Heliyon 10 (2024) e34591

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

New compatible pair of TCM: Paeoniae Radix Alba effectively alleviate Psoraleae Fructus-induced liver injury by suppressing NLRP3 inflammasome activation

Yingjie Xu [a,b,c,1], Xianling Wang [a,b,1], Yan Wang [a,b,1], Longxin Guo [a,b,c], Xiaomei Zhao [a,b], Ming Dong [a,b], Jincai Wen [a,b], Zhixin Wu [a,b], Chenyi Li [a,b,c], Wenqing Mu [a,b], Yuming Guo [e], Zhaofang Bai [a,b,c,d,*], Xiaohe Xiao [a,b,c,d,**]

[a] Senior Department of Hepatology, The Fifth Medical Center of PLA General Hospital, Beijing, 100039, China
[b] China Military Institute of Chinese Materia, The Fifth Medical Centre, Chinese PLA General Hospital, Beijing, 100039, China
[c] School of Pharmacy, Dali University, Dali, 671000, China
[d] National Key Laboratory of Kidney Diseases, Beijing, 100039, China
[e] Department of Infectious Diseases, The Fifth Medical Center of Chinese PLA General Hospital, National Clinical Research Center for Infectious Diseases, Beijing, 100039, China

ARTICLE INFO

Keywords:
Psoraleae Fructus
Paeoniae Radix Alba
NLRP3 inflammasome
Drug-induced liver injury
Detoxification by compatibility

ABSTRACT

Objective: Drug-induced liver injury (DILI), a type of acute inflammation, has sparked significant concern owing to its unpredictability and severity. Psoraleae Fructus (PF), an edible Chinese herb widely used in traditional Chinese medicine (TCM), causes liver injury. Therefore, the elucidation of the mechanism underlying PF-induced liver injury and the search for more effective means of detoxification using herbal compatibility has become an urgent issue. This study evaluated the hepatoprotective effects of Paeoniae Radix Alba (PRA), a hepatoprotective Chinese medicine, on PF-induced liver injury and explored the underlying mechanisms.

Methods: A rat model of lipopolysaccharide (LPS)-induced immune stress was established to evaluate the hepatotoxicity of PF and the detoxifying effect of PRA. Subsequently, inflammatory pathways were identified using network pharmacology. Finally, the molecular mechanism by which PRA alleviates PF-induced liver injury was validated using an inflammasome activation model in bone marrow-derived macrophages (BMDMs).

Results: In vivo, hepatocytes in rats treated with LPS + PF exhibited massive inflammatory infiltration and apoptosis, and the expression of liver injury indicators and inflammatory factors was significantly upregulated, which was reversed by PRA pretreatment. Network pharmacology showed that PRA alleviated PF-induced liver injury and was associated with the NOD-like receptor signaling pathway. Moreover, PF directly induced inflammasome activation in LPS-primed BMDMs which in turn induced caspase-1 activation and the secretion of downstream effector cytokines such as IL-1β. PRA pretreatment inhibited PF-induced activation of the NLRP3 inflammasome by mitigating the accumulation of mitochondrial reactive oxygen species (mtROS).

* Corresponding author. Senior Department of Hepatology, The Fifth Medical Center of PLA General Hospital, Beijing, 100039, China.
** Corresponding author. Senior Department of Hepatology, The Fifth Medical Center of PLA General Hospital, Beijing, 100039, China.
E-mail addresses: baizf2008@hotmail.com (Z. Bai), pharmacy_302@126.com (X. Xiao).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.heliyon.2024.e34591
Received 25 March 2024; Received in revised form 11 July 2024; Accepted 12 July 2024
Available online 14 July 2024

Conclusions: The present study demonstrates that PRA alleviated PF induced-liver injury by inhibiting NLRP3 inflammasome activation. The results of this study are expected to inform the prevention and control of PF-induced hepatotoxicity in clinical practice.

1. Introduction

Drug-induced liver injury (DILI) is the result of acute or chronic overdosing of drugs or herbal medicines and is a major cause of acute liver failure [1,2]. Traditional Chinese medicines (TCMs) are generally considered non-toxic [3]; however, recent research has revealed that some non-toxic herbs and dietary supplements, such as Psoraleae Fructus (PF), Densefruit Pittany Root-bark, Radix Bupleuri, and Szechwan Chinaberry Fruit, may potentially induce DILI, particularly when administered at high doses or over an extended period of time [4–6]. The National Center for Adverse Reactions (NCAR) has reported multiple cases of hepatic injury caused by TCM preparations containing PF, such as Zhuang Guan Jie Wan (ZGW) and Xianling Baogu (XLGB) [7–9]. To explore whether PF alone can cause liver injury in a previous study on dismantling the ZGW, our group found that PF, when administered alone, induced liver injury in rats under immunological stress [10]. PF is the desiccated fruit of the legume, *Psoralea corylifolia* Linn. It is extensively used for the prevention and treatment of various diseases, including vitiligo, osteoporosis, and psoriasis [11]. However, PF has severe hepatotoxicity. Tian et al. systematically analyzed 84 cases of adverse reactions to PF between 1978 and 2016. A total of 48 patients were diagnosed with liver injury, accounting for 57.14 % of all cases, In addition, case reports of patients dying from acute liver failure and multi-organ dysfunction after PF administration have been published [12]. This restricts the application of PF in clinical settings. Therefore, the elucidation of the mechanism underlying PF-induced liver injury and the search for more effective means of detoxification by herbal compatibility are essential.

Herb compatibility is a practice that has been extensively used in TCM for the prevention and treatment of diseases [13] and involves preparing TCM concoctions that reduce the toxicity and side effects of toxic herbs [14,15]. Paeoniae Radix Alba (PRA) is known to relieve pain, calm the liver, and restrain the liver yang [16]. According to Chinese medicine theory, liver yin has nourishing and moisturizing effects and can limit excess liver yang. A balance of yin and yang can maintain the normal physiological function of the liver. In addition, it has been reported that Total Glucosides of Paeony from PRA may improve fatty liver by ameliorating mitochondrial damage and protecting mitochondrial functions [17]. In addition, PRA alleviates common three-wingnut root-induced hepatotoxicity by reducing oxidative stress [18]. In addition, paeoniflorin, an index constituent of PRA, ameliorates lipopolysaccharide (LPS)-induced acute liver injury by inhibiting mitochondrial damage and suppressing the NLRP3 inflammasome activation via SIRT3/FOXO1a/SOD1 signaling [19]. PRA plays important roles in the development of various liver diseases.

The liver and immune system function synergistically to prevent exogenous antigens from causing infections. Inflammasomes are vital innate immune sensors that enable the immune system to regulate cellular health. They are cytoplasmic multiprotein complexes comprising three components: nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), an effector cysteine-containing aspartate protein hydrolase (pro-caspase-1), and apoptosis-associated speck-like protein (ASC) [20]. The inflammasome is activated in response to various pathogen-associated molecular patterns and damage-associated molecular patterns (PAMPs and DAMPs, respectively) [21]. Several pattern recognition receptors (PRRs), including NLRP1, NLRP3, AIM2, NLRC4, and IFI16, can form inflammasomes [22], of which the NLRP3 inflammasome is the most studied. Formation of the NLRP3 inflammasome involves the recruitment of pro-caspase-1 and ASC by NLRP3. Once activated, caspase-1 cleaves pro-IL-1β and pro-IL-18, resulting in the secretion of pro-inflammatory cytokines such as IL-1β and IL-18 [23,24]. Abnormal inflammasome activation plays a crucial role in liver disease and is a key risk factor for DILI.

In this study, we assessed the therapeutic effects of PRA on PF-induced liver injury. We investigated the prevention, treatment, and pharmacological mechanisms of PRA in PF-induced liver injury using network pharmacology and verification *in vitro*. The results provide evidence and a reference for clinical protocols.

2. Methods

2.1. Analysis of constituents in PF and PRA alcohol extracts

The extracts of both herbs were dissolved and diluted with 20 % methanol, shaken well, filtered, and the samples were analyzed using a Waters Ultra High Performance Liquid Chromatograph. The results obtained were compared with those of PF and PRA composition retrieved from databases. The separation was conducted using an ACQUITY UPLC® HSS C18 1.7 μm 2.1 × 150 mm column. Acetonitrile was used as mobile phase A, and a 0.1 % phosphoric acid solution served as mobile phase B. PF was detected at 246 nm, whereas PRA was detected at 230 nm. The column temperature was maintained at 30 °C, and the flow rate was set at 0.2 mL/min.

