

Multi-target mechanisms of paeoniflorin in inflammatory regulation: A network pharmacology (Review)

JIN ZHANG^{1,2}, JUNJIE YE², KUN JIN², XUAN SUN¹, XINYU YU¹, YUAN ZHAO³ and NI ZHU^{1,2}

¹School of Stomatology and Ophthalmology, Xianning Medical College, Hubei University of Science and Technology, Xianning, Hubei 437100, P.R. China; ²Hubei Key Laboratory of Environmental Risks and Related Diseases Precision Control, School of Basic Medicine Sciences, Hubei University of Science and Technology, Xianning, Hubei 437100, P.R. China; ³School of Biomedical Engineering, Xianning Medical College, Hubei University of Science and Technology, Xianning, Hubei 437100, P.R. China

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Abstract. Paeoniflorin (PF), a monoterpene glycoside from *Paeonia* roots, shows low toxicity and broad anti-inflammatory activity. A systematic review (PubMed, through 2025) with network pharmacology indicates that PF restores mitochondrial function, raises anti-inflammatory mediators (IL-10 and arginase-1), reduces TNF- α , IL-1 β and IL-6, and eases oxidative stress. Its effects hinge on intertwined routes, dampening TLR4/MyD88, blocking NF- κ B nuclear entry, lowering MAPK phosphorylation, reshaping JAK-STAT, and disrupting NLRP3 inflammasome assembly. Network mapping singles out a conserved core module (MAPK1, MAPK3, HSP90AA1, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2), with HSP90AA1 and ESR1 as disease-tailored effectors in rheumatoid arthritis and osteoarthritis (OA). PF absorbs fast and gathers in the liver with minimal toxicity, yet oral availability stays poor. Preclinical promise spans OA, colitis, and atherosclerosis, while clinical data remain thin. Synergistic pairing with other agents and structural refinement of PF broaden its reach, though such computational leads need

experimental corroboration. Future efforts should improve its bioavailability, develop targeted delivery systems, and conduct rigorous clinical trials to move PF from tradition to evidence-based medicine.

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1. Introduction

Inflammation is a central pathological process in numerous diseases and plays a critical role in the pathogenesis of conditions such as atherosclerosis (AS), arthritis, and liver injury. Inflammation encompasses both acute and chronic responses and represents a defensive reaction to diverse injurious stimuli (1). Resolution of inflammation involves a complex network of signaling molecules, including interleukin-6 (IL-6) and interleukin-8 (IL-8), that regulate cellular activities involved in the removal of inflammatory cells from affected tissues and the restoration of tissue homeostasis (2). Dysregulation of these processes can contribute to persistent inflammation and disease progression. The development of safe and effective agents capable of regulating inflammatory responses and their underlying signaling networks remains clinically important.

Paeonia lactiflora Pall. is a traditional Chinese medicine with ~1,200 years of documented use (3). Its medicinal use has been recorded in Chinese herbal classics across successive dynasties, including the *Essential Prescriptions of the Golden Chamber* (late Eastern Han Dynasty), the *Illustrated Materia Medica* (Song Dynasty), and the *Compendium of Materia Medica* (Ming Dynasty). These texts describe classical herbal formulas containing peony root, including Jin Gui Shen Qi Pill, Da Huang Mu Dan Decoction, and Wen Jing

Correspondence to: Professor Ni Zhu, School of Stomatology and Ophthalmology, Xianning Medical College, Hubei University of Science and Technology, 88 Xianning Road, Xianning, Hubei 437100, P.R. China
E-mail: zhuni@whu.edu.cn

Abbreviations: AA, adjuvant-induced arthritis; AKI, acute kidney injury; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CHOL, cholestatic liver diseases; CP-25, paeoniflorin-6'-O-benzenesulfonate; CUMS, chronic unpredictable mild stress; FLS, fibroblast-like synoviocytes; HIRI, hepatic ischemia-reperfusion injury; IBD, inflammatory bowel disease; NP, network pharmacology; OA, osteoarthritis; PF, paeoniflorin; PF-PLC, PF-phospholipid complex; PPI, protein-protein interaction; RA, rheumatoid arthritis; TCM, Traditional Chinese Medicine; TGP, total glucosides of peony; UC, ulcerative colitis

Key words: paeoniflorin, inflammatory diseases, Chinese herbal medicine, anti-inflammatory, network pharmacology

Decoction. According to traditional Chinese medicine theory, peony root has the properties of clearing heat and cooling blood, activating blood circulation, and resolving blood stasis. It has traditionally been used to treat gynecological disorders, such as dysmenorrhea and amenorrhea, cardiovascular conditions associated with blood stasis syndrome, and disorders involving pain and inflammation, such as abdominal pain (4). Research on classical herbal formulations has provided further evidence for the pharmacological effects associated with peony root. For example, Shao Yao Gan Cao Decoction, derived from Zhang Zhongjing's *Treatise on Cold Damage and Miscellaneous Diseases* (Han Dynasty), was historically used to alleviate spasmodic pain (5). Contemporary studies have shown that this formula suppresses the progression of inflammatory diseases through inhibition of the TLR4/NF- κ B and JAK2/STAT6 pathways, which are established regulators of inflammatory responses (6). These findings provide a basis for examining the active constituents of *Paeonia lactiflora*, particularly paeoniflorin (PF), as potential mediators of its anti-inflammatory effects.

PF, isolated from the roots of *Paeonia lactiflora* Pall. or *Paeonia suffruticosa*, is regarded as a major bioactive substance underlying the pharmacological effects of peony. PF was first isolated in 1963 and was approved for drug production by the China National Medical Products Administration in 1998 (3). PF has also been investigated clinically for rheumatoid arthritis (RA) (7), AS (8), vasculitis (9), pancreatic cancer (10) and other diseases. In AS, for example, increasing PF doses have been reported to enhance serum superoxide dismutase (SOD) activity and reduce serum total phospholipid (TP) and free cholesterol (FC) levels. PF also exerts protective effects on key cells involved in AS, including endothelial cells and smooth muscle cells, and ameliorates pathological changes associated with their dysfunction, including endothelial injury, chronic inflammation and abnormal platelet activation (8). These findings suggest that the biological effects of PF extend beyond the modulation of individual inflammatory mediators and involve multiple cellular processes relevant to disease progression. Further investigation of the molecular structure and physicochemical properties of PF may support its application in drug design and development. PF has been investigated in several preparation forms, which introduces an important consideration when interpreting evidence across studies. To maintain consistency in evidence evaluation, the present review categorizes the included studies into six groups according to the intervention used: i) Purified PF, which is a high-purity PF monomer, identified by reported purity, explicit designation as a monomer compound, or procurement from recognized high-purity suppliers such as Sigma-Aldrich, MedChemExpress, or the National Institutes for Food and Drug Control; ii) total glucosides of peony (TGP), which are extracts containing PF as the major component together with other analogues; iii) PF-containing herbal extracts, which are aqueous or ethanolic extracts prepared from *Paeonia* raw materials; iv) PF derivatives, which are chemically modified PF products; v) PF-containing compound formulas, formulations in which PF is combined with other drugs; and vi) PF nano-formulations, which are PF incorporated into liposomes, nanoparticles, or other delivery systems. Purified PF studies are considered the primary evidence for mechanistic

interpretation, whereas other intervention categories are identified when they first appear. This distinction is important because effects observed with TGP, herbal extracts, compound formulas, or delivery systems cannot necessarily be attributed to PF alone. Available evidence indicates that PF may suppress inflammatory responses through several interconnected mechanisms:

i) Amelioration of mitochondrial dysfunction: PF increases mitochondrial membrane potential and improves the functional activity of electron transport chain complexes, thereby alleviating mitochondrial dysfunction (11). Maintenance of mitochondrial function may contribute to the anti-inflammatory effects of PF by limiting cellular disturbances associated with inflammatory activation.

ii) Promotion of anti-inflammatory factors: PF significantly promotes the secretion of the anti-inflammatory factors interleukin-10 (IL-10), arginase-1 (Arg-1) and transforming growth factor- β (TGF- β). PF also restores olanzapine-suppressed components of the TGF- β 1 pathway, including transforming growth factor- β receptor II (T β RII), T β RI, and phosphorylated SMAD family member 3 (p-SMAD3), in tissues and cells, with histological findings confirming reduced inflammation (12,13).

iii) Suppression of proinflammatory mediators: PF suppresses the secretion of inflammatory effectors, particularly tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), through disruption of signal transduction in the toll-like receptor 4/nuclear factor kappa-B (TLR4/NF- κ B) pathway (14,15). PF also reduces proinflammatory cytokine levels by directly decreasing IL-6 and inhibiting interleukin-22 (IL-22) expression through suppression of the p38 MAPK pathway, contributing to its anti-inflammatory effects (1,16).

iv) Attenuation of oxidative stress: PF promotes the transcriptional activation of Nrf2 and its downstream target HO-1, thereby reducing oxidative stress (17).

v) Regulation of immune responses: PF also exerts immunomodulatory effects, including regulation of dendritic cell activity (14).

