



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

# Panax Quinquefolii Radix glucan ameliorates liver fibrosis by modulating macrophage polarization and suppressing inflammation via the TLR4/MyD88/NF- $\kappa$ B signaling pathway

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**ABSTRACT:** Liver fibrosis is a critical pathological stage in chronic liver disease progression, with effective clinical therapeutics remaining limited. Herein, a homogeneous glucan (XYS1-1, 12.59 kDa) was isolated and purified from *Panax quinquefolius* Radix roots—a widely used medicinal and functional food ingredient. Structural characterization via monosaccharide composition, IR, methylation analysis, and 2D-NMR confirmed YYS1-1 has a  $\rightarrow 4$ - $\alpha$ -D-Glcp-(1 $\rightarrow$  backbone with terminal  $\alpha$ -D-Glcp branches at C-6 positions, distinguishing it from commercial starches as a potential functional hydrocolloid. *In vitro* (TGF- $\beta$ 1-induced LX-2 cells) and *in vivo* (CCl<sub>4</sub>-induced fibrotic mice) assays demonstrated that YYS1-1 alleviated liver fibrosis in a concentration-dependent manner-outperforming the clinical reference Fuzheng Huayu Capsule in reducing  $\alpha$ -SMA/collagen expression, serum ALT/AST levels, and hepatic pathological injury. Mechanistically, YYS1-1 exerted antifibrotic effects by suppressing macrophage inflammatory responses, regulating M1/M2 polarization, and inhibiting the TLR4/MyD88/NF- $\kappa$ B pathway. These findings highlight YYS1-1, as a promising candidate for functional foods targeting liver health, while expanding insights into natural polysaccharide-based strategies against liver fibrosis.

**Keywords:** *Panax quinquefolius*; Glucan; Liver fibrosis; TLR4/NF- $\kappa$ B pathway; Macrophage polarization

## 1. Introduction

Liver fibrosis (LF) is a pivotal pathological intermediate in chronic liver disease progression, characterized by excessive extracellular matrix (ECM) deposition (predominantly collagen) driven by persistent hepatic injury and dysregulated wound healing [1,2]. Globally, approximately 25% of the population is affected by fibrosis-related diseases, and fibrosis contributes to nearly 45% of all deaths in industrialized nations—underscoring its severe public health burden [3,4]. Advanced LF is irreversible, progressing to cirrhosis or hepatocellular carcinoma (HCC) and drastically reducing treatment success and patient survival, highlighting an urgent need for effective early interventions [5-7].

Hepatic stellate cells (HSCs) are the primary effector cells in LF: quiescent HSCs remain fibrosis-inert,

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Received 15 October 2025

Received in revised form 19 November 2025

Accepted 1 April 2026

but hepatic injury triggers their activation into myofibroblast-like cells (MFBs) that secrete excessive ECM [8, 9]. Chronic inflammation has been established as a key pathogenic driver in LF [10], with mechanistic studies revealing that immune cell-derived pro-inflammatory induce HSCs activation and promote the progression of fibrosis [11–13].

Among immune regulators, macrophages-critical components of the hepatic microenvironment-undergo context-dependent polarization into pro-inflammatory M1 or anti-inflammatory M2 subsets, a process governed by pathways including TLR4/NF- $\kappa$ B, JAK/STATs, and TGF- $\beta$ /Smads [14–17]. M1 macrophage polarization amplifies inflammation, further activating HSCs and perpetuating ECM accumulation, while dysregulated macrophage plasticity is implicated in LF pathogenesis [18, 19]. Targeting macrophage polarization thus emerges as a promising strategy to halt or reverse LF.

Polysaccharides from Traditional Chinese Medicine herb, with diverse structures, low toxicity, and excellent biocompatibility, exhibit robust hepatoprotective potential [20–22]. For example, a polysaccharide, APFC-2, isolated from *Cornus officinalis*, mitigates lipid accumulation in alcoholic fatty liver disease [23]. While a homogeneous polysaccharide (YJ3A1) from *Rosa chinensis* exerts antioxidant, anti-inflammatory, and anti-fibrotic effects in non-alcoholic steatohepatitis (NASH) [24]. Moreover, due to pathological transmission via the liver-gut axis, a close pathological link exists between colitis and hepatic injury [25, 26]. Polysaccharides isolated from *Dictyophora indusiata* (SE-DIP) exert anti-inflammatory activity by blocking the activation of gut-derived inflammatory signaling pathways [27]. Their mechanism of action aligns with the regulatory requirements of the liver-gut axis, demonstrating potential therapeutic value for treating hepatic injury. Polysaccharide CP isolated from *Cistanche deserticola* alleviates alcohol-induced liver injury by regulating the gut-liver axis homeostasis [28].

*Panax quinquefolius Radix* as a traditional herbal medicine, is widely used in the health supplement and biopharmaceutical industries due to its significant pharmacological activities, including antitumor, cardiovascular protection, antioxidant, and anti-inflammatory effects [29, 30]. However, research on the active compounds of ginseng has primarily focused on saponins [31, 32], a type of nanoparticle, AGVNs, formulated from *Panax quinquefolius Radix* saponins exerts anti-inflammatory effects by reducing macrophage migration in zebrafish and modulating the secretion of inflammatory mediators in RAW 264.7 cells [33], Zhang et al. discovered that three ginsenosides unique to *Panax quinquefolius Radix* American ginseng can inhibit lung adenocarcinoma by targeting Hsp90 $\alpha$  [34], the rare ginsenosides in *Panax quinquefolius Radix* can modulate oxidative stress pathways, hereby alleviating oxidative damage to hepatocytes [35], with relatively little attention given to ginseng polysaccharides.

Herein, we isolated and purified a novel glycan (XYS1-1) from *Panax quinquefolius Radix*, characterized its fine structure, evaluated its anti-LF effects revealed its underlying mechanisms. This study provides the first experimental evidence for the therapeutic potential of *Panax quinquefolius Radix* polysaccharides in LF, while proposing macrophage polarization regulation as a viable LF treatment strategy.

## 2. Materials and methods

## 2.1 Chemicals and reagents

*Panax quinquefolius Radix* was sourced from Wendeng, Shandong, China and was identified by Prof. Jianfeng Xu (Department of Marine Pharmacology, College of Food Science and Technology, Shanghai Ocean University, Shanghai, China). DEAE Sepharose Fast Flow and Sephadex S-100 High Resolution were obtained from Cytiva Biotechnology Co., Ltd. 1-Phenyl-3-methyl-5-pyrazolone (PMP) was purchased from Shanghai Yuanye Biotechnology Co., Ltd. The BCA assay kit, RIPA cell lysis buffer, neutral red staining solution, and NO assay kit were supplied by Biyun Tian Biotechnology. Monosaccharide standards, including L-rhamnose, L-fucose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose, and galacturonic acid, were procured from Fluka Corporation (USA). Fetal bovine serum (FBS) and DMEM high-glucose culture medium were obtained from Gibco Corporation (USA). Phosphate buffered saline (PBS), Cell Counting Kit-8 (CKK-8), and trypsin digestion solution (containing EDTA) were purchased from Adamas. The Evo M-MLV reverse transcription kit, SYBR Green Pro Taq HS premix, and qPCR kit were sourced from Acori (Hunan, China). Primary antibodies included  $\alpha$ -SMA (#P62736n) from CST Corporation (USA) and Fibronectin (Ag8004) from Proteintech. The mouse interleukin (IL-6) and tumor necrosis factor (TNF- $\alpha$ ) assay kits were obtained from Jingmei Biotechnology (Jiangsu, China). The SPAREasy Cell RNA Kit, used for rapid RNA extraction, was supplied by Sikejie (Shandong, China). Fuzheng huayu Jiaonang (FZHY), Shanghai Huanghai Pharmaceutical Co., Ltd. Other analytical-grade reagents were purchased from Sinopharm Chemical Reagent Co. Ltd.

## 2.2 Preparation and purification of XYS1-1

Crude polysaccharides were extracted from *Panax quinquefolius Radix* using a complex enzyme extraction method. After defatting the *Panax quinquefolius Radix* powder with anhydrous ethanol, crude polysaccharides were extracted using a composite enzyme method, which involved sequential addition of papain and trypsin. Following extraction, the supernatant solution was concentrated and dialyzed (3500 Da) for three days. The resulting crude polysaccharides were then freeze-dried and designated as XYS. The XYS sample was fractionated using a DEAE Sepharose Fast Flow column with a linear NaCl gradient elution protocol. This process yielded three distinct fractions: XYS0 (deionized water fraction), XYS1 (0.1M NaCl fraction), and XYS2 (0.2M NaCl fraction). The main component, XYS1, was further purified using Sephadex S-100 High Resolution gel column, resulting in a refined polysaccharide fraction designated as XYS1-1.

## 2.3 Chemical Characterization of XYS1-1

### 2.3.1 Physicochemical properties

Total sugar content was determined using glycose as standard, while galacturonic acid was employed for uronic acid quantification. Protein levels in the polysaccharides were measured with a BCA assay kit. The presence of a triple-helix conformation in XYS1-1 was evaluated using the Congo Red assay.

### 2.3.2 Homogeneity and molecular weight analysis

The purity and molecular weight of XYS1-1 were determined by the HPGPC method [36]. The

chromatographic columns were TOSOH TSK PWXL G4000 and TSK PWXL G2500 in tandem, injecting at a flow rate of 0.5 mL/min with a flowability of 0.15mol/L NaNO<sub>3</sub>. Data were processed using Empower 3 (Waters) software.

### 2.3.3 Determination of monosaccharide composition

The polysaccharides' monosaccharide profile was determined using high-performance liquid chromatography (HPLC) with PMP pre-column derivatization [37]. 20 µL aliquots from the aqueous phase were passed through a 0.22 µm filter membrane prior to HPLC analysis. Monosaccharide standard mixtures were well prepared with concentrations of 100 µg/mL.

### 2.3.4 Linkage pattern analysis

The methylation method was illuminated according to the minor modified method [38]. The completely methylated production was prepared into partially methylated alditol acetates (PMAA) by hydrolyzation, reduction and acetyliztion for gas chromatography-mass spectrometry (GC-MS) analysis.

### 2.3.5 Analysis of FT-IR spectrum

Fourier-transform infrared (FT-IR) spectra were recorded over the wavenumber range of 400-4000 cm<sup>-1</sup> to characterize the functional groups present in the polysaccharide sample.

### 2.3.6 NMR analysis

For NMR analysis, XYS1-1 was dehydrated, exchanged and dissolved in 0.5 mL D<sub>2</sub>O under vacuum. Using acetone as the internal standard, NMR spectra were recorded using a Varian Mercury 500 NMR spectrometer [39].

## 2.4 Cell culture and treatment

### 2.4.1 LX-2 cells

LX-2 cells (Beijing Beina Chuanglian Biotechnology) were cultured in DMEM (10% FBS, penicillin/streptomycin) at 37 °C, 5% CO<sub>2</sub>. For the *in vitro* LF model, cells were cultured for 24 h, then starved in serum-free medium for another 24 h. Post-starvation, 10 ng/mL TGF-β1 was added to induce activation (blank control excluded).

### 2.4.2 RAW264.7 macrophages

RAW264.7 cells (Servicebio) were cultured in DMEM supplemented with 10% fetal bovine serum. Cells were stimulated with 1 µg/mL lipopolysaccharide (LPS) or 10 ng/mL interleukin-4 (IL-4) to establish the model.

### 2.5 Cell counting kit-8 (CCK8) determination

Cell viability was evaluated via Cell Counting Kit-8 (CCK-8). After 48 h of treatment with different polysaccharide concentrations, CCK-8 reagent was added and incubated in the dark for 2 h. Absorbance was measured at 450 nm.

### 2.6 Cell Migration Assay of LX-2

LX-2 cells were uniformly seeded in 6-well plates (3 parallel reference lines marked on plate undersides). At 80% confluence, a scratch was made with a 200  $\mu$ L pipette tip. Cells were washed with PBS to remove debris, and the initial wound area was recorded (0 h). Media with different polysaccharide concentrations were added, and after 24 h incubation, images were captured to evaluate cell migration.

### 2.7 Flow Cytometric Detection of RAW264.7

To assess YYS1-1's impact on macrophage phenotype, cells were seeded in 6-well plates, stimulated (as in 2.4.2), and treated with YYS1-1 (different concentrations). Fluorescently labeled antibodies were added (30-minute incubation); post-resuspension, cells were analyzed via flow cytometry.

### 2.8 Real-time quantitative PCR

RNA was quickly extracted using the SPAREasy Cell RNA Kit (SparkJade) according to the manufacturer's protocol. A reverse transcription program was then set up for reverse transcription. The primers used are shown in Table 1.

**Table 1** Primers used for RT-PCR analysis

|               | forward primer         | reverse primer         |
|---------------|------------------------|------------------------|
| Fibronectin   | CCATCGCAAACCGCTGCCAT   | AACACTTCTCAGCTATGGGCTT |
| $\alpha$ -SMA | CATGAAGTGTGACATCGACATC | TGATCTTGATCTTCATGCTGCT |
| COL1A1        | CCTGGATGCCATCAAAGTCT   | CGCCATACTCGAACTGGAAT   |
| GAPDH         | ACTTTGGTATCGTGGAAGGACT | GTAGAGGCAGGGATGATGTTCT |

### 2.9 Western blot analysis

Cellular proteins were extracted and subjected to gel electrophoresis, then transferred to PVDF membranes. Membranes were blocked with 5% BSA, incubated with specific primary antibodies, followed by corresponding secondary antibodies (room temperature, shaking) to form complexes. ECL reagent was added for fluorescence signal generation, which was recorded via darkroom exposure or chemiluminescence imaging. Band grayscale values were quantified using ImageJ.

### 2.10 Animal experiment

The study utilized 8-week-old male C57BL/6J mice (SPF grade, 18–20 g), procured from Sibeifu (Suzhou) Biotechnology Co. Ltd. (license number: SCXK (Suzhou) 2022-0006). All animal procedures were approved by the Shanghai Marine Medical Ethics Committee (ethical batch number: shou-DW-2022-008), and humane care was provided throughout the study. As shown in Fig. 5a, 60 male C57BL/6J mice (1-week acclimation) were randomized into 6 groups (n=10/group): Control, Model, YYS1-1-L (low-dose), YYS1-1-M (medium-dose), YYS1-1-H (high-dose), and FZHY (positive control). Except Control, all groups received intraperitoneal 10% CCl<sub>4</sub> (3 $\times$ /week, 8 weeks); Control received saline. From week 3 of CCl<sub>4</sub> administration, treatment groups received corresponding interventions (6 weeks); Control/Model received saline. Body weight was monitored; in week 9, mice were fasted for 12 h (water available) before sampling.

