Acupuncture and Herbal Medicine

Paeoniae Radix Alba effectively attenuates *Polygonum multiflorum* Thunb.-induced idiosyncratic liver injury by modulating M2 macrophage polarization

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Abstract

Objective: *Polygonum multiflorum* Thunb. (PM) is a commonly used tonic herb known to cause idiosyncratic drug-induced liver injury (IDILI). This study explored the detoxification effects and potential mechanisms of action of Paeoniae Radix Alba (PRA) on PM-induced IDILI.

Methods: Network pharmacology analysis was utilized to predict the related targets of "PRA-PM-innate immunity." A non-hepatotoxic lipopolysaccharide (LPS) and PM-induced IDILI model was used to evaluate the detoxification effects of PRA by measuring liver function indicators, pathological examinations, and macrophage-related factors. Bone marrow-derived macrophages (BMDMs) were stimulated with IL-4 to differentiate into M2 macrophages, and the effects of PM and PRA on M2 macrophage polarization were explored.

Results: Target screening of "PRA-PM-innate immunity" identified 21 intersecting targets, most of which were closely associated with macrophage polarization. In rat models of IDILI induced by PM, the combined use of PRA significantly reduced the extent of liver damage and the levels of inflammatory factors, while promoting the expression of M2 macrophage-related factors such as interleukin (IL)-4, IL-10, arginase 1 (Arg1), and CD206. *In vitro*, PM dose-dependently inhibited the expression of the Arg1 protein and M2 macrophage-related genes, whereas PRA exhibited the opposite effect. When used in combination, PRA ameliorated the inhibitory effect of PM on M2 macrophage polarization.

Conclusions: Our results demonstrate that PRA has a therapeutic effect on PM-induced IDILI; its mechanism may involve alleviating liver injury by promoting M2 macrophage polarization, thus reducing the expression of inflammatory factors.

Keywords: IDILI, M2 macrophage polarization, Paeoniae Radix Alba, *Polygonum multiflorum* Thunb.

Graphical abstract: http://links.lww.com/AHM/A120.

Introduction

Drug-induced liver injury (DILI) is the direct or indirect damage to the liver caused by drugs or their metabolites[1]. DILI is generally classified as intrinsic or idiosyncratic DILI (IDILI)[2]. IDILI typically occurs in a small subset of susceptible individuals, is unpredictable, and is not influenced by drug dosage or administration timing[3,4]. As research on IDILI has advanced, potential mechanisms of IDILI have been preliminarily elucidated, possibly involving liver metabolic dysfunction, inflammatory stress, and immune homeostasis imbalance[5–7]. In recent years, incidents of liver damage induced by traditional Chinese medicines (TCM) such as *Polygonum multiflorum* Thunb. (PM), *Epimedii Folium* (EF), and *Psoraleae Fructus* (PF) have become increasingly common[8–10]. PM is traditionally used in clinical practice as a nontoxic tonic with purported effects of darkening hair, nourishing the liver and kidneys, and enriching the essence and blood, according to TCM theory[11,12]. However, in recent years, liver injury caused by PM and its preparations has been frequently reported. The pathogenesis of PM remains unclear and

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effective therapeutic approaches are lacking in clinical practice.

Macrophages are critical components of the body's immune system. They are distributed throughout all tissues and are indispensable for immune defense and inflammation[13]. Macrophages exhibit remarkable plasticity and can differentiate into various phenotypes in response to various stimuli[14]. These distinct macrophage phenotypes manifest different characteristics and functions in the physiological and pathological activities of the body, a phenomenon known as macrophage polarization[15]. M1 macrophages, also known as pro-inflammatory macrophages, primarily secrete tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and other inflammatory cytokines. In contrast, M2 macrophages, also known as anti-inflammatory macrophages, predominantly produce IL-4, IL-10, and arginase 1 (Arg1), which inhibit inflammatory reactions and promote tissue damage and repair[16]. Recently, numerous studies have demonstrated the crucial role of macrophage polarization in many pathological and physiological processes, including inflammation, tissue repair, and metabolism[17]. Interestingly, these pathological processes often coincide with liver diseases, indicating that macrophage polarization may play a key role in the occurrence and reversal of various liver diseases, such as acute liver injury (ALI), fatty liver, hepatitis, and fibrosis[18,19].

The compatibility of TCM for detoxification has been an important aspect of TCM theory since ancient times. Paeoniae Radix Alba (PRA), the root of the perennial herbaceous plant Paeonia lactiflora Pall., is known for its efficacy in nourishing blood, softening the liver, relieving pain, and astringing yin to stop sweating[20]. It is commonly used in clinical practice to treat inflammatory and liver diseases. Numerous studies have shown that PRA and its main components effectively ameliorate liver damage induced by thioacetamide, carbon tetrachloride (CCL4), and α-naphthyl isothiocyanate[21].

Medicinal pairs refer to fixed combinations of two drugs that have been clinically proven to be effective, with a certain theoretical basis, and in accordance with certain legal regulations. They are commonly used in clinical prescriptions in TCM, characterized by their simplicity and compatibility with Chinese herbal medicines. Research has revealed that a data mining analysis of 61 formulated preparations containing PM in the 2015 edition of the "Chinese Pharmacopoeia" resulted in the association of 36 pairs of PM medicinal pairs through the Apriori algorithm. Among the compatible medicines, PRA and PM form a classic nourishing pair[22]. Furthermore, experimental semi-quantitative analysis showed that after combination with PRA, the content of most of the components in PM significantly decreased, with only a few compounds showing significant increases in content. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH)-free radical-scavenging ability also decreased. These studies provide a basis for further exploration of the detoxification effect and mechanism of action of PRA in PM-induced IDILI[23].

In this study, we utilized network pharmacology to unveil the close relationship between the shared targets of "PRA-PM-natural immunity" and macrophage polarization. In a PM-induced IDILI rat model and *in vitro* experiments, PM suppressed the activation of M2 macrophages, leading to elevated inflammation and the subsequent occurrence of IDILI. However, when PM was combined with PRA, it mitigated the inhibitory effect of PM on M2 macrophage polarization and reduced the expression of inflammatory factors, thereby alleviating PM-induced liver injury. These findings provide new scientific evidence for the development of therapies for PM-induced IDILI.

Materials and methods

Animals

Female C57BL/6 mice aged 6 to 8 weeks, weighing 18 to 22 g, and male SD rats aged 10 weeks, weighing 180 to 200 g, were purchased from Beijing Specific Pathogen-Free (SPF) Biotechnology Company Limited. They were housed in a sterile laboratory mouse room for 1 week, with a 12-hour day and night cycle, had access to clean drinking water, and were given fresh mouse food daily. The room was maintained at a temperature of (25 ± 2) °C. All animal experiments were approved by the Animal Health Committee of the Fifth Medical Center of the Chinese General Hospital of the People's Liberation Army (Animal use license number: SCXK2019-0010, Animal Ethics Number: IACUC-2023-0013).

Cell culture

Bone marrow-derived macrophages (BMDMs) were isolated from the femur of an 8-week-old female C57BL/6 mice and cultured in Dulbecco modified eagle medium (DMEM). All the media contained 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The incubation conditions were maintained at 37°C and 5% CO_2.

Reagents and antibodies

DMEM (PYG0073) was purchased from BOSTER (Wuhan, China). Certified FBS was purchased from Viva Cell (Shanghai, China). Murine macrophage colony-stimulating factor (M-CSF) was purchased from MedChem Express (Shanghai, China). IL-4 (214-14) was purchased from PeproTech (Hebei, China). Lipopolysaccharide (LPS) (12880) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Color-stained protein markers were purchased from Vazyme (Beijing, China). HSP90 polyclonal antibody (1:5,000, 13171-1-AP) was obtained from Proteintech (Chicago, IL, USA). Arg1 (D4E3M™) XP® Rabbit mAb (86352) was obtained from Cell Signaling Technology (Boston, MA, USA).

Preparation of PRA and PM extract

The sliced PRA was extracted twice with 75% ethanol at a 1:10 herb-to-ethanol volume ratio and soaked for more than 10 h each time. The ethanol extracts were then combined, recovered, and concentrated using a vacuum rotary evaporator at 50°C. Subsequently, they were dried in a vacuum oven for more than 12 h to obtain alcoholic extracts[24]. An appropriate amount of raw PM was weighed and cold extraction was performed using eight times the amount of 50% ethanol for 48 h. The

extracts were then combined, and ethanol was concentrated under reduced pressure to obtain the crude extract through vacuum drying[25].

Determination of the composition in PM and PRA

The PM extract was determined by high-performance liquid chromatography (HPLC). Chromatographic column: ACQUITY UPLC® HSS C_{18} 1.7 μm 2.1 mm × 150 mm Column; mobile phase: acetonitrile as mobile phase A, 0.1% phosphoric acid solution as mobile phase B; flow rate: 0.2 mL/min; column temperature: 30°C; detector: electrochemical detector; detection wavelength: 280 nm. Three monosaccharide standards (2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, Emodin, Physcion) were used as standards for PM.