The close relationship between inflammatory and immune responses provides a mechanistic basis for considering PF as a regulator of interconnected inflammatory processes rather than as an agent acting on a single inflammatory mediator. Despite the availability of diverse therapeutic strategies, long-term management of inflammatory diseases remains challenging because of adverse effects, inadequate efficacy in some patients, and limited durability of therapeutic responses. These limitations support continued investigation of agents capable of regulating multiple inflammatory and immune pathways.

Network pharmacology (NP) is an emerging discipline that investigates drug-biological network interactions by integrating multiple disciplines and aims to elucidate the multi-target and multi-signaling mechanisms of therapeutic agents. The multi-component and multi-target characteristics of traditional Chinese medicine (TCM) make NP particularly applicable to the study of TCM formulations. TCM formulations are considered to exert therapeutic effects through coordinated regulation of multiple biological targets and pathways, with the overall aim of restoring physiological balance, strengthening healthy *qi*, and eliminating pathogenic factors (18). Advances in artificial intelligence and large-scale data analysis have

further expanded the application of NP to TCM research, providing approaches for investigating treatment mechanisms at the systems level (19). NP applications in TCM include efficacy evaluation, mechanism elucidation, target prediction, safety assessment, drug repurposing and drug design (20). The integration of NP with TCM research has shifted the focus from the conventional 'single target, single drug' paradigm toward a 'network target, multi-component' framework. This approach enables the identification of potentially synergistic components in herbal formulas, analysis of the compatibility principles of Chinese herbal medicines, characterization of network-level regulatory mechanisms, and investigation of the multi-target basis of TCM therapeutic effects (21).

As a major active compound of *Paeonia lactiflora*, PF exhibits broad anti-inflammatory and immunomodulatory activities. By applying NP, the present review comprehensively examines the multi-target and multi-pathway mechanisms through which PF may regulate inflammatory responses. The analysis provides a scientific basis for understanding the potential application of PF in inflammation-related disorders, including arthritis and colitis. By focusing on the interactions between PF, inflammatory targets, and signaling pathways, the present review also provides a framework for further investigation of the anti-inflammatory mechanisms and therapeutic potential of PF.

The present review was based on a search of the PubMed database using 'paeoniflorin' as the search term. As of December 2025, the initial search identified 1,990 records. After exclusion of articles considered irrelevant to the anti-inflammatory mechanisms of PF, non-research articles, and articles published before 2019, 964 articles remained. A further 833 articles were excluded because of insufficient essential information, including unclear targets, incomplete data, or undefined pathways. This screening process reportedly yielded 131 articles for analysis. Additionally, articles unrelated to PF or those involving NP only were also excluded. Finally, 123 articles were included in this review (Fig. S1).

Previous reviews have summarized the pharmacological effects of PF in individual disease models. The present review focuses on the broader relationship between PF and inflammatory regulation and provides three specific contributions: i) Construction of a multi-target/multi-pathway inflammatory regulatory network of PF by integrating NP and molecular docking; ii) a pan-disease comparison to identify conserved core modules while distinguishing general inflammatory hubs from disease-specific effectors; and iii) comprehensive synthesis of emerging evidence on PF derivatives and nano-formulations as potential strategies for therapeutic development, which have received less comprehensive coverage in previous reviews.

2. Pharmacokinetics

PF, $C_{23}H_{28}O_{11}$, is a monoterpene glycoside from *Paeonia* species. Its pinane-type skeleton carries a β -D-glucosyl moiety at C-1 and a benzoyl group esterified at C-9, along with multiple hydroxyl groups. These features give PF high water solubility, beneficial for formulation, but restrict membrane permeability and limit oral bioavailability. In animal studies, oral bioavailability of PF is ~3-4% (22). A human pharmacokinetic

study reported that after an oral dose of an extract containing 108.18 mg of PF, plasma C_{max} reached only 6.97 ng/ml (23). Although the physicochemical and pharmacokinetic properties of PF have been described, how its solubility relates to absorption, distribution and pharmacological efficacy is not fully understood (24,25).

In the molecular structure diagram, the red-highlighted region corresponds to the C-ring of the core scaffold, the central pharmacophore required for biological activity. The blue box covers the benzoyl and glucosyl moieties, both critical to PF's anti-inflammatory and analgesic effects. Specifically, the carbonyl oxygen in the benzoyl group (yellow) forms hydrogen bonds with enzymes or receptors *in vivo*, improving target-binding affinity and serving as the primary site for anti-inflammatory action. The orange regions mark functional groups that increase hydrophilicity and support dissolution, absorption, and systemic transport. These include the hydroxymethyl group ($-CH_2OH$ at C-6 of the glucose unit), which increases molecular hydrophilicity and promotes dissolution and transmembrane transport, and the hydroxyl groups ($-OH$) on the glucopyranose ring, which enhance water solubility through higher polarity and hydrogen-bonding capacity, improving diffusion in biological fluids and drug action (Fig. 1). That said, direct experimental data are still insufficient to clarify the hydrolysis mechanism, the exact metabolite profile, and their quantitative impact on the duration of pharmacological effect and oral bioavailability (26). In addition, the hydroxyl groups of PF can form non-covalent interactions with biological targets (enzymes or receptors), including hydrogen bonds and van der Waals forces, as supported by molecular docking and pharmacological studies (27).

Pharmacokinetic data indicate that although PF concentrations after oral administration are markedly lower than those after intravenous injection, rapid absorption occurs, with peak plasma levels reached within 10 min (28). Orally administered PF is poorly absorbed in the gastrointestinal tract; most is metabolized by gut microbiota in the distal small intestine, cecum, and colon, sites where hydrolysis and biotransformation are accelerated, producing a range of metabolites (29). Given intravenously, PF crosses the blood-brain barrier (BBB) and distributes to the hippocampus and cerebral cortex (28). Both the parent compound and its intestinal metabolites then undergo hepatic biotransformation, including oxidation, reduction, hydroxylation, methylation and conjugation reactions (for example, glucuronidation and sulfation). Metabolism is mediated mainly by cytochrome P450 enzymes via hydroxylation and demethylation. Unchanged PF accounts for only a minor fraction *in vivo*; most is detected in plasma, urine, and feces as metabolites (30,31). Benzoic acid, a major metabolite, crosses the BBB and produces antidepressant effects, whereas the distribution and functional roles of other metabolites remain to be determined (29).

Taken together, PF is extensively transformed *in vivo*, hydrolyzed by gut microbiota and then processed by hepatic oxidative and conjugative metabolism, generating metabolites with distinct biological activities. This low systemic exposure after oral administration contrasts with the concentrations used in *in vitro* studies, pointing to a gap between experimental findings and clinical conditions. These metabolites not only mediate PF's anti-inflammatory efficacy but also provide

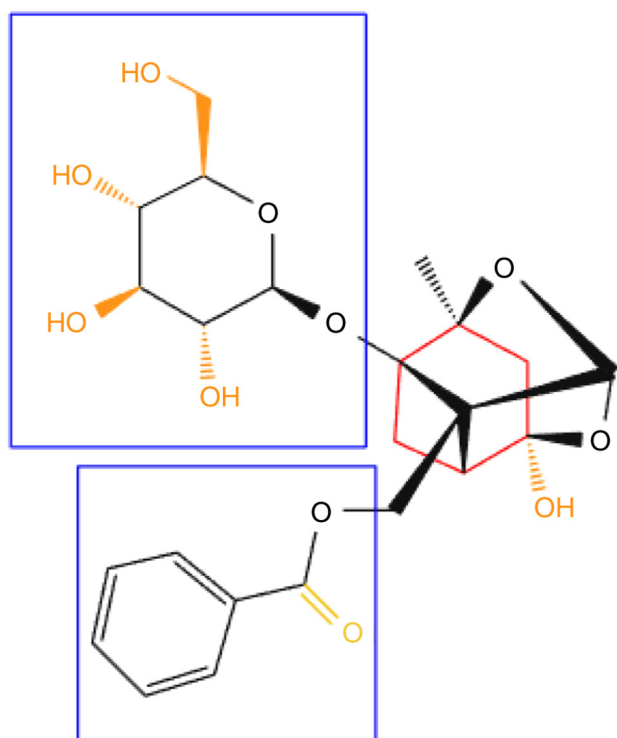


Figure 1. Chemical structure of paeoniflorin.

the molecular basis for its multi-target and multi-cascade pharmacological actions.

3. Molecular mechanisms of PF for modulating inflammation

Inhibitory effect of PF on the NF- κ B signaling pathway. NF- κ B is a family of transcription factors that plays crucial roles in various physiological and pathological processes. NF- κ B signaling is primarily mediated through two distinct pathways, the canonical (classical) and non-canonical (alternative) pathways, which differ in their activation mechanisms and biological functions. The canonical NF- κ B pathway is a central regulator of inflammatory responses and is therefore an important target of PF in the modulation of inflammation (32).