2.2. Animal handling and experimental design

A total of 48 SD rats, aged 8 weeks and weighing 180–200 g were obtained from SPF Biotechnology Co., Ltd. (Beijing, China). All rats were randomly divided into cages and maintained at a constant temperature under a 12-h light/dark cycle. All experiments were performed in accordance with relevant guidelines and regulations. All experiments were approved by the Laboratory Animal Center of

the Fifth Medical Center of the Chinese PLA General Hospital.

For this animal experiment, we created an immunological stress model by administering LPS via tail vein injection. We selected a dose of 3.6 g kg^{-1} for PF and a dose of 3.6 g kg^{-1} for PRA based on published literature [25]. Following a 7-day adaptation period, the 48 rats were divided into six groups, each consisting of eight rats, based on their body weight. These groups included control, LPS, PF, LPS + PRA, LPS + PF (model), and LPS + PF + PRA groups. After 3 days of pretreatment with PRA and simultaneous gavage of PF and PRA on the last day, saline was administered to the control group after 3 h and LPS (2.8 mg kg^{-1}) was injected intravenously into the tail veins of the LPS, LPS + PRA, LPS + PF, and the LPS + PF + PRA groups. The control and PF groups were intravenously administered an equivalent volume of saline via the tail vein. After 8 h, the rats were anesthetized with 1 % sodium pentobarbital at a dose of 50 mg kg^{-1}. Venous blood was obtained from the rats, and the liver tissue was collected. Liver tissue was placed in frozen storage tubes containing 4 % paraformaldehyde.

Liver tissue was used for histological examination using hematoxylin-eosinstaining (HE) staining and dUTP nick-end labeling (TUNEL). ALT, AST, and ALP levels in rat serum were measured according to the manufacturer's instructions provided with the kits. RNA extraction from the liver tissue samples was performed using TRIzol reagent. Subsequently, cDNA was generated using a reverse transcription reagent. The levels of gene expression for IL-1β, TNF-α, IL-6, and IL-18 were detected in each sample. The primers were as follows:

IL-1β F:CCTCTGACAGGCAACCACTTA R:GCACTGGTCCAAATTCAATTC

TNF-α F:CTGGCGTGTTCATCCGTTCT R:GCCACTACTTCAGCGTCTCGT

IL-6 F:ACTTCCAGCCAGTTGCCTTCTTG R:TGGTCTGTTGTGGGTGGTATCCTC

IL-18 F:GGATCTTGCGTCAATTCAAGG R:TTGGCTGTCTTTTGTCAACGA

β-Actin F:GCTGTGCTATGTTGCCCTAGACTTC R:GGAACCGCTCATTGCCGATAGTG

2.3. Network pharmacology analysis

Using ETCM (http://www.tcmip.cn/ETCM/), the active chemical compounds present in Bai Shao and Bu Gu Zhi were identified. Additional key chemical ingredients were incorporated by referencing the relevant literature. Ingredients were imported into PubChem (https://pubchem.ncbi.nlm.nih.gov/) to obtain SMILE numbers. SMILES strings of the matched active compounds were imported into SwissTargetPrediction (http://swisstargetprediction.ch/) for target prediction. IDILI targets were acquired from 2 databases, GeneCards (https://www.genecards.org/) and Online Mendelian Inheritance in Man (OMIM, https://omim.org/) using the keyword "idiosyncratic drug induced liver injury". The STRING (https://cn.string-db.org/) analysis tool (metascape.org/gp/index.html#/main/step1) was used for the PPI analysis. Gene Ontology (GO) analyses and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses were performed to enrich the functions of the potential targets of PRA in the pretreatment of PF-induced liver injury using the DAVID database (https://david.ncifcrf.gov/). Finally, the data were processed, and pathway-target network maps were constructed using Cytoscape 3.9.1 (http://cytoscape.org/).

2.4. Antibodies and reagents

The PF and PRA were purchased from Shanghai Winherb Medical Technology Co., Ltd. Fetal bovine serum was purchased from VivaCell (Shanghai, China). Mouse colony-stimulating factor (MCSF; HY-P7085), Nigerian (HY-12815A) and MCC950 (HY-12815A) were obtained from MedChemExpress (MCE, USA). DMEM (PYG0073) and dimethyl sulfoxide (DMSO; PYG0040) were purchased from BOSTER (Wuhan, China). MitoSOX was purchased from Invitrogen (State of New Jersey, USA). Caspase-1 antibody (1:1000, AG-20B-0042-C100) and NLRP3 (1:2000, AG-20B-0014-C100) were acquired from AdipoGen (San Diego, CA, USA). The IL-1β antibody (1:2000, AF-401-NA) was bought from R&D. The ASC antibody (1:1000, 67834T) was obtained from CST, whereas the LaminB antibody (1:3000, 66095-1-Ig) was procured from Proteintech. DSS (ab141274) was obtained from Abcam. RT-MIX (R323-01) and QPCR MIX (Q712-02) reagents were purchased from Vazyme (Nanjing, China). Mouse IL-1β (1210122) enzyme-linked immunosorbent assay (ELISA) kits and Rat IL-1β (1310122) ELISA kits were obtained from Dakewe (China). The CytoTox 96 Non-Radioactive Cytotoxicity Assay (G1780) was obtained from Promega. ALT (C009-2-1), AST (C010-2-1), and ALP (A059-2-2) kits were purchased from Nanjing Jiancheng Biological Technology Co., Ltd. (China).

2.5. Cell culture

Bone marrow-derived macrophages (BMDMs) were obtained from the femurs of 8-week-old male SPF-grade C57 mice. BMDMs were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1 % penicillin/streptomycin and 10 % FBS, cultured in Petri dishes, shaken well, and placed in a constant-temperature CO_2 incubator. After 5 days, the differentiation of BMDMs was completed. These cells were used for further experiments.

2.6. Western blot

The supernatant was mixed with one-fourth of the total volume of trichloroacetic acid (TCA) per tube, and the protein extraction step was performed as previously described. Protein samples were separated using 10 % or 12 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis in running buffer. These membranes were blocked for 1 h, incubated with primary antibodies at 4 °C, and then incubated with secondary antibodies at room temperature as previously described [26].