The chemical properties of PRA were determined using HPLC. Chromatographic conditions: ACQUITY UPLC® HSS C_{18} 1.7 μm 2.1 mm × 150 mm Column; mobile phase: acetonitrile (A) and 0.1 % phosphoric acid solution (B); flow rate: 0.2 mL/min; column temperature: 30°C; detector: electrochemical detector; detection wavelength: 230 nm. Six monosaccharide standards (Gallic acid, Protocatechuic aldehyde, (+)-Catechin, Alibiflorin, Paeoniflorin, 1, 2, 3, 4, 6-O-Pentagalloylglucose.) were used as the standards for PRA.

Western blot

We determined the protein expression of Arg1 by western blotting, with HSP90 as a loading control. Whole-cell lysates of BMDMs were prepared using a 1× and 5× SDS-polyacrylamide gel electrophoresis (SDS-PAGE)-loaded radioimmunoprecipitation assay buffer (RIPA) buffer composed of 50 mM Tris-HCl (pH 7.45), 150 mM NaCl, 1% Triton X-100, and 1% sodium deoxycholate. The protein samples were heated at 105°C on a metal bath, separated using 10 % SDS-PAGE, and transferred onto a polyvinylidene fluoride (PVDF) membrane. The samples were then incubated with 5% skimmed milk for 1 h, followed by overnight incubation with primary antibodies at 4°C. This was followed by three tris buffered saline with tween 20 (TBST) washes, incubation with the corresponding secondary antibodies, and three additional TBST washes. Finally, a commercial ECL kit with an enhanced chemiluminescence assay membrane was used according to the manufacturer's instructions to measure the protein expression levels.

Enzyme-linked immunosorbent assay (ELISA)

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and superoxide dismutase (SOD) assay kits were supplied by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). ELISA measurements of rat TNF-α (EK382HS), IL-6 (EK306), IL-1β (EK301B), IL-10 (EK310), IL-4 (EK304) were obtained from Multisciences (Lianke) Biotech (Hangzhou, China).

Table 1

Quantitative PCR primer sequences

Target gene	Sequence (5′–3′)
Mouse actin	GGCTGTATTCCCCTCCATCG CCAGTTGGTAACAATGCCATGT
Mouse Arg1	GTGAAGAACCCACGGTCTGT CTGGTTGTCAGGGGAGTGTT
Mouse CD206	CTTCGGGCCTTTGGAATAAT TAGAAGAGCCCTTGGGTTGA
Mouse PPAR-γ	GGAAGACCACTCGCATTCCTT GTAATCAGCAACCATTGGGTCA
Mouse Fizz1	ACCTTTCCTGAGATTCTGCCCC CAGTGGTCCAGTCAACGAGTAAGC
Mouse IRF4	CTTTGAGGAATTGGTCGAGAGG GAGAGCCATAAGGTGCTGTCA
Mouse Ym1	GGCTACACTGGAGAAAAT AGTCCCC CCAACCCACTCATTACCCTGATAG
Rat actin	GCTGTGCTATGTTGCCCTAGACTTC GGAACCGCTCATTGCCGATAGTG
Rat Arg1	AGTGTGGTGCTGGGTGGAGAC GCGGAGTGTTGATGTCAGTGTGAG
Rat CD206	TCAACTCTTGGACTCACGGC CATGATCTGCGACTCCGACA
Rat TNF-α	AGTTCCCAAATGGGCTCCCT TGGTGGTTTGCTACGACGTG
Rat IL-6	ACTTCCAGCCAGTTGCCTTCTTG TGGTCTGTTGTGGGTGGTATCCTC

PCR: Polymerase chain reaction; TNF-α: Tumor necrosis factor-alpha.

Quantitative real-time polymerase chain reaction (qRT-PCR)

To generate complementary DNA (cDNA), the total RNA was first isolated using the RNA extraction method. Total RNA was extracted using the reverse StarScript III All-in-one RT Mix (A230-10, Genstar) and 2 RealStar Fast SYBR (A304-10, Genstar). The primer sequences are listed in Table 1.

Rats and animal experiment design

The male SD rats weighing 180 to 200 g were divided into six groups: (1) control, (2) PM, (3) LPS, (4) LPS + PRA, (5) LPS + PM, and (6) LPS + PM + PRA, with six rats in each group. Prior to modeling, groups (2), (5), and (6) were administered intragastric PM at a dosage of 2.16 g/kg, whereas groups (4) and (6) were administered intragastric PRA at the same dose. Groups (1) and (3), on the other hand, were administered an equivalent volume of physiological saline intragastrically. The raw drug dosage of PM is calculated based on twice the clinical dosage and is compatible with PRA in equal proportions. The corresponding dose was calculated based on the extraction conversion ratio. After 3 h of administration, groups (3), (4), (5), and (6) groups were given 2.8 mg/kg LPS through the tail vein. The other groups received equal amounts of physiological saline *via* tail vein injections. Rat serum and liver samples were collected 7 h post-LPS administration. A segment of each excised liver

was immersed in a 10% formalin-neutral buffer solution for fixation, and the liver lobules were preserved in cryopreserved tubes. The extent of liver injury was evaluated through histopathological assessment using hematoxylin and eosin (H&E) staining, TdT-mediated dUTP NickEnd Labeling (TUNEL) staining, as well as the quantification of the serum levels of IL-1β, TNF-α, IL-6, IL-4, IL-10, ALT, AST, ALP, and SOD.

Network pharmacology analysis

Through the utilization of HIT 2.0 (http://hit2.badd-cao.net), the active chemical compounds in PM and PRA were screened. Innate immunity-related targets were acquired from GeneCards (https://www.genecards.org/) using the keyword "Innate immunity." These databases were used to search for all the target genes. Subsequently, UniProt was used to rectify the names of the target genes. A network depicting the interaction between the active components and predicted targets of PM-PRA was established based on their interaction data and visualized using the Cytoscape software (version 3.9.1). The Network Analyzer plugin in Cytoscape was employed to calculate the degree, betweenness, and closeness centralities, which were utilized to assess the topological significance of the nodes in the network.

A Venn diagram was constructed to show the intersecting targets of PM-PRA and innate immunity. STRING (https://string-db.org/) is an interactive gene database search tool that provides protein-protein interaction (PPI) information. The screened common targets were imported into the STRING 11.0 database, the species was selected as "Homo Sapiens," and the following species were selected "Interaction Score" is set to "Minimum required interaction score = 0.4" Cytoscpace 3.9.1 was applied to generate the PPI network diagram of the common targets, with adjustments made to the node size and color based on the degree in the diagram.

Gene ontology (GO) analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

Combining GO and KEGG enrichment analyses can provide comprehensive functional information about a large number of genes from a macro perspective and identify drug-disease signaling pathways. The common targets identified after STRING processing were uploaded to the Metascape database for GO and KEGG analyses, yielding relevant data on cellular components (CC), molecular function (MF), biological processes (BP), and KEGG pathways. The top 10 GO terms and the top 20 KEGG pathways with the smallest *P* values were chosen and imported into the bioinformatics mapping website (http://www.bioinformatics.com.cn/) to create bar and bubble graphs.

Constructing the network of the main compound pathways and targets of PM-PRA and innate immunity

The top 20 signaling pathways with the smallest *P* value, identified through KEGG enrichment analysis, along with their corresponding targets and compounds, were imported into Ctyoscape 3.9.1. Subsequently, a network diagram illustrating the main compound-pathway-target interactions between PM-PRA and innate immunity was created.

Statistics

GraphPad Prism 8 software was used to analyze the data. One-way analysis of variances (ANOVAs) and Dunnett *post hoc* test were used to compare multiple groups. *P* values <0.05 were regarded as statistically significant for all the experimental results, which were all expressed as the mean ± standard error of mean (SEM).

Results

Target screening for PM-PRA-innate immunity

First, network pharmacology analysis was used to explore the relevant targets and components of PRA in combination with PM. HIT 2.0 screened the effective active compounds and targets of PM and various types of PRA. Upon entering the active ingredients and potential targets into Cytoscape 3.9.1, a network diagram illustrating the interactions between the active ingredients and targets (Figure 1A) was generated. The network contained 294 nodes and 365 edges. Network analysis tools indicated a network centralization of 0.76 and a network heterogeneity of 5.45, suggesting that certain nodes in the network are more concentrated and influential than others. Through the interactions between these components and their targets, we found that the combination of PRA and PM involved multiple components and targets.

Previous studies have shown that IDILI usually occurs in a small number of susceptible individuals and is primarily mediated by innate immunity. Therefore, we investigated the relevant targets of innate immunity. From the GeneCards database, 1,783 innate immunity targets were obtained and standardized using UniProt. A Venn diagram of the targets associated with "PM-PRA-innate immunity" was drawn, and 21 common targets were obtained (Figure 1B). Twenty-one intersection targets in the figure were input into STRING 11.0 and used to create a PPI network diagram in Cytoscape (Figure 1C) consisting of 20 nodes and 298 edges. The top five scoring nodes based on degree were ALB, MMP9, TNF, HIF1A, PTGS2, IL1B, STAT3, PPARG, PTEN, BCL2, ICAM1, MAPK1, and RELA (Table 2). A review of previous studies and literature research revealed that the targets are closely related to macrophage polarization[26,27].

