Exploring the Therapeutic Mechanism of the Mongolian Medicine Scabiosa Against Hepatic Fibrosis Based on UHPLC-TOF-MS/MS Combined with Network Pharmacological Methods and Experimental Verification

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Exploring the therapeutic mechanism of the Mongolian medicine scabiosa against hepatic fibrosis based on UHPLC-TOF-MS/MS combined with network pharmacological methods and experimental verification

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Abstract: Objective To systematically elucidate the chemical composition of Mongolian medicine scabiosa, explore its key targets and related pathways by network p harmacology, and further clarify its against hepatic fibrosis mechanism through experiments. Methods The Mongolian medicine scabiosa was determined by UHPLC -TOF-MS/MS and its components were analyzed; The intersection of Mongolian medicine scabiosa was screened in the Swiss target prediction database to obtain the against hepatic fibrosis target of Mongolian Medicine Scabiosa. The protein interaction was analyzed through a string database, and the GO function and KEGG path way were analyzed on the Metascape platform. Meanwhile, we selected the PI3K/Akt and PI3K, Akt, p-Akt, p38 and p-p38 targets in MAPK signaling pathway for v erification. The mechanism against hepatic fibrosis of Mongolian medicine scabiosa was verified by in vitro and in vivo experiments. Results A total of 120 chemical constituents were identified, including flavonoids, alkaloids, coumarins, terpenoids, phenols, and fatty acids. According to the prediction, there are 63 against hepatic fibrosis targets, including 20 core targets. GO enrichment analysis involves three aspects: biological process (BP), cell component (CC), and molecular function (MF). KEGG enrichment results show that PI3K/Akt, EGFR, Rap1, HIF-1, Ras, MAPK are significant pathways. In the in vivo experiment, Mongolian medicine scabiosa can significantly reduce serum biochemical indexes (P<0.05), hydroxyproline (P<0.05); Masson staining showed that the proliferation of abnormal fibrous tissue w as reduced, and the infiltration of inflammatory cells was reduced, which improved the fibrotic liver tissue; RT-qPCR results showed that the expression of α-SMA, c ollagen, PI3K, Akt, p38 mRNA decreased(P<0.05). The results of Western blot showed that compare with the model group, the Mongolian medicine scabiosa could i nhibit the expression of markers of hepatic fibrosis α-SMA, collagen, PI3K, Akt, p-Akt, p38,p-p38decreased(P<0.01); In the in vitro experiment, MTT results showe d that the inhibition rate of serum containing Mongolian medicine scabiosa on HSC-T6 was the highest at 24 hours; Western blot results showed that compared with t he control group, the protein expression levels of α-SMA, collagen, PI3K, Akt, p-Akt, p38,p-p38 in high, medium and low dose groups were significantly decreased

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(P< 0.05); Mongolian medicine scabiosa containing serum can significantly increase the apoptosis rate of HSC-T6; Conclusion It revealed that Mongolian medicine scabiosa plays an anti-hepatic fibrosis role by regulating PI3K, Akt, MAPK14, and other key targets.
Keywords: Mongolian medicine Scabiosa; Hepatic fibrosis; UHPLC-TOF-MS/MS; Network pharmacology; Signaling pathway

Introduction

Hepatic fibrosis (HF) is the result of hepatocyte regeneration, collagen activation, and extracellular matrix (ECM) deposition after chronic liver injury[1]. Alcohol, viral hepatitis, drugs, toxins, nonalcoholic steatohepatitis (NASH)can lead to hepatic fibrosis[2]. After the liver injury, hepatic stellate cells (HSCs) are activated, and HSCs are gradually transformed into myofibroblasts, resulting in α-SMA, collagen I, and collagen III increased, resulting in scar deposition and promoting the occurr ence of HF[3, 4]. Inhibition of HSC proliferation and activation is an accurate treatment strategy for hepatic fibrosis. Therefore, the search for hepatic fibrosis drugs has become a hot spot in the current research[5]. Mongolian Medicine is mostly compound, with small side effects, multi-component, multi-target, and multi-channel adv antages. It shows the unique advantages of ethnic medicine is against hepatic fibrosis.

Mongolian Medicine names of Scabiosa are "Taosen taorima", "Taosen taorimo" and " Ukhryin - Shurusu ", also known as "Mongolian mountain radish flower"[6]. M ongolian medicine scabiosa is mainly produced in Inner Mongolia, Heilongjiang, Jilin, and other places. Narrow-leaf scabiosa and North scabiosa are widely studied in Inner Mongolia. The medicine has sweet, astringent, dull, dry, greasy, heavy, cool, and other sexual flavors, and has the functions of clearing heat and " Zaoxieriwu su ". The inflorescence has antipyretic[7], antioxidants[8], against liver injury[9],hepatic fibrosis[10], renal function protection, and cancer effects[11]. The central compon ent of scabiosa has the effect of against hepatitis. Among the classic prescriptions of Mongolian Medicine such as DE DU SAFFLOWER-7 and E LI GEN-7, it can b e used for the treatment of hepatic fibrosis.

Mongolian medicine scabiosa has a good anti-hepatic fibrosis effect in the practice of Mongolian medicine, but the specific mechanism is still unclear. Therefore, it is of great significance to further clarify the anti-hepatic fibrosis mechanism of Mongolian medicine scabiosa for the prevention of hepatic fibrosis. Network pharmacol ogy integrates system biology, traditional pharmacology, network analysis, and computer analysis techniques to clarify the mechanism of action of drugs at multiple l evels, which is characterized by integrity, systematization, and novelty[12, 13]. At present, most of the anti-liver fibrosis methods highlight the effect on a cell or a signa ling molecule, without considering that hepatic fibrosis is a complex process[12]. From the current research, it is limited to use traditional biological experimental met hods to explore the mechanism of Mongolian medicine. Network pharmacology provides a new way to solve this problem. Therefore, in this paper, the UHPLC-TOF -MS/MS method was used to identify the components of Mongolian Medicine Scabiosa, and the network pharmacological method was used to screen the pathways a nd targets. In vitro and in vivo experiments were conducted to verify the accuracy of the mechanism of action of Mongolian medicine scabiosa against hepatic fibrosi

s.

1 Material and Methods

1.1 Materials

Chemical analysis was conducted on a connected system of UHPLC (Nexera X2 LC-30A, Shimadzu Corp, Japan)-hybrid triple quadruple time-of-flight mass spectrometer (Triple TOF™ 5600+, AB Sciex, Forster City, CA, USA) with an electrospray ionization source (ESI). KQ 5200de ultrasonic cleaner were purchased from Kunshan Ultrasonic Instrument Co., Ltd. Mongolian medicine scabiosa were purchased from Inner Mongolia Tiansheng Mongolian Medicine Co., Ltd. alanine transaminase (ALT), aspartate aminotransferase(AST), alkaline phosphatase (AKP), and hydroxyproline assay kits were purchased from Nanjing Jianjian (China, Nanjing).MTT cell proliferation and cytotoxicity test kit，Masson trichrome staining kit，RIPA lysate, phenylmethylsulfonyl fluoride (PMSF), 5 × Protein loading buffer were purchased from Solarbio (Beijing, China).α-SMA、collagenI、GAPDH 、PI3K、Akt、p-Akt、p38MAPK/ MAPK14、 p-p38、IgG、were purchased from Cell Signaling Technology (Danvers, United States). Fastking RT Kit, Super real Premium Plus，Annexinv FITC apoptosis Kit were purchased from Beyotime(Beijing, China), 10% fetal bovine serum (Gibco, New York, United States), DMEM high glucose medium (Gibco ,New York, United States).

1.2 Method

1.2.1 Chemical compositions identification

0.3g sample of Mongolian medicine scabiosa was put into a 15ml centrifuge tube, soaked in 5ml 50% methanol for 4h, and extracted by ultrasonic at 40 ℃ for 40min. After centrifugation at 4 ℃ and 12000rpm for 10min, take the supernatant and put it into a 25ml volumetric flask. Repeat the operation once. Combine the two supernatants, shake well and take 10 μ L supernatant was detected by HPLC. Gradient elution was carried out in 0.2mol/L ammonium acetate solution containing 0.1% formic acid and acetonitrile mass spectrometry. The raw data of UHPLC-TOF-MS/MS is imported into MS-DIAL4.12 software for preprocessing, which mainly includes extraction, denoising, deconvolution, peak alignment, and other important steps. Finally, the CSV format needs to be exported. The extracted peak information was searched and compared in MassBank, Respect, and GNPs to obtain the possible molecular formula.

1.2.2 Enrichment of key targets

The structure of components is retrieved in PubChem database, and transformed into canonical SMILES structure in chemdrawv14.00.117 software, and stored in Swiss Target Prediction database（http://www.swiss target prediction.Ch/) to screen the related targets of Mongolian Medicine Scabiosa; At OMIM(https://omim.org/)and GeneCards(https://www.GeneCards.org/) database screen hepatic fibrosis-related targets. Map the against hepatic fibrosis target of Mongolian medicine scabio

sa on Venny. 2.1 website.

1.2.3 Network construction and analysis

The anti-liver fibrosis target of Mongolian medicine scabiosa was imported into STRING (https://www.String-db.org/) database for protein interaction. Cytoscape3.6.0 software was used for Network Analyzer Network topology index analysis to screen the core targets against hepatic fibrosis. The active ingredients and targets of Mongolian medicine scabiosa were introduced into Cytoscape3.6.0 to construct an active ingredients-targets network map.

1.2.4 Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

The GO and KEGG enrichment targets of Mongolian medicine scabiosa against hepatic fibrosis were carried out on the Metascape platform was screened and visualized on the website of Wei sheng xin (www.bioinformatics.com.cn).

1.2.5 In vivo experimental verification

Fifty clean male Wistar rats weighing 200-220g for 7 weeks, were divided into blank, HF model groups and low, medium, and high dose groups (50 mg/kg, 100 mg/kg, and 200 mg/kg). In addition to the blank group, the other groups were gavaged with 2ml / kg 50% carbon tetrachloride peanut oil solution twice a week for 10 weeks. In the process of modeling, both the blank group and the model group were gavaged with 0.5% CMC Na, and the remaining three groups were gavaged with low, medium, and high doses. After 10 weeks, serum and liver tissues were taken for follow-up experiments.

1.2.5.1 ALT,AST,ALP and hydroxyproline content

After the blood was taken from the abdominal aorta, it was allowed to stand at room temperature for 2 min, 4 ℃, 4000r/min, centrifuged for 10 min, and the bleeding serum was separated. The changes of ALT and AST in serum were detected by Wright's method and UV spectrophotometer, and the content of ALP was detected by visible light colorimetry and UV spectrophotometer; The rat liver was washed with phosphate buffer (PBS), and the content of hydroxyproline in liver tissue was detected by acid hydrolysis and ultraviolet spectrophotometer.

1.2.5.2 Pathological observation of liver tissue

Take an appropriate amount of liver tissue and fix it in a container containing 4% formaldehyde for 48 hours. After the liver tissue is treated with 70%, 80%, 90%, and 95% gradient alcohol, it is then dehydrated in absolute ethanol, embedded in paraffin, sliced, stained with HE and Masson, and observed under a microscope.

1.2.5.3 Quantitative Real-Time PCR

Trizol reagent was used to extract total RNA from liver tissue. Total RNA extract was dissolved in RNase-free water. The final RNA purity was detected by mea suring OD260/280 values with a Nucleic Acid/Protein Analyzer, and the RNA integrity was verified by Type 1 nucleic acid dye on 1% agose gel. The cDNA was obta ined by one-step reverse transcription according to the instructions, and 2 μ L was amplified by PCR. The amplification conditions were predenaturation at 95℃ for 1 5min, 1 cycle. After 40 cycles of denaturation at 95℃ for 10s and annealing at 60℃ for the 30s. The relative expression of RNA after amplification was calculated by 2- ΔΔ Ct(Table1).