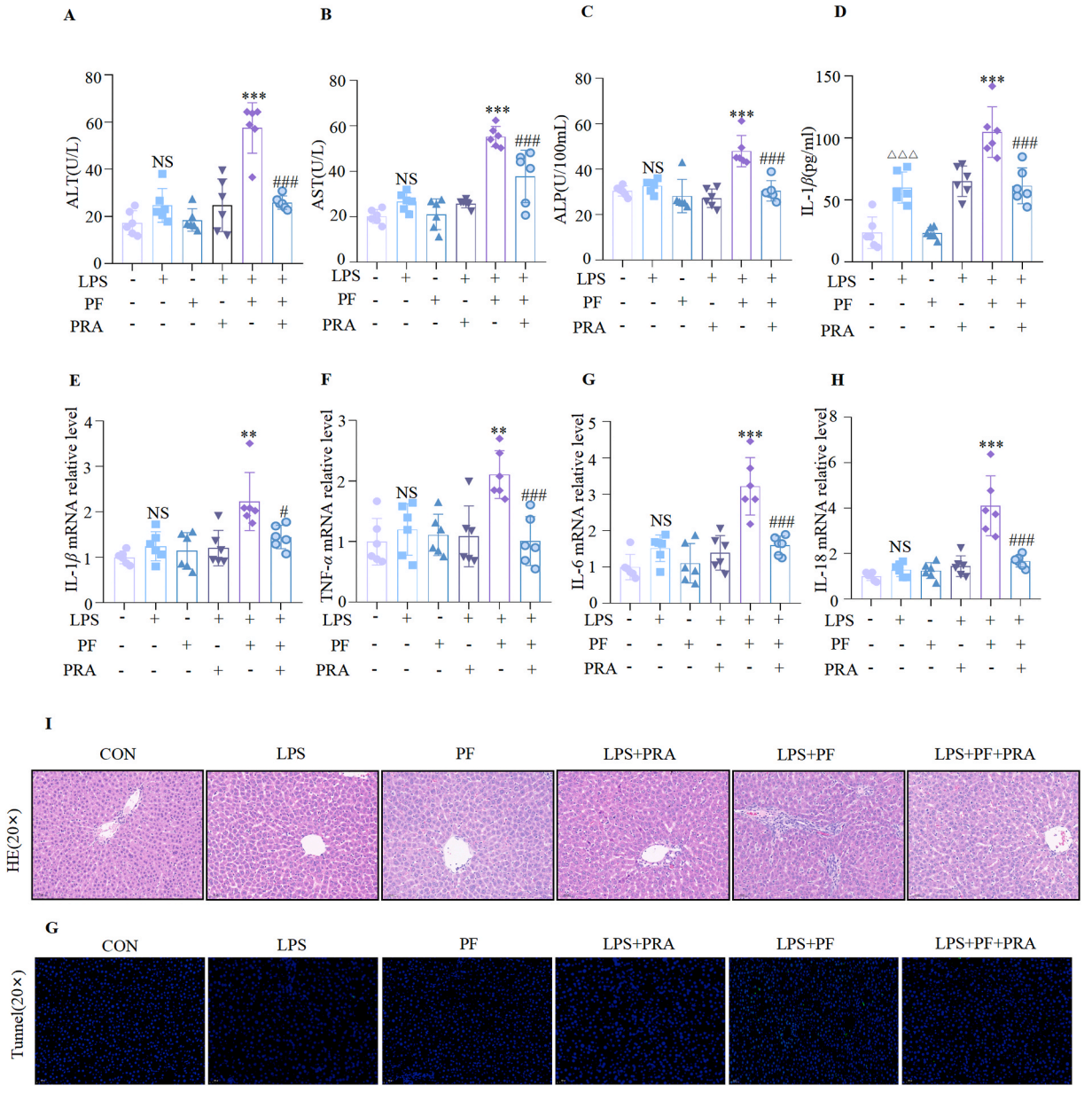


Fig. 1. PRA pretreatment reverses PF-induced liver injury in rats. PRA pre-treatment was administered for 3 days, followed by a treatment with PF and PRA on the last day. After 3 h, 2.8 mg/kg of LPS was injected into the tail vein of male SD. Serum levels of ALT (A), AST (B), ALP (C), and IL-1β (D) were measured using assay kits. Detection of IL-1β (E), TNF-α (F), IL-6 (G), and IL-18 (H) gene expression in liver tissue. (I) H&E staining was performed to observe liver injury. (G) TUNEL staining was conducted to observe apoptosis of hepatocytes. One-way ANOVA was used to compare the groups. $^{\triangle\triangle\triangle}P < 0.001$ vs. the control group. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the LPS group. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. the LPS + PF group. NS: not significant.

2.7. Lactate dehydrogenase (LDH) assay

Detection of LDH released from the cell supernatants was performed according to the instructions provided.

2.8. ELISA

IL-1β and TNF-α levels in cell supernatants and IL-1β in rat serum were determined following the ELISA kit manufacturer's instructions.

2.9. Inflammasome activation

To stimulate inflammasome activation, BMDMs were seeded in 12-well plates at a density of 1.2×10^6 cells/well and incubated overnight. The next day, the fluid was replaced, and the cells were activated by dissolving 50 ng/mL LPS in DMEM for 4 h. The cells were first exposed to PRA at concentrations of 250, 500, and 1000 μg ml^{-1} for 1 h. Subsequently, the cells were stimulated with Nigericin for 30 min or PF at a concentration of 125 μg ml^{-1} for 1.5 h. Cell status was observed, and both the supernatant and cell lysates were collected. The supernatant was transferred to an EP tube and centrifuged for 5 min at 5000 g. The supernatant was removed and stored.

2.10. ASC oligomerization assay

BMDMs were seeded in 12-well plates at a density of 1.2×10^6 cells/well. After 4 h of pretreatment with LPS, the cells were exposed to various doses of PRA (250, 500, or 1000 μg ml^{-1}) for 1 h. Subsequently, the cells were subjected to stimulation with PF at a concentration of 125 μg·ml-1 for 1.5 h. Supernatant was removed after observing the cell status and cells were lysed with Triton buffer. Subsequent procedures were performed as previously described [27].

2.11. Detection of mitochondrial reactive oxygen species (mtROS) release

The experiment was performed as described in Section 2.9. Samples were collected, stained with MitoSOX (Invitrogen) at 37 °C for 10 min, and rinsed with HBSS three times. The samples were then subjected to flow cytometry using a FACSCanto II Cell Analyzer.

2.12. Statistical analysis

Comparison of data between multiple groups was performed using one-way ANOVA with Dunnett's post hoc multiple tests, and comparisons between two groups were performed using unpaired Student's *t*-tests. Differences were plotted using Prism software (version 8.0) and were considered statistically significant at $P < 0.05$.

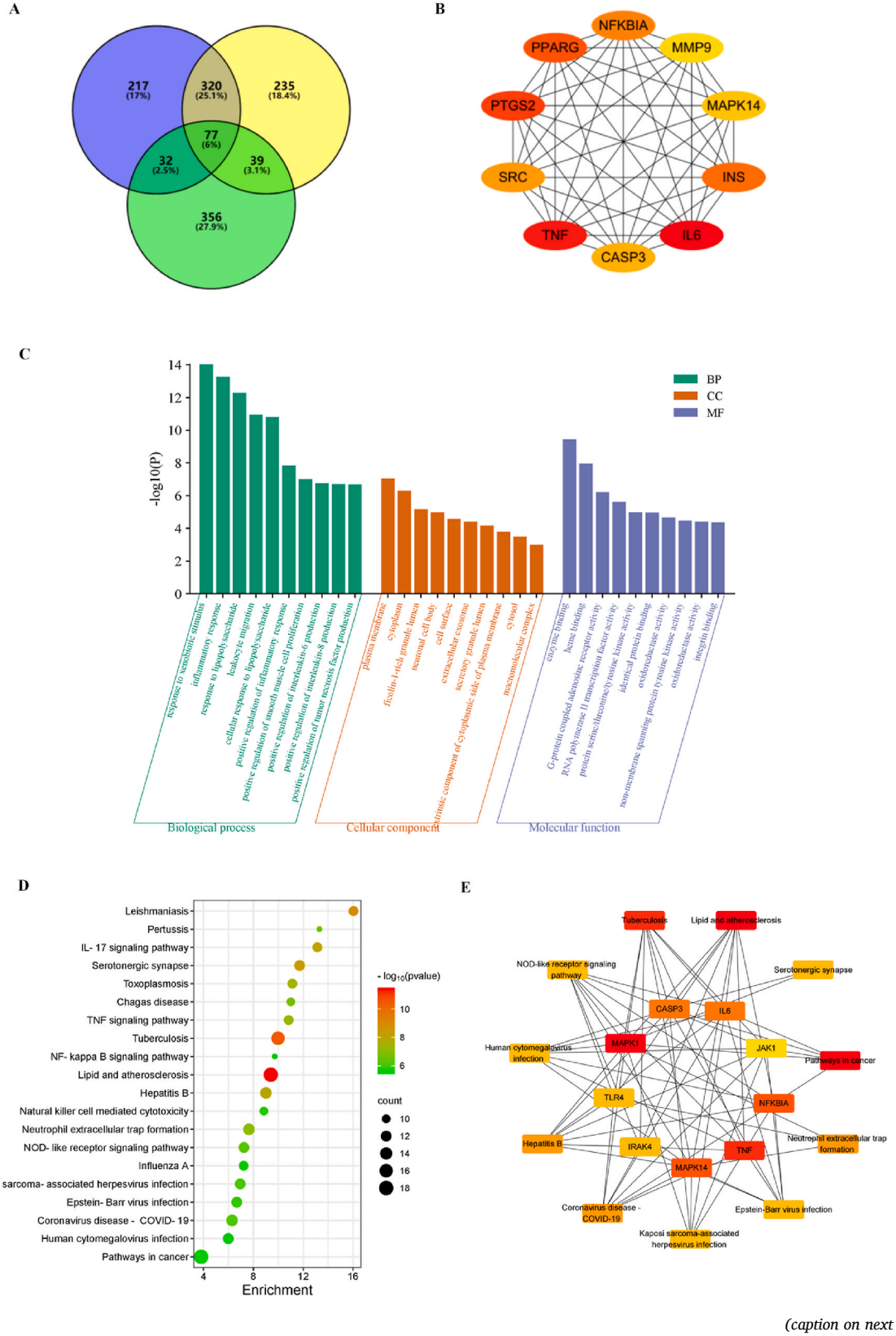
3. Results

3.1. Pretreatment of DILI rats with PRA reversed PF-induced liver injury

To investigate whether PRA had a preventive effect on PF-induced liver injury, we developed an immune stress rat model and pretreated the rats with PRA for 3 days. On the last day, the rats were treated with PRA + PF. Next, LPS was intravenously administered to the rats through the caudal vein. Blood serum ALT (U/L), AST (U/L), and ALP (U/100 mL) levels were measured and used as indicators of the extent of liver injury [28]. As shown in Fig. 1A–C, the results demonstrated that there were no increases in the levels of these indicators in the LPS and PF groups, suggesting that neither LPS, PF, nor the LPS + PF administration were sufficient to cause liver injury. However, the rats that received the combination of LPS and PF had significantly increased serum ALT, AST, and ALP levels, suggesting that PF caused liver injury in rats under LPS-induced immunological stress. Pretreatment of Rat with PRA significantly reduced serum levels of ALT, AST, and ALP, suggesting that PRA alleviated PF-induced liver injury.