### 2.11 Enzyme-linked immunosorbent assay (ELISA)

Using the ELISA kit according to its operating instructions to detect the secretion level of inflammatory

factors.

### 2.12 Serum biochemistry and hepatic TG assays

Collect blood samples and centrifuge at 4 °C and 3500 rpm for 10 min. Collect the supernatant and follow the kit instructions to determine ALT and AST levels in the serum using Lai's microplate method, analyzing liver function. Take serum samples and measure triglyceride (TG) content directly using the reagent kit method. Calculate triglyceride content (mmol/L) using the formula: Triglyceride content (mmol/L) = (ASample well-Ablank well)/ (AStandard well-Ablank well) × Cstandard Well.

### 2.13 Histological analysis

Excise mouse liver specimens with approximate dimensions of 1 cm × 1 cm × 1.5 cm. Process the specimens through routine dehydration, paraffin embedding, and microtomy to obtain 5 µm-thick serial sections for subsequent HE and Masson staining.

### 2.14 Immunohistochemical (IHC) staining

Dewaxed tissue sections were treated with 3% H<sub>2</sub>O<sub>2</sub> to block endogenous peroxidase, then washed with PBS. Non-specific binding was blocked with goat serum, followed by overnight incubation with primary antibody at 4 °C (humidified box). Secondary antibody was added and incubated as required. Antigens were visualized via DAB staining, with 3-min hematoxylin counterstaining. Sections were dehydrated, mounted, imaged with a digital panoramic scanner, and data processed using NDP View 2.9.22.

### 2.15 Immunofluorescence staining

RAW264.7 cells were seeded onto cell slides in a 12-well plate. After polysaccharide treatment, cells were fixed with paraformaldehyde, permeabilized with 1% Triton X-100, and blocked with 5% BSA at room temperature. Following primary and secondary antibody incubations, the cells were stained with DAPI for 10 minutes to visualize the nuclei. The slides were then sealed with an anti-fade fluorescent mounting medium. Finally, images were captured under a fluorescence microscope for analysis.

### 2.16 Construction of sugar chain structure fragments and molecular docking

The sugar chain structure fragment of YYS1-1 was constructed using Chem3D and subjected to energy minimization to obtain its lowest-energy conformation. The 3D structure of the TLR4 protein (PDB ID: 2Z62) was downloaded from the Protein Data Bank (PDB). Molecular docking was performed using AutoDock, with the Grid Box set to fully encompass the entire TLR4 protein. All other parameters were maintained at default settings. The docking conformation with the lowest binding energy was selected as the final result, representing the most stable interaction between YYS1-1 and TLR4.

### 2.17 16S rRNA sequencing

Genomic DNA was extracted from samples using a kit. Specific primers targeting the 16S rRNA V3-V4 region (with 5'-end barcodes for sample differentiation) were used for PCR. Low cycles (optimized via pre-experiments to reduce bias and ensure sufficient amplification) were run with TransStart FastPfu DNA Polymerase (TransGen AP221-02) on an ABI GeneAmp® 9700. Purified PCR products were quantified via

QuantiFluor™-ST (Promega) and equimolarly pooled for balanced sequencing.

### 2.18 Statistical analysis

Data analysis was conducted using Excel, GraphPad Prism 9.5, OriginPro 8.5.1, ImageJ, and Adobe Illustrator 2023. The results were expressed as mean  $\pm$  standard error (Mean  $\pm$  SEM), with all experiments performed in triplicate. Statistical significance was assessed using appropriate methods, with significance levels represented as follows: no significant difference is shown as ns, \*  $P < 0.05$  indicates significant differences \*\*  $P < 0.01$  indicates a significant difference \*\*\*  $P < 0.001$  indicates a significant difference \*\*\*\*  $P < 0.0001$  indicates a very significant difference.

## 3 Results

### 3.1 Preparation and structural characterization of XYS1-1

The polysaccharide extraction procedure is illustrated in Fig. 1a. After ethanol defatting, defatted powder was obtained with a yield of 71.3%. The crude polysaccharide designated as XYS was obtained with a yield of 9.2%. As shown in Fig. 1b, XYS was purified using a DEAE column, yielding three major polysaccharide fractions: XYS0 (deionized water fraction), XYS1 (0.1 mol/L NaCl fraction), and XYS2 (0.2 mol/L NaCl fraction), with yields of 4%, 48.5%, and 3.75%, respectively. The main fraction XYS1 was further purified due to its higher yield (Fig. 1c). The purity and homogeneity of XYS1-1 was confirmed by HPGPC, revealing a relative molecular weight of 12.59 kDa (Fig. 2a). The total carbohydrate content of XYS1-1 was determined to be 99.06%. Protein content results showed negligible protein presence. The monosaccharide composition analysis of XYS1-1 (Fig. 2b) revealed that XYS1-1 was mainly composed of glucose (95.12%).

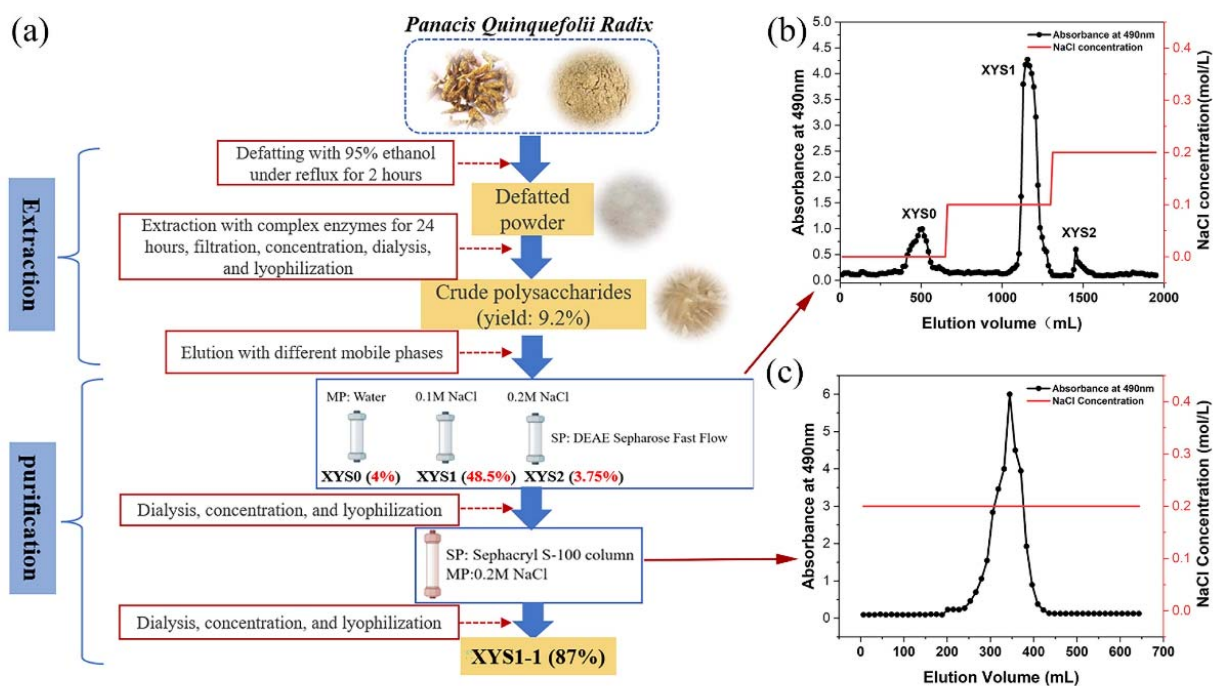


**Fig. 1** Extraction, isolation, and purification of XYS1-1. (a) Flowchart of the purification process of XYS1-1. (b) Elution curve of DEAE Cellulose column. (c) Elution curve of Sephadex S-100 gel column.

The FT-IR spectrum of YYS1-1 (Fig. 2c) exhibited characteristic polysaccharide absorption bands at 3325, 2935, 1647, and 1017  $\text{cm}^{-1}$  [40,41]. The peak at 934  $\text{cm}^{-1}$  may result from the angular deformation (bending) vibration of the C-H bond in the  $\alpha$ -D-glucan  $\alpha$ -pyranose isomer [42]. Generally, absorption peaks in the 800–950  $\text{cm}^{-1}$  range are commonly used to determine the configuration of glycosidic bonds. The absorption peak near 850  $\text{cm}^{-1}$  is often associated with  $\alpha$ -glycosidic linkages, indicating the possible presence of monosaccharide units connected by  $\alpha$ -glycosidic bonds in the polysaccharide structure [43].

If a polysaccharide possesses a triple helix structure, it will undergo a complexation reaction when mixed with Congo Red reagent, causing the maximum absorption wavelength of the mixed solution to shift towards longer wavelengths, i.e., a red shift [44]. As shown in Fig. 2d, after mixing YYS1-1 with Congo Red solution, there was no significant red shift in the maximum absorption wavelength. Moreover, as the concentration of sodium hydroxide solution increased, the trend of changes in the maximum absorption wavelength of the mixed solution was similar to that of the Congo Red solution alone. Therefore, it can be concluded that YYS1-1 does not contain a triple helix structure.

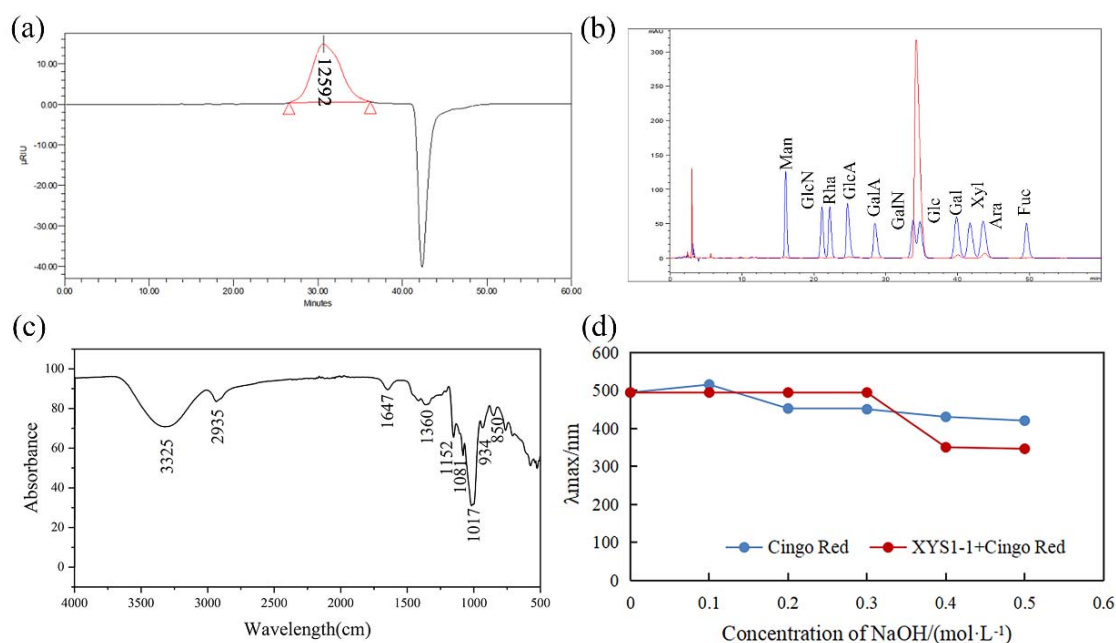


**Fig. 2** Characterization of YYS1-1. (a) HPGPC results of YYS1-1. (b) Monosaccharide composition results of YYS1-1. (c) IR spectrum of YYS1-1. (d) Congo Red binding results.

To further elucidate the glycosidic linkage types in YYS1-1, methylation analysis was performed using GC-MS, with the results presented in Fig. S1 and Table 2. The total ion chromatogram revealed five major peaks which belong to different linkage types. Based on the monosaccharide composition results and CCRC mass spectral database comparisons, these signals are respectively attributed to T-Glcp (6.87%), 1,4-Glcp (60.02%), 1,6-Glcp (11.33%), 1,3,4-Glcp (3.31%), and 1,4,6-Glcp (18.47%). Major branching points were identified at 1,3,4-linked Glcp (3.31%), and 1,4,6-linked Galp (18.47%). The ratio of three main linkage types of 1,4-Glcp, 1,6-Glcp, and 1,4,6-Glcp was 6: 1: 2, suggesting that one putative structure presence of YYS1-1 wherein the backbone consists of six  $\alpha$ -1,4-linked glycoses and one  $\alpha$ -1,6-linked glucose. Every third  $\alpha$ -1,4-linked glycoses have a branch at the C-6 position, with a terminal glucose substituent.