Table 1The primer sequences for RT-qPCR

Genes	Forward primer	Reverse primer
α-SMA	F:GCGTGGCTATTCCTTCGTGACTAC	R:CCATCAGGCAGTTCGTAGCTCTTC
CollagenI	F:TGTTGGTCCTGCTGGCAAGAATG	R:GTCACCTTGTTCGCCTGTCGCAGC
PI3K	F:GCTG TTGA TAGA CCAC CGCT TCC	R:TGCC CTGT TCCT CTTCC
Akt	F:CAAG CACG GTGT GACC ATGA	R:TCAG TAAG CGTC TGGG CAAC
MAPK14(p38)	F:AGGAGAGGCCCACGTTCTAC	R:CCGGGGACAGGTTCTGGTAT

1.2.5.4 Western blot analysis

50mg of liver tissue was added to PMSF and RIPA, which was crushed in a homogenizer and centrifugation for 10min at 12000r/min. The supernatant was taken to measure the protein concentration with BCA, then 5× protein loading buffer was added, boiled at 98℃ for 5 min, and stored in the refrigerator at -80℃. The conc entrated glue and the separated glue were prepared, and 10μl of protein was loaded for SDS-page electrophoresis. The membrane was transferred at 200mA and seale d with 5% skim milk powder at room temperature for 1h. Rabbit anti-α -SMA, rabbit anti-CollagenI, rabbit anti-PI3K, rabbit anti-Akt, rabbit anti-p-Akt, rabbit anti-P 38, rabbit anti-p-p38 were incubated at room temperature for 2h, and washed with TBST 3 times, 10 min each time.

Dylight 800-labeled goat anti-rabbit immunoglobulin G (IgG) antibody was incubated at room temperature for 2h and washed with TBST 3 times, 10min each time. An automatic chemiluminescence image analysis system was used to develop, and the protein gray value was analyzed by ImageJ.

1.2.6 In vitro validation

1.2.6.1 Preparation of medicated serum

Twenty Wistar rats, male, 160-180g, were divided into the blank group (n = 10) and Mongolian medicine scabiosa group (n = 10). The dosage of Mongolian me

dicine scabiosa was 500mg/(kg) after 10 times of the lowest adult dose d) On the 7th day, fasting 12 hours before gavage, anesthesia within 2 hours after gavage, bl ood was collected from the abdominal aorta by negative pressure blood sampling container, and centrifuged at 3000 r/min for 15 minutes. After centrifugation, the su pernatant was inactivated in a 56 ℃ water bath for 30 minutes, 0.22 μ The serum containing the drug was obtained after filtration with a 10 mmol membrane and stor ed in a refrigerator at - 80 ℃. The blank group was given normal saline by gavage.

1.2.6.2 Cell culture

HSC-T6 cells were cultured in DMEM high glucose medium containing 10% fetal bovine serum at 37 ℃ in a 5% CO2 incubator.

1.2.6.3 Cytotoxicity test

MTT assay was used to detect the cytotoxicity of Mongolian medicine scabiosa to HSC-T6 cells. HSC-t6 cells were inoculated in 96-well plates (6×103 cells/we ll) for 24 h. Zero control group, control group, low, medium, and high dose groups of drug-containing serum were set, and each group was set with 4 compound Well s. The administration group was treated with 10%, 20%, and 30% drug-containing serum for 24, 48, and 72 hours, respectively. The supernatant was then discarded a nd 10μLMTT solution was added to each well. After continuous culture for 4 h, discard the supernatant, add 110μLFormazan to each well, and place in a shaker for 1 0min. Finally, the absorbance was read at 490 nm using a microplate reader. Inhibition rate(%)=1-(OD_{drug}/$OD_{control}$)×100%.

1.2.6.4 Western blot analysis

HSC-T6 in the six-well plate was digested and the cells were dissolved with PMSF and RIPA at 4℃. Centrifugation at 1000r/min for 15min, the supernatant was determined with BCA, 5× protein loading buffer was added, boiled at 98℃ for 5min, and Western blot analysis was performed. Rabbit anti-α -SMA, rabbit anti-Coll agenI, rabbit anti-PI3K, rabbit anti-Akt, rabbit anti-p-Akt, rabbit anti-P38, rabbit anti-p-p38 primary antibody were incubated at 4℃ overnight. The membrane was w ashed with 0.1%(v/v)Tween-20 in Tris buffer saline (pH 7.4) and then incubated with a second antibody for 2h. An automatic chemiluminescence image analysis syst em was used to develop, and the protein gray value was analyzed by ImageJ.

1.2.6.5 Cell apoptosis assay

There were no staining holes, single staining holes, control group, low, medium, and high dose groups of medicated serum of Mongolian Medicine Scabiosa, wit h 5 multiple holes in each group. After HSC-T6 adherent culture for 12 hours, the corresponding concentrations (10%, 15%, 20%) of serum-containing drugs were ad ded to each group for continuous culture for 24 hours. The cells were digested at 12 000 r/m and centrifuged for 5 minutes. After discarding the supernatant, the cells

were resuspended with annexin V-FITC binding solution and then mixed with annexin V-FITC and PI. Flow cytometry was performed after incubation at room tempe rature for 20 min.

1.2.7 Statistical analysis

The data were analyzed by SPSS22.0 software, and the results were expressed as mean ± standard deviation (SD). Comparison between groups larger than two group s was performed using one-way analysis of variance (ANOVA). Comparison between groups with normality and homogeneity of variance was performed using the L SD test. It was considered statistically significant when $P<0.05$.

2 Results

2.1 Identification of the Chemical Constituents in Scabiosa by UHPLC-TOF-MS/MS

According to the total ion flow diagram, the first and second fragment information, the accurate molecular weight of the compound was obtained. The chemical constituents were identified by retention time, mass charge ratio, and adduct ion. A total of 120 compounds were identified, including flavonoids, alkaloids, coumarin s, terpenoids, phenols, and fatty acids (Table 2).

Table 2 Identification of the chemical constituents of Mongolian medicine scabiosa by UHPLC-TOF-MS/MS

NO	Identity	Rt(min)	Mz	Adduct ions	Formula	M/z	MS/MS
1	Choline	1.189	104.1093	[M+H]+	C5H14NO	105.1126	58.06887:935、 60.08254:389
2	Succinic acid	5.333	119.0329	[M+H]+	C4H6O4	120.0363	68.99759:402
3	Nicotinic acid	1.091	124.0236	[M+H]+	C6H5NO2	120.0269	53.02594:126、 82.99405:259
4	Cinnamaldehyde	5.34	133.0677	[M+H]+	C9H8O	134.0711	77.03212:129 、105.07906:126
5	Spermidine	1.047	146.1654	[M+H]+	C7H19N3	147.1688	72.0854:680、112.11783:385
6	Coumaric acid	2.955	147.0447	[M+H-H2O]+	C9H8O3	148.0452	119.04906:125、147.08626:125
7	P-coumaric acid	3.675	163.0188	[M-H]-	C9H8O3	163.0188	163.03569:125
8	Methionine	1.601	150.0575	[M+H]+	C5H11NO2S	150.0575	57.05724:258、102.05367:130
9	Dopamine	1.904	154.0835	[M+H]+	C8H11NO2	155.0869	113.06096:126
10	Rosmarinic acid	4.506	163.0364	[M+H]+	C18H16O8	164.0397	117.03635:135、163.04413:266
11	3-Hydroxycinnamic acid	1.632	165.0526	[M+H]+	C9H8O3	166.056	119.06104:127
12	Phenylalanine	2.698	166.0858	[M+H]+	C9H11NO2	167.0892	51.03143:126、93.08544:267
13	Gallic acid	2.129	171.0271	[M+H]+	C7H6O5	172.0304	171.02708:9580、171.12753:126
14	Trans-4-hydroxy-3-methoxycinnamate	2.114	195.067	[M+H]+	C10H10O4	196.0703	195.06697:2328、196.07032:300

15	4-Hexylresorcinol	10.089	195.1379	[M+H]+	C12H18O2	196.1413	195.13791:4018、196.14126:396
16	Spermine	1.022	203.2234	[M+H]+	C10H26N4	204.2267	203.22339:1604、204.22674:200
17	Pilocarpine	9.698	209.1486	[M+H]+	C11H16N2O2	210.1519	209.16098:252
18	Prolintane	7.495	218.1559	[M+H]+	C15H23N	219.1592	218.15643、218.17102
19	Ellipticine	1.696	247.0785	[M+H]+	C17H14N2	248.0818	218.15643:126 、247.0847:2746
20	Matrine	9.664	249.1834	[M+H]+	C15H24N2O	250.1867	215.07059:285、249.1875:256
21	Benzoylecgonine	1.598	290.1354	[M+H]+	C16H19NO4	291.1358	58.06772:126、161.1129:126
22	Tectorigenin	2.07	301.0895	[M+H]+	C16H12O6	302.0928	301.14716:518
23	Isoxsuprine	6.937	302.1375	[M+H]+	C18H23NO3	303.1409	112.08042:393、284.12814:256
24	Quercetin	4.114	303.0501	[M+H]+	C15H10O7	304.0539	112.08042:393、247.07854:259
25	Hesperetin	6.294	303.0844	[M+H]+	C16H14O6	304.0878	145.02951:126、268.13083:126
26	Pergolide	6.968	315.1842	[M+H]+	C19H26N2S	316.1876	315.19397:259
27	Isorhamnetin	4.606	317.0655	[M+H]+	C16H12O7	318.0671	239.04169:126、285.05463:383
28	3'-Methoxy-4',5,7-trihydroxyflaonol	6.321	315.0494	[M-H]-	C16H12O7	315.0518	107.02756:126、300.04877:1104
29	Kaurenic acid	7.941	325.2354	[M+H]+	C20H30O2	326.2387	324.19269:267、 325.24524:2596
30	Berberine	5.93	336.0943	[M+H]+	C20H18NO4	337.0977	202.43413:126、336.12827:916
31	Yohimbic acid	3.645	341.1953	[M+H]+	C20H24N2O3	342.1987	107.08719:126、169.12019:126
32	Lauroylcarnitine	10.086	344.2781	[M+H]+	C19H37NO4	345.2815	71.08508:126、344.22876:256
33	Chelerythrine	1.589	348.1262	[M+H]+	C21H18NO4	349.1295	287.09702:126
34	Asterric acid	3.484	349.0899	[M+H]+	C17H16O8	350.0933	185.05644:126、349.1329:498
35	Camptothecin	2.603	349.1077	[M+H]+	C20H16N2O4	350.1111	149.03424:126 、349.12958:514
36	Chlorogenic acid	3.341	355.1038	[M+H]+	C16H18O9	356.1072	62.7101:252、165.71355:410
37	Corynanthine	4.229	355.17	[M+H]+	C21H26N2O3	356.1733	137.1469:126、355.1843:1538
38	Laudanosine	9.624	358.1979	[M+H]+	C21H27NO4	359.2002	188.09511:126、323.22943:126
39	Stigmasterol	13.59	395.368	[M+H]+	C29H48O	396.3714	191.18109:125
40	S-adenosyl-methionine	2.476	399.1489	[M+H]+	C15H22N6O5S	400.1523	391.92365:259、399.14612:918
41	Dehydrocholic acid	11.258	403.2324	[M+H]+	C24H34O5	404.2357	129.01974:252、185.10208:126
42	Mangiferin	3.04	423.1055	[M+H]+	C19H18O11	424.1079	149.02409:126、378.18411:126
43	Cosmosiin	4.449	433.1121	[M+H]+	C21H20O10	434.1155	271.06418:13981、272.14539:1261
44	Apigenin-7-O-glucoside	4.666	433.1139	[M+H]+	C21H20O10	434.1172	137.13525:126、276.24005:384
45	Apigenin 8-C-glucoside	4.196	433.1174	[M+H]+	C21H20O10	434.1208	210.17685:126、371.25336:133
46	Quercetin-3-O-xyloside	3.392	435.0845	[M+H]+	C20H18O11	436.0872	184.98349:126、435.3205:133
47	Ursolic acid	6.272	439.3549	[M+H]+	C30H48O3	440.3582	191.17844:130
48	Biochanin-7-O-glucoside	3.859	447.129	[M+H]+	C22H22O10	448.1323	179.045:126、447.20813:4357