GO and KEGG databases enrichment analysis and compounds-targets-pathways (C-T-P) network construction

Metascape was used to conduct GO analysis of the targets in the PPI network. The results indicated that 15 CC, 31 MF, and 670 BP were identified ($P < 0.05$, the bar graph of the top 10 is shown in Figure 2A). After KEGG pathway analysis, 108 signaling pathways were identified ($P < 0.05$; the bubble chart of the top 20 is shown in Figure 2B). Several pathways related to macrophage polarization were significantly enriched, including the TNF-α and nuclear factor kappa-B (NF-κB) signaling pathways.

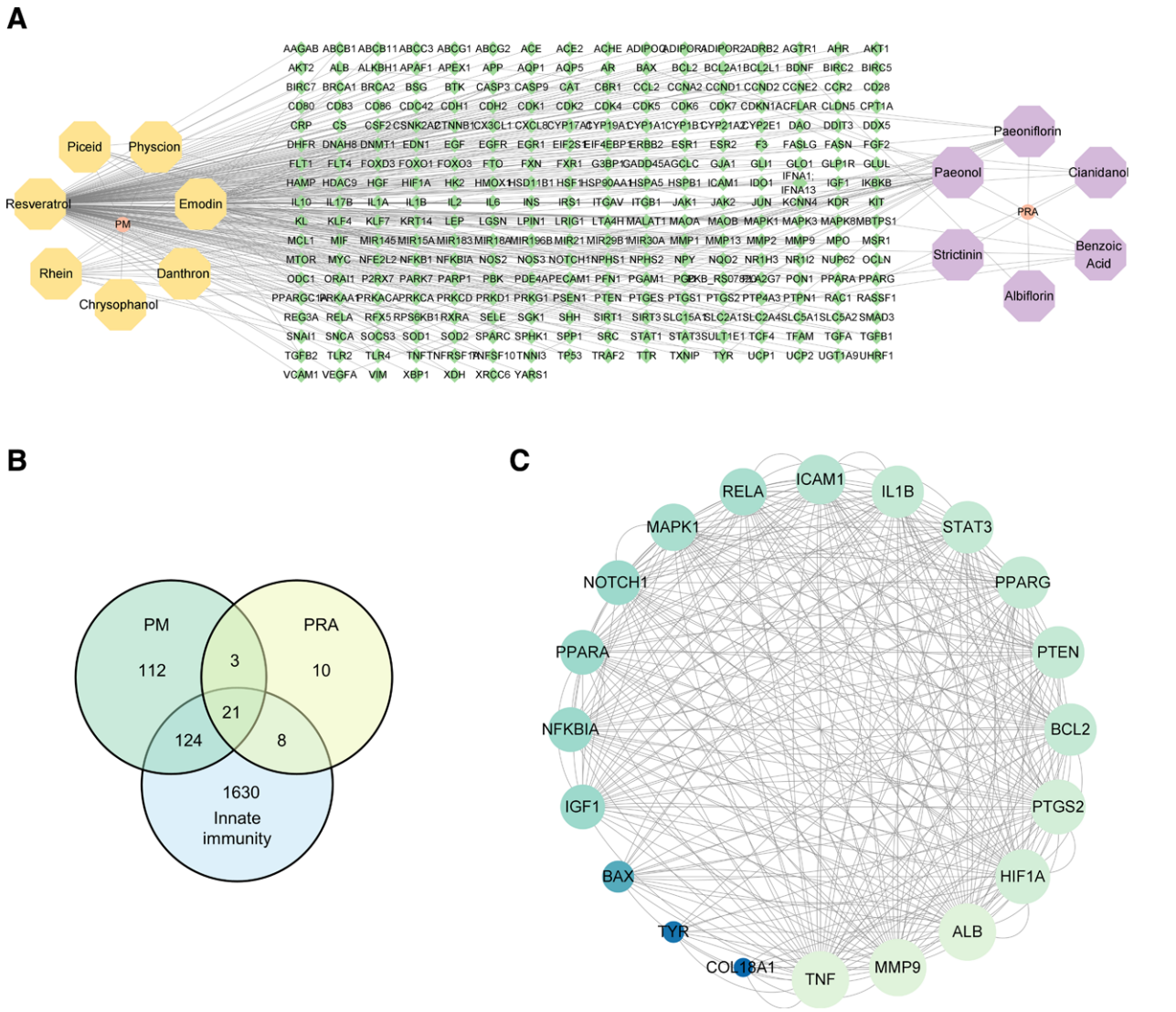

Figure 1. Acquisition of overlapping targets and PPI analysis. (A) PM-PRA active ingredient-target network. The yellow and purple octagons represent the PM and PRA active compounds, respectively. The green diamonds represent the targets related to PM-PRA. (B) The Venn diagram shows 21 overlapping targets between the PM-PRA active compounds and innate immunity. (C) The network diagram shows the PPI network of 20 overlapping targets. The color depth is related to the degree. The darker the color, the higher the degree. PM: *Polygonum multiflorum* Thunb.; PPI: protein-protein interaction; PRA: Paeoniae Radix Alba.

Next, we constructed a C-T-P network linking compounds, pathways, and targets to elucidate the relationship between them (Figure 2C), which consisted of 45 nodes and 206 edges. Important compounds in PRA, such as paeonol, act on IL1B, TNF, and PTGS2, whereas paeoniflorin acts on MMP9, TNF, and PTGS2. Moreover, rhein in the PM can act on MMP9, TNF, and STAT3, whereas resveratrol acts on MMP9, TNF, IL1B, PTGS2, STAT3, NFKB1A, and PPARA. Therefore, we hypothesized that the intrinsic mechanism of PRA-paired PM involves the synergistic effects of multiple components and targets.

PRA alleviates PM-induced liver injury in LPS-mediated rat models of inflammation

To further elucidate whether the PM and PRA components used in the experiment were consistent with the requirements of the Chinese Pharmacopoeia, we used HPLC to analyze them. The results indicated that the peak times of the PM and PRA components were consistent with the reference standards [Supplementary Figure S1A, B, http://links.lww.com/AHM/A119]. Previous studies by our research team have demonstrated that the ingestion of PM alone does not cause liver injury, but co-administration with LPS at a non-hepatotoxic dose can induce IDILI[28]. Therefore, we evaluated the therapeutic effects of PRA on PM-induced hepatotoxicity in an LPS-induced IDILI-susceptible rat model. The results showed that compared to the LPS group, rats in the LPS + PM group exhibited significantly increased serum levels of ALT, AST, and ALP and significantly decreased SOD activity. When PM was combined with PRA, the levels of ALT, AST, and ALP significantly decreased, and SOD activity increased (Figure 3A–D). Subsequently, we conducted a histological analysis of the rat liver using H&E and TUNEL staining. The results indicated that co-treatment with LPS and PM led to a disordered hepatic cord arrangement, hepatocyte swelling with foam-like degeneration, and substantial infiltration of

Table 2

Targets in protein-protein interaction network of PM-PRA-innate immunity

Target name	Degree
ALB	38
MMP9	38
TNF	38
HIF1A	36
PTGS2	36
IL1B	34
STAT3	34
PPARG	34
PTEN	34
BCL2	34
ICAM1	32
MAPK1	30
RELA	30
NFKBIA	28
NOTCH1	28
PPARA	28
IGF1	28
BAX	18
TYR	10
COL18A1	8

MAPK: Mitogen-activated protein kinase; PM: *Polygonum multiflorum* Thunb.; PRA: Paeoniae Radix Alba.

inflammatory cells in the liver parenchyma, which were not observed in the control, PM, LPS, and LPS + PRA groups. The degree of liver injury significantly reduced after combination therapy with PRA (Figure 3E). Additionally, TUNEL fluorescence staining showed that TUNEL-positive signals increased significantly in rats co-treated with LPS and PM and decreased after combination with PRA (Figure 3F). Taken together, these findings indicate that PRA alleviates PM-induced IDILI.

PRA inhibits LPS and PM-induced inflammatory responses and promotes M2 macrophage polarization

In the network pharmacology study, we found that the intersection of "PM-PRA-innate immune" targets is closely related to macrophage polarization. Therefore, we investigated factors relevant to macrophage polarization. The results showed that co-treatment with PM and LPS not only elevated the expression levels of the inflammatory factors TNF-α, IL-6, and IL-1β in the serum but also reduced the levels of the anti-inflammatory factors IL-4 and IL-10. Interestingly, after combination treatment with PRA, the levels of inflammatory factors significantly decreased, while IL-10 and IL-4 levels increased (Figure 4A–E). Furthermore, using quantitative polymerase chain reaction (qPCR) to determine the expression of TNF-α, IL-6, Arg1, and CD206 mRNA in the rat liver tissues, we found that compared to the LPS + PM group, the co-administration of PRA resulted in a decreased expression of TNF-α and IL-6 mRNA in the liver tissues, and increased expression levels of Arg1 and CD206 mRNA (Figure 4F–I). These findings revealed that co-treatment with PM and LPS induced the transformation of macrophages into M1 macrophages, thereby increasing the degree of inflammation. However, PRA can induce the transformation of pro-inflammatory M1 macrophages into anti-inflammatory M2 macrophages, thus alleviating inflammation and reducing PM-induced liver injury.