**Table 2** Methylation analysis of YYS1-1

| Retention time (min) | Compound Name | Major mass fragments (m/z)               | Area (%) |
|----------------------|---------------|------------------------------------------|----------|
| 17.674               | T-Glcp        | 71,87,102,118,129,145,162,205            | 6.87     |
| 18.806               | 1,4-Glcp      | 71,87,99,118,129,142,173,187             | 60.02    |
| 18.961               | 1,6-Glcp      | 71,87,99,102,118,129,143,162,173,189,233 | 11.33    |
| 19.414               | 1,3,4-Glcp    | 87,118,129,185,231,305                   | 3.31     |
| 19.848               | 1,4,6-Glcp    | 87,102,118,127,142,159,187,201,261       | 18.47    |

Combining the monosaccharide composition, methylation analysis, and relevant references [45, 46], the glucoside linkage types of YYS1-1 were further conducted using 1D-NMR and 2D-NMR. The main glycosidic linkages of YYS1-1 are identified as A-D in Table 3. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of YYS1-1 are shown in Fig. 3a and Fig. 3b. The  $^1\text{H}$  and  $^{13}\text{C}$  signals in Table 3 are identified through  $^1\text{H}$ - $^1\text{H}$ -COSY (Fig. 3c), HSQC (Fig. 3d), and HMBC (Fig. 3e) spectra. Four major anomeric carbon signals are present in the  $^{13}\text{C}$ -NMR spectrum, which are identified as residue A (97.56 ppm), B (99.60 ppm), C (98.04 ppm), and D (99.88 ppm). They are determined by the chemical shift signals and were assigned as C1 of T- $\alpha$ -Glcp, 1,4-linked- $\alpha$ -D-Glcp, 1,6-linked- $\alpha$ -D-Glcp, and 1,4,6-linked- $\alpha$ -D-Glcp, respectively. Combined with the information of HSQC spectrum in Fig. 3d, the signals of the anomeric proton signals can be attributed in the  $^1\text{H}$ -NMR spectrum. The obvious peaks at 5.30 ppm and 5.28 ppm belong to the H1 of 1,4-linked- $\alpha$ -Glcp, and 1,4,6-linked- $\alpha$ -Glcp, while the signal in a relatively high field at 4.88 ppm was assigned to the H1 of T- $\alpha$ -Glcp and 1,6-linked- $\alpha$ -Glcp. Further, according to the  $^1\text{H}$ - $^1\text{H}$ -COSY spectrum of YYS1-1, the H2-H5 signals are also assigned respectively. Based on  $^1\text{H}$ - $^1\text{H}$ -COSY and HSQC spectra analysis, all other  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of residues are identified and listed in Table 3. The down-field chemical shift at 76.74 ppm and 77.86 ppm belong to C4 of 1,4-linked- $\alpha$ -Glcp and 1,4,6-linked- $\alpha$ -Glcp. The down-field chemical shift at 65.47 ppm and 65.84 ppm belong to C6 of 1,6-linked- $\alpha$ -Glcp and 1,4,6-linked- $\alpha$ -Glcp, while the signals at 60.72 ppm and 60.23 ppm are assigned to C6 of T- $\alpha$ -Glcp and 1,4-linked- $\alpha$ -Glcp. The HMBC spectra are used to provide valuable insights into the relationships between different sugar residues, aiding in the determination of linkage types. In the HMBC spectrum of YYS1-1, the cross-peak signals at H1 (5.30 ppm)/C4 (76.74 ppm), H1 (5.28 ppm)/C4 (77.86 ppm), and H1 (4.88 ppm)/C6 (65.47 ppm) are suggested the presence of  $\rightarrow 4$ )- $\alpha$ -D-Glcp $\rightarrow$ linkage,  $\rightarrow 4,6$ )- $\alpha$ -D-Glcp $\rightarrow$ linkage, and  $\rightarrow 6$ )- $\alpha$ -D-Glcp $\rightarrow$ linkage, respectively. Further, the presence of cross-peak signals between H4 /residue B (3.55 ppm) and C1 /residue D (99.88 ppm) suggests that 1,4-linked- $\alpha$ -D-Glcp residue is attached to the 1,4,6-linked- $\alpha$ -Glcp at C4 position, while, the cross-peak signals at H4/residue B (3.55 ppm) and C1 /residue C (98.04 ppm) can be speculated that 1,4-linked- $\alpha$ -D-Glcp residue is attached to the 1, 6-linked- $\alpha$ -Glcp at the C4. Plus, the cross-peak signals at H6/residue D (3.88 ppm) and C1 /residue A (97.56 ppm) indicates the terminal residue  $\alpha$ -D-Glcp is attached to the 1,4,6-linked- $\alpha$ -Glcp at C6 position. When considering the methylation results in conjunction with the data presented, the analysis further indicates that YYS1-1 possesses a 1,4-linked-D-Glcp backbone, with a terminal residue of  $\alpha$ -D-Glcp- (1  $\rightarrow$  attached to the  $\rightarrow 4,6$ )- $\alpha$ -D-Glcp-(1  $\rightarrow$  at the C-6 position. Based on this analysis, Fig. 3f illustrates potential repeating units for YYS1-1.

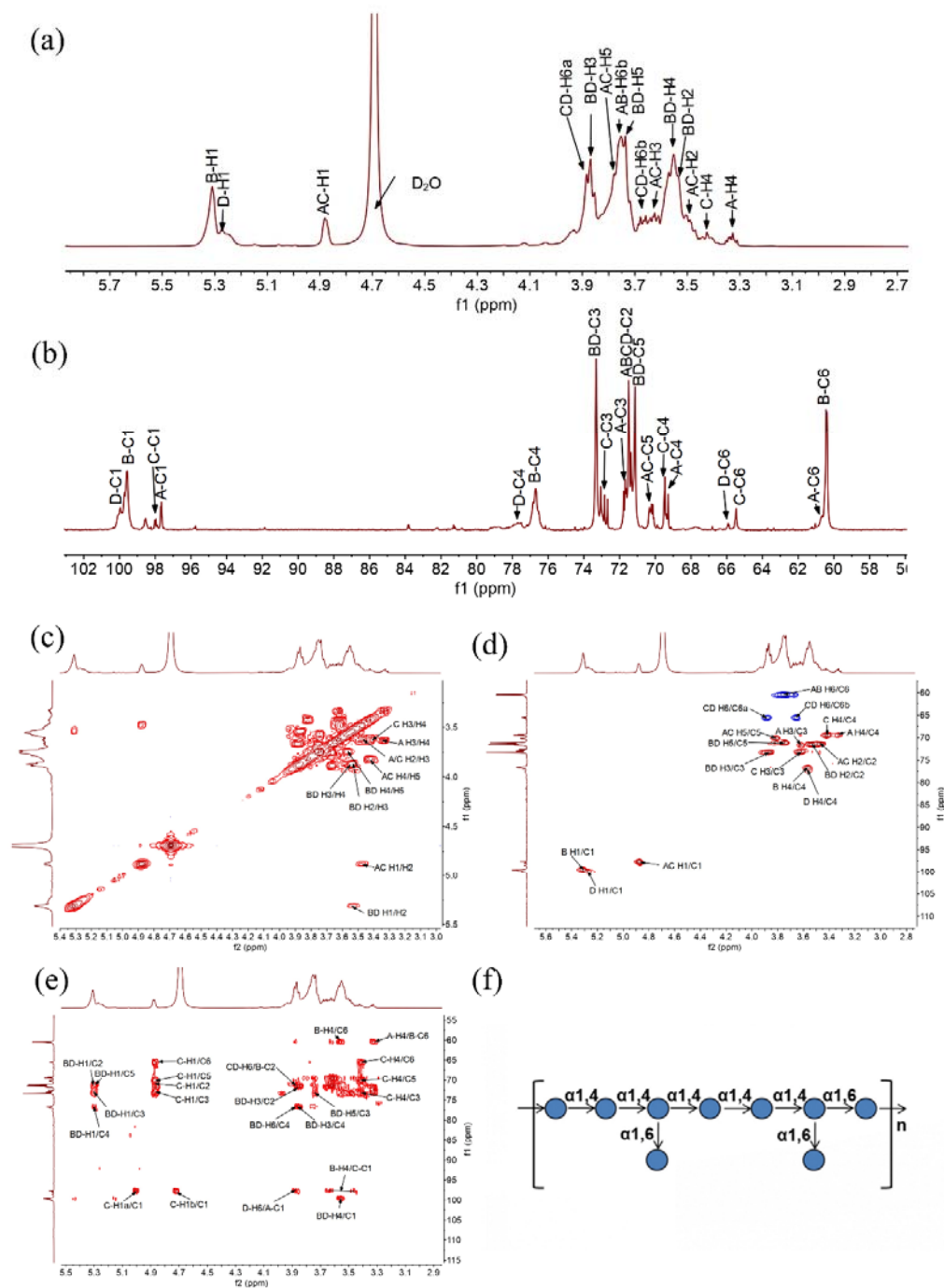


**Fig. 3** 1D-NMR and 2D-NMR spectra of XYS1-1. (a) The  $^1\text{H}$ -NMR spectrum of XYS1-1. (b) The  $^{13}\text{C}$ -NMR spectrum of XYS1-1. (c) 2D-NMR spectra of XYS1-1.  $^1\text{H}$ - $^1\text{H}$  COSY spectrum of XYS1-1. (d) HSQC spectrum of XYS1-1. (e) HMBC spectrum of XYS1-1. (f) Structural formula of XYS1-1.

**Table 3**  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts of XYS1-1

| Residues |                                          |   | 1     | 2     | 3     | 4     | 5     | 6a          |
|----------|------------------------------------------|---|-------|-------|-------|-------|-------|-------------|
| A        | T- $\alpha$ -Glc <sub>p</sub>            | H | 4.87  | 3.48  | 3.63  | 3.32  | 3.80  | 3.73        |
|          |                                          | C | 97.56 | 71.62 | 71.83 | 69.39 | 70.17 | 60.72       |
| B        | 1,4-linked- $\alpha$ -Glc <sub>p</sub>   | H | 5.30  | 3.53  | 3.87  | 3.55  | 3.74  | 3.75        |
|          |                                          | C | 99.60 | 71.35 | 73.25 | 76.74 | 71.08 | 60.23       |
| C        | 1,6-linked- $\alpha$ -Glc <sub>p</sub>   | H | 4.88  | 3.48  | 3.62  | 3.41  | 3.80  | 3.88a/3.65b |
|          |                                          | C | 98.04 | 71.56 | 73.13 | 69.30 | 70.32 | 65.47       |
| D        | 1,4,6-linked- $\alpha$ -Glc <sub>p</sub> | H | 5.28  | 3.53  | 3.85  | 3.55  | 3.73  | 3.88a/3.65b |
|          |                                          | C | 99.88 | 71.41 | 73.25 | 77.86 | 71.08 | 65.84       |

### 3.2 *XYIS1-1 inhibited TGF- $\beta$ 1-induced LX-2 activation, proliferation, migration and fibrotic marker expression*

Activated HSCs are the primary producers of ECM, and TGF- $\beta$ 1 acts as a key factor in promoting HSC activation [47]. To evaluate XYIS1-1's effect on HSC activation, we stimulated LX-2 cells with TGF- $\beta$ 1, treated them with different XYIS1-1 concentrations, and assessed outcomes. LX-2 cells exhibited obvious morphological alterations after induction with TGF- $\beta$ 1 (Fig. 4a).

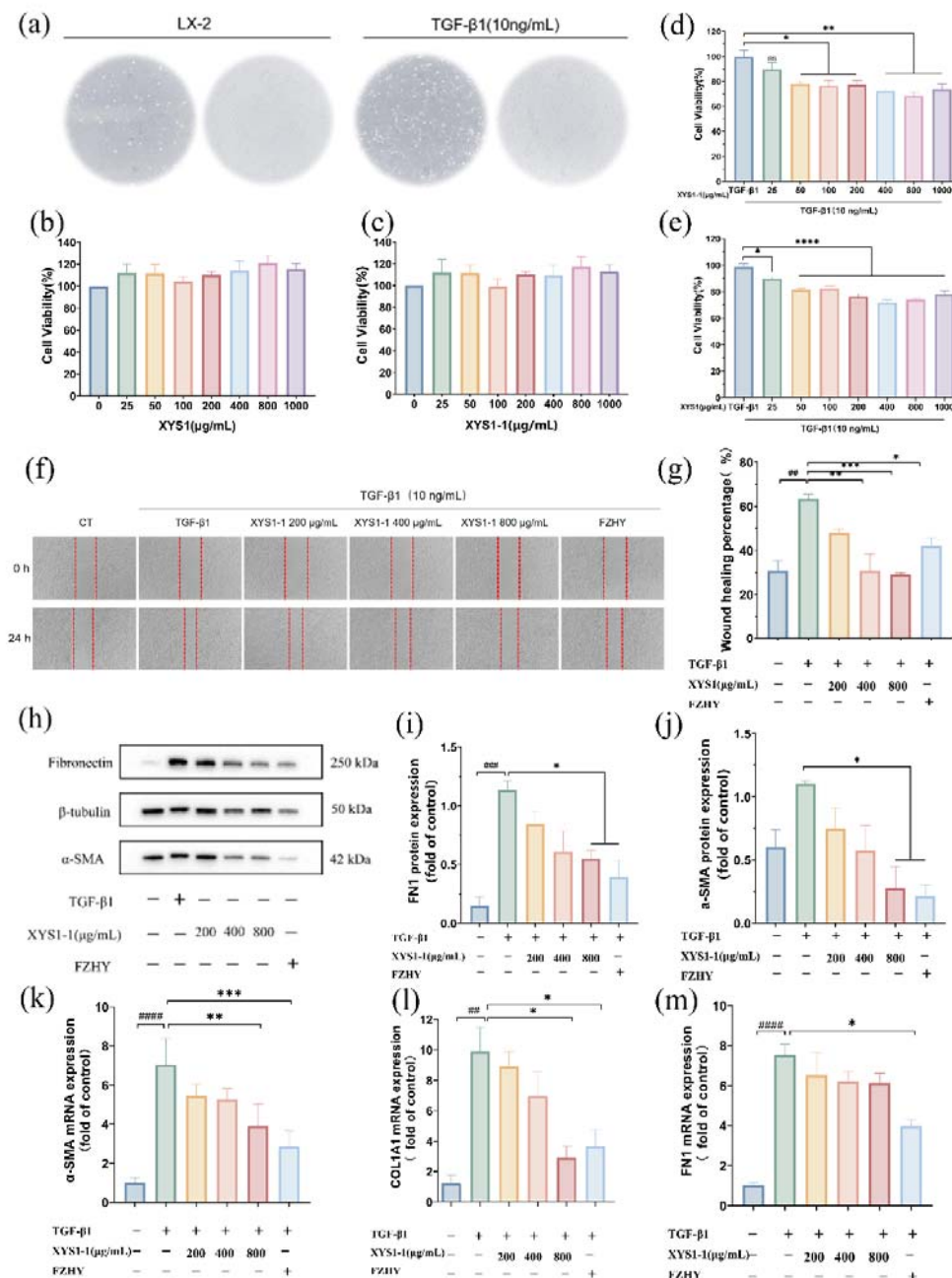


**Fig. 4** Inhibitory effects of XYIS1-1 on TGF- $\beta$ 1-induced activation of LX-2 cells. (a) Representative images of normal LX-2 cells and LX-2 cells activated by TGF- $\beta$ 1. (b-e) Cell viability of LX-2 cells before and after activation. (f) Effects of different concentrations of XYIS1-1 on the migration ability of LX-2 cells. (g) Scratch wound healing rate. (h) Effects of different concentrations of XYIS1-1 on the expression of fibrosis-related proteins in LX-2 cells. (i-j) Quantification of FN1 and  $\alpha$ -SMA expression levels using ImageJ software. (k-m) Effects of different concentrations of XYIS1-1 on the expression of fibrosis-related genes in LX-2 cells. Data are presented as mean  $\pm$  SD (n = 3). # $P$  < 0.05, ### $P$  < 0.01 compared with the control group; \* $P$  < 0.05, \*\* $P$  < 0.01 compared with the TGF- $\beta$ 1 group.