49	Luteolin-6-C-glucoside	3.817	449.1089	[M+H]+	C21H20O11	450.1122	131.05202:126、449.508:690
50	Cyanidin-3-glucoside	4.561	449.1096	[M]+	C21H21O11	449.1086	107.04773:126、449.14621:382
51	Betulinic acid	10.012	457.3692	[M+H]+	C30H48O3	458.3725	377.29407:252、457.39832:511
52	Peonidin-3-O-β-D-glucoside	4.814	463.1247	[M+H]+	C22H23O11	464.128	74.5218:126、429.09894:568
53	Delphinidin-3-O-β-glucopyranoside	4.3	465.0998	[M+H]+	C21H21O12	466.1032	73.48975:256、465.14166:390
54	Kaempferol-3-O-glucoside	4.565	471.0926	[M+H]+	C21H20O11	472.0959	217.13036:126、471.11038:4094
55	Corosolic acid	10.033	473.3586	[M+H]+	C30H48O4	474.362	427.36542:126、473.36124:126
56	Vicriviroc m alate	7.214	534.3106	[M+H]+	C28H38F3N5O2	535.314	277.23386:126 、479.30133:126
57	Amentoflavone	4.503	539.1166	[M+H]+	C30H18O10	540.12	163.0365:383、 539.55432:1008
58	Biflavonoid-flavone base + 3O and flavanone base + 2O + 1MeO	3.102	555.1318	[M+H]+	C31H22O10	556.1352	325.09689:126、555.23743:324
59	Naringenin-7-O-rutinoside	1.676	581.1826	[M+H]+	C27H32O14	582.1859	375.19989:126、581.2337:952
60	Kaempferol-3-O-glucoside-6''-p-coumaroyl	5.353	595.1423	[M+H]+	C30H26O13	596.1457	147.0484:1271、 595.18781:536
61	Rutin	4.116	611.16	[M+H]+	C27H30O16	612.1622	85.03064:126、611.20917:1396
62	Kaempferol-3-O-rutinoside	4.362	617.1459	[M+H]+	C27H30O15	618.1493	183.02946:126、617.60309:344
63	Flavonol base + 3O, 1MeO, O-Hex-dHex	4.408	625.1837	[M+H]+	C28H32O16	626.187	235.07053:256、625.51349:773
64	Acarbose	3.321	646.2262	[M+H]+	C25H43NO18	647.2296	370.14111:129、646.24945:396
65	Fumaric acid	1.301	115.0046	[M-H]-	C4H4O4	115.0046	56.07348:126
66	2-Hydroxybenzaldehyde	4.357	121.0284	[M-H]-	C7H6O2	123.0351	92.06061:126、 121.0385:1084
67	Salicylic acid	2.478	137.0253	[M-H]-	C7H6O3	138.0287	137.04158:125
68	P-hydroxybenzeoic acid	3.689	137.0268	[M-H]-	C7H6O3	138.0286	65.03075:126、137.03703:2428
69	Gentisic acid	3.135	153.0207	[M-H]-	C7H6O4	154.0241	93.06663:63、153.033:189
70	Coumarin + 1O	3.34	161.0238	[M-H]-	C9H6O3	161.0234	133.03888:252
71	P-coumaric acid	3.675	163.0188	[M-H]-	C9H8O3	164.0222	163.03569:125
72	L-(-)-phenylalanine	2.698	164.073	[M-H]-	C9H11NO2	165.0764	72.02854:263、101.05108:261
73	Lauric acid	10.783	199.17	[M-H]-	C12H24O2	200.1733	143.06599:126 、199.1897:126
74	2,6-Di-tert-butyl-4-methylphenol	4.001	219.173	[M-H]-	C15H24O	220.1764	127.0936:126、219.21745:433
75	Myristic acid	12.301	227.1999	[M-H]-	C14H28O2	228.2071	227.22791:378
76	6-Hydroxyflavone	5.393	237.056	[M-H]-	C15H10O3	238.0593	193.07635:256、219.06946:256
77	Pentadecanoic acid	12.969	241.2161	[M-H]-	C15H30O2	242.2194	225.05496:388
78	Uridine	1.63	243.0609	[M-H]-	C9H12N2O6	244.0643	122.04195:126、200.08321:126
79	Chrysin	7.467	253.0496	[M-H]-	C15H10O4	254.0528	151.01828:126、253.07574:1360
80	Palmitoleic acid	12.456	253.2157	[M-H]-	C16H30O2	254.2191	181.14389:126、253.22858:256
81	9-Trans-Palmitelaidic acid	13.04	253.2193	[M-H]-	C16H30O2	254.2227	123.08641:126、253.2569:126
82	Magnolol	1.955	265.0942	[M-H]-	C18H18O2	266.0975	158.73671:297、255.2596:7401
83	Apigenin	6.111	269.0465	[M-H]-	C15H10O5	270.0497	85.03926:126、265.11005:3485

84	Naringenin chalcone	6.199	271.0595	[M-H]-	C15H12O5	272.0651	93.04588:257
85	a-Linolenic acid	9.627	277.2145	[M-H]-	C18H30O2	278.2182	119.06125:252、271.08728:655
86	y-Linolenic acid	11.881	277.2187	[M-H]-	C18H30O2	278.2199	277.23846:126
87	9Z,12Z-Linoleic acid (NMR)	12.716	279.2335	[M-H]-	C18H32O2	280.2351	261.93088:126、279.25555:6512
88	Oleic acid	13.686	281.2486	[M-H]-	C18H34O2	282.252	261.93088:126、279.25555:6512
89	Cyanidin	2.337	285.0358	[M-H]-	C15H11O6	286.0392	112.98975:126、168.03923:126
90	6-Gingerol	8.355	293.173	[M-H]-	C17H26O4	294.1763	136.10315:126、192.14954:516
91	7,4'-Dimethoxy-5-hydroxyflavanone	4.6	299.0927	[M-H]-	C17H16O5	300.096	253.23056:126
92	2-Chloroadenosine	2.7	300.0452	[M-H]-	C10H12ClN5O4	301.0486	112.99426:257 、232.08847:394
93	Ellagic acid	1.093	300.9892	[M-H]-	C14H6O8	301.9926	120.96051:126、254.89232:522
94	Flavanone base + 3O, 1MeO	6.295	301.0705	[M-H]-	C16H14O6	302.0739	123.02272:126、301.10571:648
95	Arachidonic acid	12.456	303.2312	[M-H]-	C20H32O2	304.2346	163.08835:126、259.26151:525
96	Flavone base + 3O, 2MeO	3.754	329.0683	[M-H]-	C17H14O7	330.0717	168.27313:126、269.07382:261
97	Gallic acid hexoside	2.062	331.0654	[M-H]-	C13H16O10	332.0687	331.11612:252
98	Docosatetraenoic acid	6.225	331.2509	[M-H]-	C22H36O2	332.2543	298.07492:126、331.28625:639
99	N-2-Hydroxycyclopentyladenosine	8.348	350.1246	[M-H]-	C15H21N5O5	351.128	265.18106:252
100	Caffeoyl quinic acid	2.728	353.0851	[M-H]-	C16H18O9	355.0918	146.09454:126、353.0986:126
101	S-adenosyl-homocysteine	1.643	383.114	[M-H]-	C14H20N6O5S	384.1173	248.08559:126、321.13257:378
102	9-Nitro-20(S)-camptothecin	2.348	392.0976	[M-H]-	C20H15N3O6	393.1009	62.00884:126
103	Daidzein-8-C-glucoside	2.721	415.1041	[M-H]-	C21H20O9	416.1075	188.97481:126、415.19547:126
104	Puerarin	2.915	415.1042	[M-H]-	C21H20O9	416.1075	112.99891:126、415.25009:126
105	Formononetin-7-O-glucoside	3.833	429.1198	[M-H]-	C22H22O9	430.1231	62.00551:63、429.31219:63
106	Kaempferol-3-O-α-L-rhamnoside	2.716	431.1151	[M-H]-	C21H20O10	432.1184	145.04587:126、431.03604:126
107	Epicatechin-3-O-gallate	3.504	441.1011	[M-H]-	C22H18O10	442.1044	179.07037:256、441.21112:378
108	Luteolin-7-O-glucoside	5.946	447.0898	[M-H]-	C21H20O11	449.0939	282.26071:126、285.06256:395
109	α-Boswellic acid	11.701	455.3523	[M-H]-	C30H48O3	457.3584	391.27481:126、455.40399:395
110	Epigallocatechin-3-gallate	4.754	457.0926	[M-H]-	C22H18O11	458.0959	269.06985:398、457.15945:809
111	Isoflavone base + 2O, O-Hex	2.916	461.1099	[M-H]-	C21H20O9	462.1132	165.06392:126、
112	18-β-glycyrrhetinic acid	9.627	469.3295	[M-H]-	C30H46O4	470.3328	423.27933:256、425.40698:272
113	Maslinic acid	7.103	471.3098	[M-H]-	C30H48O4	472.3134	471.34167:126、471.39069:84
114	Coumarin + 1O + 1MeO, O-Hex-Hex	3.083	515.1375	[M-H]-	C22H28O14	516.1408	191.07942:258、323.10324:390
115	Kaempferol-3-glucoside-3"-Rhamnoside	5.492	593.1331	[M-H]-	C27H30O15	594.1339	255.05511:126、284.08432:258
116	Datiscetin-3-O-rutinoside	5.353	593.1339	[M-H]-	C27H30O15	594.134	135.05669:126、231.00208:126
117	Quercetin-3-O-vicianoside	6.314	595.1348	[M-H]-	C26H28O16	596.1382	285.06277:521、285.1033:262
118	Kaempferol 3-O-sophoroside	5.27	609.122	[M-H]-	C27H30O16	610.1254	145.05121:126、561.55334:126

119	Dihydrohesperetin-7-O-neohesperidoside	3.309	611.1633	[M-H]-	C28H36O15	612.1667	281.10199:126、611.26764:711
120	Isorhamnetin-3-O-galactoside-6"-rhamnoside	5.414	623.1454	[M-H]-	C28H32O16	624.1487	315.06528:254、623.20099:390

No: Number; Mz: Z-average molecular weight; Rt: Relative retention time; M/z: Mass-to-charge ratio; MS/MS: Mass spectrometry/Mass spectrometry

2.2 Enrichment of key targets

The canonical SMILES number of chemical components was input into Swiss Target Prediction to select the species as "Homo sapiens", and a total of 239 target s related to Mongolian medicine scabiosa were obtained. Input "Hepatic Fibrosis" into OMIM, GeneCards, and database to obtain a total of 1199 hepatic fibrosis-rela ted targets. Through venny.2.1.0 website, 63 targets of Mongolian medicine scabiosa against hepatic fibrosis were obtained.(Figure 1)

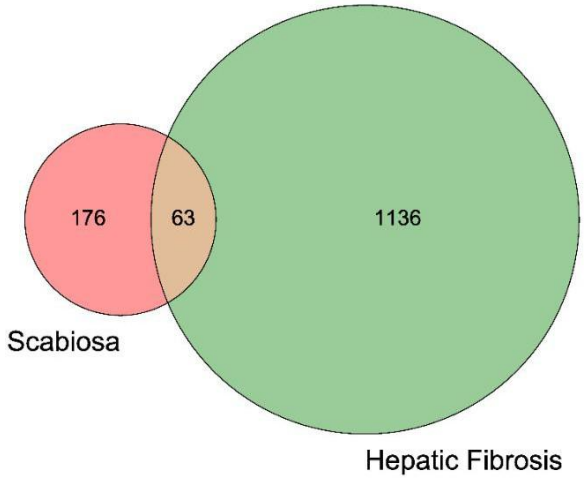


Figure 1 Venn plots of composition-related targets and disease-related targets

2.3 Construction of PPI network

The use of the STRING database to establish a PPI protein interaction network can better explain the anti-hepatic fibrosis mechanism of Mongolian Medicine Scabiosa. The anti-liver fibrosis target was uploaded to the STRING database, the "Multiple Protein" mode was selected, the species was "Homo sapiens", and the confidence level was set to 0.9 to obtain the interaction information of PPI proteins, and the information was imported to Cytoscape3.6.0 for visual analysis. In the figure, the network consists of 63 nodes and 133 edges, and the average node degree is 4.29. "Node" represents the target, "edge" represents the interaction between the target and the target, and "combine Score" represents the thickness of the edge. The greater the combined Score value, the stronger the degree of combination (figure 2).