PM inhibits the expression of M2 macrophage markers and related genes induced by IL-4

We investigated the effects of PM on the phenotype of M2 macrophages *in vitro*. IL-4 induces the differentiation of BMDMs into M2 macrophages[29]. We co-treated BMDMs with IL-4 and different concentrations of PM, and the western blot results revealed a dose-dependent decrease in the protein levels of the M2 macrophage marker Arg1 (Figure 5A). To further explore the effect of PM on M2 macrophage polarization, we used qPCR to study the expression levels of the M2 macrophage-related genes PPAR-γ, Fizz1, Ym1, and IRF4 mRNA after PM treatment. The results indicated that PM inhibited the mRNA levels of these factors in a dose-dependent (Figure 5B–G). They also showed that PM may reduce the anti-inflammatory capacity of the body by inhibiting M2 macrophage polarization, thereby resulting in severe liver damage in the presence of LPS.

PRA promotes M2 macrophage polarization

PRA exhibits strong anti-inflammatory properties, and in our previous *in vivo* experiments, we found that it promoted M2 macrophage differentiation. When we stimulated IL-4 and different concentrations of PRA, the results showed a dose-dependent increase in Arg1 protein expression in BMDMs by PRA. Additionally, PRA had a significant promoting effect on the expression of the M2 macrophage-related genes Arg1, CD163, PPAR-γ, Fizz1, and Ym1 mRNA (Figure 6A–G). These results further confirmed the ability of PRA to regulate macrophage transformation into anti-inflammatory M2 macrophages.

PRA ameliorates the inhibitory effect of PM on IL-4-induced M2 macrophage polarization

To further investigate the combined effect of PRA and PM on the IL-4-induced differentiation of M2 macrophages, we administered different concentrations of PM and PRA to the BMDMs. Western blotting results showed that with different concentrations of PRA, while maintaining the PM concentration constant, the expression of the Arg1 protein was higher than that of PM alone. Additionally, the expression of M2 macrophage-related genes, including Arg1, CD163, PPAR-γ, Fizz1, and Ym1 mRNA, was also increased compared to PM alone, with the most effective outcome observed when PM and PRA were administered at equal concentrations (Figure 7A–G). In summary, PRA ameliorated the inhibitory effect of PM on M2 macrophages and promoted their differentiation into M2 macrophages, thereby generating more anti-inflammatory factors, alleviating liver inflammation, and ultimately mitigating PM-induced liver injury.

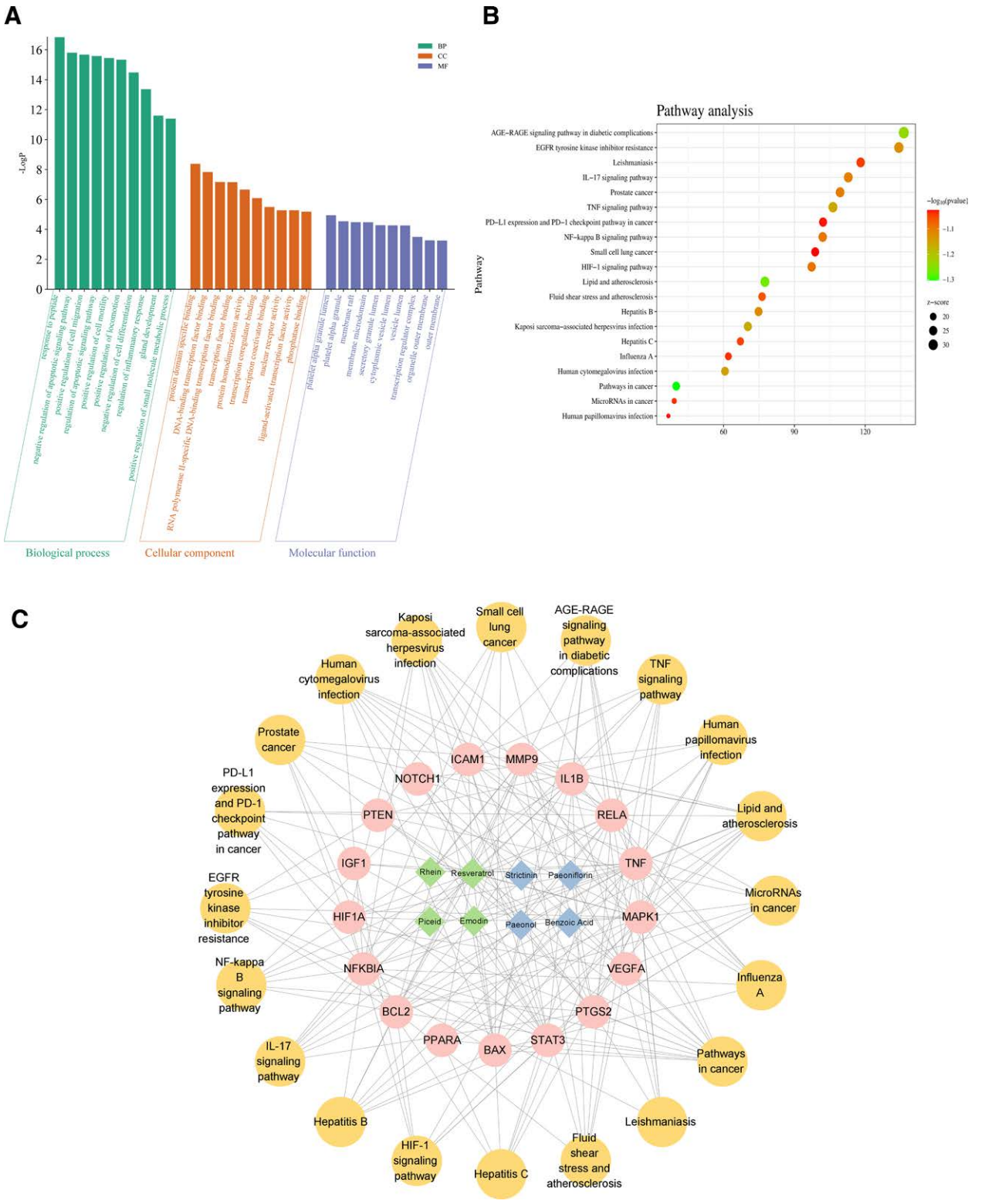

Figure 2. Analysis of gene ontology and KEGG enrichment, and the network of the main pathways and targets of PM-PRA and innate immunity. (A) Top 10 significantly enriched terms in BP, CC, and MF. (B) Display of the pathways related to 21 overlapping targets between the PM-PRA active compounds and innate immunity in KEGG enrichment analysis. (C) C-T-P network of the PM-PRA active compounds regulating innate immunity. The pink nodes represent 21 overlapping targets and the yellow nodes represent the pathways related to the 21 targets. The green and blue diamonds represent PM and PRA, respectively. BP: Biological processes; C-T-P: Compound-target-pathway; CC: Cellular components; KEGG: Kyoto encyclopedia of genes and genomes; MF: Molecular function; PM: *Polygonum multiflorum* Thunb.; PRA: Paeoniae Radix Alba.

Discussion

With the widespread clinical application of traditional non-toxic Chinese medicines and their preparations, traditional non-toxic Chinese medicine-induced IDILI is becoming increasingly serious. Therefore, awareness of the safety precautions for Chinese medicine is gradually increasing. In recent years, IDILI has become a focus and challenge in domestic and foreign research. Current research on the mechanism of IDILI mainly focuses on immune stress, abnormal individual immune