Inhibition of nuclear transcription factor activity. In the hippocampus of mice with depression induced by the chronic unpredictable mild stress (CUMS) model, western blotting and immunofluorescence analyses showed that high-dose PF significantly inhibited the stress-induced increase in NF- κ B p65 protein expression and nuclear fluorescence intensity (33). As a pivotal transcription factor, NF- κ B regulates the production and release of diverse inflammatory cytokines. PF effectively inhibited NF- κ B activation in this model, providing experimental evidence for its ability to regulate inflammation-related transcriptional activity (33).

Inhibition of IKK activity. IKK (I κ B kinase), the key kinase responsible for the phosphorylation of NF- κ B inhibitor alpha (I κ B α), plays a central role in NF- κ B activation. PF inhibits IKK activity, thereby reducing I κ B α phosphorylation and subsequent proteasome-mediated degradation. This preserves cytoplasmic retention of the NF- κ B p65/p50 dimer and limits its nuclear translocation and transcriptional activity, contributing to the anti-inflammatory effects of PF (34).

Inhibiting the degradation of I κ B α . I κ B α is a pivotal negative regulator of the NF- κ B pathway. By binding NF- κ B subunits such as p65 in the cytoplasm, I κ B α prevents their nuclear translocation and limits the transcription of proinflammatory genes. PF inhibits I κ B α phosphorylation, thereby suppressing its ubiquitin-proteasome-mediated degradation, increasing I κ B α stability, and restricting NF- κ B nuclear translocation. Experiments in activated T lymphocytes and a mouse model of acute contact dermatitis induced by 1-chloro-2,4-dinitrobenzene (DNCB) demonstrated that PF treatment dose-dependently inhibited I κ B α phosphorylation and NF- κ B p65 activation while reducing interferon- γ secretion. These findings support a mechanism in which PF suppresses inflammatory responses by stabilizing I κ B α and inhibiting NF- κ B signaling (35).

Inhibition of nuclear translocation of the p65/p50 complex. The p65/p50 heterodimer is the primary functional form of NF- κ B involved in canonical pathway signaling. PF reduces NF- κ B nuclear translocation by suppressing its activation. In activated T lymphocytes, PF (10 μ M) significantly reduced NF- κ B p65 expression and dose-dependently inhibited I κ B α phosphorylation. In a mouse model of acute contact dermatitis induced by DNCB, oral administration of PF (75 or 150 mg/kg/day) dose-dependently reduced NF- κ B p65 expression and I κ B α phosphorylation in T lymphocytes. These findings indicate that PF suppresses NF- κ B activation and nuclear translocation by reducing p65 expression and maintaining I κ B α -mediated cytoplasmic retention of the NF- κ B complex, thereby limiting the transcription of downstream proinflammatory genes (34).

Inhibitory effects of PF on the MAPK signaling pathway. The MAPK pathway constitutes a three-tiered kinase cascade in which MAPK kinase kinases (MAPKKKs) respond to diverse extracellular and intracellular signals and activate MAPK kinases (MAPKKs) through phosphorylation. MAPKKs then phosphorylate MAPKs at specific sites, leading to their activation. Activated MAPKs regulate various downstream target proteins and, in response to extracellular stimuli, control cell fate and cellular responses (36). The MAPK pathway is also involved in the production of inflammatory mediators, activation of immune cells, and regulation of tissue inflammatory responses. Aberrant MAPK activation is associated with several inflammatory diseases, including RA, neuroinflammation and cardiovascular inflammation.

Inhibition of MAPKK (MKK4). Western blot analysis demonstrated that tributyltin chloride (TBTC) treatment significantly increased the phosphorylation of MAPKK 4 (MKK4) in a TBTC-induced neurotoxicity model, indicating activation of the MKK4 pathway. Pretreatment with PF at 50 and 100 μ M dose-dependently suppressed this increase, with p-MKK4 expression in the 100 μ M PF group significantly lower than that in the TBTC model group. These findings indicate that PF suppresses upstream stress signaling by inhibiting MKK4 phosphorylation and activation, which may contribute to its neuroprotective effects (37).

Inhibition of MAPKs [Jun N-terminal kinase (JNK), p38, ERK1/2, ERK5]

PF inhibits JNK phosphorylation. PF's regulation of JNK is context-dependent rather than uniformly inhibitory. JNK can

act as an upstream regulator of Nrf2 under oxidative stress conditions. Compared with the H₂O₂-only group, PF enhanced JNK activation in an oxidative stress model (38). In chronic inflammatory models, PF inhibits JNK phosphorylation, which may reduce AP-1-mediated inflammatory signaling. By contrast, under acute oxidative stress, PF can enhance JNK activation and promote Nrf2 nuclear translocation and HO-1 expression, supporting an antioxidant response. In both the spontaneously hypertensive rat (SHR) cardiac model and adjuvant-induced arthritis (AA) rat fibroblast-like synoviocyte (FLS) experiments, PF significantly reduced p-JNK levels and was associated with reduced inflammatory responses together with inhibition of p38/ERK signaling. In an H₂O₂-induced melanocyte oxidative stress model, PF enhanced JNK activation, promoted Nrf2 nuclear translocation and HO-1 upregulation, and exerted a protective effect that was reversed by the JNK inhibitor SP600125. These findings support a context-dependent role of JNK in PF-mediated cellular protection and inflammatory regulation. PF may suppress sustained JNK-mediated inflammatory signaling under chronic inflammatory conditions while promoting JNK-dependent antioxidant responses under oxidative stress (39).

PF inhibits p38 MAPK phosphorylation. PF exerts anti-inflammatory effects in part through inhibition of p38 MAPK phosphorylation, thereby reducing the production of p38-dependent proinflammatory mediators, including TNF- α , IL-6 and IL-1 β . In models including bupivacaine-induced neurotoxicity in human neuroblastoma SH-SY5Y cells, the SHR cardiac remodeling model, and the AA rat model, PF markedly suppressed p38 activation and alleviated neuroinflammation, myocardial fibrosis and other pathological changes. Its inhibitory effect was comparable to that of the specific p38 inhibitor SB203580. Given the central role of p38 in inflammatory signaling, suppression of p38 activation represents an important component of the anti-inflammatory effects of PF (39).

PF significantly inhibits ERK phosphorylation. In FLS derived from AA rats, western blotting confirmed that PF significantly inhibited ERK phosphorylation (p-ERK). The proposed mechanism involves inhibition of the aberrant translocation of G protein-coupled receptor kinase 2 (GRK2) to the cell membrane, thereby restoring the normal interaction between GRK2 and phosphorylated ERK (p-ERK) and reducing pathological accumulation of p-ERK in the nucleus. This effect was particularly evident under inflammatory stimulation, such as prostaglandin E₂ (PGE₂) exposure, during which PF reversed both increased GRK2 membrane localization and p-ERK nuclear translocation. PF may thus regulate inflammatory signaling through the GRK2/p-ERK axis (40). ERK5 is a distinct MAPK family member activated through phosphorylation by MAPKK 5 (MEK5). Activated ERK5 regulates transcription factors, including myocyte enhancer factor, cellular-myelocytomatosis oncogene homolog, and cellular-FBJ murine osteosarcoma viral oncogene homolog, which are involved in the regulation of cell-cycle progression and apoptosis. PF activates endoplasmic reticulum stress and induces calpain-1 activation, which promotes the proteolytic cleavage and degradation of ERK5 and thereby suppresses ERK5-mediated signaling (41).

PF applies anti-inflammatory impacts via broad inhibition of the MAPK pathway. MAPKs are central components of inflammatory signal transduction. The three major MAPK subtypes, p38, JNK and ERK, are frequently activated under inflammatory conditions and can phosphorylate downstream transcription factors that promote inflammatory mediator production and tissue injury. p38 is strongly associated with the production of inflammatory mediators, JNK contributes to inflammatory signaling and apoptosis, and ERK participates in the progression of several inflammatory processes. Inhibition of MAPK phosphorylation can therefore attenuate inflammatory responses. For example, PF reduced p-p38, p-JNK and p-ERK levels in SHR models and alleviated inflammatory responses. In RA models, the PF derivative PF-6'-O-benzenesulfonate (CP-25) inhibited activation of all three major MAPK subtypes in synovial cells through partially distinct mechanisms. CP-25 inhibits GRK2 membrane translocation, thereby reducing ERK nuclear translocation and downstream inflammatory mediator production. It also suppresses p38 and JNK through pathways that are independent of GRK2 (41,42).

Inhibitory effect of PF on the JAK-STAT signaling pathway. The Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway is a central regulatory system involved in inflammatory homeostasis. Activation of this pathway by cytokines regulates the expression of factors such as suppressor of cytokine signaling 3 and B-cell lymphoma 3-encoded protein, which provide negative feedback to inflammatory signaling and can limit NF- κ B activity and the production of proinflammatory mediators such as TNF- α and IL-6 (43). PF and its structurally modified derivatives have been reported to regulate this pathway at several levels, providing another mechanism through which PF may modulate inflammatory responses.