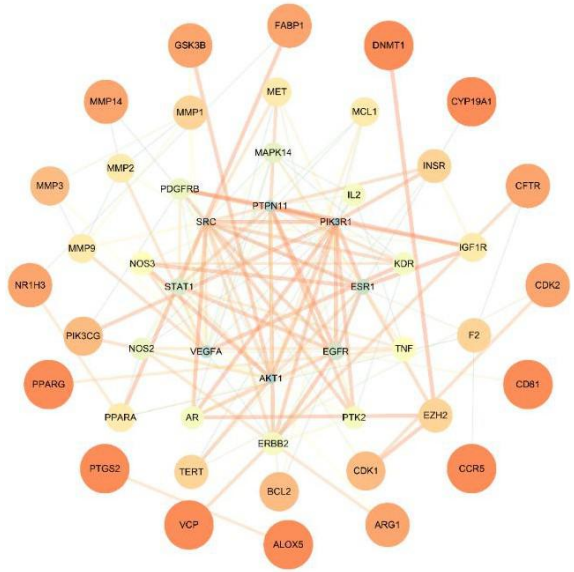


Figure 2 PPI network of Mongolian medicine scabiosa in the treatment of hepatic fibrosis

2.4 Construction of “Drugs-Compositions-Targets-Pathways” network

The drugs, active components, targets, and pathways of Mongolian medicine scabiosa were introduced into cytoscape3.6.0 to construct the active components target pathway network diagram (Figure 3). Network analysis was used to calculate the network topology parameters, in which green represents the target, red represen

ts the active components and purple represents the pathway. Node size is determined by Degree value. Based on the results of topology heterogeneity analysis, condit ions such as node compactness ≥ median, Degree value ≥2 times median, node Mediation Centrality ≥ median are set as important parameters for screening core targ ets. From the analysis of topological heterogeneity, we can get core targets, including Akt1、PIK3R1、EGFR、PPARA、IGF1R、INSR、MAPK14、TNF、PPA RG、BRAF(Table 3). According to the order of degree, Quercetin、Ellagic acid、Apigenin、Epicatechin-3-O-gallate、Epigallocatechin-3-gallate、9-Trans-Palmit elaidic acid、Oleic acid、Chrysin、4-Hexylresorcinol、Cyanidin were found to have relatively high activity(Table 4).

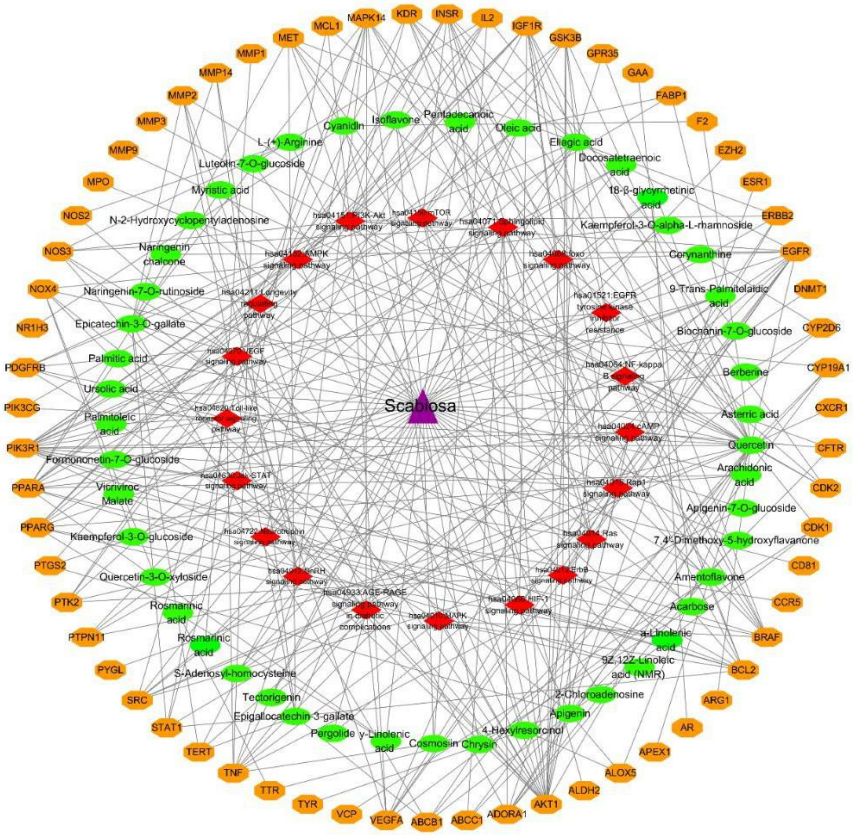


Figure 3 The "Drugs-active ingredients - targets - pathways" network of Mongolian medicine scabiosa in the treatment of hepatic fibrosis

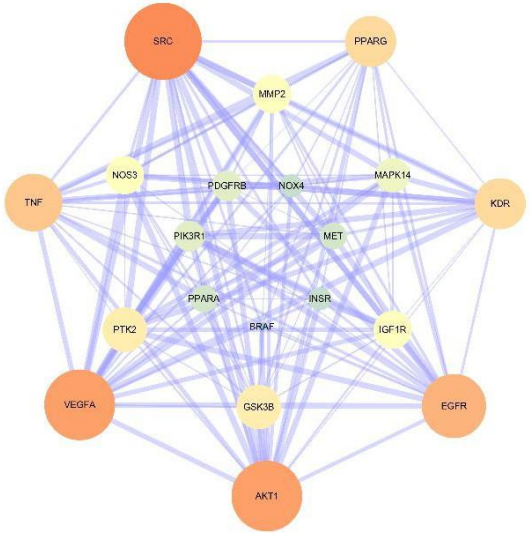


Figure 4 Core targets enrichment

Table.3 Core targets of Mongolian medicine scabiosa in the treatment of hepatic fibrosis

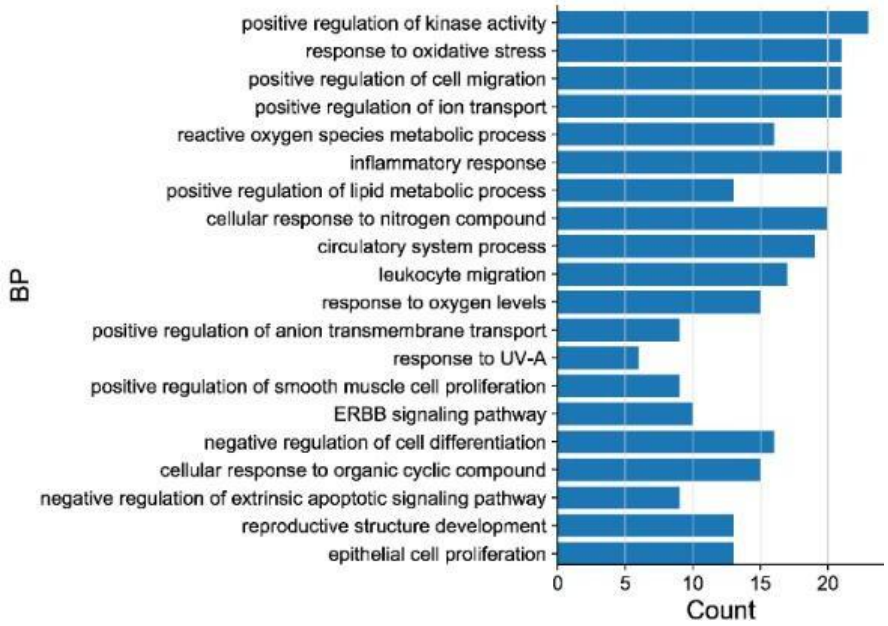
Gene symbol	Degree	Mediation entrality	Compactness	Gene symbol	Degree	Mediation entrality	Compactness
Akt1	20	0.058237	0.404984	GSK3B	8	0.017131	0.372493
PIK3R1	18	0.04448	0.402362	VEGFA	8	0.011406	0.343008
EGFR	13	0.033815	0.379009	SRC	7	0.009045	0.358127
PPARA	12	0.012796	0.313253	KDR	7	0.007186	0.362117
IGF1R	11	0.016921	0.376812	MET	7	0.007972	0.339426
INSR	10	0.015087	0.37464	NOX4	6	0.01284	0.356164
MAPK14	10	0.012624	0.344828	NOS3	6	0.00589	0.337662
TNF	9	0.018696	0.329114	PDGFRB	6	0.002621	0.330789
PPARG	9	0.013265	0.308789	PTK2	5	0.004557	0.356164
BRAF	9	0.007155	0.33419	MMP2	5	0.007889	0.352304

Table 4 Active ingredient parameters of Mongolian Medicine Scabiosa

Identity	Degree	Mediation entrality	Compactness	Identity	Degree	Mediation entrality	Compactness
Quercetin	29	0.251465	0.485075	Naringenin chalcone	2	0.004149	0.380117
Ellagic acid	15	0.081451	0.439189	Tectorigenin	2	0.004149	0.380117
Apigenin	13	0.070685	0.411392	Kaempferol-3-O-glucoside	2	0.001513	0.363128
Epicatechin-3-O-gallate	9	0.042614	0.411392	Quercetin-3-O-xyloside	2	0.001513	0.363128
Epigallocatechin-3-gallate	8	0.030982	0.401235	Kaempferol-3-O-alpha-L-rhamnoside	2	0.001513	0.363128
9-Trans-Palmitelaidic acid	5	0.011116	0.373563	Naringenin-7-O-rutinoside	2	0.001709	0.361111
Oleic acid	5	0.011116	0.373563	7,4'-Dimethoxy-5-hydroxyflavanone	3	0.018759	0.363128
Chrysin	4	0.005547	0.365169	2-Chloroadenosine	2	0.001707	0.365169
4-Hexylresorcinol	3	0.017595	0.363128	N-2Hydroxycyclopentyladenosine	2	0.001707	0.365169
Cyanidin	3	0.018759	0.363128	Pergolide	2	0.001707	0.365169
Cosmosiin	3	0.007741	0.380117	Berberine	9	0.042614	0.411392
Luteolin-7-O-glucoside	3	0.007741	0.380117	Corynanthine	8	0.030982	0.401235
Amentoflavone	3	0.022777	0.377907	Asterric acid	2	0.005049	0.361111
L-(+)-arginine	3	0.015348	0.373563	Apigenin-7-O-glucoside	2	0.005049	0.361111
Palmitoleic acid	3	0.005548	0.365169	Biochanin-7-O-glucoside	2	0.005049	0.361111
a-Linolenic acid	3	0.002981	0.369318	Formononetin-7-O-glucoside	2	0.007072	0.365169
y-Linolenic acid	3	0.002981	0.369318	Ursolic acid	3	0.007741	0.380117
9Z,12Z-Linoleic acid (NMR)	3	0.002981	0.369318	Vicriviroc Malate	2	0.004979	0.373563
Arachidonic acid	3	0.002981	0.369318	Acarbose	3	0.007741	0.380117
Docosatetraenoic acid	3	0.002981	0.369318	Myristic acid	2	0.002388	0.365169
S-Adenosyl-homocysteine	3	0.03065	0.363128	Pentadecanoic acid	2	0.002388	0.365169
18-β-glycyrrhetinic acid	3	0.023055	0.369318	Palmitic acid	2	0.015385	0.361111
Rosmarinic acid	2	0.015385	0.361111	Isoflavone	2	0.015385	0.361111