Subsequently, we examined the levels of several inflammatory factors in rat sera. As shown in Fig. 1D–a notable increase in IL-1β levels in the serum of the rats in the LPS group compared to the control group was observed. This observation indicated that the administration of LPS alone led to an increase in systemic IL-1β levels. Furthermore, the levels of IL-1β were significantly higher in the LPS + PF group compared to the LPS group. However, the administration of PRA in conjunction with PF resulted in a significant reduction in the serum levels of IL-1β compared to the LPS + PF group. After dissecting and homogenizing the liver tissues, the mRNA expression levels of inflammatory factors were examined by qPCR. As shown in Fig. 1E–H, the mRNA expression levels of IL-1β, TNF-α, IL-6, and IL-18 were notably elevated in the LPS + PF group compared to the LPS group. Coincidentally, this inflammation was ameliorated by PRA.

Histological changes in the liver tissue were observed under a microscope after HE and TUNEL staining. As shown in Fig. 1I and G, the liver tissue of rats in the control group contained hepatocytes with normal structures. The hepatocytes in the liver tissue extracted from rats in the LPS, PF, and LPS + PRA groups did not exhibit clear pathological changes or apoptosis. However, in the LPS + PF group, extensive inflammatory infiltration into the tissue was observed, and the hepatocytes suffered significant focal necrosis and apoptosis in the liver parenchyma. These pathological changes were significantly ameliorated by pretreatment with PRA. Taken



(caption on next page)

Fig. 2. Network pharmacology analyses of PRA treatment in PF induced-liver injury. (A) Venn diagram of the active ingredients of PRA, toxic ingredients of PF, and idiosyncratic drug-induced liver injury (IDILI) targets. Blue circles indicate targets of PF, yellow circles indicate targets of PRA, and green circles indicate targets of IDILI. (B) Protein-protein interaction (PPI) network of crossover targets. The node represents the target protein and the line represents the interaction. (C–D) GO terms and KEGG pathway enrichment. (E) The inclusion of PRA treatment in PF induced-liver injury pathway-target network. The top 10 terms in all categories of GO and significant changes in the top 20 pathways based on $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

together, these results demonstrate that PRA prevents PF-induced liver injury.

3.2. Network pharmacology-based analysis

To investigate the mechanism of action of PRA in ameliorating PF-induced liver injury, we conducted a network pharmacology analysis. The ETCM and related literature were used to obtain 24 PRA-active compounds and 34 PF-toxic compounds (detailed compound information is provided in Table S1). Using two databases, GeneCards and OMIM, we identified 65 and 430 confirmed and potential IDILI targets, respectively. The intersection of active ingredients and disease targets was visualized using a Venn diagram (Fig. 2A), which revealed 77 common targets. To further explore the mechanism of PRA in the pretreatment of PF-induced DILI at the protein level, we constructed a PPI network model with the 77 common targets, The top 10 key targets were screened using the McCreight algorithm. Inflammation-related proteins, such as TNF and IL-6, were the key targets (Fig. 2B). GO analysis was performed to enrich the functions of the PRA-PF-IDILI target network, and the top 10 Biological Process (BP), molecular function (MF), and CellularComponent (CC) terms are displayed using bubble charts (Fig. 2C). Following KEGG pathway analysis, we identified 119 signaling pathways (the bubble chart of the top 20 is shown in Fig. 2D). As shown in Fig. 2E, the pathway–target network demonstrated that MAKP1, MAKP14, IL-6, NFKB1, and JAK1 were the top five key targets in terms of degree value, and the NOD-like receptor signaling pathway interacted with most targets, excluding disease pathways.

3.3. PF activated the NLRP3 inflammasome in LPS-primed BMDMs

As shown in Fig. 3A, Ultra-high performance liquid chromatography (UHPLC) showed that Psoralen, Isopsoralen, Neobavaisoflavone, Bavachin, Corylin, Psoralidin, Isobavachalcone, Bavachinin A, and Bakuchiol were present in the alcoholic extract of PF. Multiple studies have shown that inflammasomes play a role in the development of liver injury [29–31]. To investigate whether PF-induced liver injury resulted from inflammasome activation, we pretreated BMDMs with LPS for 4 h and then treated them with PF for 1.5 h. As shown in Fig. 3B, caspase-1 p20 and IL-1β p17 protein expression levels in the supernatant increased in a dose-dependent manner after the treatment of in BMDMs with PF. In addition, the levels of secreted IL-1β (Fig. 3C) and LDH (Fig. 3D) released from the BMDMs were considerably higher following treatment with PF. Next, we investigated whether PF triggered the secretion of IL-1β and maturation of caspase-1 by activating the NLRP3 inflammasome. To accomplish this, we pretreated the BMDMs with MCC950, a specific inhibitor of the NLRP3 inflammasome, for 1 h and then measured caspase-1 and IL-1β protein expression, as well as the levels of IL-1β and LDH in supernatant. As shown in Fig. 3E, MCC950 effectively suppressed PF-induced caspase-1 activation as well as the secretion of IL-1β and the release of LDH (Fig. 3F and G). These results demonstrated that PF triggered the activation of the NLRP3 inflammasome.

3.4. PRA inhibited the activation of the NLRP3 inflammasome in LPS-primed BMDMs

As shown in Fig. 4A, UHPLC showed that Gallic acid, Protocatechuicaldehyde, (+)-Catechin, Alibiflorin, Paeoniflorin and 1,2,3,4,6-*O*-Pentagalloylglucose were present in the alcoholic extract of PRA. NLRP3 inflammasome activation can be triggered by multiple external stimuli, including nigericin, ATP, Poly(I:C), and SiO_2 [32]. Nigericin bacteriocins trigger the assembly and activation of the NLRP3 inflammasome by increasing intracellular K^+ efflux. ATP leads to pore formation or altered cell membrane permeability through activation of the P2X7 receptor, a calcium-permeable ion channel, which activate the NLRP3 inflammasome. SiO_2 crystals induce intracellular oxidative stress and increase ROS production, which activates the NLRP3 inflammasome [33]. It has been reported transfection of the dsRNA analogue poly (I:C) activates the NLRP3 inflammasome via a pathway requiring endosomal acidification [34]. To investigate the impact of PRA on the activation of the NLRP3 inflammasome, we assessed the ability of PRA to inhibit caspase-1 activity and the secretion of IL-1β. We pretreated LPS-primed BMDMs with PRA and stimulated them with nigericin to trigger NLRP3 inflammasome activation. As shown in Fig. 4B, PRA effectively inhibited caspase-1 maturation in the supernatant, indicating that PRA suppresses nigericin-induced NLRP3 inflammasome activation. Moreover, PRA suppressed the secretion of IL-1β and LDH release (Fig. 4C and D). To further investigate whether PRA inhibited other stimuli inducing NLRP3 inflammasome activation, we stimulated LPS-primed BMDMs with NLRP3 inflammasome activators and observed that PRA suppressed ATP, SiO_2, and poly(I:C)-stimulated maturation of caspase-1 (p20) and reduced IL-1β secretion (Fig. 4E and F).