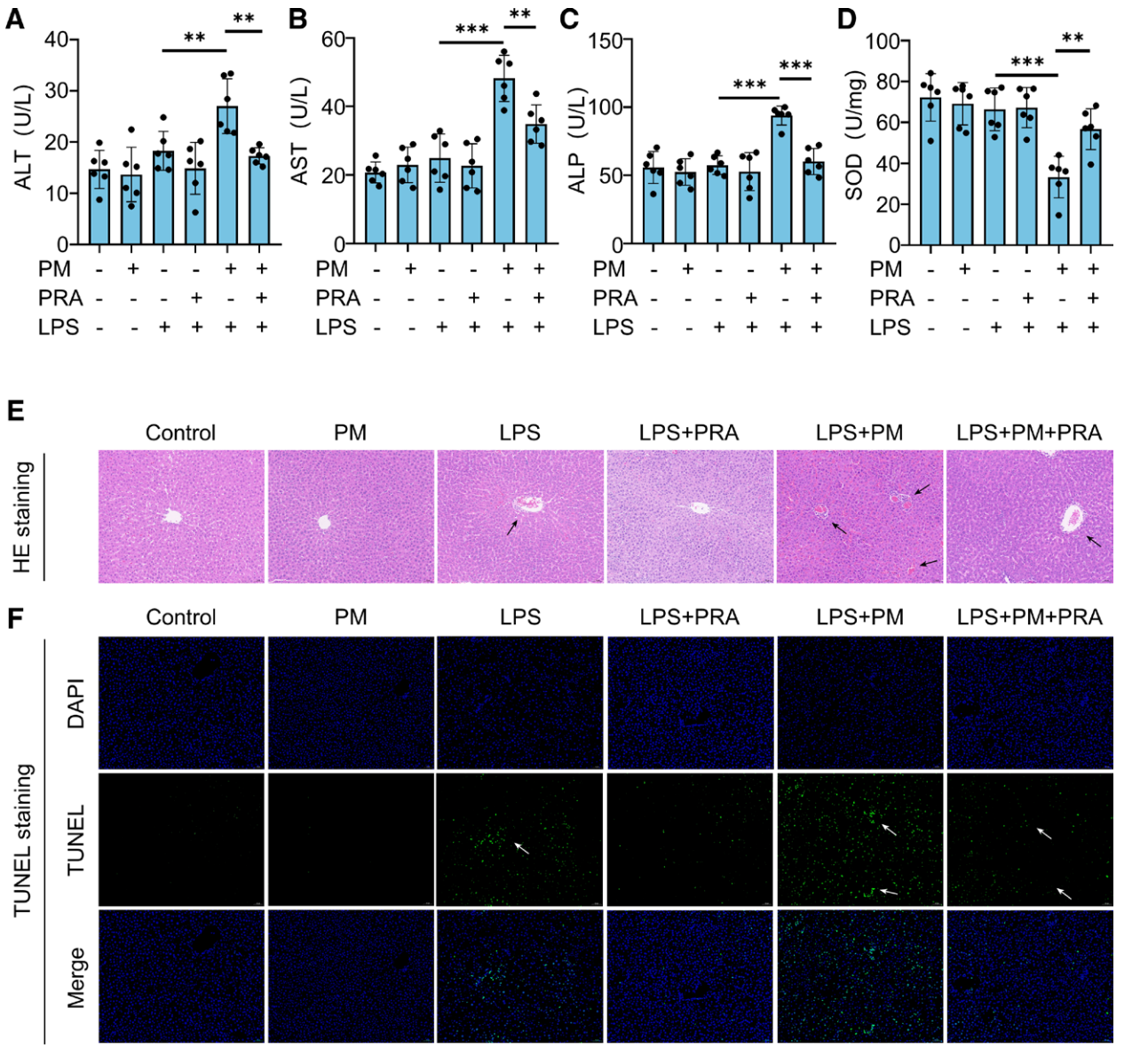

Figure 3. PRA attenuates PM-induced liver injury in an LPS-mediated rat model. Prior to modeling, the rats were administered intragastrically with corresponding doses of PM (2.16 g/kg), PRA (2.16 g/kg), and physiological saline. Subsequently, LPS (2.8 mg/kg) was injected into the tail vein 3 h later, and serum and liver tissues were collected 7 h later. (A–D) ELISA kits were employed to measure the activity of ALT, AST, ALP, and SOD in the rat serum. (E) Histological images of liver tissue sections stained with H&E (20× magnification, scale bar = 50 μm) are presented. (F) Representative images of TUNEL staining (original magnification 20×, scale bar = 50 μm) are displayed. Statistical differences were evaluated using one-way ANOVA and Dunnett *post hoc* test. The data were presented as the means ± SEM ($n = 6$ for each group), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; ANOVA: Analysis of variance; AST: Aspartate aminotransferase; DAPI: 4',6-diamidino-2-phenylindole; ELISA: Enzyme-linked immunosorbent assay; H&E: Hematoxylin and eosin; LPS: Lipopolysaccharide; PM: *Polygonum multiflorum* Thunb.; PRA: Paeoniae Radix Alba; SEM: Standard error of mean; SOD: Superoxide dismutase; TUNEL: TdT-mediated dUTP NickEnd Labeling.

responses, and decreased activity of related metabolic enzymes[30,31]. Immune stress is an important inducing factor for IDILI, and the body is more prone to IDILI under immune stress[7,32]. In recent decades, several research teams in China have found that PM, EF, PF, and their related preparations, such as Qibao Meiran Dan, Xianling Gubao capsule, and Zhuanggu Guanjie pills, can cause adverse reactions related to liver injury and produce IDILI under immune stress[33–35]. Therefore, the scientific evaluation of IDILI induced by traditional non-toxic Chinese medicines has become one of the most challenging issues in the field of TCM safety research[4]. Previous studies using immune stress models and component knock-in and knock-out methods have found that cis-stilbene glycoside (cis-SG) is the main susceptible component of PM-induced immune liver injury, whereas trans-stilbene glycoside (trans-SG) enhances immune activity and induces liver injury caused by cis-SG, confirming that PM-induced liver injury is a result of the synergistic effect of the abnormal immune activation state of the body, immune-promoting substances in PM, and potential liver injury-prone components[36,37]. This has also provided scientific answers to key issues such as liver injury properties, objectivity, and the causative mechanisms of PM-induced liver injury.

It is well known that the clinical use of TCM primarily involves compatibility or compound formulations, with compatibility detoxification being a commonly used detoxification method in TCM clinical practice. In earlier studies conducted by our research team, we found

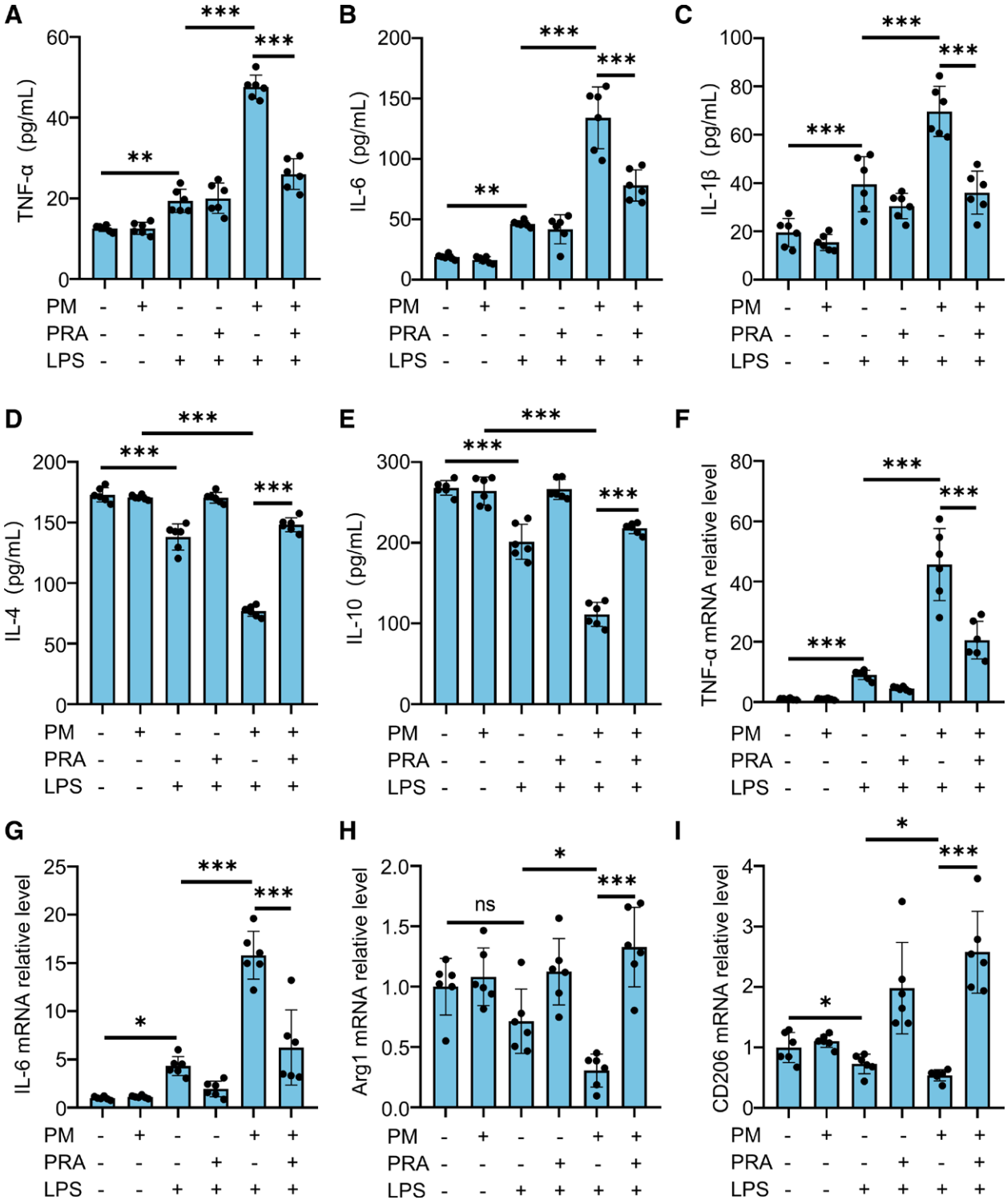

Figure 4. Effects of PM, PRA, and their combined administration on pro-inflammatory and anti-inflammatory factors. ELISA was utilized to determine the levels of (A) TNF-α, (B) IL-6, (C) IL-1β, (D) IL-4, and (E) IL-10 in the rat serum. Real-time quantitative PCR was employed to detect the expression levels of (F) TNF-α, (G) IL-6, (H) Arg1, and (I) CD206 mRNA in the rat liver tissue. Statistical differences were evaluated using one-way ANOVA and Dunnett *post hoc* test. The data were presented as the mean ± SEM ($n = 6$ for each group), *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ANOVA: Analysis of variance; ELISA: Enzyme-linked immunosorbent assay; IL: Interleukin; LPS: Lipopolysaccharide; PCR: Polymerase chain reaction; PM: *Polygonum multiflorum* Thunb.; PRA: Paeoniae Radix Alba; SEM: Standard error of mean; TNF-α: Tumor necrosis factor-alpha.

that Chinese medicinal herbs with efficacy in clearing heat, cooling blood, detoxifying, and promoting diuresis, such as poria, could reduce PM-induced IDILI by targeting the natural immune pathway[38]. PRA is a traditional Chinese herbal medicine with a long history in China, and its anti-inflammatory and hepatoprotective effects have been confirmed in many studies[39].