2.5 GO function analysis

The anti-hepatic fibrosis target of Mongolian medicine scabiosa was input into the meta landscape platform for de annotation analysis. The research background was that "Homo sapiens" selected biological process biological process (BP), cell component(CC), molecular function(MF), and visually analyzed the top-ranking information of each group on the bioinformatics website. BP mainly involves positive regulation of kinase activity, response to oxidative stress, positive regulation of cell migration, positive regulation of lipid metabolic process, positive regulation of smooth muscle cell proliferation, and ERBB signaling pathway; CC mainly involves vesicle lumen, perinuclear region of cytoplasm, extracellular matrix, glutamatergic synapse and so on; MF mainly involves protein kinase activity, nuclear receptor activity, protein serine/threonine kinase activity, G protein-coupled chemoattractant receptor activity, ATPase-coupled transmembrane transporter activity(Figure 5).



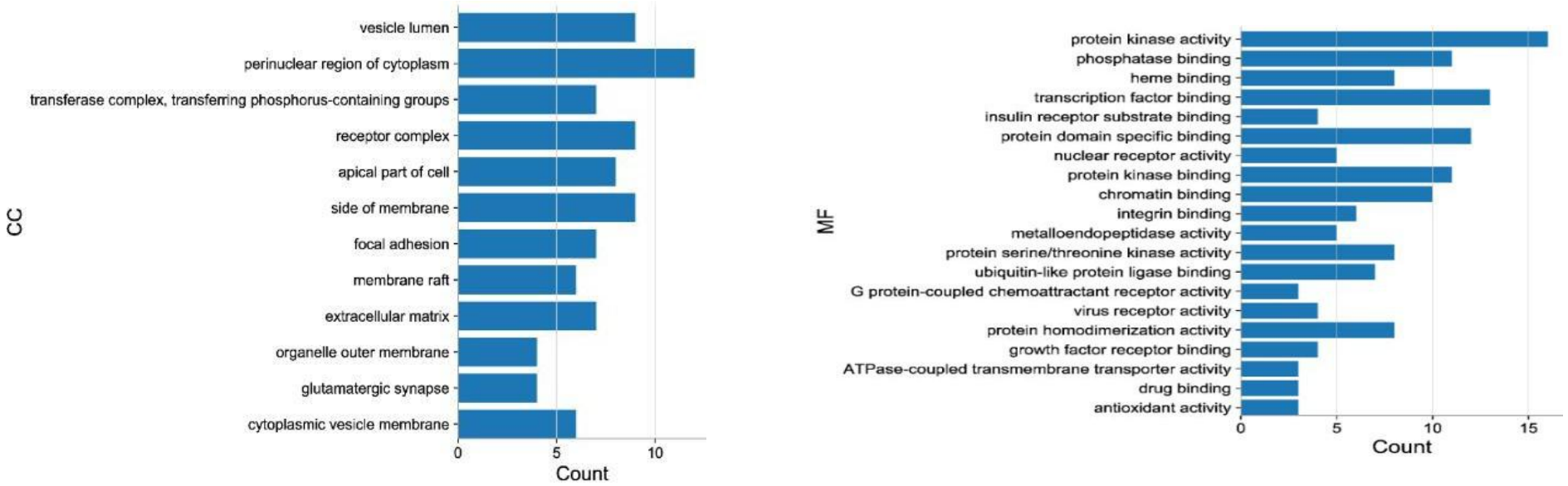


Figure 5 BP、CC、MF Enrichment of biological function

2.6 KEGG pathway analysis

KEGG pathway enrichment analysis was conducted in the Metascape database to screen out statistically significant pathways (P<0.05). After ranking according to P-value, the top 20 pathways were visually analyzed on the bioinformatics website. As shown in(Figure 6), the enriched pathways are mainly involved in apoptosis, inflammation, and cancer. For example, PI3K/Akt signaling pathway, EGFR signaling pathway, Rap1 signaling pathway, HIF-1 signaling pathway, Ras signaling pathway, and MAPK signaling pathway are all typical anti-fibrosis related pathways. Through enrichment results and literature review, we found that PI3K/Akt and MAPK signaling pathways can be used to study the occurrence and

development of hepatic fibrosis.

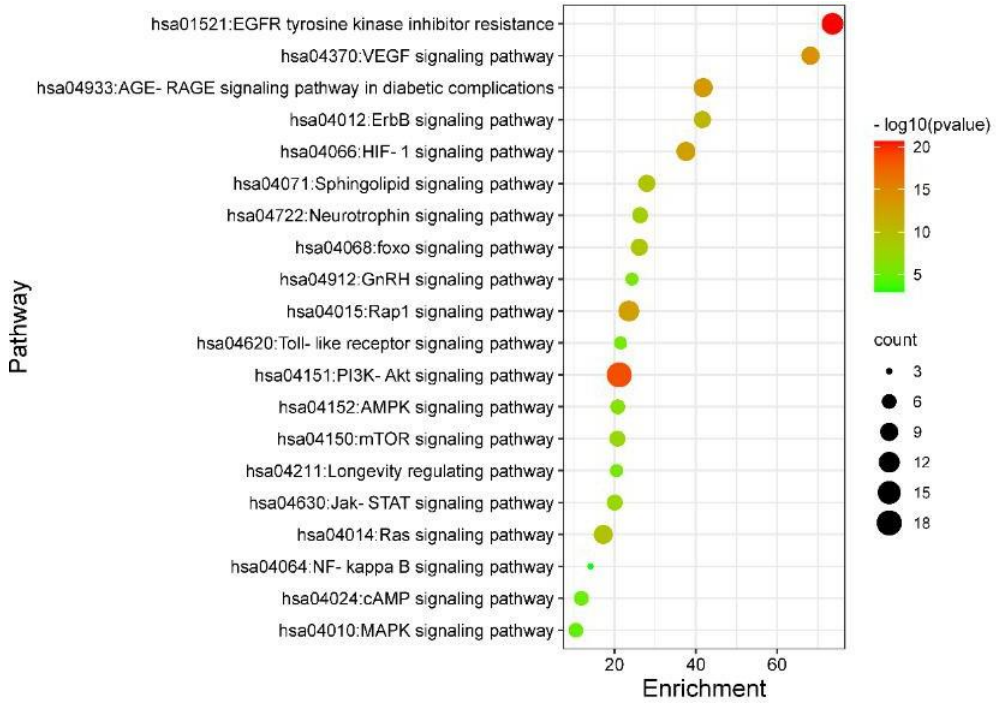


Figure 6 KEGG Pathway enrichment analysis

2.7 In vivo validation

2.7.1 Serum biochemical indexes, hydroxyproline detection results

The results of serum biochemical indexes showed that ALT, AST, and ALP values in the model group were significantly increased compared with the blank grou p ($P< 0.01$). Compared with model group, ALT ($P< 0.01$), AST and ALP ($P< 0.05$) were significantly decreased in low-dose group, and ALT ($P< 0.01$), AST ($P< 0. 01$) and ALP ($P< 0.01$) were significantly decreased in medium-dose group. ALT ($P< 0.01$), AST and ALP ($P< 0.01$) were significantly decreased in the high-dose gr

oup. The hydroxyproline results showed that compared with the blank group, the hydroxyproline content in the model group was significantly increased ($P< 0.01$); C ompared with the model group, the content of hypdroxyproline in the low-dose group was significantly decreased, and the content of hydroxyproline in the medium-d ose and high-dose groups was significantly decreased ($P< 0.01$). All the above results showed that Mongolian medicine scabiosa had an obvious anti-hepatic fibrosis effect(Table 5).

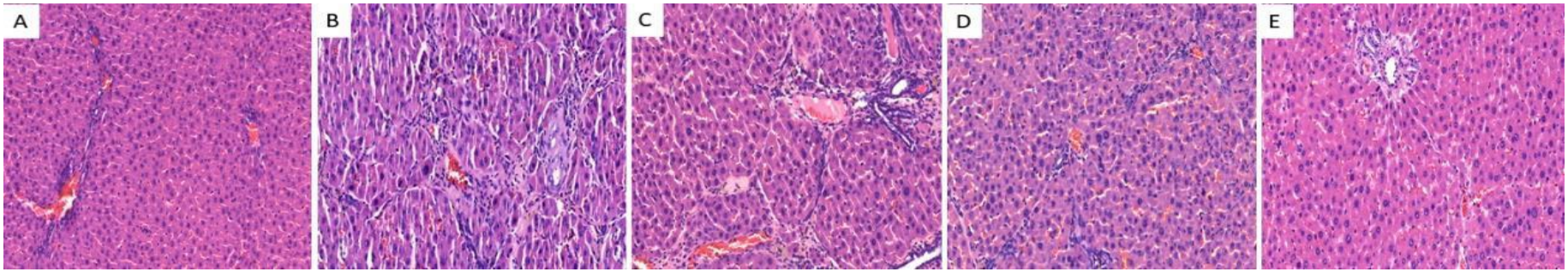
Table 5 Determination results of serum liver function index, and hydroxyproline

Group	ALT/（U·L-1）	AST/（U·L-1）	AKP/（U·L-1）	hydroxyproline (μ g · g-1)
Blank group	9.18±5.42	14.65±4.27	14.46±3.68	342±42.41
Model group	32.63±8.20##	35.41±16.06##	40.58±21.85##	1452.95±232.06##
Low-dose group	18.46±7.12**	29.51±10.95	22.40±6.32*	1012.80±232.22
Medium-dose group	14.50±5.34**	18.24±6.53**	15.21±11.33**	723.34±121.90**
High-dose group	16.35±8.01**	26.35±10.50	12.52±11.36**	725.36±217.84**

# $P< 0.05$, # $P< 0.01$ compared with Blank group, * $P< 0.05$, * * $P< 0.01$ compared with Model group

2.7.2 Pathological observation of liver tissue

We observed the results of HE and Masson staining and found that there was no fibrous proliferation in the blank group, there was a large amount of collagen depos ition, fibrous proliferation, and hepatocyte necrosis in the model group. Compared with the model group, the pathological changes of Mongolian medicine scabiosa tr eatment groups recovered. The results showed that Mongolian medicine scabiosa had a significant effect on anti-hepatic fibrosis (Figure 7).