CCK-8 assay showed YYS1 and YYS1-1 (0-1000  $\mu\text{g/mL}$ ) had no cytotoxicity on normal LX-2 cells (Figs. 4b-c), but exerted dose-dependent inhibition on TGF- $\beta$ 1-activated LX-2 cells (0-800  $\mu\text{g/mL}$ ), peaking at 800  $\mu\text{g/mL}$  (Figs. 4d-e). The clinical anti-liver fibrosis (LF) agent FZHY (positive control) showed maximal inhibition at 400  $\mu\text{g/mL}$ , which was used in subsequent experiments (Fig. S2). Furthermore, YYS1-1 dose-dependently suppressed activated LX-2 migration (800  $\mu\text{g/mL}$  optimal; Fig. 4f), and scratch wound healing assay revealed significantly reduced wound closure rate in YYS1-1-treated groups vs. control after 24 h ( $P < 0.05$ ; Fig. 4g).

HSC activation is characterized by elevated  $\alpha$ -SMA and ECM (e.g., collagen, fibronectin) expression. The model group (TGF- $\beta$ 1-stimulated) had significantly higher mRNA levels of fibrosis markers ( $\alpha$ -SMA, COL1A1, FN1) vs. control ( $P < 0.01$ ; Figs. 4k-m), confirming successful LF model induction. YYS1-1 dose-dependently reduced these markers vs. model ( $P < 0.05$ ), with similar effects in FZHY-treated cells. Consistently, Western blot showed higher  $\alpha$ -SMA and fibronectin protein levels in the model group, which YYS1-1 significantly attenuated (Figs. 4h-j). These data indicate YYS1-1 mitigates LF by inhibiting HSC activation and ECM overproduction, consistent with RT-qPCR results.

### 3.3 YYS1-1 alleviates the symptoms of CCl<sub>4</sub>-induced liver damage in a mouse model

To further investigate the therapeutic effects of YYS1-1 on LF, a well-established CCl<sub>4</sub>-induced LF mouse model was employed in this study [48, 49]. Mice were intraperitoneally injected with a 10% (v/v) CCl<sub>4</sub>-olive oil mixture three times weekly for 8 weeks; from the second week onward, YYS1-1 or the clinical anti-LF agent FZHY (positive control) was administered daily via oral gavage (Fig. 5a). Gross liver morphology (Fig. 5b) revealed that livers from the model group exhibited severe adhesions to adjacent tissues, rounded edges, and diffuse small nodules on both the surface and cross-sections. In contrast, YYS1-1-treated mice had livers with a smoother surface, softer texture, and no obvious swelling. As depicted in CCl<sub>4</sub> injection, which was markedly ameliorated by YYS1-1 treatment. Notably, the model group exhibited a significant reduction in body weight, which was effectively reversed by YYS1-1 treatment (Fig. 5d). Compared to the control group, the liver weight and liver-to-body weight ratio were significantly increased in the model group ( $P < 0.01$ ), while these parameters were significantly reduced in the YYS1-1 and FZHY groups ( $P < 0.01$  or  $P < 0.05$ ) (Figs. 5e-f). H&E staining revealed that the liver tissue structure in the control group was intact, with well-organized hepatic lobules and no significant pathological changes. In contrast, the model group exhibited severe pathological damage, including hepatocyte edema, vacuolization, and disorganized cell arrangement, indicating substantial liver injury (Fig. 5c). Serum levels of ALT, AST, and TG were significantly elevated in the model group compared to the control group ( $P < 0.01$ ). However, these levels were significantly reduced in the YYS1-1 and FZHY groups ( $P < 0.01$ ), with the high-dose YYS1-1 group showing more pronounced effects (Figs. 5g-i). In mice treated with different doses of YYS1-1 or FZHY, only mild hepatocyte edema was observed, with no disruption of hepatic lobular structure or severe necrosis. These results demonstrate that the CCl<sub>4</sub>-induced LF mouse model exhibited severe liver injury, while treatment with YYS1-1 and FZHY significantly alleviated the extent of liver damage.

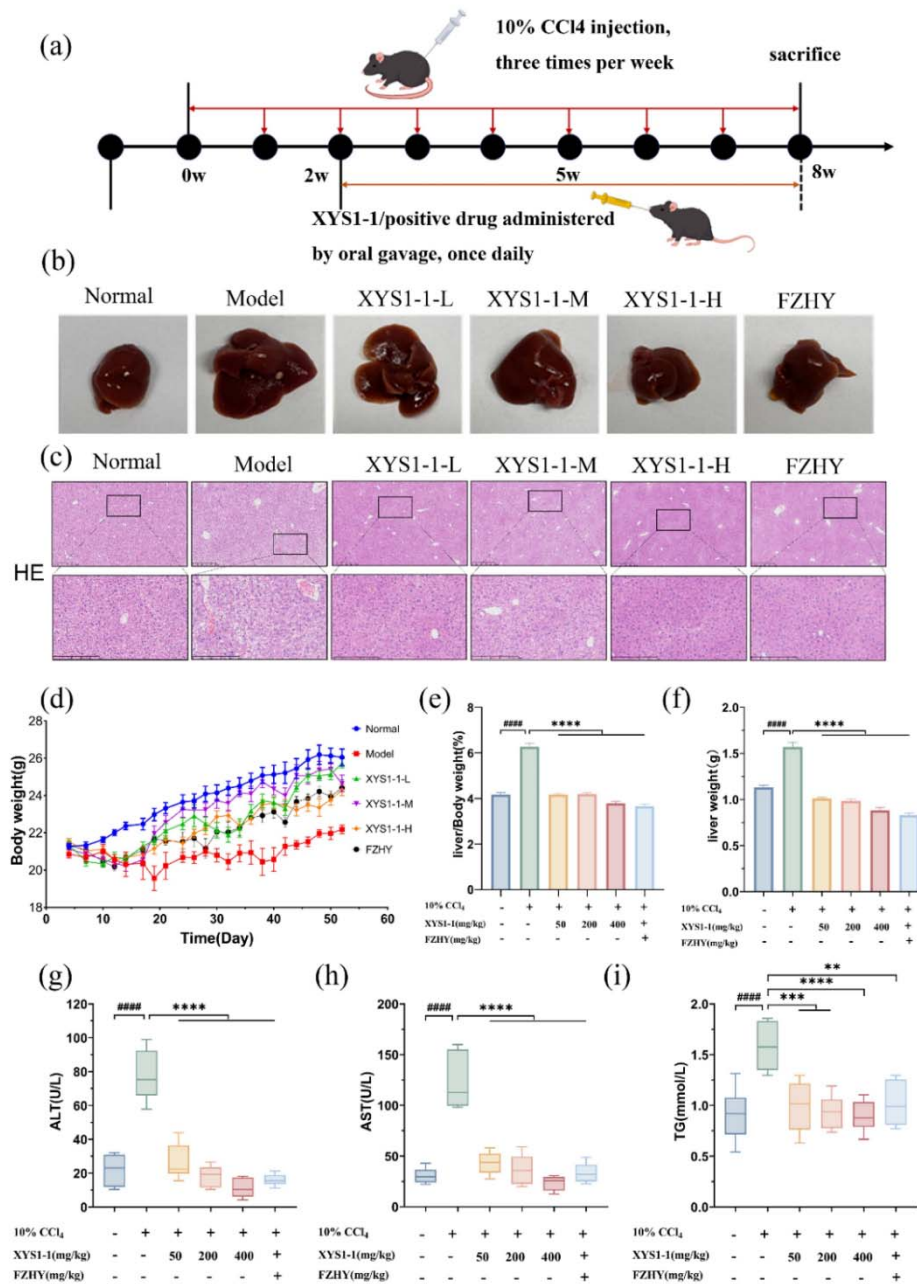


**Fig. 5** YYS1-1 alleviates the symptoms of CCl<sub>4</sub>-induced LF in a mouse model. (a) Schematic diagram of the mouse model establishment and treatment process. (b) Representative images of livers from each experimental group. (c) H&E staining of liver sections. (d) Changes in mouse body weight over the treatment period. (e) Liver/Body Weight Ratio. (f) Liver weight measurements. (g-i) Effects of YYS1 on serum levels of (g) aspartate aminotransferase (AST), (h) alanine aminotransferase (ALT), and (i) triglycerides (TG) in CCl<sub>4</sub>-induced LF mice, n=6.

### 3.4 YYS1-1 ameliorated LF induced by CCl<sub>4</sub> and inhibited HSC activation in vivo

Masson staining (Fig. 6a) showed sparse, evenly distributed collagen fibers in normal livers, whereas the model group exhibited extensive blue collagen deposition around central/portal veins (a hallmark of fibrosis). Low-, medium-, and high-dose YYS1-1, as well as FZHY, significantly reduced hepatic collagen content; ImageJ quantification (Fig. 6c) confirmed these effects, indicating improved LF. Immunohistochemistry (Fig. 6b) revealed markedly higher expression of fibrotic markers ( $\alpha$ -SMA, COL1A1, FN1) in the model group vs. controls (validating LF model establishment). YYS1-1 dose-dependently downregulated these markers, as did

FZHY (Figs. 6d-f), demonstrating XYS1-1 inhibits HSC activation and excessive ECM deposition.

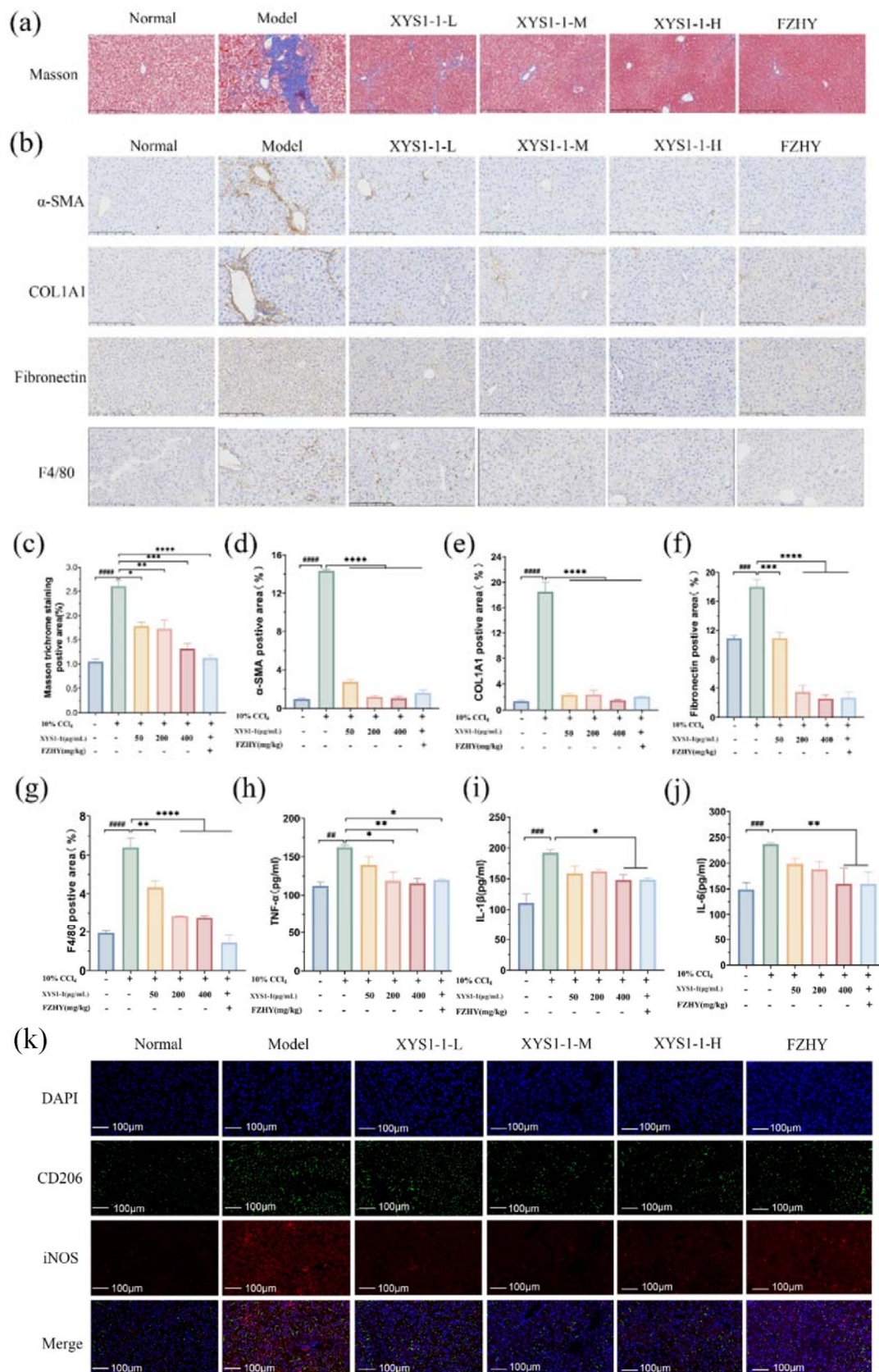


**Fig. 6** XYS1-1 ameliorates LF induced by CCl<sub>4</sub> and inhibits HSC activation *in vivo*. (a) Effect of XYS1-1 on collagen deposition in CCl<sub>4</sub>-induced LF in mice. (b) Immunohistochemical detection of  $\alpha$ -SMA, COL1A1, FN1, and F4/80 expression levels in liver tissue of mice from each group. (c) Quantitative analysis results of Masson staining, n=6. (d-g) Quantitative analysis results of immunohistochemistry for  $\alpha$ -SMA, COL1A1, FN1, and F4/80 expression, respectively, n=6. (h-j) Effect of XYS1 on the levels of inflammatory cytokines (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ) *in vivo*, n=6. (k) Immunofluorescence data of CD206 and iNOS in liver paraffin sections from each group of mice.