From the perspective of network pharmacology, we constructed a target network related to the analysis of PRA-PM innate immunity, which yielded 21 related targets. Most of these targets are closely associated with M2 macrophage polarization. Macrophages are essential components of the first line of defense against adverse environments. They play a crucial role in innate

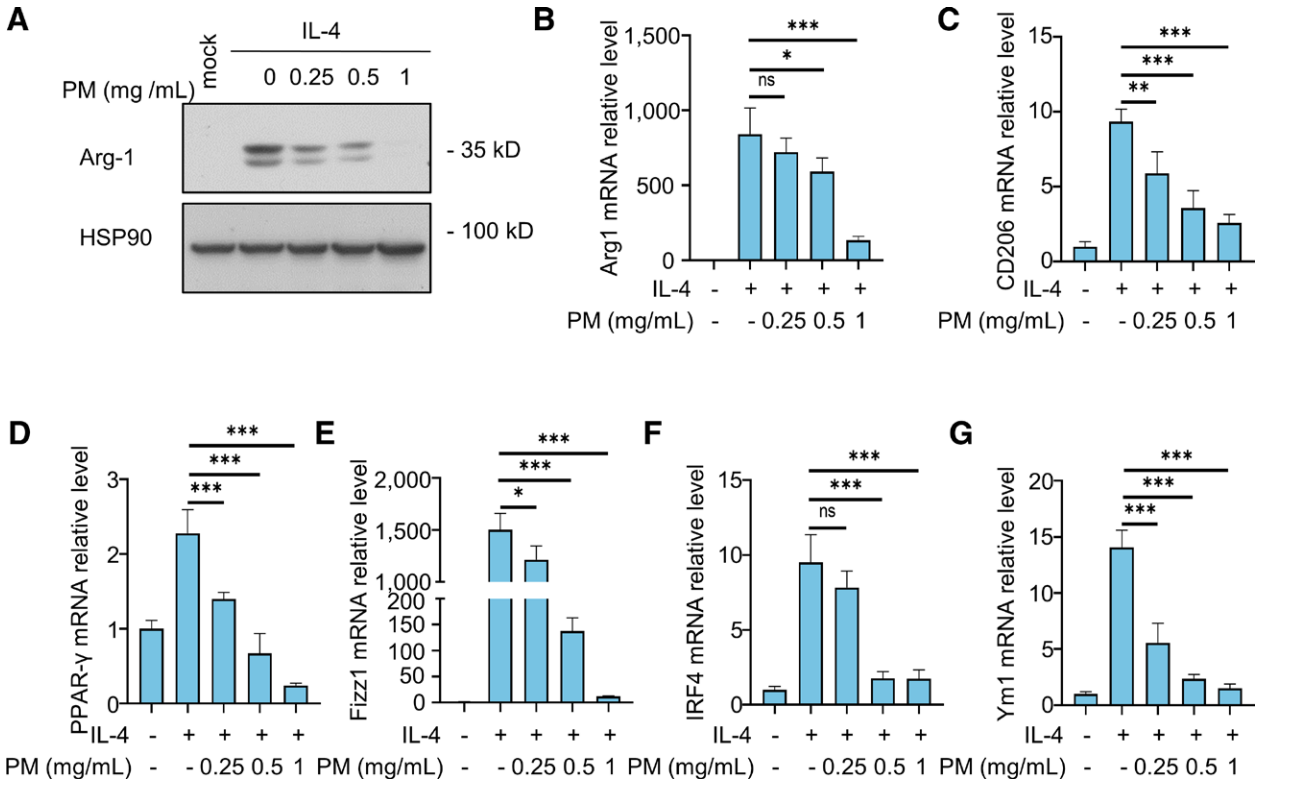

Figure 5. PM suppresses the expression of M2 macrophage markers and related genes induced by IL-4. (A) BMDMs were pretreated with different concentrations of PM (0.25, 0.5, 1 mg/kg) for 1 h, followed by stimulation with IL-4 (20 ng/mL) for 24 h. The expression levels of Arg1 and HSP90 proteins were then detected *via* western blot. (B–G) BMDMs were pretreated with different concentrations of PM (0.25, 0.5, 1 mg/kg) for 1 h, followed by stimulation with IL-4 (20 ng/mL) for 24 h. The expression levels of Arg1, CD206, PPAR-γ, Fizz1, IRF4, and Ym1 were measured using qPCR. Statistical differences were evaluated using one-way ANOVA and Dunnett *post hoc* test. The data were presented as the mean ± SEM ($n = 3$ for each group), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ANOVA: Analysis of variance; BMDMs: Bone marrow-derived macrophages; IL: Interleukin; PM: *Polygonum multiflorum* Thunb.; qPCR: Quantitative polymerase chain reaction; SEM: Standard error of mean.

immunity and contribute to the initiation of adaptive immune responses. MMP9, PTGS2, STAT3, and PPARG may also be involved in the regulation of macrophage polarization and inflammation by PRA. For example, overexpression of MMP9 can promote M1 phenotype transformation in LPS-induced murine pulmonary macrophages, whereas inhibition of MMP9 expression can promote M2 phenotype transformation[26,27]. The KEGG pathway enrichment analysis revealed various pathways related to macrophage polarization, including the TNF signaling pathway, mitogen-activated protein kinase (MAPK) signaling pathway, Toll-like receptor signaling pathway, NF-κB signaling pathway, and phosphoinositide 3-kinase-Akt (PI3K/Akt) signaling pathway. Therefore, PRA may influence macrophage polarization through multiple targets and signaling pathways, thereby promoting differentiation into M2 macrophages and alleviating PM-induced IDILI.

Research indicates that immune stress-induced inflammatory liver injury primarily involves macrophages, neutrophils, and other cells involved in innate immunity[40]. When the body is in a state of mild inflammation, macrophages are activated, leading to their differentiation into M1 macrophages, which in turn secrete pro-inflammatory factors such as TNF-α and IL-6, thereby promoting inflammation in the body. In contrast, M2 macrophages secrete anti-inflammatory factors, such as IL-4, IL-10, and Arg1, which alleviate inflammation[41]. The literature also reports that components of PRA, such as total glucosides of paeony (TGP), can reverse pro-inflammatory M1 macrophages to M0 macrophages while promoting the transformation of M0 macrophages into anti-inflammatory M2 macrophages, thereby exerting therapeutic effects[42].

Our *in vivo* results demonstrated that in the LPS- and PM-induced IDILI model, the co-administration of PRA led to a decrease in serum ALT, AST, and ALP liver injury markers in the rats and increased SOD oxidative activity. H&E and TUNEL results revealed reduced inflammatory infiltration and positive cell counts in the liver tissue, indicating alleviated damage. These preliminary findings validated our hypothesis that PRA can mitigate PM-induced IDILI. The measurement of M1/M2 macrophage-associated factors in rat serum revealed that LPS stimulation alone resulted in elevated pro-inflammatory factors TNF-α, IL-6, and IL-1β, with a slight decrease in the anti-inflammatory factors IL-4 and IL-10, as well as reduced expression of Arg1 and CD206 mRNA in the tissue, confirming that LPS stimulation induces macrophage polarization towards M1 macrophages, thereby eliciting inflammation. Compared to LPS stimulation alone, co-administration of PM and LPS led to a more pronounced increase in pro-inflammatory factor expression in both the serum and liver tissue, along with a significant decrease in anti-inflammatory factors. Consequently, we inferred that the combined use of PM and LPS inhibited *in vivo* macrophage polarization toward M2 macrophages, leading to a reduced secretion