Inhibition of JAK phosphorylation and activation. The PF derivative CP-25 selectively inhibited the phosphorylation of JAK1, JAK2 and JAK3 (p-JAKs) in human umbilical vein endothelial cells (HUVECs) exposed to interleukin-17A (IL-17A; 50 ng/ml), without altering total JAK protein levels. This inhibition was accompanied by significant reductions in p-STAT3 and secretion of the inflammatory adhesion molecule intercellular adhesion molecule-1 (ICAM-1) (9). Time-course experiments showed that suppression of JAK phosphorylation preceded the reduction in STAT3 phosphorylation, supporting JAK inhibition as an upstream event in CP-25-mediated regulation of STAT3. These findings indicate that CP-25 can modulate JAK activation without reducing total JAK protein abundance. The same study reported concentration-dependent inhibition of JAK1, JAK2 and JAK3 phosphorylation by CP-25 in IL-17A-stimulated HUVECs, together with reductions in p-STAT3 and ICAM-1, providing consistent evidence for regulation of the JAK-STAT axis (9). Evidence from PF-containing formulations also supports the involvement of this pathway. In Buyang Huanwu Decoction, a formula containing PF and other glycosides, the glycoside fraction attenuated AS in ApoE^{-/-} mice and reduced inflammatory responses, accompanied by decreased phosphorylation of JAK2, STAT1 and STAT3. Molecular docking further suggested interactions between PF and proteins associated with the JAK-STAT pathway (44). Because these findings were obtained with a PF derivative or

a multi-component formulation rather than purified PF alone, they provide indirect evidence for PF-associated JAK-STAT regulation. Direct experiments using purified PF are required to establish whether inhibition of JAK phosphorylation is an intrinsic property of PF.

Inhibition of STAT protein phosphorylation and nuclear translocation. STAT proteins are key transcriptional effectors downstream of JAKs. Following phosphorylation, STAT proteins dimerize and translocate to the nucleus, where they regulate the transcription of target genes involved in cytokine signaling and inflammatory responses (43). Evidence indicates that PF can regulate STAT signaling at the levels of gene expression, phosphorylation and downstream cellular function. At the level of STAT expression, droplet digital PCR and immunoblotting showed that PF significantly reduced both *Stat1* and *Stat3* mRNA and protein expression while also inhibiting JAK2 phosphorylation (9). These findings suggest that PF can influence both the expression and activation of components of the JAK-STAT pathway. In an LPS-induced macrophage polarization model, PF and its nano-formulation also modulated STAT phosphorylation by suppressing p-STAT1, a mediator associated with M1 macrophage polarization, while promoting recovery of p-STAT6, which is associated with M2 polarization. The targeted nano-formulation Pae-PPNPs-DS produced stronger changes in p-STAT1 and p-STAT6 than the parent compound and other tested carriers, suggesting that the delivery system can enhance the effects of PF on STAT signaling (12).

Overall, these findings indicate that PF and PF-derived interventions can regulate the JAK-STAT pathway at multiple levels, including JAK phosphorylation, STAT expression, STAT phosphorylation and immune-cell function. The available evidence also suggests that these effects may contribute to macrophage polarization and suppression of inflammatory responses. Since some of the reported effects involve CP-25 or PF nano-formulations rather than purified PF, the extent to which the complete regulatory pattern represents an intrinsic property of PF requires further investigation.

Inhibitory effect of PF on NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome activation. The NLRP3 inflammasome is a multiprotein signaling complex composed primarily of NLRP3 as the sensor, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) as the adaptor, and caspase-1 as the downstream effector protease. Upon activation, NLRP3 promotes ASC oligomerization and caspase-1 activation, leading to maturation and release of IL-1 β and IL-18 and, in numerous settings, gasdermin D (GSDMD)-mediated pyroptosis (45). PF has been reported to inhibit NLRP3 inflammasome activation through regulation of both upstream inflammatory signaling and inflammasome-associated components.

Inhibition of NLRP3 inflammasome assembly and activation. In both *in vivo* CUMS-induced depressive mice and *in vitro* LPS/ATP-stimulated BV2 microglia models, PF treatment significantly reduced the protein levels of NLRP3, ASC and caspase-1 in hippocampal tissue and cells. These changes were accompanied by reduced IL-1 β secretion and GSDMD-mediated pyroptosis. Mechanistically, PF (100 μ M) increased Sirtuin 1 (SIRT1) expression and inhibited NF- κ B

p65 nuclear translocation, whereas inhibition of SIRT1 with EX-527 abolished the protective effects of PF. These findings support involvement of the SIRT1/NF- κ B/NLRP3 axis in PF-mediated suppression of inflammasome activation (33). A separate study identified a metabolic signaling pathway linking succinate accumulation to NLRP3 activation. In a Complete Freund's Adjuvant-induced model, succinate accumulation increased succinate receptor 1 (SUCNR1) and hypoxia-inducible factor 1- α (HIF-1 α) expression and was associated with NLRP3 inflammasome activation. Immunofluorescence and qPCR showed increased NLRP3 and caspase-1 protein expression and increased *Nlrp3* and *Caspase-1* mRNA levels in dorsal root ganglion neurons, accompanied by increased IL-1 β release and neuroinflammation. PF suppressed SUCNR1 and HIF-1 α expression, reduced inflammasome activity, and decreased IL-1 β production. These findings indicate that PF may alleviate neuropathic pain through modulation of the succinate-SUCNR1-HIF-1 α -NLRP3 axis (46).

Suppression of caspase-1 activation and mature IL-1 β secretion. PF also regulates downstream components of NLRP3 inflammasome signaling. In CUMS-induced mice, hippocampal caspase-1 protein levels were markedly higher than those in control mice, whereas PF treatment reduced caspase-1 expression in a dose-dependent manner, with the strongest effect observed at the highest dose tested. The associated reduction in IL-1 β production and pyroptosis suggests that PF can attenuate caspase-1-dependent inflammatory signaling (33).

Inhibition of the TLR4/MyD88 signaling pathway by PF. TLR4 is a transmembrane pattern-recognition receptor that detects bacterial lipopolysaccharide (LPS) and initiates signaling cascades involved in innate immune and inflammatory responses (47,48). PF has been reported to attenuate inflammatory responses partly through suppression of TLR4/MyD88 signaling.

Inhibition of TLR4 activation. In AA rats, TLR4 surface expression on peritoneal macrophages was increased together with MyD88, NLRP3, ASC, caspase-1 and GSDMD-N, indicating activation of TLR4-associated inflammatory and pyroptotic signaling. PF treatment reduced the expression of these markers. Flow cytometry further showed increased TLR4 fluorescence in macrophages from AA rats, which was reduced by PF at 1 and 10 μ M. *In vitro*, LPS/ATP stimulation increased TLR4 expression, whereas PF reduced the corresponding fluorescence intensity. Western blotting produced consistent findings, supporting the inhibitory effect of PF on TLR4 expression and downstream signaling (49).

Suppression of MyD88-dependent signaling. In a model of cholestatic liver injury induced by 17 α -ethinyl-estradiol, PF and its micellar formulation (PF-PLC) suppressed the TLR4/MyD88/NF- κ B axis. PF-PLC markedly reduced MyD88 protein expression and was associated with decreased NF- κ B nuclear translocation and inflammatory cytokine production. These effects were supported by western blotting and a reduction in NF- κ B-positive cells detected by immunofluorescence. The findings indicate that modulation of MyD88-dependent signaling may contribute to the hepatoprotective effects of PF-PLC (50).

Multi-pathway crosstalk in the anti-inflammatory network of PF. The aforementioned signaling pathways are interconnected rather than functioning as independent pathways. PF has been reported to inhibit both NF- κ B nuclear translocation and MAPK phosphorylation, including JNK, p38 and ERK, suggesting that upstream signaling nodes may contribute to the coordinated regulation of these pathways. TAK1 is one such convergence point because TLR4/MyD88 signaling can activate TAK1, which in turn contributes to activation of IKK and downstream NF- κ B signaling as well as MKK4/7 and JNK signaling. The observed effects of PF on NF- κ B and MAPK pathways are therefore consistent with regulation at shared upstream signaling nodes, although direct evidence demonstrating TAK1 as a molecular target of PF remains necessary. PF also affects the SIRT1/NF- κ B/NLRP3 axis. Increased SIRT1 activity can reduce NF- κ B p65 transcriptional activity, thereby limiting the priming of NLRP3 inflammasome components. PF-mediated suppression of the succinate-SUCNR1-HIF-1 α -NLRP3 axis provides another connection between metabolic stress and inflammasome activation. HIF-1 α can regulate inflammatory gene expression, including NLRP3-associated responses, allowing metabolic and inflammatory signals to converge at the inflammasome (51). These observations suggest that PF may influence inflammatory signaling through several interconnected upstream regulators rather than through a single molecular target. The JAK-STAT pathway provides another point of interaction within this network. CP-25 inhibits JAK1/2/3 phosphorylation and reduces STAT3 activation, whereas PF has been shown to suppress NF- κ B signaling. JAK-STAT and NF- κ B pathways can interact at the level of shared inflammatory mediators and cellular responses, including IL-6-associated signaling and endothelial inflammatory activation.