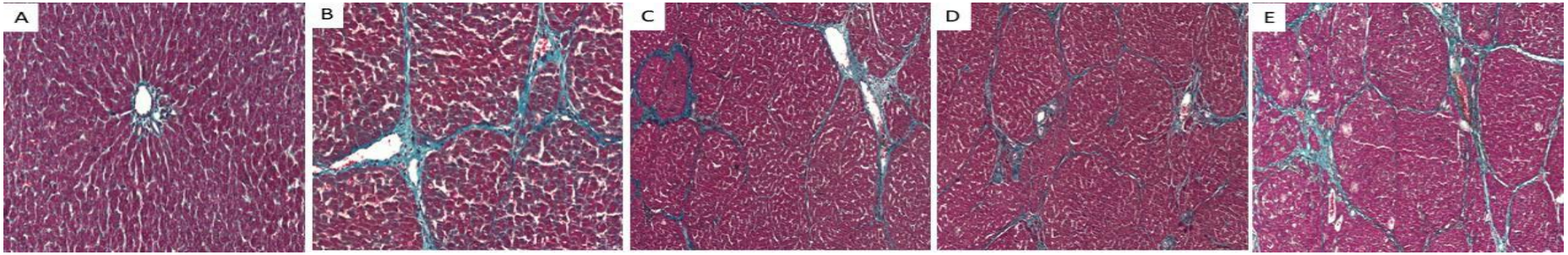
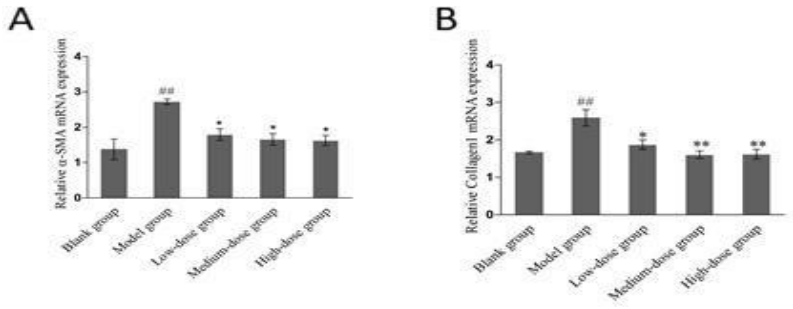


Figure 7 Effect of scabiosa on pathological changes of liver in mice (HE and Masson staining, original magnification: ×200). A：Blank group；B：Model group；C：Low-dose group；D：Medium-dose group；E：High-dose group

2.7.3 RT-QPCR results of α-SMA, CollagenI,PI3K, Akt ,p38 in liver tissue

Compared with the blank group, the mRNA relative expressions of α-SMA, CollagenI, PI3K ,Akt and p38mRNA in model group were significantly increased ($P< 0.01$). Compared with model group, the mRNA relative expression levels of α-SMA($P< 0.05$), CollagenI ($P< 0.05$), PI3K($P< 0.01$), Akt($P< 0.01$) and p38 in low-dose group were decreased. The mRNA relative expression levels of α-SMA($P< 0.05$), CollagenI ($P< 0.01$) PI3K($P< 0.01$), Akt($P< 0.01$) p38 ($P< 0.01$) were decreased in the medium dose group. The mRNA relative expression levels of α-SMA($P< 0.05$), CollagenI ($P< 0.01$) PI3K($P< 0.01$), Akt($P< 0.01$) p38 ($P< 0.01$) in the high-dose group were significantly decreased.



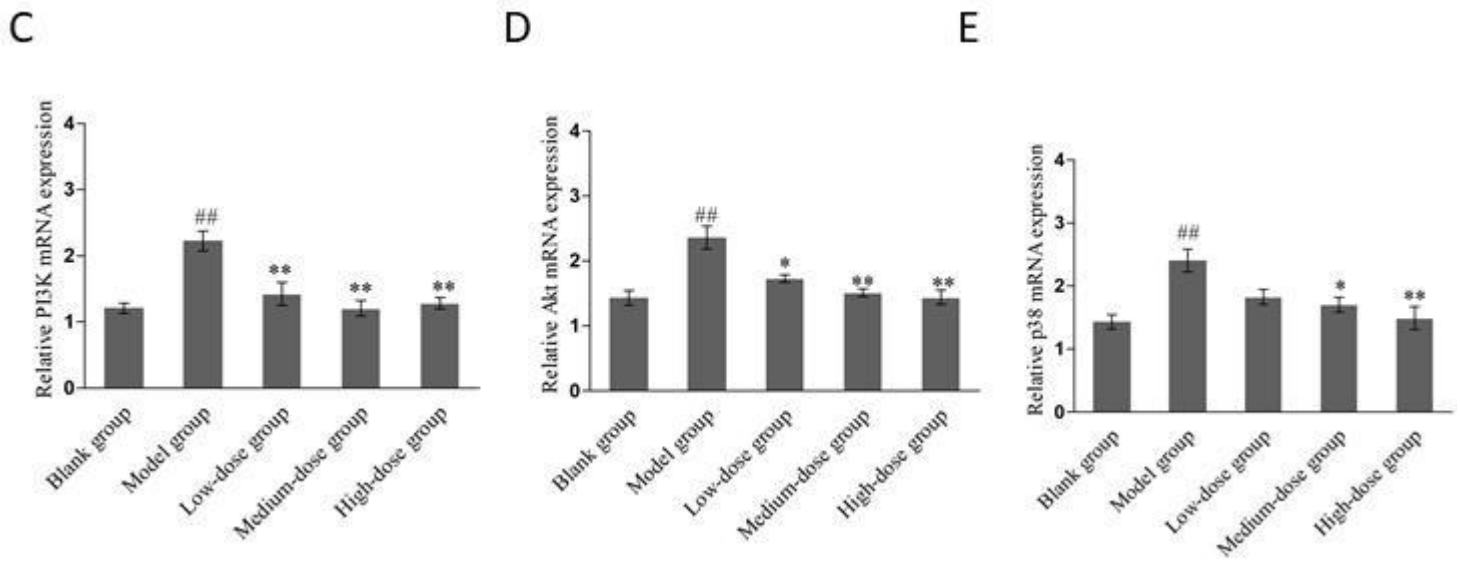
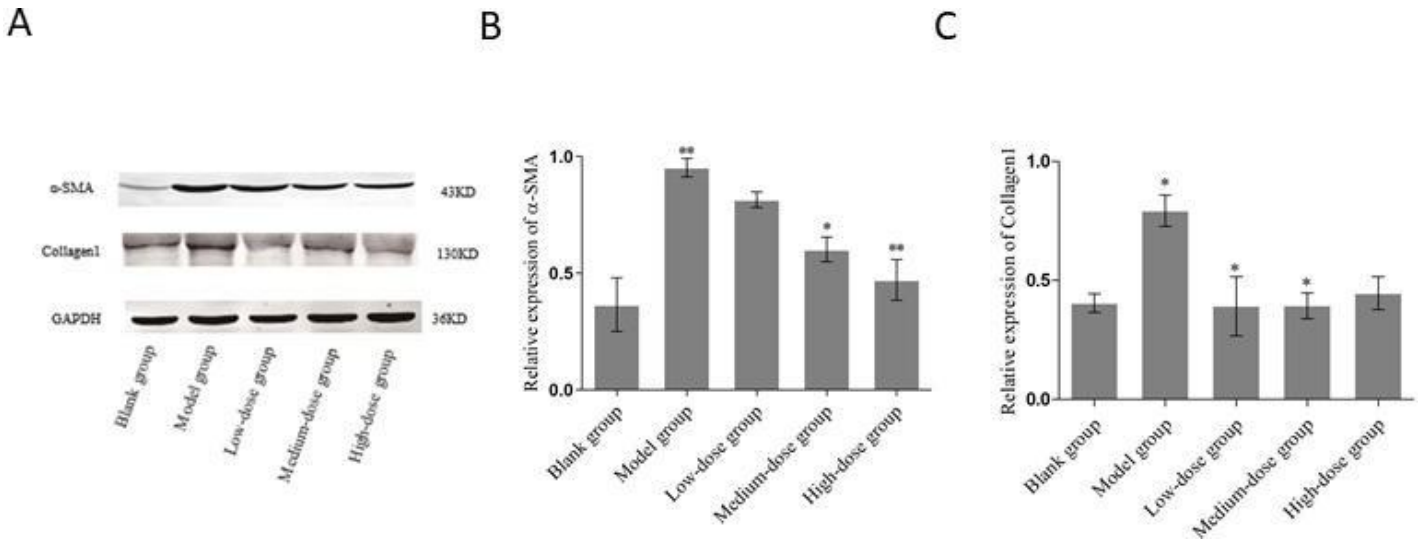


Figure 8 Effect of different doses of scabiosa on liver tissue α-SMA、CollagenI 、PI3K、Akt、p-Akt、p38、p-p38mRNA expression. A-E: Levels of α-SMA、CollagenI 、PI3K、Akt、p-Akt、p38、p-p38 were measured RT-QPCR and quantitatively analyzed (n=3/group).#P<0.05，##P<0.01 compare with Blank group；*P<0.05，**P<0.01 compare with Model group

2.7.4 Western blot detection α-SMA、CollagenI 、PI3K、Akt、p-Akt、p38、p-p38 protein expression

According to the results of KEGG pathway enrichment and screened core targets in Table 3, PI3K/Akt signaling pathway and MAPK signaling pathway which is closely related to hepatic fibrosis were screened. The core targets of PI3K, Akt, p-Akt, P38, and p-p38 were verified. At the same time, α-SMA and CollagenI were verified as significant markers of rat hepatic fibrosis. Compared with the blank group, the protein expressions of α-SMA ($P< 0.01$), CollagenI($P<0.05$), PI3K($P<0.01$), Akt($P<0.05$), p-Akt($P<0.01$), p38 ($P<0.05$) and p-p38 ($P<0.05$) in liver tissues of the model group were increased. Compared with the model group, the expression levels of α-SMA, CollagenI($P< 0.05$), PI3K($P< 0.05$), Akt, p-Akt, P38, and p-p38 were decreased in the low-dose group. The expression levels of α-SMA ($P<0.05$), CollagenI($P<0.05$), PI3K($P< 0.01$), Akt($P<0.05$), p-Akt, P38, p-p38 ($P< 0.01$) in the medium-dose group were significantly decreased, while in the high-dose group, The expression levels of α-SMA ($P<0.01$), CollagenI, PI3K($P< 0.001$), Akt, p-Akt($P< 0.05$), p38 ($P<0.01$) and p-p38 ($P< 0.001$) were significantly decreased. The results showed that Mongolian medicine scabiosa could reduce the expression of α-SMA, CollagenI, PI3K, Akt, p-Akt, P38, and p-p38, and had a good effect on anti-hepatic fibrosis (figure 9)