To assess inflammation's role in fibrosis, serum inflammatory cytokines were measured: CCl<sub>4</sub> increased TNF- $\alpha$ , IL-6, and IL-1 $\beta$  levels vs. controls, while YYS1-1 significantly reduced these cytokines (Figs. 6h-j), suggesting anti-inflammatory effects contribute to its anti-LF activity. The innate immune system plays a critical role in the development and progression of LF. To further investigate whether YYS1-1 influences LF by mediating innate immune cells, particularly macrophages, we examined the expression of F4/80, a macrophage marker. The control group had minimal F4/80<sup>+</sup> macrophages (a macrophage marker) around hepatic sinusoids. In contrast, the model group showed significant F4/80<sup>+</sup> macrophage accumulation in portal areas; YYS1-1 downregulated F4/80 expression (Figs. 6b, g), indicating modulation of macrophage-mediated immune responses.

The immunofluorescence analysis revealed that both iNOS (M1 macrophage marker) and CD206 (M2 macrophage marker) exhibited significantly enhanced fluorescence signals in CCl<sub>4</sub>-induced fibrotic livers compared to controls (Fig. 6k), indicating active macrophage recruitment and polarization during hepatic fibrogenesis. Notably, the increased iNOS<sup>+</sup> M1 macrophage population correlated with exacerbated inflammatory responses, while the elevated CD206<sup>+</sup> M2 macrophages were associated with progressive fibrotic deposition. Importantly, YYS1-1 treatment effectively attenuated this pathological process by significantly reducing both M1 and M2 macrophage populations in hepatic tissues, suggesting its anti-fibrotic mechanism may involve modulation of macrophage polarization dynamics in the injured liver microenvironment.

In summary, YYS1-1 ameliorates CCl<sub>4</sub>-induced LF by reducing collagen deposition, inhibiting HSC activation, lowering inflammatory cytokine levels, and regulating macrophage polarization.

### 3.5 YYS1-1 suppresses gut-liver axis-mediated inflammation and HSC activation

$\alpha/\beta$  diversity analyses demonstrated that CCl<sub>4</sub>-induced liver fibrosis was accompanied by a marked reduction in gut microbial diversity and a profound alteration of microbial community structure. YYS1-1 treatment partially restored microbial diversity and shifted the overall microbial composition toward that of the control group, with effects comparable to the positive control FZHY, suggesting that modulation of gut microbiota may contribute to the anti-fibrotic effects of YYS1-1 (Figs. 7a-f).

CCl<sub>4</sub>-induced liver fibrosis in our model was associated with a clear restructuring of the gut microbiota at the phylum level: the model group showed an increased *Firmicutes/Bacteroidota* ratio relative to controls, whereas *Proteobacteria* did not show a conspicuous expansion (Fig. 7g). Alterations in the *Firmicutes/Bacteroidota* balance have been repeatedly observed in experimental and clinical contexts of liver injury and fibrosis, and such shifts are thought to reflect dysbiosis that can compromise intestinal barrier function and facilitate translocation of microbial products that drive hepatic inflammation and fibrogenesis [50]. The absence of a strong *Proteobacteria* bloom in our samples further indicates that, in this cohort and experimental setting, dysbiosis manifested primarily as changes within dominant commensal phyla rather than as an overgrowth of classical opportunistic taxa [51].

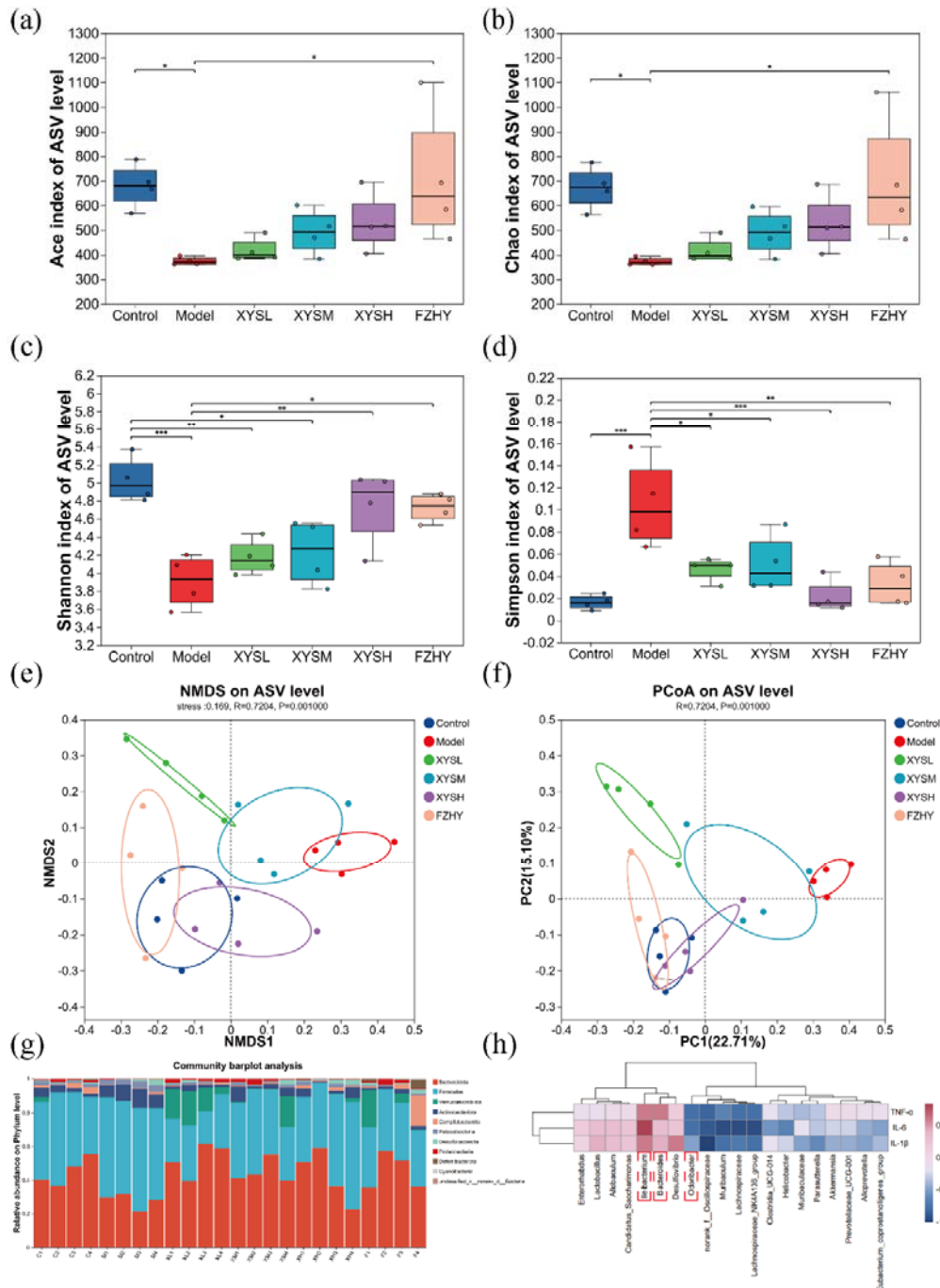


**Fig. 7** Microbial diversity analysis. (a) ACE index. (b) Chao1 index. (c) Simpson index. (d) Shannon index ( $\alpha$ -diversity). (e) PCoA analysis. (f) NMDS analysis ( $\beta$ -diversity). (g) phylum-level microbial community composition. (h) Correlation between gut microbiota and inflammatory cytokines.

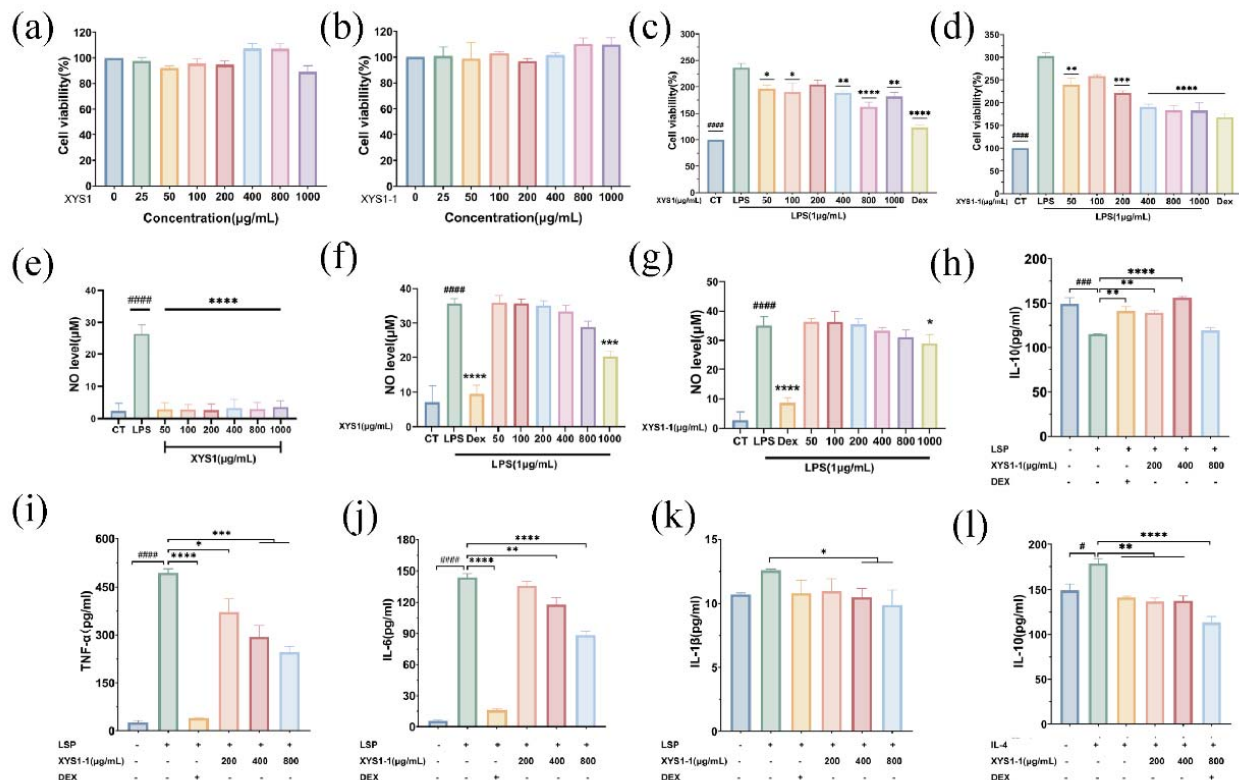
Interestingly, treatment with the XYSL-1 partially restored the microbial composition toward that of healthy controls: treated animals exhibited a decline in the *Firmicutes/Bacteroidota* ratio and an increased relative abundance of *Verrucomicrobiota*. Notably, members of *Verrucomicrobiota* (principally *Akkermansia muciniphila*) have been linked to improved mucosal barrier integrity and beneficial metabolic and anti-inflammatory effects in multiple preclinical studies. Mechanistically, *A. muciniphila* or its components have been shown to strengthen the gut barrier and modulate host responses that reduce systemic inflammation, which can translate into protection against liver injury in experimental models. These observations are consistent with recent work demonstrating that *A. muciniphila* enrichment or administration ameliorates

hepatic pathology in murine cirrhosis/fibrosis models [52, 53]. Taken together, these phylum-level data suggest that the hepatoprotective effect of the YYS1-1 may be mediated, at least in part, via restoration of a healthier gut microbial ecology and improved intestinal barrier function.

To further elucidate the relationship between inflammatory responses and gut microbial dysbiosis, we integrated the correlation results between key inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ ) and gut microbial genera with the observed shifts in microbial community composition (Fig. 7h). Interestingly, we observed an enrichment of pro-inflammatory taxa, specifically *Bacteroides* [54] and *Ileibacterium* [55], in the model group, which coincided with elevated levels of related inflammatory cytokines. Conversely, the abundance of these genera was downregulated in the treatment group (Fig. S3). Simultaneously, the proportions of anti-inflammatory bacteria, such as *Odoribacter* [56], were restored following YYS1-1 administration, suggesting a subsequent reduction in inflammatory markers.

### 3.6 YYS1-1 inhibits LPS-induced M1 macrophage polarization in vitro

YYS1's and YYS1-1's cytotoxicity in RAW264.7 cells was assessed via CCK-8 (48 h, 50-1000  $\mu$ g/mL). No tested concentration showed significant cytotoxicity ( $p > 0.05$  vs. control), with the 400  $\mu$ g/mL and 800  $\mu$ g/mL groups demonstrating mild proliferative effects (Figs. 8a-b). Based on these findings, subsequent experiments employed concentrations between 200-800  $\mu$ g/mL.