PF may also influence inflammatory responses through interactions between different cell types. PF has been reported to regulate microglia-astrocyte crosstalk through the HSP90AA1/HMGB1 pathway in neuroinflammation and macrophage-intestinal stem cell (ISC) crosstalk through Clq in chronic colitis. These findings extend the network concept beyond intracellular signaling to communication between immune and tissue-resident cells. Ferroptosis may represent another process linked to inflammatory regulation because lipid peroxidation and ferroptotic cell death can interact with inflammatory and oxidative stress pathways. Studies of related herbal formulas have shown that CXCR4 upregulation can suppress GPX4-mediated ferroptosis (52). Such findings provide a possible connection between antioxidant activity and inflammatory regulation, but evidence directly linking PF to this mechanism remains limited. Overall, the available evidence indicates that PF can influence interconnected intracellular and intercellular processes, although the contribution of individual nodes and their causal relationships require further validation.

PF exhibits anti-inflammatory activity through coordinated regulation of several signaling pathways. It suppresses TLR4/MyD88 signaling, inhibits NF- κ B activation and nuclear translocation, attenuates phosphorylation of MAPK components including JNK, p38, ERK1/2 and ERK5, modulates JAK-STAT signaling, and suppresses NLRP3 inflammasome activation (Fig. 2A-E). These pathways are interconnected

through shared upstream regulators and inflammatory mediators, providing a mechanistic basis for the multi-target effects of PF. The available evidence supports a network-level mode of action involving TLR4/MyD88, NF- κ B, MAPK, JAK-STAT and NLRP3 signaling rather than a single dominant target. Further studies using purified PF and direct target-validation approaches are needed to define the molecular nodes responsible for these coordinated effects.

4. Mechanisms of PF for modulating inflammatory diseases

Molecular targets prediction of PF's anti-inflammatory effects

NP analysis. NP analysis was performed using standard workflows. PF compounds were screened via TCMSP (v2.3, OB $\geq$ 30%, DL $\geq$ 0.18); disease targets were retrieved from GeneCards (v5.18; <https://www.genecards.org/>), OMIM (v2024-08; <https://omim.org/>), TTD (v2024-10; <https://db.idrblab.net/ttd/>), Malacards (v6.1, <https://www.malacards.org/>), HPO (v2025-01; <https://hpo.jax.org/>), PharmGKB (accessed May 2025; <https://www.pharmgkb.org/>), and Open Targets (v25.03; <https://platform.opentargets.org/>), all accessed in May 2025, with GeneCards filtered by Relevance score $\geq$ median and other databases by default thresholds. Targets were standardized via UniProt (<https://www.uniprot.org/>). PPI networks were constructed using STRING (v12.0; <https://string-db.org/>; species: *Homo sapiens*, confidence $\geq$ 0.400, max $\leq$ 20 interactors), and hub genes were predicted via Cytoscape (v3.10.2; <http://www.cytoscape.org/>) with the CytoHubba-MCC algorithm (top 10 by MCC score). Hub molecules, including TNF, IL1B, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-alpha (NFKBIA), and AKT serine/threonine kinase 1 (AKT1), emerged as core nodes within PF's action network. These targets, particularly TNF and IL1B, were consistently identified across models of rheumatic and inflammatory diseases, including RA, chronic kidney injury and colitis.

Molecular docking was performed using AutoDock Vina (version 1.2.7; <https://github.com/ccsb-scripps/AutoDock-Vina>). Three-dimensional structures of target proteins (TNF, IL1B, NFKBIA, and AKT1) were retrieved from the RCSB Protein Data Bank (PDB, <https://www.rcsb.org/>, accessed May 2025), and input files were prepared using AutoDockTools (version 1.5.7). The three-dimensional structure of PF was generated using Open Babel and prepared with PyMOL (including water molecule removal, hydrogen addition and adjustment of symmetric bi-molecules). For each target, the grid box was set individually according to the position and size of its predicted active pocket. Exhaustiveness was set to the default value of 8, and the number of binding modes (num_modes) was set to 9. A binding energy $\leq$ -7.0 kcal/mol was considered the threshold for strong binding potential. The docking results predicted highly negative binding energy values for these targets, suggesting stable occupancy of protein active sites and potential interference with inflammatory signaling. In disease models including RA, diabetic complications, and liver injury, PF showed comparable or more favorable binding energies with TNF and AKT1 than several reference anti-inflammatory compounds (53,54). Of note, molecular docking is a computational prediction tool

Paeoniflorin (PF) Multi-Target Anti-Inflammatory Mechanisms

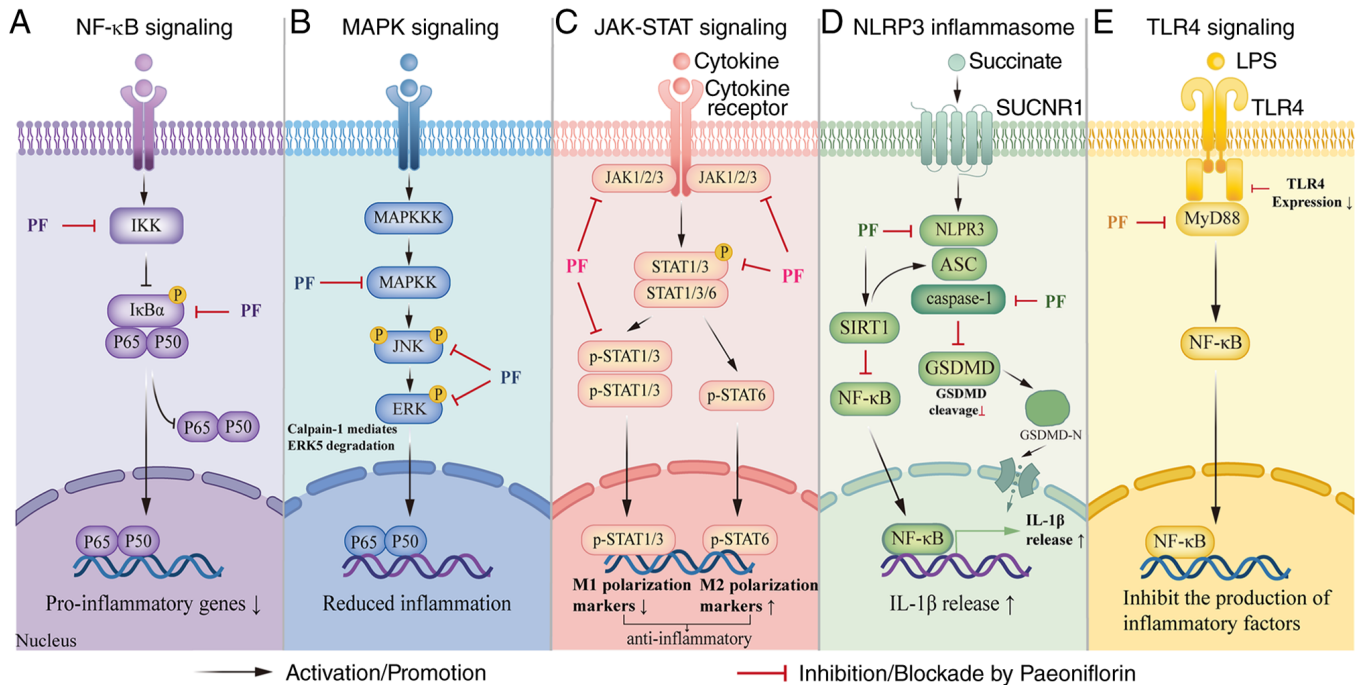


Figure 2. Anti-inflammatory mechanisms of PF. PF inhibits ($\perp$) five signaling cascades: (A) TLR4/MyD88, (B) NF- κ B activation, (C) MAPK phosphorylation (JNK/p38/ERK1/2/ERK5), (D) JAK-STAT signaling and (E) NLRP3 inflammasome assembly. Arrows ($\rightarrow$) indicate activation; T-bars ($\perp$) indicate inhibition by PF. PF, paeoniflorin; ASC, apoptosis-associated Speck-like Protein containing a CARD; CAPN1, Calpain-1; caspase-1, Caspase-1; ERK, Extracellular signal-regulated kinase; ERK5, Extracellular signal-regulated kinase 5; GSDMD, Gasdermin D; IKK, I κ B Kinase; JAK, Janus kinase; JNK, Jun N-terminal kinase; MAPK, Mitogen-activated protein kinase; MyD88, Myeloid differentiation factor 88; NLRP3, NOD-, LRR- and Pyrin Domain-containing Protein 3; p38, p38 Mitogen-activated protein kinase; STAT, Signal transducers and activators of transcription; STAT, signal transducers and activators of transcription; TLR4, Toll-like receptor 4.

with inherent limitations, including rigid protein models, simplified solvent treatments, and empirical scoring functions. Accordingly, these docking results serve as preliminary conformational support for NP predictions, rather than experimental evidence of binding or causality.

Collectively, NP and molecular docking predicted TNF, IL1B, NFKBIA, AKT1 and related proteins as key molecular targets of PF. The predicted binding affinity of PF for these targets provides a preliminary molecular basis for its multi-level regulation of inflammatory responses. This multi-target, multi-pathway profile offers a theoretical basis for further exploring PF's therapeutic potential.