3.5. PRA inhibited PF-induced inflammasome activation by inhibiting mtROS accumulation

As shown in Fig. 5A and B, administration of PF alone in LPS-primed BMDMs led to an increase in the expression of caspase-1 p20 protein and the secretion of IL-1β, which was reversed by pretreatment with PRA (250, 500, and 1000 μg mL^{-1}). After demonstrating

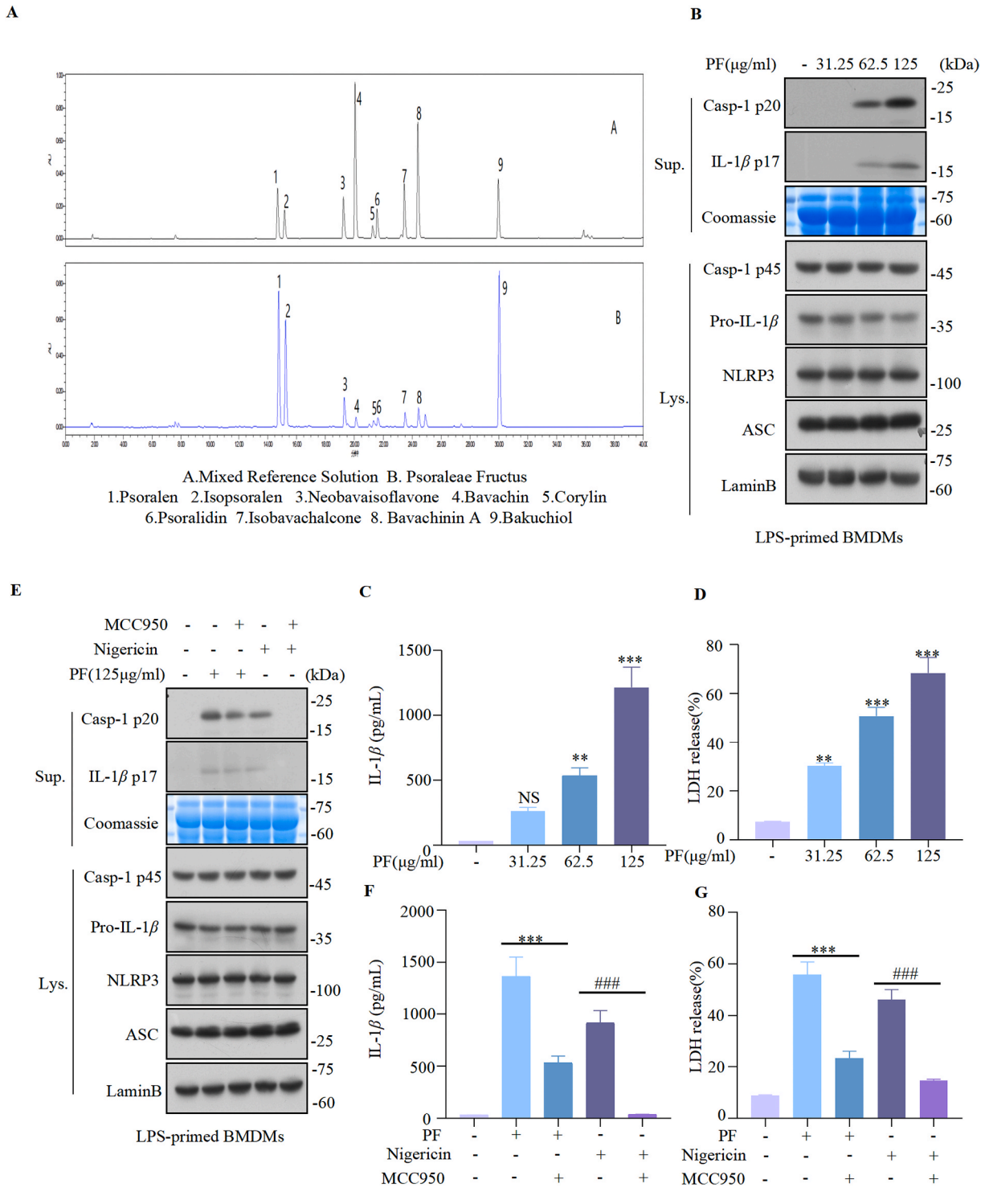
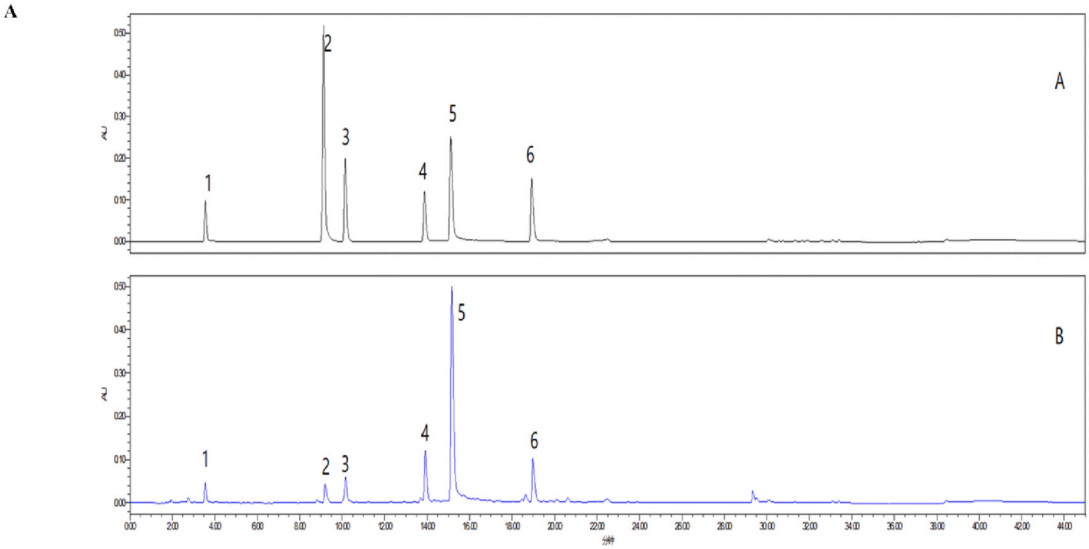
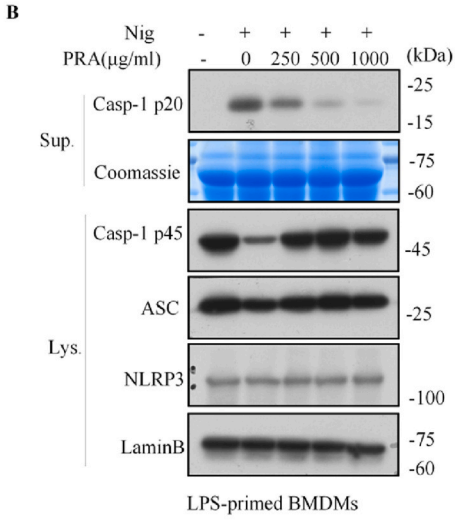


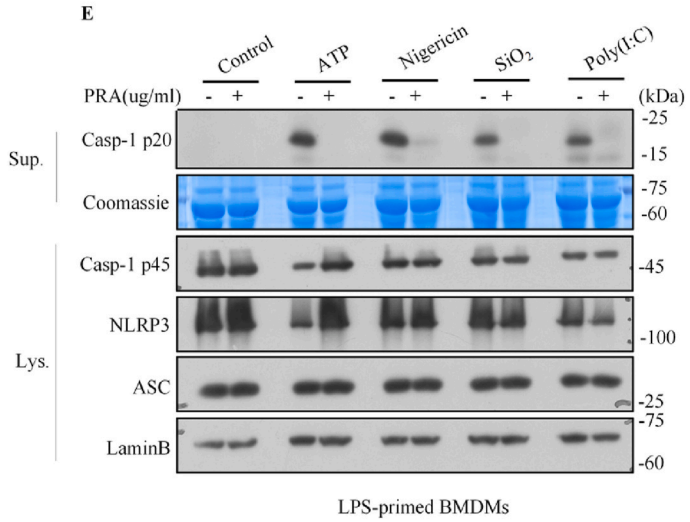
Fig. 3. PF promoted NLRP3 inflammasome activation. (A) Compositional analysis of the alcoholic extract of PF. (B–D) LPS-primed BMDMs treated with PF for 1.5 h. (B) Western blot analysis to investigate the expression levels of caspase-1 (p20) and IL-1β (p17) in the supernatant, as well as caspase-1 (p45), Pro-IL-1β (p35), NLRP3, and ASC in the lysate (C) Secretion of IL -1β and (D) LDH release in the supernatant were assessed. (E–G) MCC950 (5 μmol/L) inhibited the activation of inflammasomes induced by PF (1.5 h) in LPS-primed BMDMs. (E) Western blot analysis of caspase-1 (p20) in the supernatant and caspase-1 (p45), pro-IL-1β (p35), NLRP3 and ASC in the lysate (F) IL -1β secretion and LDH release in the supernatant were assessed (G). One-way ANOVA was used to compare the groups. The unedited images are referenced in Fig. S1 and Fig. S2. *P < 0.05, **P < 0.01, ***P < 0.001 vs. the control group. ###P < 0.001 vs. the Nigericin group.