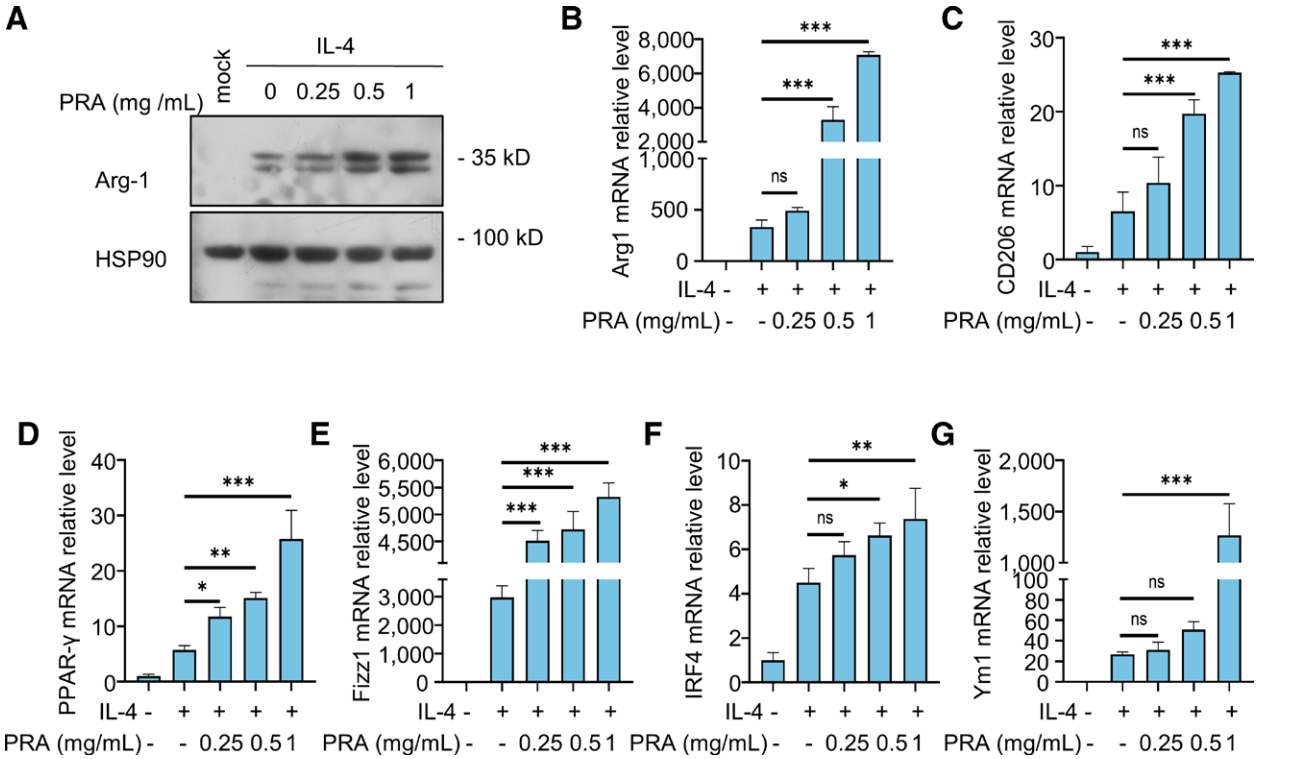

Figure 6. PRA promotes the expression of M2 macrophage markers and related genes induced by IL-4. (A) BMDMs were pretreated with different concentrations of PRA (0.25, 0.5, 1 mg/kg) for 1 h, followed by stimulation with IL-4 (20 ng/mL) for 24 h. The expression levels of Arg1 and HSP90 proteins were then detected *via* western blot. (B–G) BMDMs were pretreated with different concentrations of PRA (0.25, 0.5, 1 mg/kg) for 1 h, followed by stimulation with IL-4 (20 ng/mL) for 24 h. The expression levels of Arg1, CD206, PPAR-γ, Fizz1, IRF4, and Ym1 were measured using qPCR. Statistical differences were evaluated using one-way ANOVA and Dunnett *post hoc* test. The data were presented as the means ± SEM ($n = 3$ for each group), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ANOVA: Analysis of variance; BMDMs: Bone marrow-derived macrophages; IL: Interleukin; PRA: Paeoniae Radix Alba; qPCR: Quantitative polymerase chain reaction; SEM: Standard error of mean.

of anti-inflammatory factors and exacerbation of LPS-mediated inflammation, ultimately resulting in IDILI. However, co-administration of PRA led to a noticeable decrease in inflammatory factors in the rat serum compared to the combined use of PM and LPS, accompanied by a significant increase in the expression of IL-4, IL-10, Arg1, and CD206 mRNA in the tissue. Our *in vitro* studies revealed that PM inhibits M2 macrophage polarization in a dose-dependent manner, whereas PRA mitigates the inhibitory effect of PM on M2 macrophage polarization, which is consistent with our *in vivo* experimental results.

In summary, our network pharmacology analysis, as well as *in vivo* and *in vitro* phenotypic experiments, provides preliminary evidence that PRA can promote M2 macrophage polarization, leading to increased production of anti-inflammatory factors in the body and effectively alleviating the severity of PM-induced IDILI. This study provides new insights into the safe clinical use of PM. Furthermore, the results underscore the importance of TCM compatibility in detoxification and provide new ideas and strategies for addressing other TCM that may also cause IDILI. These findings contribute to our future exploration of the pathways through which PRA alleviates PM-induced IDILI.

Conflict of interest statement

Xiaohe Xiao is editorial board members of this journal. None of the other authors declare any conflicts of interest.

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Author contributions

Xiaohe Xiao designed the study. Ye Xiu performed most of the experiments and wrote the manuscript. Zhixin Wu helped design the project and was responsible for editing and revising the manuscript. Yichong Chen and Wenqing Mu performed the animal experiments. Xiaomei Zhao and Yurong Li performed the cell experiments and analyzed and visualized the data. Xiaohe Xiao and Zhaofang Bai provided funding. All the authors have read and approved the final version of the manuscript.

Ethical approval of studies and informed consent

All animal experiments were approved by the Animal Health Committee of the Fifth Medical Center of the Chinese General Hospital of the People's Liberation Army (Animal use license number: SCXK2019-0010, Animal Ethics Number: IACUC-2023-0013).

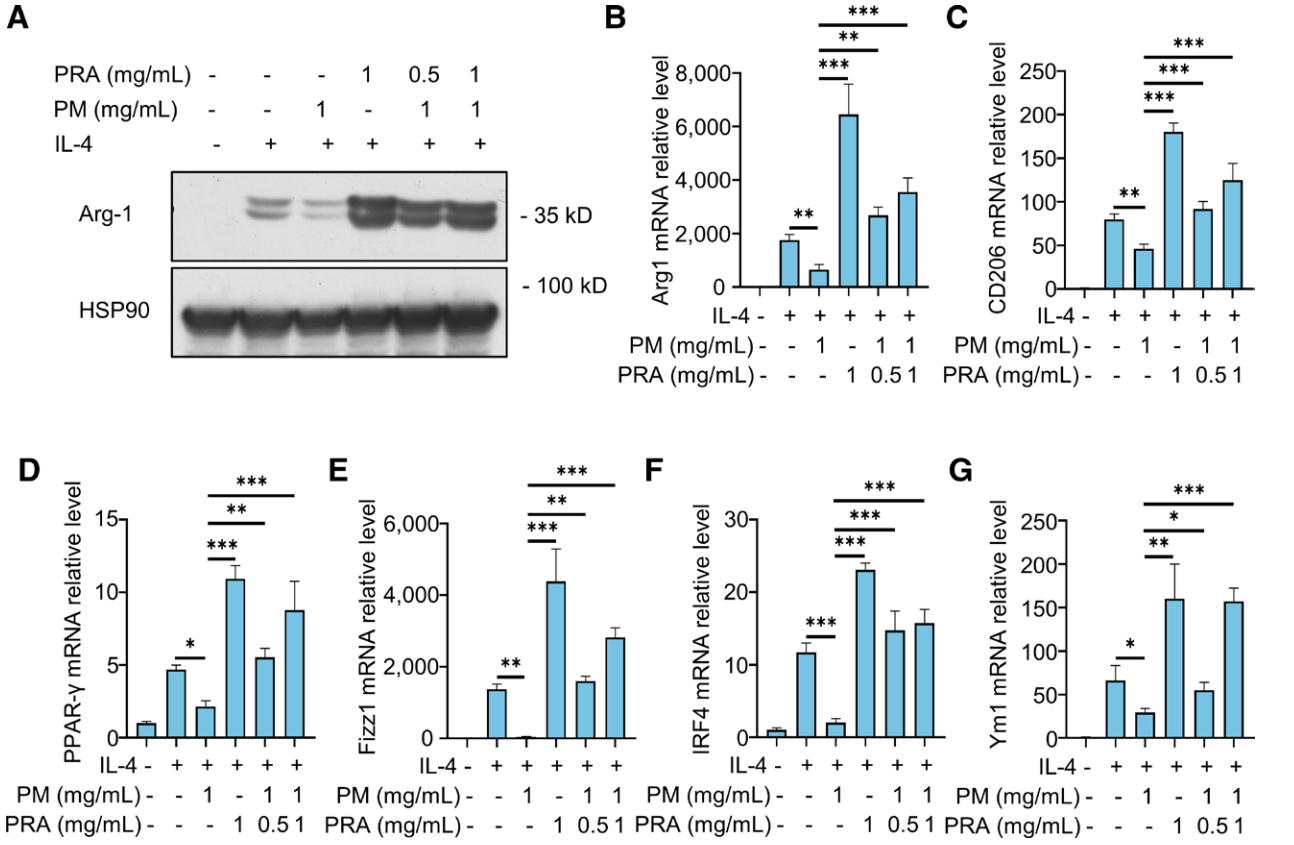

Figure 7. PRA ameliorates the inhibitory effect of PM on the expression of M2 macrophage markers and related genes induced by IL-4. (A) BMDMs were pretreated with different concentrations of PM, PRA, and their combined administration for 1 h, followed by stimulation with IL-4 (20 ng/mL) for 24 h. The expression levels of Arg1 and HSP90 proteins were then detected *via* western blot. (B–G) BMDMs were pretreated with different concentrations of PM, PRA, and their combined administration for 1 h, followed by stimulation with IL-4 (20 ng/mL) for 24 h. The expression levels of Arg1, CD206, PPAR-γ, Fizz1, IRF4, and Ym1 were measured using qPCR. Statistical differences were evaluated using one-way ANOVA and Dunnett *post hoc* test. The data were presented as the means ± SEM ($n = 3$ for each group), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. ANOVA: Analysis of variance; Arg1: Arginase 1; BMDMs: Bone marrow-derived macrophages; IL: Interleukin; PM: *Polygonum multiflorum* Thunb.; PRA: Paeoniae Radix Alba; qPCR: Quantitative polymerase chain reaction; SEM: Standard error of mean.