Protein-protein interaction (PPI) network of the molecular targets. PPI networks of these predicted targets were constructed to reveal the molecular relationship. Across disease models, including depression, diabetes, RA, chronic hepatitis and autoimmune disorders, core PF targets encompassed AKT1, SRC, HSP90AA1, TNF, CASP3, MAPK1, VEGFA and EGFR, all critically involved in inflammatory signaling, cell survival and apoptosis (55-58).

Within disease-specific PPI networks, PF-targeted proteins often occupied hub positions, serving not only within individual pathways but also as convergence points for multiple inflammatory and apoptotic cascades. The high connectivity of AKT1, SRC and MAPK1 suggests that PF's modulation of these hubs concurrently affects diverse downstream processes, enabling multi-level control of inflammation (59,60). Disease-specific PPI analyses were further conducted to delineate the core

regulatory nodes and hierarchical mechanisms underlying PF's anti-inflammatory actions.

PF and its predicted targets in inflammatory diseases

AS. AS involves plaque formation within the arterial intima, driven by various cells, lipids and cellular debris. It is recognized as a chronic vascular inflammatory disease mediated by traditional and non-traditional risk factors, accounting for most myocardial infarctions, numerous strokes, and disability in peripheral arterial occlusive disease. Historically considered as a disorder of cholesterol accumulation triggered by retention of low-density lipoprotein (LDL) in the arterial intima, scavenger receptor-mediated uptake of LDL promotes sustained immune cell infiltration into atherosclerotic plaques (61-64). Current therapeutic strategies targeting inflammatory modulation include suppression of proinflammatory cytokines, disruption of critical inflammatory signaling cascades, and facilitation of inflammation resolution. Animal experiments demonstrated that in a high-fat diet-induced AS rat model, escalating doses of PF-containing plant extract significantly increased SOD activity while decreasing serum levels of TP and FC. PF also exerted protective effects on key target cells, including vascular endothelial cells and smooth muscle cells. It alleviated core pathological processes such as endothelial injury, chronic inflammatory responses, and abnormal platelet activation resulting from cellular dysfunction (8).

For NP-targets prediction in AS, a total of 4,815 AS-related targets were retrieved. After cross-matching with PF targets,

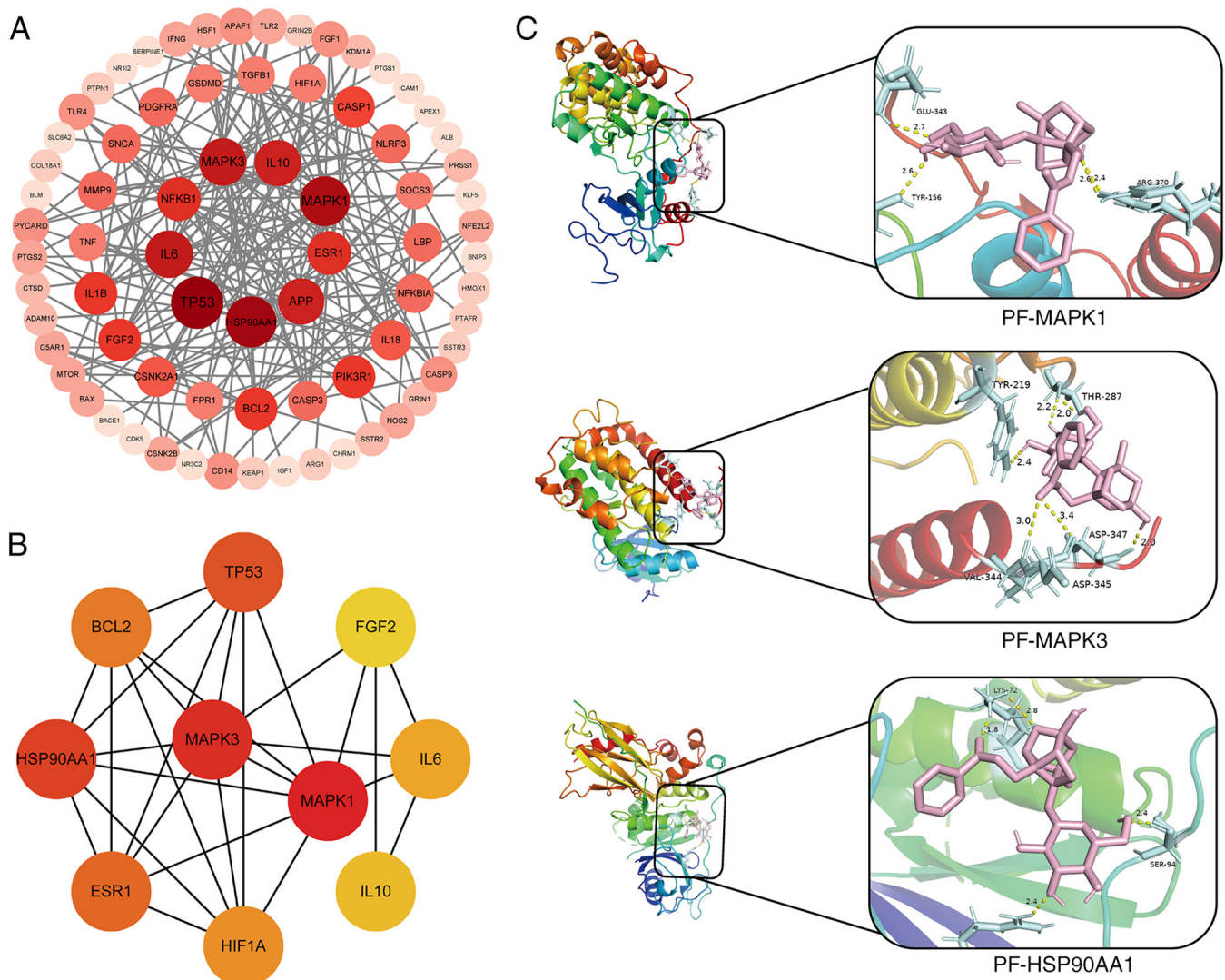


Figure 3. Network pharmacology analysis of PF against AS. (A) Protein-protein interaction network of AS-associated targets. (B) Hub targets identified in AS. (C) Molecular docking analysis of PF with the top three core target proteins in AS. AS, atherosclerosis; PF, paeoniflorin.

a PPI network was constructed (Fig. 3A), and 10 hub genes were prioritized: MAPK1, MAPK3, HSP90AA1, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 3B). Molecular docking of the top three hub targets (MAPK1, MAPK3, HSP90AA1) revealed that PF formed conventional hydrogen bonds with Glu343, Tyr156, Tyr219, Thr287, Ser94, Arg370 and Asp345; a carbon-hydrogen bond with Val344; and salt bridges with Lys72 and Asp347 (Fig. 3C). Molecular docking across all hub genes (Fig. S2A-J) further suggested high binding affinity between PF and its targets.

Respiratory diseases. Pneumonia is the most common cause of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (65). ‘Phlegm and blood stasis’ form the pathological basis of ALI. With few effective treatments available, ALI and ARDS remain severe, life-threatening conditions (66). A recent study highlighted macrophages as key contributors to the dysregulated cytokine response in virally triggered ALI (67). As central components of pulmonary innate immunity, macrophages support pathogen clearance through phagocytosis or secretion of pro-inflammatory cytokines, mechanisms essential for limiting initial viral spread. However,

prolonged viral exposure may trigger a self-amplifying inflammatory cascade that disrupts immunological balance and leads to aberrant immune activation. This pathological shift manifests as hyperactivated macrophages secreting an excess of inflammatory mediators, including IL-6, TNF- α , IL-1 β , IFN- γ , IFN- α , CCL2, IL-8 and IP-10. The resulting systemic cytokine surge compromises the structural and functional integrity of the alveolar-capillary barrier, increases vascular hyperpermeability, triggers pulmonary edema, impairs alveolar architecture, and drives pathological progression toward ALI/ARDS (68). NP screening of the Chuanxiong-Danshen herb pair identified PF as a potential active component against LPS-induced ALI. In LPS-stimulated RAW264.7 macrophages and BALB/c mice, the PF-containing fraction from this herb pair suppressed inflammatory responses and oxidative stress, reducing ROS, NO, TNF- α , IL-6 and IL-1 β levels, with mechanistic studies pointing to involvement of the NF- κ B and MAPK pathways (69,70). The antiviral effect of PF in ALI is also noteworthy. In a mouse model infected with influenza virus, oral treatment with PF (50 and 100 mg/kg) increased the survival rate of infected mice (by 40% in the

50 mg/kg group and 50% in the 100 mg/kg group) and alleviated early-stage pulmonary edema and pathological changes caused by direct viral damage (71). Its antiviral mechanism may involve a unique pathway: PF inhibits expression of $\alpha\beta 3$ integrin in lung tissue. $\alpha\beta 3$ serves as a potential co-receptor for viral entry and intracellular signal transduction. Inhibiting it may interfere with viral infection and subsequent pathological signal amplification at the source, thereby mitigating initial damage. Subsequently, by inhibiting pathways such as NF- κ B/p38 MAPK, PF curbs the resulting excessive inflammation and fibrotic cascade, achieving overall protection against viral ALI. In this context, PF demonstrates a synergistic 'antiviral-anti-inflammatory-anti-damage' effect, making it a promising multifunctional drug candidate (70).