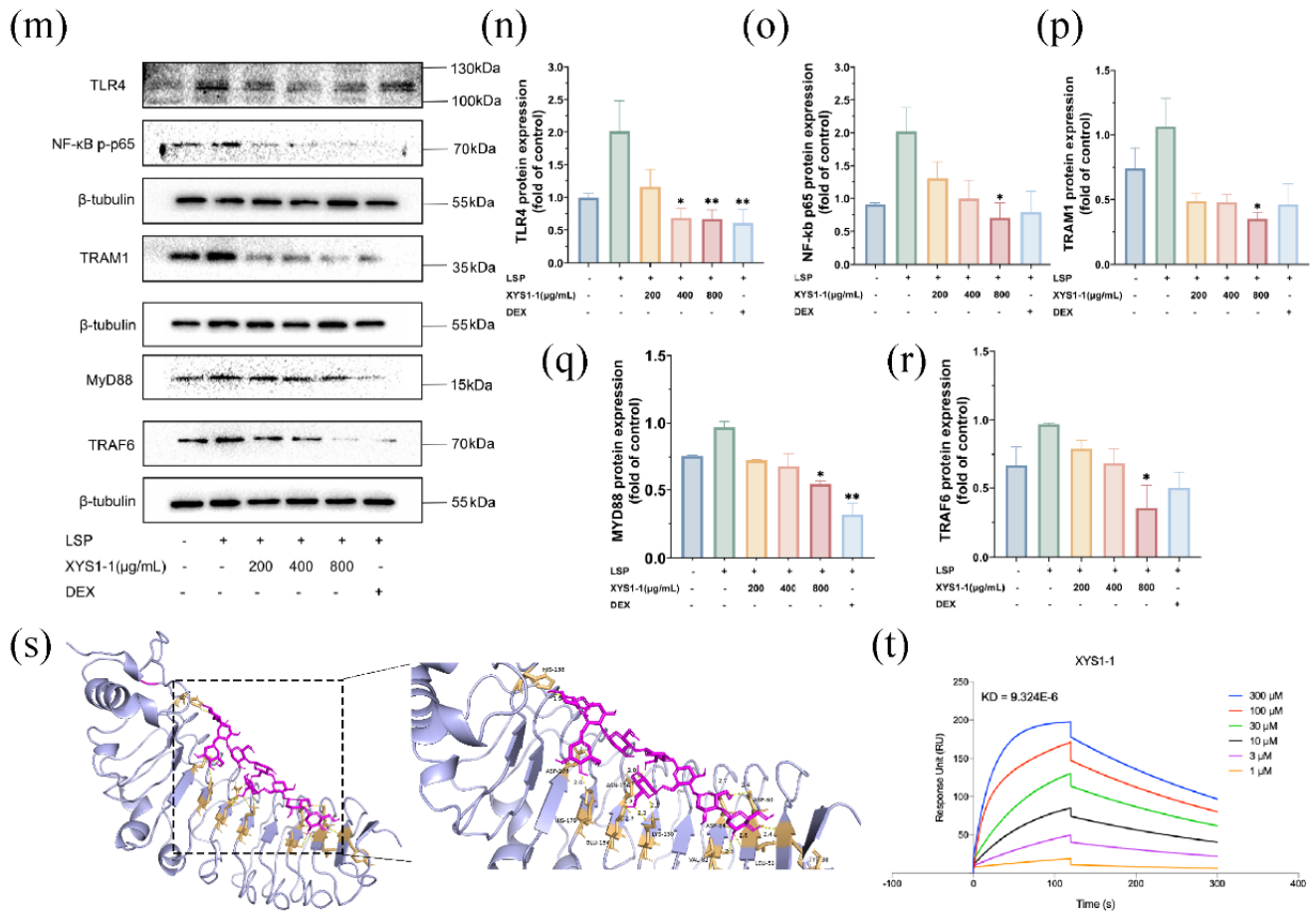


**Fig. 8** YYS1-1 inhibits LPS-induced M1 macrophage polarization in RAW264.7 cells *in vitro*. (a-b) Effects of (a) YYS1 and (b) YYS1-1 on viability of naive RAW264.7 macrophages. (c-d) Effects of (c) YYS1 and (d) YYS1-1 on viability of LPS-stimulated M1 macrophages. (e) Effect of YYS1 on NO secretion in naive RAW264.7 macrophages. (f-g) Effects of (f) YYS1 and (g) YYS1-1 on NO production in LPS-induced M1 macrophages,  $n=3$ . (h-k) Effects of YYS1-1 on cytokine secretion in LPS-stimulated RAW264.7 cells: (h) IL-10, (i) TNF- $\alpha$ , (j) IL-6, and (k) IL-1 $\beta$ . (l) Effect of YYS1-1 on IL-10 secretion in M2-polarized macrophages,  $n=3$ . (m) Effect of YYS1-1 on protein expression in TLR4/MyD88/NF- $\kappa$ B signaling pathway. (n-r) Quantification of the aforementioned proteins. (s) The repetitive sequence of YYS1-1 undergoes molecular docking with TLR4. (t) SPR analysis of the binding affinity between YYS1-1 and TLR4.

In LPS-induced M1 macrophage polarization models, YYS1 and YYS1-1 displayed concentration-dependent immunomodulatory properties. While lower concentrations (50–100  $\mu$ g/mL) showed negligible effects on cell viability, higher concentrations (400–1000  $\mu$ g/mL) significantly suppressed viability ( $P<0.01$ , Figs. 8c-d). Both YYS1 and YYS1-1 could suppress LPS-induced NO production. Notably, YYS1-1 exhibited potent inhibitory effects on LPS-stimulated overproduction of both NO and ROS in a dose-dependent manner (Figs. 8e-g, 9b). Cytokine profiling demonstrated YYS1-1's dual regulatory capacity: it significantly attenuated LPS-induced elevations in pro-inflammatory mediators TNF- $\alpha$  and IL-6 ( $P<0.01$ ), while paradoxically upregulating the anti-inflammatory cytokine IL-10 ( $P<0.01$  versus LPS group, Figs. 8h-k). This bidirectional modulation suggests YYS1-1's unique role in maintaining inflammatory homeostasis. Interestingly, in IL-4-polarized M2 macrophages, YYS1-1 dose-dependently suppressed IL-10 overexpression ( $P<0.01$ ), indicating nuanced control over macrophage plasticity.

### 3.7 YYS1-1 target TLR4 and inhibits TLR4/MyD88/NF- $\kappa$ B pathway

The Toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88)/nuclear factor- $\kappa$ B (NF- $\kappa$ B)

signaling axis serves as a central regulator of innate immune responses and inflammatory pathogenesis [57, 58]. Studies have demonstrated that various polysaccharides can specifically bind to TLRs, exerting bidirectional immunomodulatory effects through either pro-inflammatory or anti-inflammatory mechanisms [59, 60]. To further investigate the mechanism by which YYS1-1 modulates macrophage function, we examined its effects on TLR4 expression and the TLR4/MyD88/NF- $\kappa$ B signaling pathway, and performed molecular dynamics simulations to characterize the binding interaction between YYS1-1's repeat fragment and TLR4.

Western blot analysis elucidated the molecular mechanism underlying these effects. YYS1-1 treatment substantially reduced expression of TLR4 and its downstream effectors (MyD88, TRAM1, TRAF1), consequently inhibiting NF- $\kappa$ B p65 phosphorylation (Figs. 8m-r, 9a). This TLR4/MyD88/NF- $\kappa$ B pathway inhibition represents a key anti-inflammatory mechanism.

The binding affinity score reflects the likelihood and strength of molecular interaction, with lower (more negative) values indicating stronger binding potential. AutoDock predicted a binding affinity of -7.1 kcal/mol between TLR4 and the YYS1-1's repeat fragment, suggesting a favorable and stable binding conformation (Fig. 8s). Combined with SPR experimental result that YYS1-1 binds to TLR4 with an equilibrium dissociation constant of 9.324  $\mu$ mol/L, this docking data collectively demonstrates a good binding affinity of YYS1-1 for TLR4, providing a preliminary theoretical basis for its potential role in modulating TLR4-mediated signaling pathways (Fig. 8t). The three-dimensional molecular docking results suggest that hydrogen bonding is the primary mode of interaction between YYS1-1 and the TLR4 protein. Key amino acid residues involved in the binding include HIS-258, ASP-209, HIS-179, GLU-154, ASN-156, LYS-130, ASP-84, VAL-82, ASP-60, LEU-59, and TYR-38. The main chain of YYS1-1 consists of a linear structure of  $\rightarrow 4$ )- $\alpha$ -D-Glcp-(1 $\rightarrow$ , which fits well within the binding pocket of the TLR4 receptor, enabling stable molecular recognition and interaction [61]. Meanwhile, the branched T-Glcp units are linked to the main chain via the C-6 position, thereby increasing the steric hindrance of the polysaccharide. This structural feature plays a dual role in the potential interaction with the TLR4 receptor. On one hand, the branched chains may occupy concave regions within the receptor's binding pocket, enhancing structural complementarity [62]. On the other hand, their steric bulk may restrict the binding orientation and angle of the sugar chain, contributing to greater specificity in the molecular interaction. Collectively, the linear main chain and the spatial configuration introduced by the branched chains facilitate the effective recognition and binding of YYS1-1 to the TLR4 receptor in an *in vitro* molecular model, which provides a theoretical reference for subsequent exploration of the *in vivo* interaction mechanism of YYS1-1 with TLR4.

Critically, conditioned media from YYS1-1-treated macrophages significantly attenuated  $\alpha$ -SMA and fibronectin expression in LX-2 cells (Figs. 9c-e), establishing YYS1-1's indirect anti-fibrotic potential through macrophage-mediated paracrine signaling. These findings position YYS1-1 as a multifaceted therapeutic candidate capable of modulating macrophage polarization to mitigate both inflammatory responses and fibrotic progression.

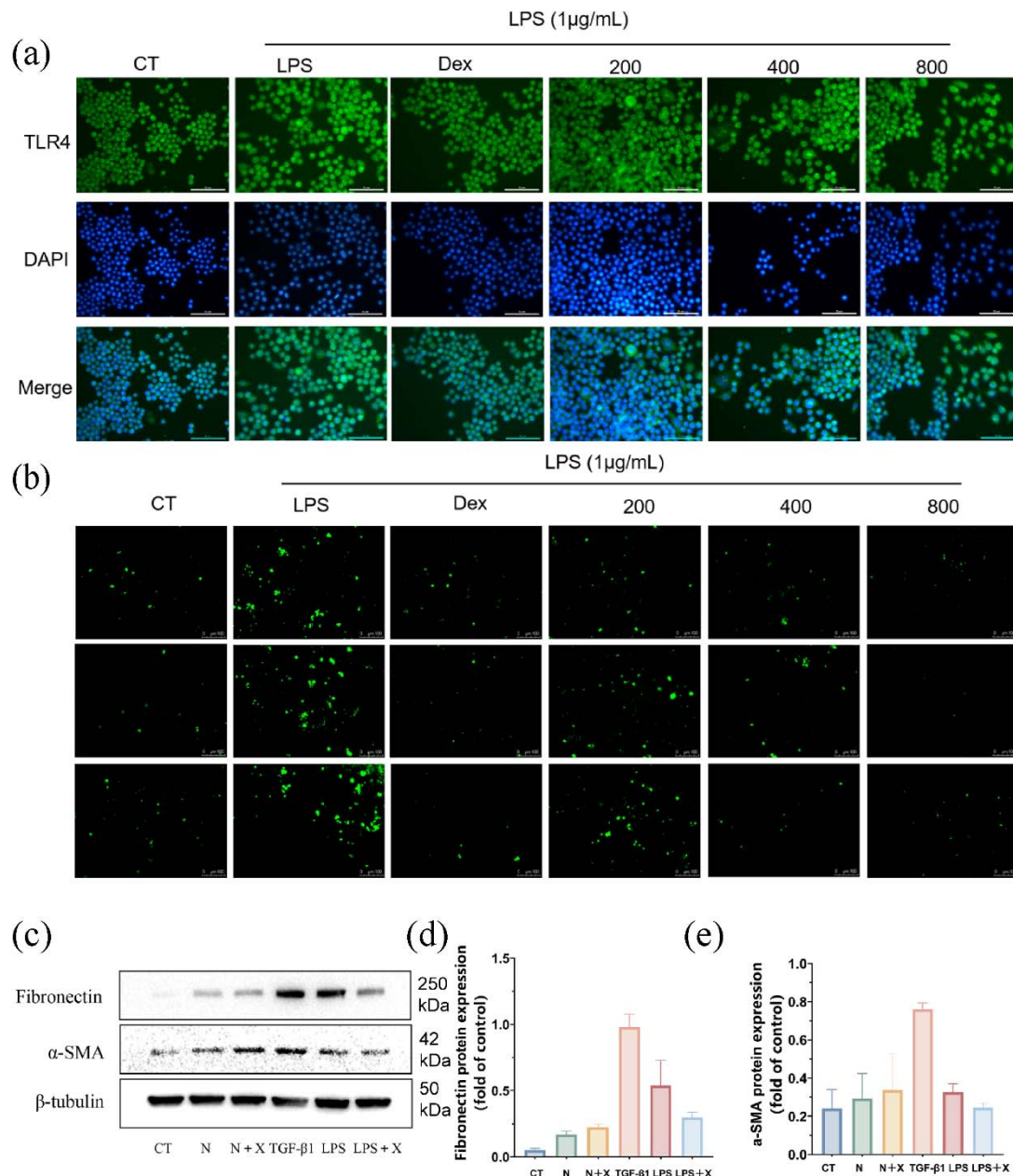


**Fig. 9** Mechanistic insights into YYS1-1 polysaccharide-mediated immunomodulation. (a) Immunofluorescence Detection of the Effects of Different Concentrations of YYS1-1 Polysaccharide on TLR4 Protein Expression in RAW264.7 Cells. (b) Quantitative assessment of ROS production in macrophages following YYS1-1 treatment. (c) Paracrine effects of macrophage-conditioned media on LX-2 cell activation: CT: Untreated LX-2 controls; N: LX-2 exposed to naive macrophage supernatant; N+X: LX-2 exposed to YYS1-1-primed macrophage supernatant (800  $\mu\text{g/mL}$ ); TGF- $\beta$ 1: Positive control (10 ng/mL TGF- $\beta$ 1); LPS: LX-2 exposed to M1 macrophage (LPS-induced) supernatant; LPS+X: LX-2 exposed to YYS1-1-modulated M1 supernatant (800  $\mu\text{g/mL}$ ). (d-e) Western blot analysis showing YYS1-1-mediated downregulation of fibrotic markers: (d) Fibronectin and (e)  $\alpha$ -SMA expression levels in LX-2 cells.

## 4 Discussion

The present study systematically investigated the anti-fibrotic potential of YYS1-1, a novel glucan isolated from *Panax quinquefolius Radix*, through structural characterization and multi-level mechanistic exploration. Our findings reveal that YYS1-1 exerts potent therapeutic effects against LF via dual modulation of macrophage polarization and direct inhibition of HSCs activation. YYS1-1 is a homogeneous neutral glucan characterized as a backbone of  $\rightarrow 4$ - $\alpha$ -D-Glcp-(1 $\rightarrow$  residues with terminal Glcp branches attached via C-6

positions, with a molecular weight of 12.59 kDa. Previous studies have revealed that branched glucans demonstrate superior immunomodulatory properties relative to linear counterparts [63], likely attributable to optimized spatial recognition patterns through pattern recognition receptors (PRRs) such as Dectin-1 and TLR4 [64]. Notably, SPR experiments and molecular docking analyses indicating a specific binding affinity between YYS1-1 and TLR4, providing a preliminary theoretical and experimental basis for the subsequent exploration of YYS1-1 regulating the TLR4-mediated signaling pathway in hepatic fibrosis.