Acknowledgments

None.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

[1] Andrade RJ, Chalasani N, Björnsson ES, et al. Drug-induced liver injury. *Nat Rev Dis Primers* 2019;5(1):58.
[2] Björnsson HK, Björnsson ES. Drug-induced liver injury: pathogenesis, epidemiology, clinical features, and practical management. *Eur J Intern Med* 2022;97:26–31.
[3] Gao Y, Xu G, Ma L, et al. Icariside I specifically facilitates ATP or nigericin-induced NLRP3 inflammasome activation and causes idiosyncratic hepatotoxicity. *Cell Commun Signal* 2021;19(1):13.
[4] Uetrecht J. Mechanistic studies of idiosyncratic DILI: clinical implications. *Front Pharmacol* 2019;10:837.
[5] Segovia-Zafra A, Di Zeo-Sánchez DE, López-Gómez C, et al. Preclinical models of idiosyncratic drug-induced liver injury (iDILI): moving towards prediction. *Acta Pharm Sin B* 2021;11(12):3685–3726.
[6] Uetrecht J. Mechanisms of idiosyncratic drug-induced liver injury. *Adv Pharmacol* 2019;85:133–163.
[7] Jee A, Sernoskie SC, Uetrecht J. Idiosyncratic drug-induced liver injury: mechanistic and clinical challenges. *Int J Mol Sci* 2021;22(6):2954.
[8] Tu C, He Q, Li CY, et al. Susceptibility-related factor and biomarkers of dietary supplement polygonum multiflorum-induced liver injury in rats. *Front Pharmacol* 2019;10:335.
[9] Wang Z, Xu G, Wang H, et al. Icariside II, a main compound in Epimedii Folium, induces idiosyncratic hepatotoxicity by enhancing NLRP3 inflammasome activation. *Acta Pharm Sin B* 2020;10(9):1619–1633.
[10] Qin N, Xu G, Wang Y, et al. Bavachin enhances NLRP3 inflammasome activation induced by ATP or nigericin and causes idiosyncratic hepatotoxicity. *Front Med* 2021;15(4):594–607.
[11] Sun ML, Chen XY, Cao JJ, et al. Polygonum multiflorum Thunb extract extended the lifespan and healthspan of Caenorhabditis elegans *via* DAF-16/SIR-2.1/SKN-1. *Food Funct* 2021;12(18):8774–8786.
[12] Xue XY, Quan YY, Gong LH, et al. A review of the processed Polygonum multiflorum (Thunb.) for hepatoprotection: clinical use, pharmacology and toxicology. *J Ethnopharmacol* 2020;261:113121.
[13] Guilliams M, Scott CL. Liver macrophages in health and disease. *Immunity* 2022;55(9):1515–1529.
[14] Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol* 2018;233(9):6425–6440.
[15] Chen YN, Hu MR, Wang L, et al. Macrophage M1/M2 polarization. *Eur J Pharmacol* 2020;877:173090.
[16] Locati M, Curtale G, Mantovani A. Diversity, mechanisms, and significance of macrophage plasticity. *Annu Rev Pathol* 2020;15:123–147.
[17] Chen S, Saeed A, Liu Q, et al. Macrophages in immunoregulation and therapeutics. *Signal Transduct Target Ther* 2023;8(1):207.
[18] Wang C, Ma C, Gong L, et al. Macrophage polarization and its role in liver disease. *Front Immunol* 2021;12:803037.
[19] Kazankov K, Jørgensen SMD, Thomsen KL, et al. The role of macrophages in nonalcoholic fatty liver disease and

nonalcoholic steatohepatitis. *Nat Rev Gastroenterol Hepatol* 2019;16(3):145–159.
[20] Jiang HJ, Li J, Wang L, et al. Total glucosides of paeony: a review of its phytochemistry, role in autoimmune diseases, and mechanisms of action. *J Ethnopharmacol* 2020;258:112913.
[21] Shin MR, Lee SH, Roh SS. The potential hepatoprotective effect of paeoniae radix alba in thioacetamide-induced acute liver injury in rats. *Evid Based Complement Alternat Med* 2022;2022:7904845.
[22] Zhang YJ, Ren XL, Sun H, et al. Establishment of fingerprint and multivariate statistical analysis of compatible herb pairs with Polygonum multiflorum as the core. *China Pharmacy* 2020;31(20):2486–2691 (in Chinese).
[23] Zhang HJ, Ren XL, Sun H, et al. Study on change of ingredients content and DPPH free radical scavenging activity of drug pairs with Polygoni Multiflori Radix Praeparata based on semi-quantitative analysis. *Chinese Traditional and Herbal Drugs* 2021;52(7):1924–1936 (in Chinese).
[24] Jo GH, Kim SN, Kim MJ, et al. Protective effect of Paeoniae radix alba root extract on immune alterations in mice with atopic dermatitis. *J Toxicol Environ Health A* 2018;81(12):502–511.
[25] Li CY, Li XF, Tu C, et al. The idiosyncratic hepatotoxicity of Polygonum multiflorum based on endotoxin model. *Yao Xue Xue Bao* 2015;50(1):28–33.
[26] Du P, Xu JB, Jiang YF, et al. Saikosaponin-d attenuates Hashimoto's thyroiditis by regulating macrophage polarization. *J Immunol Res* 2022;2022:7455494.
[27] Wang ST, Ji TS, Wang L, et al. Exploration of the mechanism by which Huangqi Guizhi Wuwu decoction inhibits LPS-induced inflammation by regulating macrophage polarization based on network pharmacology. *BMC Complement Med Ther* 2023;23(1):8.
[28] Zhao YS, Zhang Y, Kong H, et al. Carbon dots from paeoniae radix alba carbonisata: hepatoprotective effect. *Int J Nanomedicine* 2020;15:9049–9059.
[29] Vago JP, Galvão I, Negreiros-Lima GL, et al. Glucocorticoid-induced leucine zipper modulates macrophage polarization and apoptotic cell clearance. *Pharmacol Res* 2020;158:104842.
[30] Wang G, Liu Y, Hou XF, et al. Discussion on influence factors, mechanism and traditional Chinese medicine pathogenesis of idiosyncratic drug-induced liver injury. *Zhongguo Zhong Yao Za Zhi* 2017;42(16):3036–3043.
[31] Ali SE, Waddington JC, Park BK, et al. Definition of the chemical and immunological signals involved in drug-induced liver injury. *Chem Res Toxicol* 2020;33(1):61–76.
[32] Lin MM, Li YY, Cao B, et al. Bavachin combined with epimedin B induce idiosyncratic liver injury under immunological stress conditions. *Chem Biol Interact* 2023;386:110774.
[33] Li CY, Niu M, Liu YL, et al. Screening for susceptibility-related factors and biomarkers of Xianling Gubao capsule-induced liver injury. *Front Pharmacol* 2020;11:810.
[34] Gao Y, Wang Z, Tang J, et al. New incompatible pair of TCM: Epimedii Folium combined with Psoraleae Fructus induces idiosyncratic hepatotoxicity under immunological stress conditions. *Front Med* 2020;14(1):68–80.
[35] Li XY, Qu CH, He Q, et al. Acute hepatitis induced by a Chinese herbal product Qibao Meiran Wan: a case study. *Int J Clin Exp Med* 2015;8(7):11624–11627.
[36] Meng YK, Li CY, Li RY, et al. Cis-stilbene glucoside in Polygonum multiflorum induces immunological idiosyncratic hepatotoxicity in LPS-treated rats by suppressing PPAR-γ. *Acta Pharmacol Sin* 2017;38(10):1340–1352.
[37] He LZ, Yin P, Meng YK, et al. Immunological synergistic mechanisms of trans-/cis-stilbene glycosides in Heshouwu-related idiosyncratic liver injury. *Sci Bull* 2017;62(11):748–751.
[38] Gao D, Pang JY, Zhang CE, et al. Poria attenuates idiosyncratic liver injury induced by Polygoni Multiflori radix praeparata. *Front Pharmacol* 2016;7:386.
[39] Yan BF, Chen X, Chen YF, 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* 2022;294:115365.
[40] Shan Z, Ju C. Hepatic macrophages in liver injury. *Front Immunol* 2020;11:322.
[41] Watanabe S, Alexander M, Misharin AV, et al. The role of macrophages in the resolution of inflammation. *J Clin Invest* 2019;129(7):2619–2628.
[42] Meng QL, Meng WT, Bian H, et al. Total glucosides of paeony protects THP-1 macrophages against monosodium urate-induced inflammation *via* MALAT1/miR-876-5p/NLRP3 signaling cascade in gouty arthritis. *Biomed Pharmacother* 2021;138:111413.