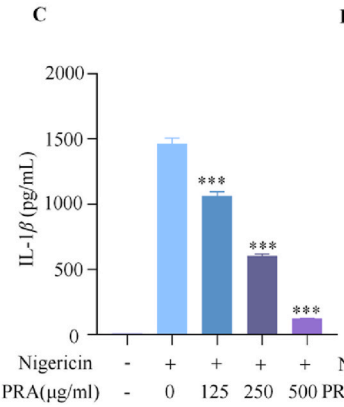
A. mixed reference solution B.Paeoniae Radix Alba
1.Gallic acid 2.Protocatechuicaldehyde 3.(+)-Catechin 4.Alibiflorin 5.Paeoniflorin
6.1,2,3,4,6-O-Pentagalloylglucose

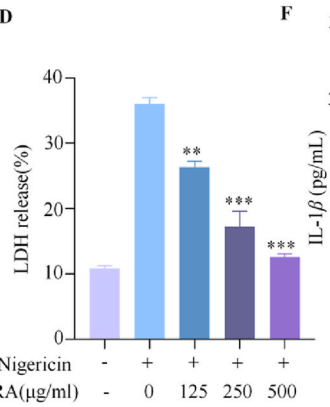


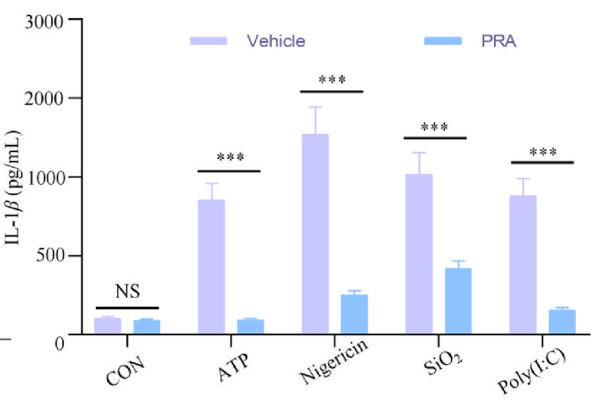
LPS-primed BMDMs



LPS-primed BMDMs







(caption on next page)

Fig. 4. PRA inhibited NLRP3 inflammasome activation in LPS-primed BMDMs. (A) Compositional analysis of the alcoholic extract of PRA. (B–D) LPS-primed BMDMs were pre-exposed to different doses of PRA and subsequently stimulated with Nigericin, (B) Western blot analysis was conducted to examine the presence of caspase-1 (p20) in the supernatant, as well as caspase-1 (p45), NLRP3, and ASC in the lysate, IL-1β secretion (C) and LDH release (D) in the supernatant were assessed. (E–F) LPS-primed BMDMs were pretreated with PRA and then stimulated with ATP, nigericin, SiO_2, and poly(I:C). (E) Western blot analysis of caspase-1 (p20) in the supernatant and caspase-1 (p45), NLRP3, and ASC in the lysate (F) IL-1β secretions in the supernatant were assessed. One-way ANOVA was used to compare the groups (C–D). An unpaired *t*-test was used to examine statistical differences (F). The unedited images are referenced in Fig. S3 and Fig. S4. *P < 0.05, **P < 0.01, ***P < 0.001. NS: not significant.

that PRA inhibited the PF-induced activation of the NLRP3 inflammasome, we investigated the mechanisms by which PRA enabled this suppression. A previous study has shown that ASC oligomerization plays an important role in inflammasome activation [35]. Fig. 5C shows that the administration of PF alone to the LPS-primed BMDMs at a dose of 125 μg L^{-1} led to a significant increase in ASC oligomerization; however, this impact was significantly suppressed by PRA. Furthermore, mtROS accumulation is a key upstream signal for inflammasome activation [36]. We observed that PF triggered the activation of the NLRP3 inflammasome by inducing the accumulation of mtROS, which was significantly inhibited by PRA (Fig. 5D and E). We then pretreated LPS-primed BMDMs with N-acetylcysteine (NAC, a ROS scavenger) to further verify whether mtROS affected PF-triggered NLRP3 inflammasome activation. The results demonstrated that PRA inhibited abnormal activation of the inflammasome by inhibiting PF-induced accumulation of mtROS.

4. Discussion

To date, no studies have identified a suitable drug to attenuate the hepatotoxicity of PF or its mechanism of action. In this study, we demonstrated that PRA could prevent or treat PF-induced liver injury in a rat model. Using network pharmacology, we found that PRA acts on the NOD-like receptor signaling pathway to alleviate PF-induced liver injury. Through *in vitro* experiments, we verified that PRA reduces the hepatotoxicity of PF by suppressing the activation of the NLRP3 inflammasome, providing valuable insights into the clinical application of PF and PRA.

Recently, there has been a notable increase in the prevalence of DILI in TCMs [37], particularly in PF administration [11]. The high prevalence of PF-induced liver injury has driven the search for a more scientific and effective means of detoxification using herbal compatibility. In the present study, we administered LPS via tail vein injection to establish a rat model of LPS-induced immunological stress. We observed that the LPS + PF group showed a significant increase in the serum expression of liver injury indicators, including ALT, AST, and ALP. Concurrently, a notable increase in the levels of inflammatory factors—e.g., IL-1β, TNF-α, IL-18 and IL-6—in the liver tissue was observed. The LPS + PF + PRA group showed reversed aberrant expression of these factors, suggesting that PRA has the potential to ameliorate PF-induced liver injury.

Network pharmacology is an interdisciplinary field that integrates systems biology and network informatics to investigate the interactions and balance within biological networks [38]. To clarify the mechanisms of action of PRA in the pretreatment of PF-induced liver injury, we used network pharmacology to analyze them. PPI network analysis comparing cross-targets revealed that TNF and IL-6 were the key targets. TNF is a pro-inflammatory cytokine with an important role in mammalian immunity and cellular homeostasis. Targeting TNF has been an effective therapeutic strategy in inflammatory diseases [39]. IL-6 is a multifunctional cytokine with well-defined proinflammatory and anti-inflammatory properties [40]. TNF and IL-6 are two key cytokines in the inflammatory response and are important targets for the treatment of inflammatory diseases. PRA alleviated PF-induced liver injury, which was possibly related to inflammation. Moreover, based on KEGG analysis and construction of a "pathway-target" network, we concluded that PRA may pretreat PF-induced liver injury by affecting the NOD-like receptor signaling pathway. The key targets of PRA enriched in the NOD-like receptor signaling pathway to attenuate PF-induced liver injury were mainly sub-signaling pathways, such as the NLRP3 inflammasome signaling pathway and the NOD/NF-KB signaling pathway. Inflammasome activation is one of the mechanisms through which the immune system induces inflammatory responses [41]. In particular, inflammasome activation is closely associated with DILI development [42]. In the present study, we developed an *in vitro* model of inflammasome activation in BMDMs. After exposing the cells to LPS and PF, caspase-1 activity, as well as the secretion of IL-1β and LDH release, all increased relative to the control group, indicating that the combination of LPS and PF induced inflammasome activation.

We observed that MCC950, an NLRP3 inflammasome inhibitor, effectively suppressed PF-induced inflammasome activation *in vitro*, suggesting that PF activates the NLRP3 inflammasome [43]. Subsequently, we demonstrated that PRA inhibited multiple stimulus-induced activation of the NLRP3 inflammasome. Finally, we found that PRA inhibited PF-induced activation of the NLRP3 inflammasome, including the maturation of caspase-1, as well as the secretion of IL-1β and release of LDH, in a dose-dependent manner. ASC oligomerization is a crucial step in inflammasome activation [44]. As the dose of PRA increased, the formation of oligomers, dimers, and multimers of ASC decreased, indicating that PRA inhibited PF-induced activation of the NLRP3 inflammasome by blocking ASC oligomerization. The production of mtROS is another important mechanism that promotes inflammasome activation [45]. Our results demonstrate that co-treatment with the ROS scavengers NAC and PF + PRA suppressed the accumulation of mtROS when the inflammasome was activated. Notably, there was no significant difference in the effect between the PF + PRA and PF + PRA + NAC groups, suggesting a redundancy in the effect arising from the simultaneous inhibition of PF-induced inflammasome activation by PRA and NAC. The results indicated that PRA effectively mitigated the accumulation of mtROS, highlighting the importance of mtROS accumulation in PF-induced inflammasome activation, and that PRA could play a preventive or therapeutic role by inhibiting this accumulation.

