Liver tissue was homogenized in 1% Triton 100 and lysed on ice to extract total protein. Mouse hepatocytes were lysed on ice to extract total protein. The protein samples were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF; #WJ002, EpiZyme, Shanghai, China) membranes. After blocking with 5% nonfat milk, the membranes were incubated with primary antibodies against Bip (#3177S), eIF2α (#5324S), and β-actin (#4970) produced by Cell Signaling Technology (Danvers, Massachusetts, USA) at 4°C overnight and then with the corresponding secondary antibodies for 2 h at room temperature. The protein bands were visualized using Omni ECL reagent (#SQ201, EpiZyme, Shanghai, China).