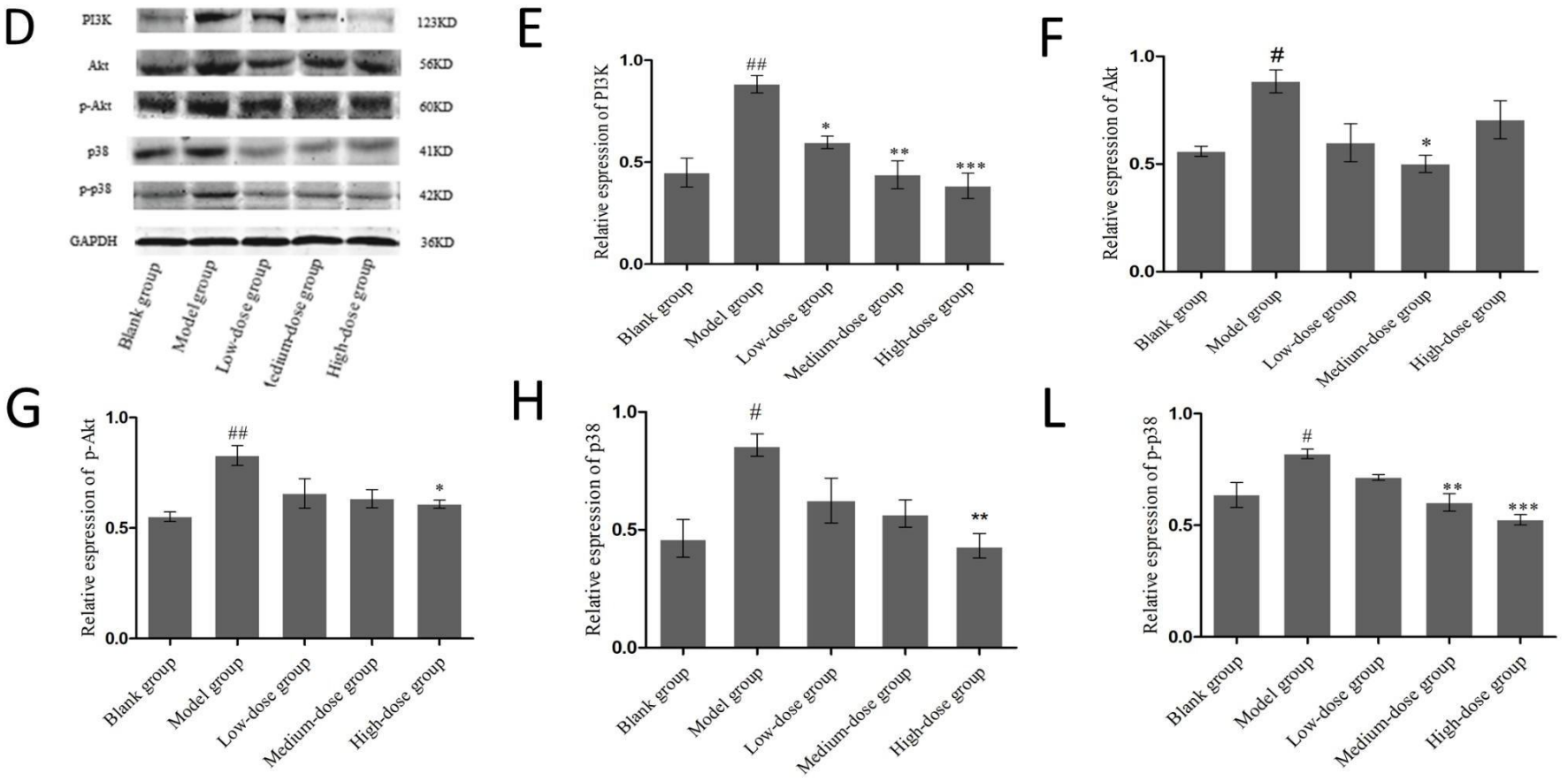


Figure 9 Effect of different doses of scabiosa on liver tissue α-SMA、CollagenI 、PI3K、Akt、p-Akt、p38、p-p38 expression. A-L: Levels of α-SMA、CollagenI 、PI3K、Akt、p-Akt、p38、p-p38 were

measured by western blotting and quantitatively analyzed (n=3/group). #P<0.05，##P<0.01 compare with Blank group；*P<0.05，**P<0.01，***P<0.001 compare with Model group

2.8 In vitro validation

2.8.1 MTT assay was used to detect the cytotoxicity of Mongolian medicine scabiosa to HSC-T6 cells.

The inhibition rates of HSC-T6 were 40.92%, 36.63%, and 73.44%, after 24 hours of treatment with low, medium, and high doses of Mongolian Medicine medicated serum; After 48 hours, the inhibition rates of HSC-T6 in serum-containing Mongolian Medicine were 32.93%, 34.90%, and 38.13%; After 72 hours, the inhibition rates of HSC-T6 in serum-containing Mongolian Medicine were 14.07%, 17.82%, and 23.03%. MTT results showed that the inhibition rate of the serum containing Mongolian medicine scabiosa on HSC-T6 was the highest at 24 hours(Figure10).

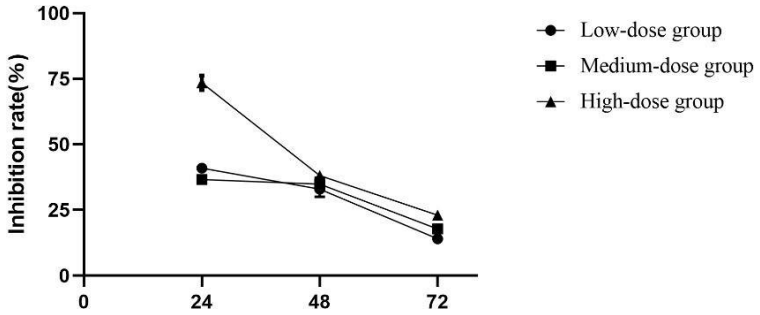
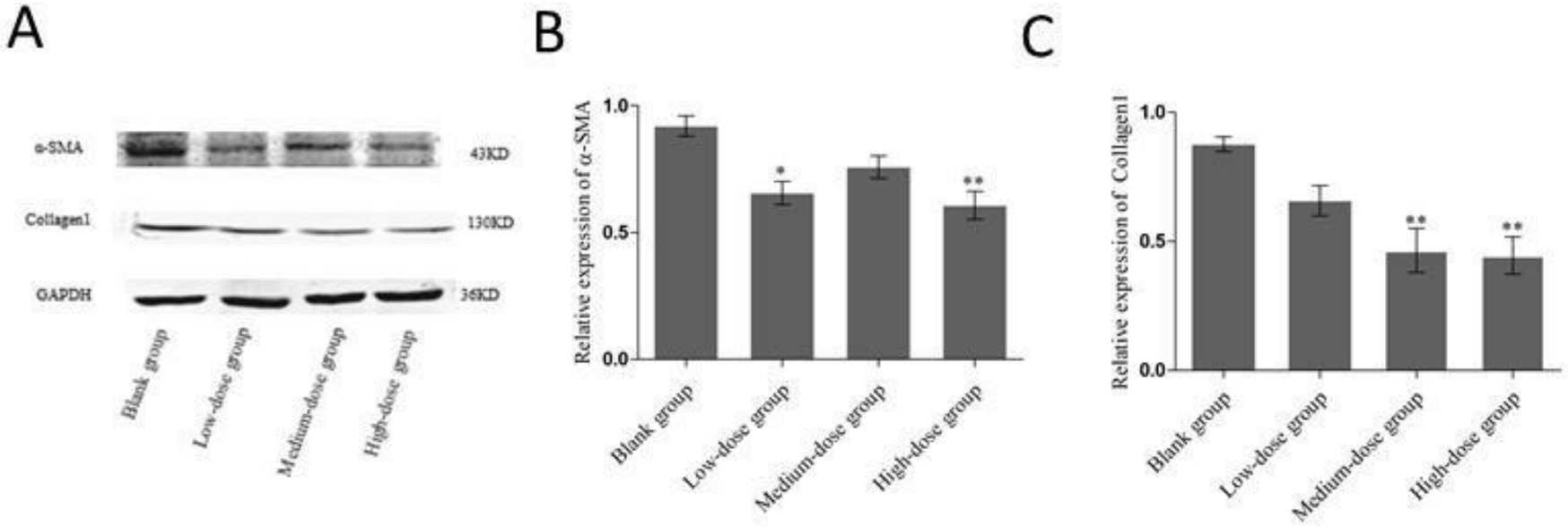


Figure 10 Inhibition rate of HSC-T6 cell proliferation

2.8.2 Protein expressions of α-SMA, CollagenI, PI3K, Akt, p-Akt, P38, and p-p38 in HSC-T6 were detected by Western blot.

Compared to the control group, The protein expression levels of α-SMA (P< 0.01), CollagenI(P<0.05), PI3K(P<0.01), Akt(P<0.05), p-Akt(P<0.01), p38 (P<0.05) and p-p38 (P<0.05) were increased in the drug-containing serum of Low dose Mongolian Medicine Scabiosa. Compared with the model group, the expression levels of α-SMA, CollagenI(P< 0.05), PI3K(P< 0.05), Akt, p-Akt, P38, and p-p38 were decreased in the low-dose group. The expression levels of α-SMA (P<0.05), CollagenI(P<0.05), PI3K(P< 0.01), Akt(P<0.05), pAkt, P38, p-p38 (P< 0.01) in the medium-dose group were significantly decreased, while in the high-dose group, T

he expression levels of α-SMA (P<0.01), CollagenI, PI3K(P< 0.001), Akt, pAkt(P< 0.05), p38 (P<0.01) and p-p38 (P< 0.001) were significantly decreased. The res ults showed that Mongolian medicine scabiosa could reduce the expression of α-SMA, CollagenI, PI3K, Akt, p-Akt, P38, and p-p38, and had a good effect on anti-liv er fibrosis (Figure 11).



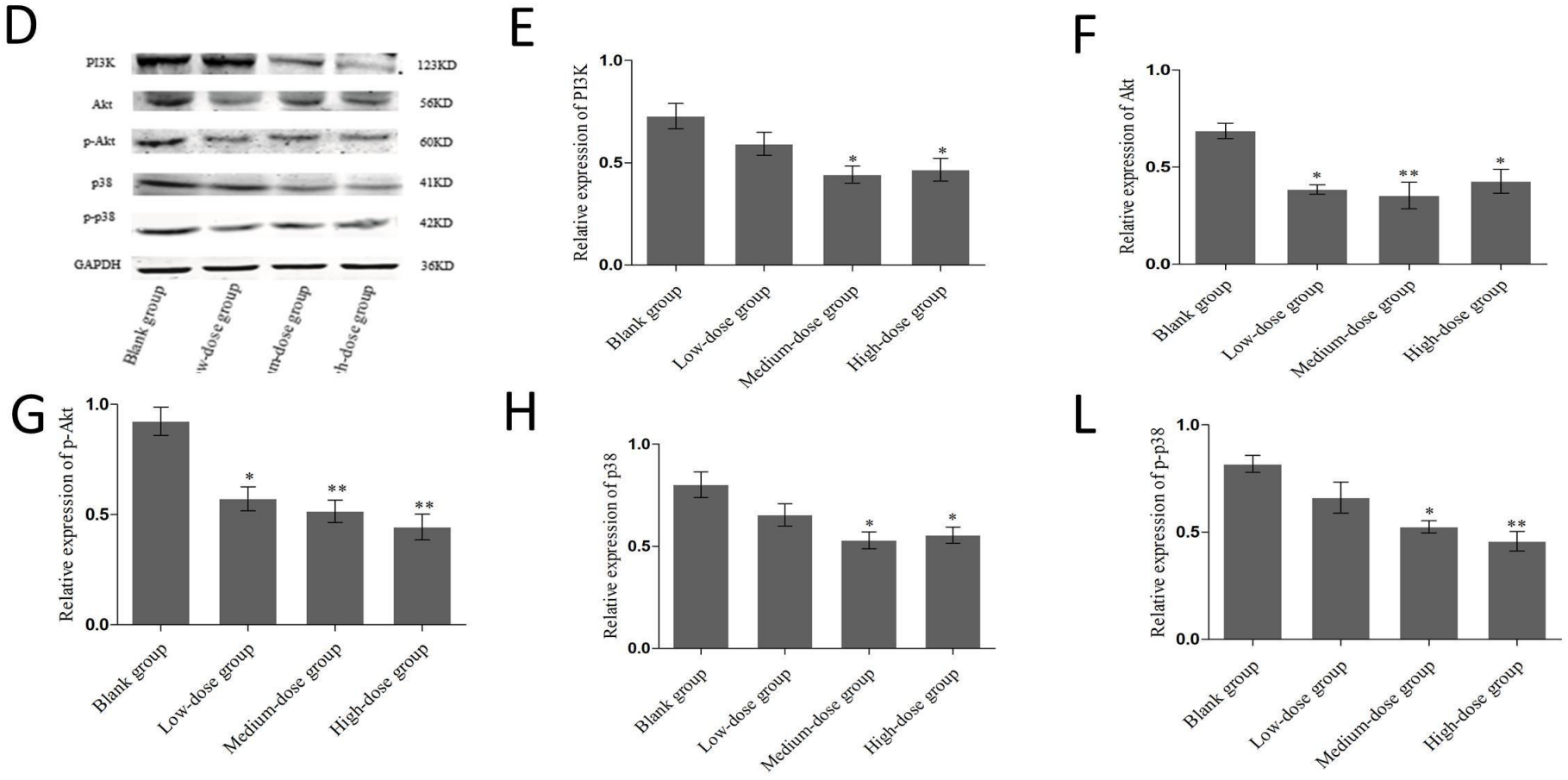


Figure 11 Effect of different doses of scabiosa in HSC-T6 cells α-SMA、CollagenI 、PI3K、Akt、p-Akt、p38、p-p38 expression. A-L: Levels of α-SMA、CollagenI 、PI3K、 Akt、p-Akt、p38、p-p38 were measured by western blotting and quantitatively analyzed (n=3/group).*P<0.05,**P<0.01 compared to the Blank group