This study has some limitations. The ability of PRA to attenuate the hepatotoxicity of PF has only been confirmed *in vitro* and *in vivo* in rats, and there are no relevant clinical studies. In addition, this study explored the mechanism by which PRA attenuates PF

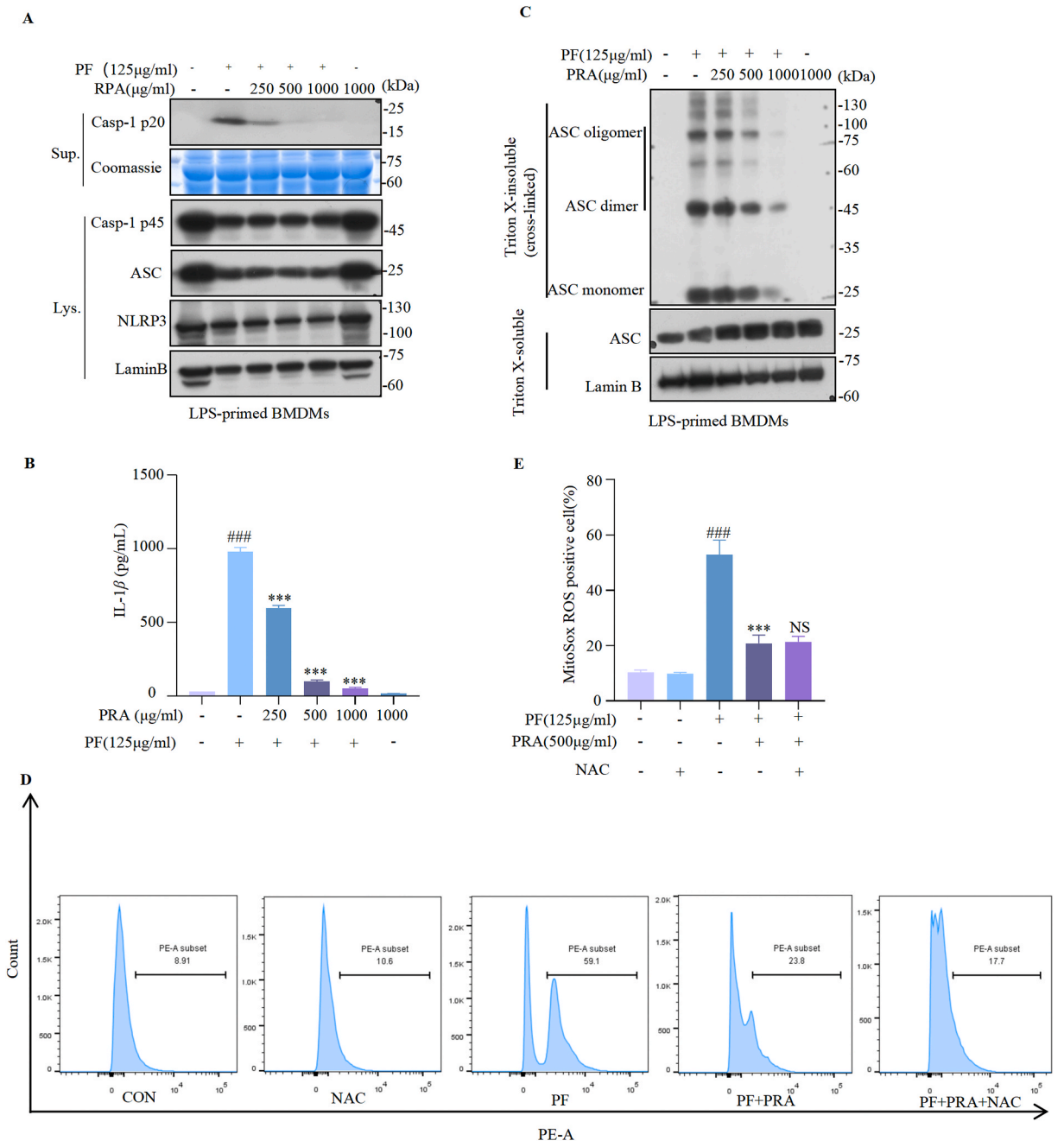


Fig. 5. PRA blocks PF-induced inflammasome activation by inhibiting mtROS accumulation. (A–C) LPS-primed BMDMs were pretreated with various doses of PRA and then stimulated with PF, (A) Western blot analysis of caspase-1 (p20) in the supernatant and pro-caspase-1 (p45), NLRP3. and ASC in the lysate, (B) Secretion of IL-1β. (C) Western blot analysis was used to detect cross-linked ASC in the Triton X-insoluble pellet. (D–E) Percentage of ROS-positive cells in LPS-primed BMDMs pretreated with PRA and NAC, stimulated with PF, and stained with MitoSox. One-way ANOVA was used to compare the groups. The unedited images are referenced in Fig. S5 and Fig. S6. $^{###}P < 0.001$ vs. the control group. ***P < 0.001 vs. the PF group. NS: not significant vs. the PF + PRA group.

hepatotoxicity; future studies should focus on the interaction of the components of the two Chinese medicines *in vivo* and *ex vivo* to reduce toxicity, which can provide a theoretical basis for the prevention of PF-induced liver injury. The results of this study may guide clinicians to consider the addition of PRA to reduce the risk of potential hepatotoxicity when prescribing PF-containing medicines. In addition, this study may provide a reference for developing or updating guidelines for the use of herbal preparations containing PF.

5. Conclusion

In conclusion, we demonstrated that PRA ameliorated PF-induced DILI caused by PF both *in vivo* by suppressing the activation of the NLRP3 inflammasome by inhibiting the accumulation of mtROS. These results not only highlight the potential of PRA in expanding the clinical applications of PF that are currently limited by its hepatotoxicity, but also provide a reference for the enhancing the safety of "toxic" TCMs.

Ethics approval and consent to participate

All animal experiments were conducted in accordance with ethical guidelines and approved by the Laboratory Animal Ethics Committee of Laboratory Animal Center of the Fifth Medical Centre, Chinese PLA General Hospital (Ethics IACUC-2023-0008).

Consent for publication

Not applicable.

Funding

This work was supported by project of the Natural Science Foundation of Beijing, China(Grant No. 7232321), the Heritage Innovation Team Project of the State Administration of Traditional Chinese Medicine (ZYYCXTD-C-202005), the project of National Natural Science Foundation of China (82230118).

Data availability

The data generated during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Yingjie Xu: Writing – original draft, Software, Methodology, Data curation. **Xianling Wang:** Project administration, Investigation, Formal analysis. **Yan Wang:** Validation, Supervision, Project administration. **Longxin Guo:** Visualization, Project administration, Investigation. **Xiaomei Zhao:** Visualization, Project administration, Investigation. **Ming Dong:** Resources, Methodology, Conceptualization. **Jincai Wen:** Validation, Supervision, Resources. **Zhixin Wu:** Project administration, Methodology, Formal analysis. **Chenyi Li:** Validation, Supervision, Methodology. **Wenqing Mu:** Validation, Methodology, Data curation. **Yuming Guo:** Visualization, Investigation, Formal analysis, Conceptualization. **Zhaofang Bai:** Writing – review & editing, Project administration, Funding acquisition, Data curation. **Xiaohe Xiao:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

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

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34591.

References

[1] N.E. Helal, M. Elkadeem, S.M. Elbastawesy, et al., Drug induced liver injury: causative agents and predictors for the outcome - a retrospective study at Tanta University Hospital, Egypt, Eur. Rev. Med. Pharmacol. Sci. 27 (2) (2023) 560–569.
[2] X. Yang, R.F. Tu, J.H. Yang, [Clinical characteristics and prognosis analysis of 498 cases with drug-induced liver injury], Zhonghua Gan Zang Bing Za Zhi 30 (7) (2022) 735–740.
[3] Y. Shi, R. Tang, F. Luo, et al., The diagnosis and management of allergic reactions caused by Chinese materia medica, Clin. Rev. Allergy Immunol. 62 (1) (2022) 103–122.
[4] N. Todorović Vukotić, J. Đorđević, S. Pejić, et al., Antidepressants- and antipsychotics-induced hepatotoxicity, Arch. Toxicol. 95 (3) (2021) 767–789.
[5] R. Teschke, A. Eickhoff, J. Schulze, et al., Herb-induced liver injury (HILI) with 12,068 worldwide cases published with causality assessments by Roussel Uclaf Causality Assessment Method (RUCAM): an overview, Transl Gastroenterol Hepatol 6 (2021) 51.