A total of 10,376 ALI-related targets were compiled. After intersecting these with PF targets, a PPI network analysis was performed using the STRING database (Fig. 4A), and ten core genes were predicted: MAPK1, MAPK3, TP53, HSP90AA1, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 4B). Molecular docking results for the top three hub genes (MAPK1, MAPK3 and TP53) suggested that PF may form conventional hydrogen bonds with glutamate at position 343, tyrosine at positions 219 and 156, arginine at 370, and aspartate at 345 (MAPK1); a carbon-hydrogen bond with valine at 344 (MAPK3); and a salt bridge with aspartate at 347 (TP53) (Figs. 3C and 4C). Analysis of the remaining seven hub genes via molecular docking also indicated strong binding potential (Fig. S2).

Liver injury. Hepatic ischemia-reperfusion injury (HIRI) refers to the tissue damage that follows reestablishment of perfusion after transient liver ischemia and is an inevitable complication during liver trauma, resection, and transplantation (72,73). The ischemic phase causes hypoxia and direct cellular injury due to inadequate blood supply. Subsequent reperfusion initiates a complement cascade, leading to secondary damage characterized by excessive ROS generation and secretion of pro-inflammatory cytokines from hepatocytes. As a result, immune cells, particularly neutrophils, are recruited from the circulation, causing further direct damage to hepatic tissue (72,74). Novel approaches, including RNA-based therapies, gene modification and progenitor cell techniques, offer potential avenues for advancing HIRI management (75). PF exerts its effects by specifically targeting the Tmem176b⁺ macrophage subset in HIRI. It suppresses excessive inflammation through activation of the THBS1-CD47 immunoregulatory axis while concurrently blocking the pro-inflammatory SPP1-CD44 signaling pathway. This two-pronged mechanism drives macrophages from an inflammation-promoting M1 state to an inflammation-resolving M2 state, remodeling the liver's immunological milieu and mitigating parenchymal damage (76). To elucidate its multi-target profile, 1,493 HIRI-related targets were retrieved, and PPI topology was assembled using STRING and analyzed by Cytoscape (Fig. S3A), with core targets MAPK1, MAPK3, HSP90AA1, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 3B). Molecular docking analysis further indicated that PF's mode of action on these IRI-related targets, including specific binding sites and interaction forces, closely aligns with findings from AS studies, offering preliminary structural insights into its broad pharmacological activity (Figs. 3C and S2).

Cholestatic liver diseases (CHOL) encompass a spectrum of hepatobiliary disorders characterized by impaired bile flow, including primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), intrahepatic cholestasis of pregnancy, IgG4-related sclerosing cholangitis, and progressive familial intrahepatic cholestasis (PFIC). The pathological process is marked by dysregulated cholangiocyte proliferation, periductal fibrotic remodeling, myofibroblast activation, accumulation of cytotoxic bile acids, and an exaggerated ductular reaction. Inflammatory cell infiltration exacerbates cholangiocyte apoptosis, leading to biliary obstruction and disrupted bile acid excretion, particularly in PBC and PSC. The accumulated bile acids then perpetuate a vicious cycle of fibrosis and inflammation (77,78). In models of cholestatic liver injury, PF significantly improves both functional and histopathological parameters. The core mechanism involves its direct interaction with the GSDMD protein, inhibiting GSDMD-mediated pyroptosis and NLRP3 inflammasome activation to block the downstream inflammatory cascade (79). In addition, PF modulates key metabolic pathways and associated signaling networks involving targets such as CDK1, CYP2C9, MAOB, mTOR and ABCB1. These modulations elevate levels of the hepatoprotective bile acid tauro-ursodeoxycholic acid and readjust linoleic acid and phosphatidylinositol metabolism. By reducing inflammatory stimuli from toxic bile acid accumulation, PF indirectly dampens overactivation of inflammatory signaling, leading to improvement in serum biochemical markers and liver histology (80). A target screening approach predicted 1,235 disease-associated targets from these databases. Intersection with PF targets and subsequent PPI network analysis (Fig. S3B) yielded ten core targets: MAPK1, MAPK3, TP53, ESR1, BCL2, HIF1A, IL6, IL1B, TNF and IL18 (MCC algorithm, Fig. S4A). Docking simulations of the top three molecules showed that their binding to PF matched the results obtained in ALI, suggesting a conserved interaction pattern as indicated by the same binding pocket and comparable free energy levels (Figs. 3C and 4C). Docking analysis of the hub genes also suggested strong binding potential (Fig. S4B-K).

Colitis. Inflammatory bowel disease (IBD) encompasses chronic, systemic inflammatory conditions affecting the gastrointestinal tract and multiple extra-intestinal sites, mainly Crohn's disease and ulcerative colitis (UC) (81,82). Despite advances in therapeutic options, IBD remains prone to relapse, profoundly impairing patient quality of life and imposing a substantial socioeconomic burden (83-85). Notably, PF has demonstrated distinctive dual functionality, simultaneously suppressing inflammation and promoting mucosal repair, in IBD therapy. In chronic colitis models, PF directly engages C1qa to orchestrate dual regulatory effects: it upregulates membrane-bound C1q to enhance macrophage-mediated bacterial clearance and accelerate inflammation resolution, while concurrently inhibiting pathological C1q secretion to suppress aberrant Wnt/ β -catenin pathway activation. This dual modulation restores ISC homeostasis, effectively addressing the intertwined challenges of inflammatory control and epithelial regeneration (86). A total of 4,384 colitis-associated targets were retrieved by NP. After cross-matching with PF targets, a PPI network was constructed (Fig. 5A), and 10 hub genes were identified: IL6, IL1B, ALB, PTGS2, CASP3, IFNG, TLR4,

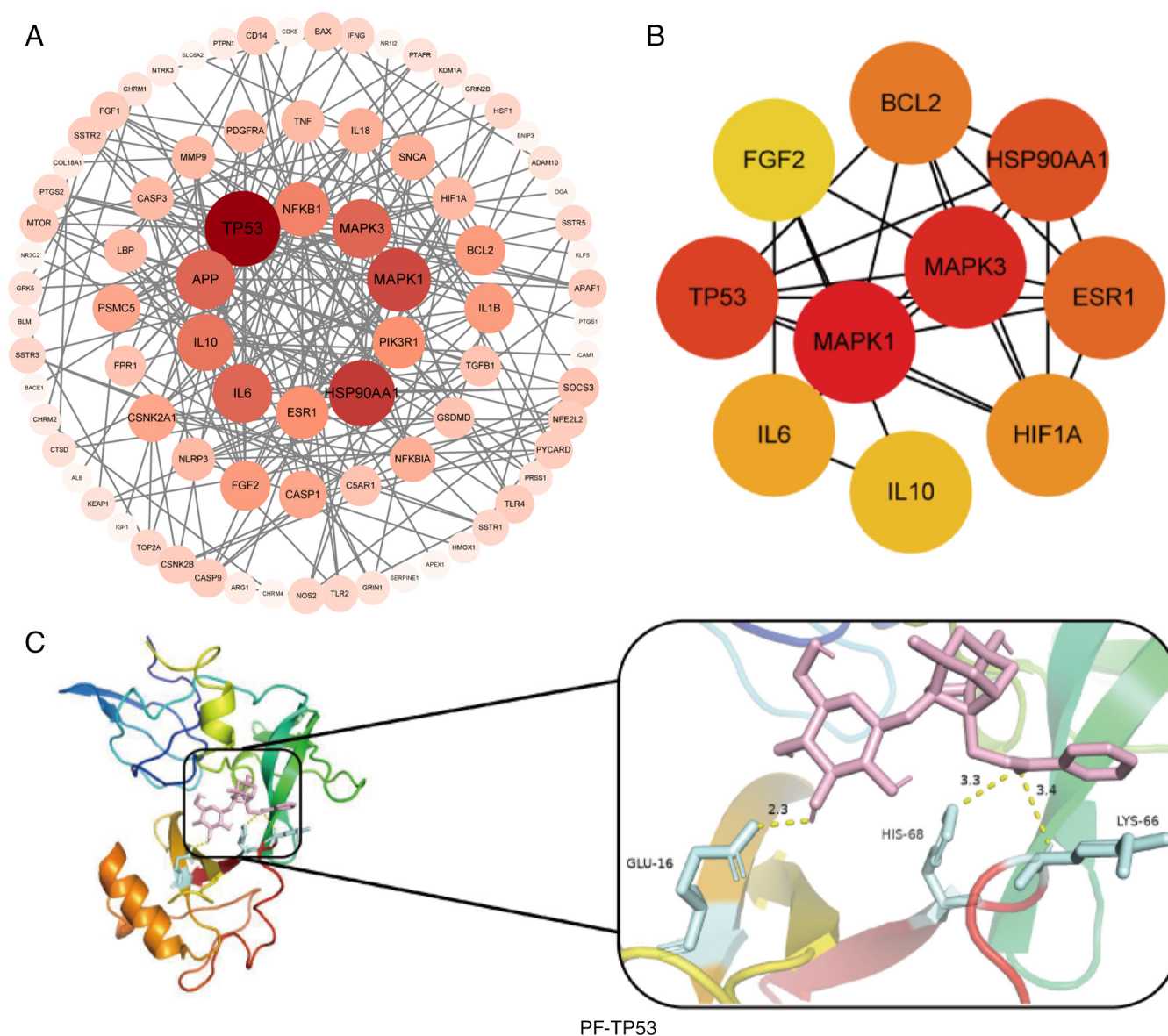


Figure 4. Network pharmacology analysis of PF against acute lung injury. (A) Protein-protein interaction network of ALI-associated targets. (B) Hub targets identified in ALI. (C) molecular docking of PF and TP53. PF, paeoniflorin; ALI, acute lung injury.