This study provides the first demonstration of the anti-fibrotic properties of *Panax quinquefolius* polysaccharide YYS1-1. *In vitro* cell experiments using intact YYS1-1 prototype were designed for the initial verification of its intrinsic biological activity, and the results showed that YYS1-1 significantly attenuated HSC activation in TGF- $\beta$ 1-stimulated LX-2 cells as evidenced by marked reductions in both protein and mRNA expression levels of  $\alpha$ -SMA and fibronectin ( $P < 0.01$ ). These findings suggest that YYS1-1 ameliorates LF by suppressing HSC activation. Using a CCl<sub>4</sub>-induced LF mouse model [2], YYS1-1 significantly reduced the serum levels of ALT, AST, and TG in the model group of mice ( $P < 0.01$ ) and effectively restored the liver-to-body weight ratio to the normal range, reflecting the comprehensive *in vivo* physiological effects of YYS1-1 (prototype and minor metabolic products). Histopathological analysis revealed that YYS1-1 treatment could markedly alleviate pathological damage characteristics such as hepatocyte edema, vacuolar degeneration, and disordered arrangement. Moreover, Masson's trichrome staining and immunohistochemical detection confirmed that YYS1-1 dose-dependently reduced collagen fiber deposition in the liver ( $P < 0.05$ ), and Western blot analysis further indicated that YYS1-1 significantly downregulated the expression of fibrosis marker proteins such as  $\alpha$ -SMA and fibronectin ( $P < 0.01$ ). Collectively, these results demonstrate that YYS1-1 exerts anti-hepatic fibrosis effects by inhibiting the activation of hepatic stellate cells and the excessive deposition of extracellular matrix.

Besides, YYS1-1 is capable of simultaneously inhibiting M1 macrophage polarization via the TLR4/NF- $\kappa$ B signaling pathway and modulating IL-10 production from M2 macrophages. This dual activity positions YYS1-1 as a “biological regulator” within the hepatic microenvironment, contrasting sharply with the unidirectional inhibitory effects of traditional anti-inflammatory compounds. *In vitro*, in LPS-stimulated RAW264.7 cells, YYS1-1 suppressed pro-inflammatory cytokines (NO, TNF- $\alpha$ , IL-6, IL-1 $\beta$ ;  $P < 0.01$ ) and upregulated anti-inflammatory IL-10 ( $P < 0.01$ ), maintaining inflammatory balance. In IL-4-induced RAW264.7 cells, it dose-dependently reduced IL-10 ( $P < 0.01$ ), modulating M2 anti-inflammatory function. *In vivo*, CCl<sub>4</sub> increased serum TNF- $\alpha$ /IL-6/IL-1 $\beta$  and hepatic macrophage recruitment; YYS1-1 reversed these, and reduced elevated M1 (iNOS) and M2 (CD206) markers (immunofluorescence). Since macrophage polarization/cytokines (e.g., IL-10) regulate HSC activation/ECM deposition, YYS1-1's bidirectional macrophage modulation and inflammatory balance maintenance contribute to anti-LF effects. Additionally,  $\alpha/\beta$  diversity analyses showed YYS1-1 enhanced gut microbiota diversity and improved community structure in CCl<sub>4</sub>-induced LF mice. These results suggest that YYS1-1 restores the disrupted gut microbiota structure and suppresses gut-liver axis-mediated inflammation and HSC activation by inhibiting pro-inflammatory taxa

while promoting anti-inflammatory commensals.

Comparative analysis with clinical antifibrotic agents has unveiled the distinct advantages of YYS1-1. While YYS1-1 demonstrated comparable efficacy to the positive control drug FZHY in inhibiting HSCs, it exhibited superior safety profiles at therapeutic concentrations. This hepatoprotective potential, coupled with its dual targeting of macrophages and HSCs, suggests that YYS1-1 may surmount the limitations of current single-target therapies. Moreover, the dose-dependent inhibition of CCl<sub>4</sub>-induced collagen deposition and F4/80<sup>+</sup> macrophage infiltration by YYS1-1 further corroborates its translational potential. Concurrently, YYS1-1 can enhance microbial diversity and ameliorate the compositional structure of the gut microbiota.

## 5 Conclusion

In conclusion, this study for the first time elucidates the anti-hepatic fibrosis activity and underlying mechanisms of YYS1-1 (Fig. 10), thereby enriching the theoretical understanding of the bioactivity of *Panax quinquefolius* polysaccharides and providing novel theoretical insights for the treatment of hepatic fibrosis. However, this study still has certain limitations. The pharmacokinetics and tissue distribution of YYS1-1 have not been characterized, which makes it difficult to systematically elucidate its *in vivo* fate and underlying mechanism. In future studies, we will employ techniques such as fecal excretion, fluorescent labeling, and radionuclide labeling, combined with *in vivo* digestive kinetics and pharmacokinetic investigations, to systematically explore the *in vivo* distribution, metabolic profiles, and structural changes of YYS1-1. Additionally, we will identify the actual active components of YYS1-1 enriched in liver tissue after oral administration, verify its tissue distribution and *in vivo* existence form, and further elucidate the *in vivo* molecular mechanism research of this polysaccharide [65, 66].

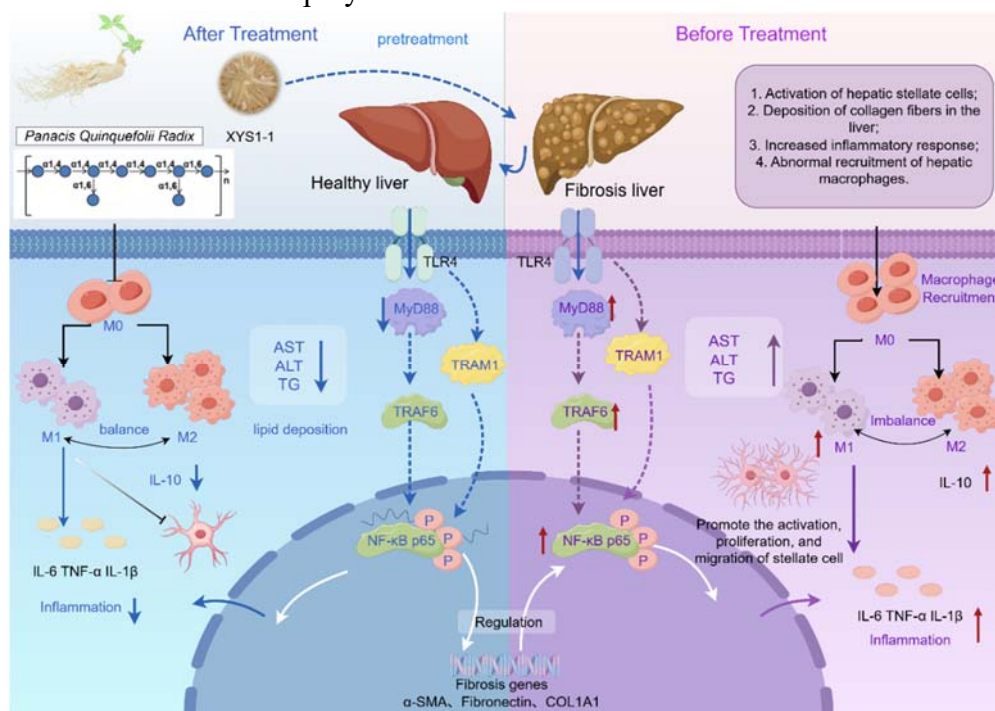


**Fig. 10** Schematic diagram of the anti-hepatic fibrosis mechanism of YYS1-1.

## Declaration of Competing Interest

The authors report no declarations of interest.

## Acknowledgments

This work was supported by Open Project Fund of Guangdong Provincial Key Laboratory of Green Processing of Natural Products and Product Safety (202502), and Open Project Fund of Marine Biomedical Science and Technology Innovation Platform of Lin-Gang Special Area (RWS-2024-002).

## Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version.

## References

- [1] X.N. Ma, W.Q. Zhou, Y.N. Nie, et al., A novel branched galacturonan from *Gardenia jasminoides* alleviates liver fibrosis linked to TLR4/NF- $\kappa$ B signaling, *Int J Biol Macromol.* 245 (2023) 125540. <https://doi.org/10.1016/j.ijbiomac.2023.125540>.
- [2] S.X. Xu, Y.E. Chen, J.D. Miao, et al., Esculin inhibits hepatic stellate cell activation and CCl<sub>4</sub>-induced liver fibrosis by activating the Nrf2/GPX4 signaling pathway, *Phytomedicine.* 128 (2024) 155465. <https://doi.org/10.1016/j.phymed.2024.155465>.
- [3] X. Zhao, J.Y.Y. Kwan, K. Yip, et al., Targeting metabolic dysregulation for fibrosis therapy, *Nat Rev Drug Discov.* 19 (2020) 57–75. <https://doi.org/10.1038/s41573-019-0040-5>.
- [4] N.C. Henderson, F. Rieder, T.A. Wynn, Fibrosis: from mechanisms to medicines, *Nature.* 587 (2020) 555–66. <https://doi.org/10.1038/s41586-020-2938-9>.
- [5] Y. Ning, X.Y. Dou, Z.C. Wang, et al., SIRT3: A potential therapeutic target for liver fibrosis, *Pharmacol Ther.* 257 (2024) 108639. <https://doi.org/10.1016/j.pharmthera.2024.108639>.
- [6] J.E. Puche, Y. Saiman, S.L. Friedman, Hepatic stellate cells and liver fibrosis, *Compr Physiol.* 3 (2013) 1473–92. <https://doi.org/10.1002/cphy.c120035>.
- [7] S.L. Friedman, M. Pinzani, Hepatic fibrosis 2022: Unmet needs and a blueprint for the future, *Hepatology.* 75 (2022) 473–88. <https://doi.org/10.1002/hep.32285>.
- [8] C. Liang, J.J. Liu, M.X. Jiang, et al., The advancement of targeted regulation of hepatic stellate cells using traditional Chinese medicine for the treatment of liver fibrosis, *J Ethnopharmacol.* 341 (2025) 119298. <https://doi.org/10.1016/j.jep.2024.119298>.
- [9] C.X. Dai, A. Yusuf, H. Sun, et al., A characterized saponin extract of *Panax japonicus* suppresses hepatocyte EMT and HSC activation *in vitro* and CCl<sub>4</sub>-provoked liver fibrosis in mice: Roles of its modulatory effects on the Akt/GSK3 $\beta$ /Nrf2 cascade, *Phytomedicine.* 93 (2021) 153746. <https://doi.org/10.1016/j.phymed.2021.153746>.
- [10] C. Ling, S.S. Liu, Y.Y. Wang, et al., Overexpression of wild-type HRAS drives non-alcoholic steatohepatitis to hepatocellular carcinoma in mice, *Zool Res.* 45 (2024) 551–66. <https://doi.org/10.24272/j.issn.2095-8137.2024.002>.
- [11] E. Seki, R.F. Schwabe, Hepatic inflammation and fibrosis: functional links and key pathways, *Hepatology.* 61 (2015) 1066–79. <https://doi.org/10.1002/hep.27332>.
- [12] T. Tsuchida, S.L. Friedman, Mechanisms of hepatic stellate cell activation, *Nat Rev Gastroenterol Hepatol.* 14 (2017) 397–411. <https://doi.org/10.1038/nrgastro.2017.38>.
- [13] M. Matsuda, E. Seki, Hepatic Stellate Cell-Macrophage Crosstalk in Liver Fibrosis and Carcinogenesis, *Semin Liver Dis.* 40 (2020) 307–20. <https://doi.org/10.1055/s-0040-1708876>.
- [14] C. Wang, C. Ma, L.H. Gong, et al., Macrophage Polarization and Its Role in Liver Disease, *Front Immunol.* 12 (2021) 803037. <https://doi.org/10.3389/fimmu.2021.803037>.
- [15] L. Wang, H.S. Zhang, L.Y. Sun, et al., Manipulation of macrophage polarization by peptide-coated gold nanoparticles and its protective effects on acute lung injury, *J Nanobiotechnology.* 18 (2020) 38. <https://doi.org/10.1186/s12951-020-00593-7>.
- [16] F. Xu, M.M. Guo, W. Huang, et al., Annexin A5 regulates hepatic macrophage polarization via directly targeting PKM2 and ameliorates NASH, *Redox Biol.* 36 (2020) 101634. <https://doi.org/10.1016/j.redox.2020.101634>.
- [17] S.L. Zhao, Y.S. Mi, B.J. Guan, et al., Tumor-derived exosomal miR-934 induces macrophage M2 polarization to promote liver metastasis of colorectal cancer, *J Hematol Oncol.* 13 (2020) 156. <https://doi.org/10.1186/s13045-020-00991-2>.
- [18] Y. Yang, Y.C. Ye, Y. Chen, et al., Crosstalk between hepatic tumor cells and macrophages via Wnt/ $\beta$ -catenin signaling promotes