[6] R. Teschke, L. Zhang, H. Long, et al., Traditional Chinese Medicine and herbal hepatotoxicity: a tabular compilation of reported cases, Ann. Hepatol. 14 (1) (2015) 7–19.
[7] Y. Cheng, Y. Liu, H. Wang, et al., A 26-week repeated dose toxicity study of Xian-ling-gu-bao in Sprague-Dawley rats, J. Ethnopharmacol. 145 (1) (2013) 85–93.
[8] J.F. Tang, X.Y. Wang, Q. Wen, et al., Idiosyncratic hepatotoxicity evaluation of Zhuangguguanjie wan mediated by immune stress, Yaoxue Xuebao 52 (2017) 1033–1040.
[9] F.L. Ge, M. Niu, Z.X. Han, et al., Landscape of hepatobiliary adverse drug reactions related to preparations containing Psoraleae Fructus and its application in pharmacovigilance, Chin. J. Integr. Med. 27 (11) (2021) 832–837.
[10] Y. Gao, Z. Wang, J. Tang, et al., New incompatible pair of TCM: epimedii Folium combined with Psoraleae Fructus induces idiosyncratic hepatotoxicity under immunological stress conditions, Front. Med. 14 (1) (2020) 68–80.
[11] Z. Shi, J. Gao, J. Pan, et al., A systematic review on the safety of Psoraleae Fructus: potential risks, toxic characteristics, underlying mechanisms and detoxification methods, Chin. J. Nat. Med. 20 (11) (2022) 805–813.
[12] W.Y. Tian, S. Lan, L. Zhang, et al., [Safety evaluation and risk control measures of Psoralea corylifolia], Zhongguo Zhongyao Zazhi 42 (21) (2017) 4059–4066.
[13] Q. Sun, M. He, M. Zhang, et al., Traditional Chinese medicine and colorectal cancer: implications for drug discovery, Front. Pharmacol. 12 (2021) 685002.
[14] X. Xu, R. Zhu, J. Ying, et al., Nephrotoxicity of herbal medicine and its prevention, Front. Pharmacol. 11 (2020) 569551.
[15] Y. Xu, J. Ding, J.N. An, et al., Effect of the interaction of veratrum nigrum with panax ginseng on estrogenic activity in vivo and in vitro, Sci. Rep. 6 (2016) 26924.
[16] B.F. Yan, X. Chen, Y.F. Chen, et al., Aqueous extract of Paeoniae Radix Alba (Paeonia lactiflora Pall.) ameliorates DSS-induced colitis in mice by tunning the intestinal physical barrier, immune responses, and microbiota, J. Ethnopharmacol. 294 (2022) 115365.
[17] S. Zhijun, Y. Yangmei, W. Guangxin, Study on the action mechanism of total glycosides of peony against non-alcoholic fatty liver in vitro, Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology 24 (7) (2022) 2748–2754.
[18] Y.C. lei, L. Qiang, N.Y. cai, et al., The combination of gastrodin and isorhynchophylline exhibits a synergistic ROS-scav-enging action in MPP $^{+}$-challenged PC12 cells, Lishizhen Medicine and Materia Medica Research 32 (5) (2021) 1042–1046.
[19] L. Li, H. Wang, S. Zhao, et al., Paeoniflorin ameliorates lipopolysaccharide-induced acute liver injury by inhibiting oxidative stress and inflammation via SIRT1/FOXO1a/SOD2 signaling in rats, Phytother Res. 36 (6) (2022) 2558–2571.
[20] J. Fu, K. Schroder, H. Wu, Mechanistic insights from inflammasome structures, Nat. Rev. Immunol. 24 (7) (2024) 518–535.
[21] F.P. Fernandes, V.N.C. Leal, D. Souza de Lima, et al., Inflammasome genetics and complex diseases: a comprehensive review, Eur. J. Hum. Genet. 28 (10) (2020) 1307–1321.
[22] X. Zhan, Q. Li, G. Xu, et al., The mechanism of NLRP3 inflammasome activation and its pharmacological inhibitors, Front. Immunol. 13 (2022) 1109938.
[23] N. Kelley, D. Jeltema, Y. Duan, et al., The NLRP3 inflammasome: an overview of mechanisms of activation and regulation, Int. J. Mol. Sci. 20 (13) (2019).
[24] Y. Chen, X. Ye, G. Escames, et al., The NLRP3 inflammasome: contributions to inflammation-related diseases, Cell. Mol. Biol. Lett. 28 (1) (2023) 51.
[25] C. Xiaofei, W. Xiaoyan, L. Weixia, et al., Buguzhi alicobol extract induces idiosyncratic liver injury in rat model of immune stress, Pharmacology and Clinics of Chinese Materia Medica 39 (3) (2023) 65–71.
[26] Z. Wang, G. Xu, Y. Gao, et al., Cardamonin from a medicinal herb protects against LPS-induced septic shock by suppressing NLRP3 inflammasome, Acta Pharm. Sin. B 9 (4) (2019) 734–744.
[27] W. Kan, Q. Li, P. Li, et al., Glycyrrhiza uralensis polysaccharides ameliorate acute lung injury by inhibiting the activation of multiple inflammasomes, J. Funct. Foods (2023) 100.
[28] M.A. Kalas, L. Chavez, M. Leon, et al., Abnormal liver enzymes: a review for clinicians, World J. Hepatol. 13 (11) (2021) 1688–1698.
[29] G. Szabo, J. Petrasek, Inflammasome activation and function in liver disease, Nat. Rev. Gastroenterol. Hepatol. 12 (7) (2015) 387–400.
[30] A. Wree, M.D. McGeough, M.E. Inzaugarat, et al., NLRP3 inflammasome driven liver injury and fibrosis: roles of IL-17 and TNF in mice, Hepatology 67 (2) (2018) 736–749.
[31] C.Y. Han, H.S. Rho, A. Kim, et al., FXR inhibits endoplasmic reticulum stress-induced NLRP3 inflammasome in hepatocytes and ameliorates liver injury, Cell Rep. 24 (11) (2018) 2985–2999.
[32] W. Shi, G. Xu, X. Zhan, et al., Carnosol inhibits inflammasome activation by directly targeting HSP90 to treat inflammasome-mediated diseases, Cell Death Dis. 11 (4) (2020) 252.
[33] T. Kodi, R. Sankhe, A. Gopinathan, et al., New insights on NLRP3 inflammasome: mechanisms of activation, inhibition, and epigenetic regulation, J. Neuroimmune Pharmacol. 19 (1) (2024) 7.
[34] J.V. Rajan, S.E. Warren, E.A. Miao, et al., Activation of the NLRP3 inflammasome by intracellular poly I:C, FEBS Lett. 584 (22) (2010) 4627–4632.
[35] Z. Zhang, S. Xie, J. Qian, et al., Targeting macrophagic PIM-1 alleviates osteoarthritis by inhibiting NLRP3 inflammasome activation via suppressing mitochondrial ROS/Cl(-) efflux signaling pathway, J. Transl. Med. 21 (1) (2023) 452.
[36] J. Zhao, J. Li, G. Li, et al., The role of mitochondria-associated membranes mediated ROS on NLRP3 inflammasome in cardiovascular diseases, Front Cardiovasc Med 9 (2022) 1059576.
[37] X. Xiao, Z. Bai, J. Wang, et al., Traditional Chinese Medicine (TCM) safety evaluation and pharmacovigilance, Chin. Sci. Bull. 66 (4) (2021) 407–414.
[38] L. Zhao, H. Zhang, N. Li, et al., Network pharmacology, a promising approach to reveal the pharmacology mechanism of Chinese medicine formula, J. Ethnopharmacol. 309 (2023) 116306.
[39] D. Brenner, H. Blaser, T.W. Mak, Regulation of tumour necrosis factor signalling: live or let die, Nat. Rev. Immunol. 15 (6) (2015) 362–374.
[40] J. Wolf, S. Rose-John, C. Garbers, Interleukin-6 and its receptors: a highly regulated and dynamic system, Cytokine 70 (1) (2014) 11–20.
[41] K.C. Barnett, S. Li, K. Liang, et al., A 360° view of the inflammasome: mechanisms of activation, cell death, and diseases, Cell 186 (11) (2023) 2288–2312.
[42] T. Liu, G. Xu, L. Liang, et al., Pharmacological effects of Chinese medicine modulating NLRP3 inflammasomes in fatty liver treatment, Front. Pharmacol. 13 (2022) 967594.
[43] J. Jiao, G. Zhao, Y. Wang, et al., MCC950, a selective inhibitor of NLRP3 inflammasome, reduces the inflammatory response and improves neurological outcomes in mice model of spinal cord injury, Front. Mol. Biosci. 7 (2020) 37.
[44] C. Chen, Y. Zhou, X. Ning, et al., Directly targeting ASC by lonidamine alleviates inflammasome-driven diseases, J. Neuroinflammation 19 (1) (2022) 315.
[45] W. Zhao, F. He, H.W. Barkema, et al., Prototheca spp. induce an inflammatory response via mtROS-mediated activation of NF-κB and NLRP3 inflammasome pathways in bovine mammary epithelial cell cultures, Vet. Res. 52 (1) (2021) 144.