2.8.3 Effects of Mongolian medicine scabiosa serum on apoptosis of HSC-T6

compared with the control group, the low-dose, medium-dose and high-dose groups (P<0.001) of Mongolian medicine scabiosa medicated serum significantly i ncreased the apoptosis rate of HSC-T6. The results showed that the higher the concentration of serum containing Mongolian medicine Scabiosa, the more obvious the anti-hepatic fibrosis effect (Figure 12)

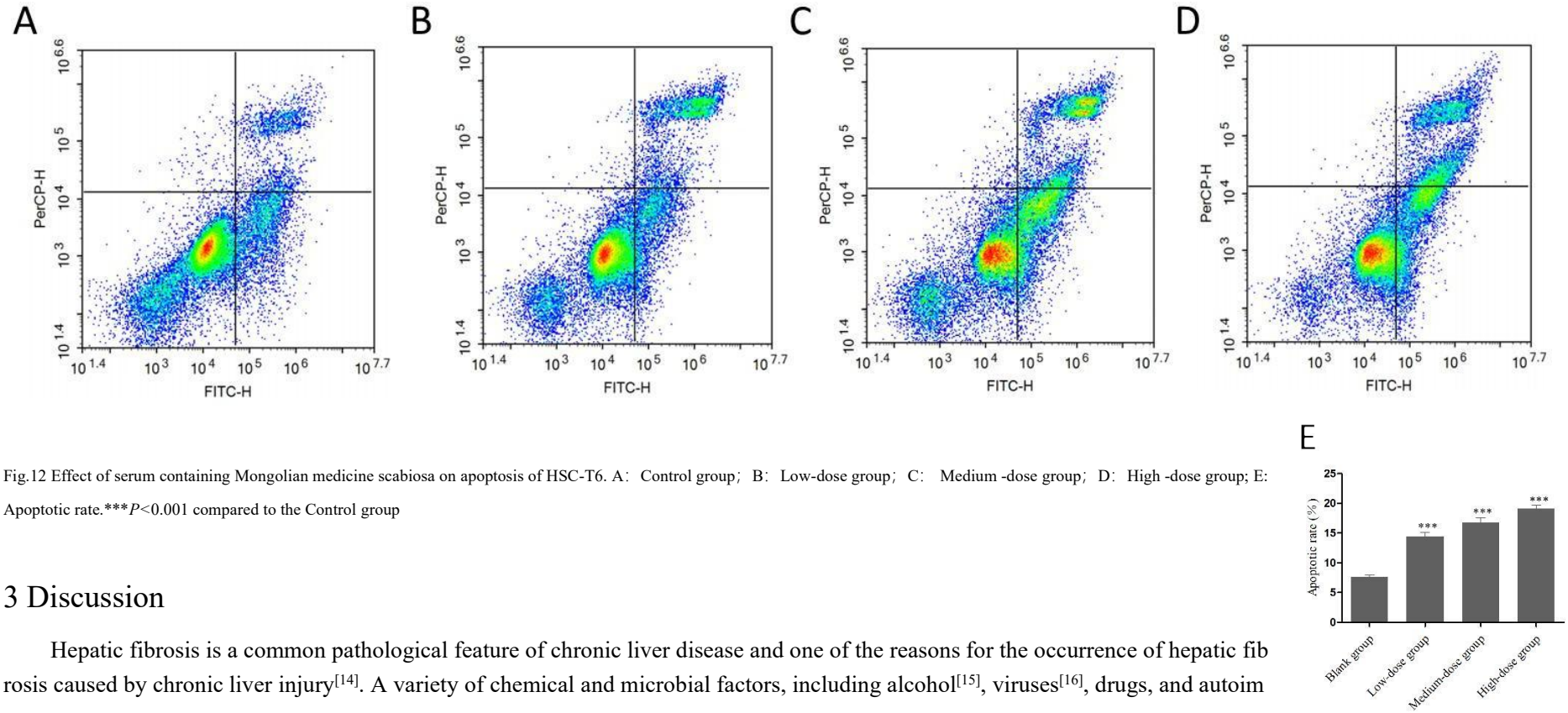


Fig.12 Effect of serum containing Mongolian medicine scabiosa on apoptosis of HSC-T6. A：Control group；B：Low-dose group；C： Medium -dose group；D：High -dose group; E: Apoptotic rate.***P<0.001 compared to the Control group

3 Discussion

Hepatic fibrosis is a common pathological feature of chronic liver disease and one of the reasons for the occurrence of hepatic fib rosis caused by chronic liver injury[14]. A variety of chemical and microbial factors, including alcohol[15], viruses[16], drugs, and autoim

mune processes[17], contribute to the occurrence of hepatic fibrosis, which is irreversible once it develops into cirrhosis[18, 19]. Seek solutions as early as possible befor e developing advanced cirrhosis or liver cancer. There were no drugs in the current study that directly alleviated or reversed hepatic fibrosis. Therefore, understanding the mechanisms behind this process is crucial for translating basic research into new clinical therapies.

In this paper, 120 constituents, including flavonoids, alkaloids, coumarins, terpenoids, phenols, and fatty acids, were identified from Mongolian medicine scabio sa by UHPLC-TOF-MS/MS technology. 63 active ingredients were selected to participate in the regulatory network, among which Quercetin, Ellagic acid, Apigenin, Epicatechin-3-o-gallate, Epigallocatechin-3-gallate, 9-trans-palmitelaidic acid, Oleic acid, Chrysin, 4-Hexylresorcinol, Cyanidin and so on have a significant effect o n anti-hepatic fibrosis. Studies have shown that Quercetin inhibits liver steatosis and fibrosis induced by cadmium chloride by down-regulating the transcription of mi R-21[20]. Apigenin alleviates hepatic fibrosis by inhibiting hepatic stellate cell activation and autophagy through TGF-β1/Smad3 and p38 /PPAR pathways[21]. Throug h network pharmacology analysis,20 core targets such as Akt1, PIK3R1, EGFR, PPARA, IGF1R, INSR, MAPK14, TNF, PPARG, and BRAF of Mongolian medicine scabiosa against hepatic fibrosis were obtained. According to GO function analysis, BP mainly involves positive regulation of kinase activity, response to oxidative s tress, positive regulation of cell migration, positive regulation of lipid metabolic process, positive regulation of smooth muscle cell proliferation, and ERBB signaling pathway; CC mainly involves vesicle lumen, perinuclear region of cytoplasm, extracellular matrix, glutamatergic synapse and so on; MF mainly involves protein kin ase activity, nuclear receptor activity, protein serine/threonine kinase activity, G protein-coupled chemoattractant receptor activity, ATPase-coupled transmembrane tr ansporter activity. According to the enrichment analysis results, PI3K/Akt, EGFR, Rap1, HIF-1, Ras, MAPK signal pathways are involved in the occurrence and deve lopment of hepatic fibrosis. PI3K/Akt signaling pathway is an important apoptosis-inhibiting signal transduction pathway [22]and plays an important role in the growt h and metabolism of gastric cancer[23], cervical cancer[24], liver cancer[25], and other tumors. After PI3K is activated, the downstream Akt protein can be activated, and Akt regulates the downstream apoptotic genes, so as to inactivate the apoptotic genes and achieve the purpose of inhibiting apoptosis[26, 27]. PI3K/Akt/mTOR signal ca scade also plays an important role in regulating cell proliferation, survival, metabolism, protein synthesis and cell cycle[26]. Dioscin alleviates BDL-and DMN-induce d hepatic fibrosis via Sirt1/Nrf2-mediated inhibition of p38 MAPK pathway[28]. Combination Figure (3) Drug-active component-target-pathway analysis, There are 1 5 key active ingredients (Quercetin, Ellagic) involved in PI3K/Akt pathway Acid, Epicatechin-3-o-gallate, epigallocatechin-3-gallate, Naringenin, Chalcone, Tectorig enin, Apigenin, Luteolin-7-o-glucoside Cosmosiin, Biochanin-7-O-glucoside, Formononetin-7-O-glucoside, and Asterric acid, L-(+) -arginine, Amentoflavone) and 1 8 key target proteins (Akt1, BCL2, CDK2, EGFR, ERBB2, GSK3B, IGF1R, IL2, INSR, KDR, MCL1, MET, NOS3, PDG,FRB, PIK3CG, PIK3R1, PTK2, VEGFA); 9 key active ingredients (Quercetin, Ellagic acid, Epicatechin-3-o-gallate, Naringenin, Chalcone, Tectorigenin, Cosmosiin, Apigenin-7-o-glucoside, Luteolin-7-O-glu coside) and 6 key target proteins (Akt1, BRAF, MAPK14, EGFR, PDGFRB, and TNF) The target proteins in these two pathways are mainly involved in substance m etabolism, cell decay, and inflammation. In vivo experiments, Mongolian medicine Mongolian medicine scabiosa can significantly reduce serum biochemical and hy droxyproline indicators; Masson staining results showed that Mongolian medicine scabiosa could reduce hepatocyte degeneration, hepatocyte necrosis, reduce the for

mation of false lobules, and improve the fibrosis of liver tissue. Mongolian medicine Mongolian medicine scabiosa can reduce the mRNA expression levels of α-SM A, Collagen1, PI3K, Akt, and p38 in liver tissue. Mongolian medicine Mongolian medicine scabiosa can reduce the protein expression levels of α-SMA, Collagen1, P I3K, Akt, p-Akt, p38, and p-p38 in liver tissue. In the in vitro experiment, the inhibition rate of serum containing Mongolian medicine Mongolian medicine scabiosa on HSC-T6 was the highest at 24 hours. Mongolian medicine scabiosa can significantly reduce the protein expression levels of α-SMA, Collagen1, PI3K, Akt, p-Akt, P38, p-p38. Flow cytometry showed that the serum containing Mongolian Scabiosa could promote the increase of apoptosis rate of HSC-T6. The higher the concentr ation in the specified range, the higher the apoptosis rate of HSC-T6, the more obvious the anti-liver fibrosis effect. It is confirmed that Mongolian medicine scabiosa plays an anti-liver fibrosis role by acting on key targets in key pathways.

In this study, UHPLC-TOF-MS/MS combined with network pharmacology and experimental verification methods were used to study the mechanism of anti-hep atic fibrosis action of Mongolian medicine Scabiosa. It has been confirmed that Mongolian medicine scabiosa plays a synergistic role in anti-hepatic fibrosis by regul ating important targets.

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ETHICS STATEMENT

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Inner Mongolia Medical University(YKD2015153). We selected experimental animals with anatomical and physiological characteristics suitable for experimental purposes. In the experiment, standardized animals were selected according to the research objectives.