- M2-like macrophage polarization and reinforces tumor malignant behaviors, *Cell Death Dis.* 9 (2018) 793. <https://doi.org/10.1038/s41419-018-0818-0>.
- [19] K. Kazankov, S.M.D. Jørgensen, K.L. Thomsen, et al., The role of macrophages in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, *Nat Rev Gastroenterol Hepatol.* 16 (2019) 145-59. <https://doi.org/10.1038/s41575-018-0082-x>.
- [20] J. Bai, Y.S. Wei, Y. He, et al., Preparation and biological activity of polysaccharide metal ion complex and feasibility analysis of *Tremella fuciformis* polysaccharide with metal ions, *Food Med Homol.* 2 (2025) 9420031. <https://doi.org/10.26599/fmh.2025.9420031>.
- [21] Z.X. Li, M. Zhang, Y.Y. Liu, et al., Advances in the Preparation, Structure-Activity Relationship, and Biological Mechanisms of *Agaricus* Polysaccharides, *Food Sci Hum Wellness.* (2025). <https://doi.org/10.26599/fshw.2025.9250590>.
- [22] Z.X. Wang, K. Huang, K.L. Pu, et al., *Naematelia aurantialba*: a comprehensive review of its medicinal, nutritional, and cultivation aspects, *Food Med Homol.* 2 (2025) 9420072. <https://doi.org/10.26599/fmh.2025.9420072>.
- [23] H.X. Bai, Y.X. Gao, S. Wang, et al., Structure characteristics of a novel pectic polysaccharide from *Fructus Corni* and its protective effect on alcoholic fatty liver, *Carbohydr Polym.* 352 (2025) 123153. <https://doi.org/10.1016/j.carbpol.2024.123153>.
- [24] X.Q. Jing, G.Q. Zhou, A.M. Zhu, et al., RG-I pectin-like polysaccharide from *Rosa chinensis* inhibits inflammation and fibrosis associated to HMGB1/TLR4/NF- $\kappa$ B signaling pathway to improve non-alcoholic steatohepatitis, *Carbohydr Polym.* 337 (2024) 122139. <https://doi.org/10.1016/j.carbpol.2024.122139>.
- [25] Z.P. Liu, W.S. Tang, G.Z. Wang, et al., Ferulic acid inhibiting colon cancer cells at different Duke's stages, *Food Med Homol.* 2 (2025) 9420063. <https://doi.org/10.26599/fmh.2025.9420063>.
- [26] H.N. Xu, C.Y. Lu, C.H. Zhao, et al., A combination of metabolomics and microbiome analyses reveal the modulation of lipid metabolism effect of *Nannochloropsis gaditana* polysaccharides on alcohol-induced dyslipidemia in mice, *J Funct Foods.* 119 (2024) 106282. <https://doi.org/10.1016/j.jff.2024.106282>.
- [27] Z.K. Zeng, L. Wang, Z.H. Zhang, et al., Steam-exploded *Dictyophora indusiata* polysaccharide protect against inflammation by regulating gut barrier, microbiota and metabolites, *Food Med Homol.* (2025). <https://doi.org/10.26599/fmh.2026.9420107>.
- [28] H.C. Wang, J.J. Yan, K. Wang, et al., The gut-liver axis perspective: Exploring the protective potential of polysaccharides from *Cistanche deserticola* against alcoholic liver disease, *Int J Biol Macromol.* 256 (2024) 128394. <https://doi.org/10.1016/j.ijbiomac.2023.128394>.
- [29] Z.N. Wu, C. Li, J.H. Li, et al., Extraction of American ginseng polysaccharide by ultrasound-assisted deep eutectic solvents-based three-phase partitioning: Process optimization, structural characterization, and anti-ulcerative colitis study, *Ultrason Sonochem.* 112 (2025) 107206. <https://doi.org/10.1016/j.ultsonch.2024.107206>.
- [30] X.Y. Xu, Z. Wang, S. Ren, et al., Improved protective effects of American ginseng berry against acetaminophen-induced liver toxicity through TNF- $\alpha$ -mediated caspase-3/-8/-9 signaling pathways, *Phytomedicine.* 51 (2018) 128-38. <https://doi.org/10.1016/j.phymed.2018.09.234>.
- [31] M.Y. Zhao, L.Q. Wang, M.Z. Wang, et al., Targeting fibrosis, mechanisms and cilinical trials, *Signal Transduct Target Ther.* 7 (2022) 206. <https://doi.org/10.1038/s41392-022-01070-3>.
- [32] L. Li, Y.H. Liu, H. Yu, et al., Comprehensive phytochemicals analysis and anti-myocardial ischemia activity of total saponins of American ginseng berry, *J Food Biochem.* 46 (2022) e14042. <https://doi.org/10.1111/jfbc.14042>.
- [33] T.P. Li, H. Wang, W.J. Bi, et al., Nano-Characterization, Composition Analysis, and Anti-Inflammatory Activity of American-Ginseng-Derived Vesicle-like Nanoparticles, *Molecules.* 29 (2024) 3443. <https://doi.org/10.3390/molecules29153443>.
- [34] X.M. Zhang, L.W. Han, P.H. Li, et al., Region-Specific Biomarkers and Their Mechanisms in the Treatment of Lung Adenocarcinoma: A Study of *Panax quinquefolius* from Wendeng, China, *Molecules.* 26 (2021) 6829. <https://doi.org/10.3390/molecules26226829>.
- [35] Y.M. Zhang, Y.F. Liu, Y.C. Wei, et al., The role of rare ginsenosides in the prevention and treatment of liver diseases, *J Ethnopharmacol.* 355 (2026) 120666. <https://doi.org/10.1016/j.jep.2025.120666>.
- [36] S.H. Zhang, C.Q. Zhang, M.X. Li, et al., Structural elucidation of a glucan from *Crataegus pinnatifida* and its bioactivity on intestinal bacteria strains, *Int J Biol Macromol.* 128 (2019) 435-43. <https://doi.org/10.1016/j.ijbiomac.2019.01.158>.
- [37] P.P. Wang, W.F. Liao, J.P. Fang, et al., A glucan isolated from flowers of *Lonicera japonica* Thunb. inhibits aggregation and neurotoxicity of A $\beta$ <sub>42</sub>, *Carbohydr Polym.* 110 (2014) 142-7. <https://doi.org/10.1016/j.carbpol.2014.03.060>.

- [38] A.N. Zhang, S.Q. Gao, C.H. Shen, et al., Bupleuri Radix polysaccharides enhance the efficacy and intestinal absorption of baicalin *via* regulating intestinal  $\beta$ -glucuronidase activity in MASH mice, *Phytomedicine*. 145 (2025) 157092. <https://doi.org/10.1016/j.phymed.2025.157092>.
- [39] P.P. Wang, L. Zhang, J. Yao, et al., An arabinogalactan from flowers of *Panax notoginseng* inhibits angiogenesis by BMP2/Smad/Id1 signaling, *Carbohydr Polym.* 121 (2015) 328-35. <https://doi.org/10.1016/j.carbpol.2014.11.073>.
- [40] H. Zhang, C.C. Li, P.F.H. Lai, et al., Fractionation, chemical characterization and immunostimulatory activity of  $\beta$ -glucan and galactoglucan from *Russula vinosa* Lindblad, *Carbohydr Polym.* 256 (2021) 117559. <https://doi.org/10.1016/j.carbpol.2020.117559>.
- [41] Z. Huang, Y.J. Zeng, X. Chen, et al., A novel polysaccharide from the roots of *Millettia Speciosa* Champ: preparation, structural characterization and immunomodulatory activity, *Int J Biol Macromol.* 145 (2020) 547-57. <https://doi.org/10.1016/j.ijbiomac.2019.12.166>.
- [42] X.B. Xu, Z.X. Gu, S. Liu, et al., Purification and characterization of a glucan from *Bacillus Calmette Guerin* and the antitumor activity of its sulfated derivative, *Carbohydr Polym.* 128 (2015) 138-46. <https://doi.org/10.1016/j.carbpol.2015.04.025>.
- [43] W.B. Neely, Infrared spectra of carbohydrates, *Adv Carbohydr Chem.* 12 (1957) 13-33. [https://doi.org/10.1016/S0096-5332\(08\)60203-9](https://doi.org/10.1016/S0096-5332(08)60203-9).
- [44] J.W. Lu, Y.X. Wang, X.J. Feng, et al., Structural characterization and immune-modulating properties of a novel  $\beta$ -galactomannan extracted from black soybean hulls, *Carbohydr Polym.* 358 (2025) 123568. <https://doi.org/10.1016/j.carbpol.2025.123568>.
- [45] X.Z. Wan, C. Ai, Y.H. Chen, et al., Physicochemical Characterization of a Polysaccharide from Green Microalga *Chlorella pyrenoidosa* and Its Hypolipidemic Activity via Gut Microbiota Regulation in Rats, *J Agric Food Chem.* 68 (2020) 1186-97. <https://doi.org/10.1021/acs.jafc.9b06282>.
- [46] E.R. Suárez, J.A. Kralovec, M.D. Nosedá, et al., Isolation, characterization and structural determination of a unique type of arabinogalactan from an immunostimulatory extract of *Chlorella pyrenoidosa*, *Carbohydr Res.* 340 (2005) 1489-98. <https://doi.org/10.1016/j.carres.2005.04.003>.
- [47] N.R. Gough, X.Y. Xiang, L. Mishra, TGF- $\beta$  Signaling in Liver, Pancreas, and Gastrointestinal Diseases and Cancer, *Gastroenterology*. 161 (2021) 434-52.e15. <https://doi.org/10.1053/j.gastro.2021.04.064>.
- [48] P. Strnad, G.Z. Tao, Q. Zhou, et al., Keratin mutation predisposes to mouse liver fibrosis and unmasks differential effects of the carbon tetrachloride and thioacetamide models, *Gastroenterology*. 134 (2008) 1169-79. <https://doi.org/10.1053/j.gastro.2008.01.035>.
- [49] Y.S. Lee, E. Seki, In Vivo and In Vitro Models to Study Liver Fibrosis: Mechanisms and Limitations, *Cell Mol Gastroenterol Hepatol.* 16 (2023) 355-67. <https://doi.org/10.1016/j.jcmgh.2023.05.010>.
- [50] Z.M. Li, M. Ni, H.Y. Yu, et al., Gut Microbiota and Liver Fibrosis: One Potential Biomarker for Predicting Liver Fibrosis, *Biomed Res Int.* 2020 (2020) 3905130. <https://doi.org/10.1155/2020/3905130>.
- [51] N.R. Shin, T.W. Whon, J.W. Bae, *Proteobacteria*: microbial signature of dysbiosis in gut microbiota, *Trends Biotechnol.* 33 (2015) 496-503. <https://doi.org/10.1016/j.tibtech.2015.06.011>.
- [52] H. Plovier, A. Everard, C. Druart, et al., A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice, *Nat Med.* 23 (2017) 107-13. <https://doi.org/10.1038/nm.4236>.
- [53] N. Oguri, J. Miyoshi, Y. Nishinarita, et al., *Akkermansia muciniphila* in the small intestine improves liver fibrosis in a murine liver cirrhosis model, *NPJ Biofilms Microbiomes.* 10 (2024) 81. <https://doi.org/10.1038/s41522-024-00564-y>.
- [54] A. Acevedo-Román, N. Pagán-Zayas, L.I. Velázquez-Rivera, et al., Insights into Gut Dysbiosis: Inflammatory Diseases, Obesity, and Restoration Approaches, *Int J Mol Sci.* 25 (2024) 9715. <https://doi.org/10.3390/ijms25179715>.
- [55] J. Li, H. Wei, Y. Zhang, et al., PM<sub>2.5</sub> exacerbates nasal epithelial barrier damage in allergic rhinitis mice: A crosstalk between gut microbiota and NLRP3 inflammasome, *Ecotoxicol Environ Saf.* 295 (2025) 118140. <https://doi.org/10.1016/j.ecoenv.2025.118140>.
- [56] C. Cui, L. Fang, L. Li, et al., *Odoribacter splanchnicus* rescues aging-related intestinal P-glycoprotein damage via GDP-L-fucose secretion, *Nat Commun.* 16 (2025) 10665. <https://doi.org/10.1038/s41467-025-65692-1>.
- [57] K.A. Fitzgerald, J.C. Kagan, Toll-like Receptors and the Control of Immunity, *Cell.* 180 (2020) 1044-66.

<https://doi.org/10.1016/j.cell.2020.02.041>.

- [58] P. Kong, Z.Y. Cui, X.F. Huang, et al., Inflammation and atherosclerosis: signaling pathways and therapeutic intervention, *Signal Transduct Target Ther.* 7 (2022) 131. <https://doi.org/10.1038/s41392-022-00955-7>.
- [59] Q. Yuan, W. Liu, W. Hao, et al., Glycosidic linkages of fungus polysaccharides influence the anti-inflammatory activity in mice, *J Adv Res.* 67 (2025) 161-72. <https://doi.org/10.1016/j.jare.2024.01.037>.
- [60] X.P. Liu, Q.Y. Wang, J. Wang, et al., Structural characterization, chain conformation and immunomodulatory activity of a heteropolysaccharide from *Inonotus hispidus*, *Int J Biol Macromol.* 260 (2024) 129187. <https://doi.org/10.1016/j.ijbiomac.2023.129187>.
- [61] J.J. Dong, Y.X. Bai, Q. Wang, et al., Insights into the Structure-Function Relationship of GH70 GtfB  $\alpha$ -Glucanotransferases from the Crystal Structure and Molecular Dynamic Simulation of a Newly Characterized *Limosilactobacillus reuteri* N1 GtfB Enzyme, *J Agric Food Chem.* 72 (2024) 5391-402. <https://doi.org/10.1021/acs.jafc.4c00104>.
- [62] M.Z. Li, J.J. Wen, X.J. Huang, et al., Interaction between polysaccharides and toll-like receptor 4: Primary structural role, immune balance perspective, and 3D interaction model hypothesis, *Food Chem.* 374 (2022) 131586. <https://doi.org/10.1016/j.foodchem.2021.131586>.
- [63] Q. Wang, X.J. Sheng, A. Shi, et al.,  $\beta$ -Glucans: Relationships between Modification, Conformation and Functional Activities, *Molecules.* 22 (2017) 257. <https://doi.org/10.3390/molecules22020257>.
- [64] R. Huo, Wuhanqimuge, M. Zhang, et al., Molecular dynamics modeling of different conformations of beta-glucan, molecular docking with dectin-1, and the effects on macrophages, *Int J Biol Macromol.* 293 (2025) 139382. <https://doi.org/10.1016/j.ijbiomac.2024.139382>.
- [65] Z. Qi, T.X. Gao, J.J. Li, et al., Structural characterization and prebiotic potential of polysaccharides from *Polygonatum sibiricum*, *Food Sci Hum Wellness.* 13 (2024) 2208-20. <https://doi.org/https://doi.org/10.26599/FSHW.2022.9250184>.
- [66] W.F. Liao, D.X. Cao, Y. Wang, et al., 1,3-and 1,4-linked polysaccharides uptake in intestinal cells relies on clathrin/dynamin 1/Rab5-dependent endocytosis, *Nat Commun.* 17 (2026) 1831. <https://doi.org/10.1038/s41467-026-68542-w>.