TGFB1, NFKB1 and HIF1A (Fig. 5B). Molecular docking suggested high-affinity binding between PF and these targets. Critical interactions involve hydrogen bonds with Pro139, Lys120/82, Gln39, Gly45, and Met44, and a salt bridge with Glu99 (Figs. 5C and S5A-J).

UC presents with recurrent abdominal pain, hematochezia and diarrhea; prolonged disease duration may induce mucosal architectural distortion and elevate colorectal neoplasia risk (87). Compelling evidence indicates that dysregulated death of intestinal epithelial cells (IECs) compromises barrier integrity, triggering inflammatory cascades central to UC progression. Current first-line pharmacotherapy employs mesalazine (5-aminosalicylic acid, 5-ASA) to suppress mucosal inflammation and induce remission (88,89); however, a significant proportion of patients show inadequate response or treatment refractoriness due to disease complexity (90). PF has been shown to drive intestinal epithelial regeneration: *in vivo*, PF markedly ameliorates dextran sulfate

sodium-induced colitis and restores mucosal architecture; *in vitro*, PF activates the PI3K/Akt/mTOR axis to counteract TNF- α -mediated growth suppression, accelerates repair of LPS-injured IEC-6 cells, and upregulates regeneration-associated molecular markers, providing multi-level mechanistic validation of its reparative efficacy (91). A total of 10,781 UC-related targets underwent identical NP processing; a PPI network was constructed (Fig. S3C), which predicted a distinct core target set: MAPK1, MAPK3, HSP90AA1, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 3B). Molecular docking analyses further suggested that PF's binding modes with these targets, including binding pockets and computed free energies, were generally comparable to those observed in the ALI model (Figs. 3C, 4C and S2).

Acute kidney injury (AKI). AKI is a heterogeneous, multifactorial syndrome driven by renal tubular injury and inflammation (92). Pathogenesis of AKI involves extensive activation of innate and adaptive immunity; specifically,

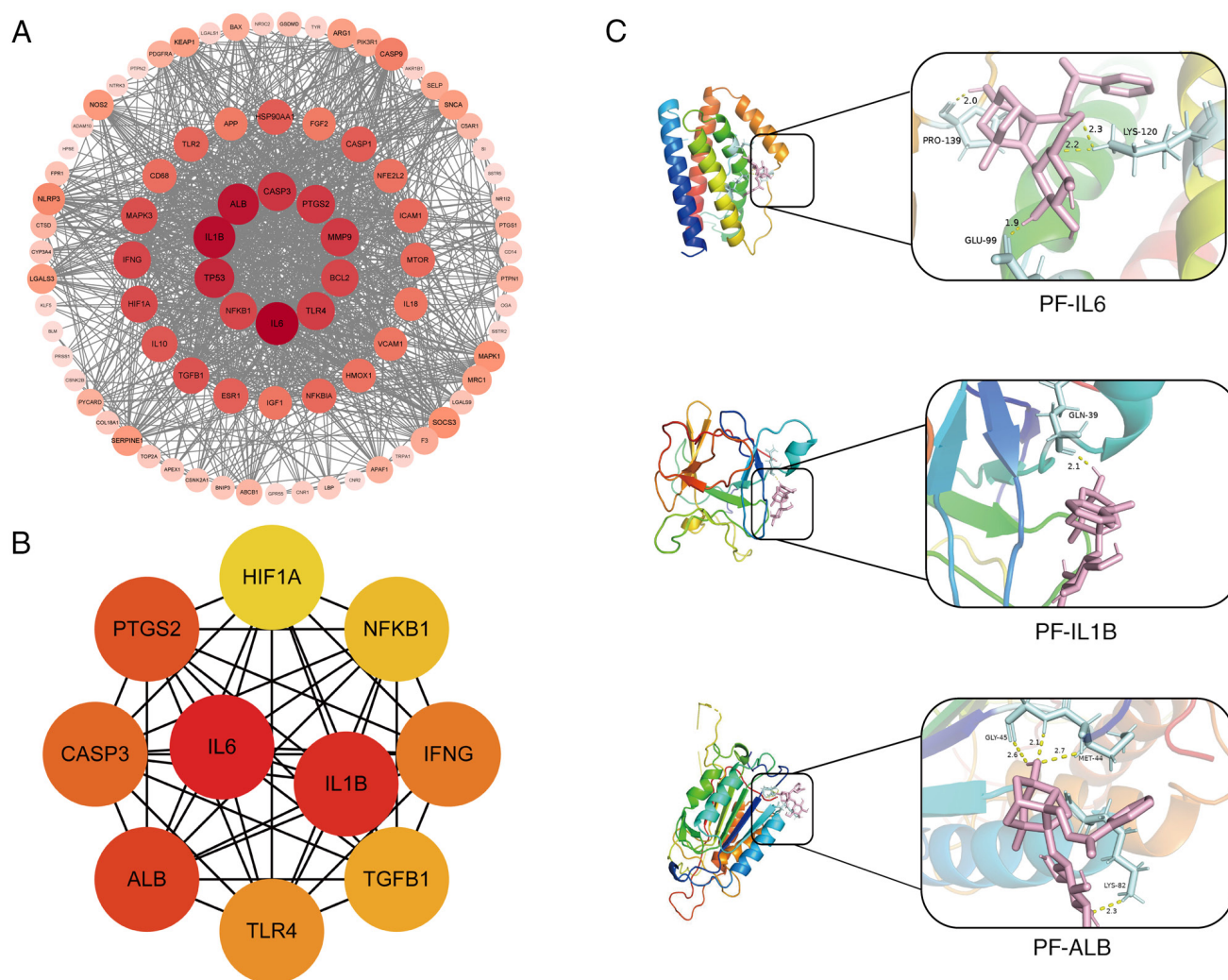


Figure 5. Network pharmacology analysis of PF against colitis. (A) Protein-protein interaction network of colitis-associated targets. (B) Hub targets identified in colitis. (C) Comparative molecular docking analysis of PF with the top three core target proteins in colitis. PF, paeoniflorin.

inflammation- and oxidative stress-induced endothelial dysfunction, coupled with coagulopathy, precipitates microcirculatory failure and a marked reduction in glomerular filtration rate (92,93). Although renal replacement therapy remains standard care, the challenge of early AKI detection underscores the need for preventive and therapeutic innovations (94,95). In a murine bilateral renal ischemia-reperfusion (IR) AKI model, PF-enriched extract dose-dependently attenuated tissue injury, inflammation and ferroptosis, findings corroborated in a hypoxia/reoxygenation model using human renal tubular epithelial cells (HK2). Mechanistically, PF upregulated solute carrier family 7 member 11 (Slc7a11 in mice; SLC7A11 in humans), enhancing glutathione biosynthesis and conferring resistance to ferroptosis, thereby enabling coordinated suppression of ferroptosis and inflammation (96). A total of 10,131 AKI-associated genes were overlapped with predicted PF targets (Fig. S3D); a PPI network was constructed using STRING, and 10 hub targets, MAPK1, MAPK3, TP53, HSP90AA1, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 4B), were predicted via MCC algorithm in Cytoscape. Ligand-receptor interaction modeling demonstrated favorable binding energies and three-dimensional poses between PF and these targets, with binding free energies and active-site

interactions highly consistent with prior ALI studies (Figs. 3C and 4C).

Diseases of the musculoskeletal system

RA. RA is a systemic autoimmune disorder characterized by synovial hyperplasia, autoantibody production, progressive bone erosion and articular symptoms (for example, joint swelling, tenderness). A core therapeutic challenge lies in rebalancing inflammatory and reparative processes (97). Pathogenically, synovial macrophages secrete proinflammatory cytokines (TNF- α , IL-1), which, alongside FLS, activate osteoclasts and drive bone destruction (98). Although disease-modifying antirheumatic drugs remain foundational, novel therapeutics are urgently needed (99). In a collagen-induced arthritis rat model, PF and its active constituents ameliorated disease by: i) Suppressing inflammation (reduced TNF- α , IL-1 β , IL-6; elevated IL-10); ii) mitigating oxidative stress (decreased malondialdehyde and nitric oxide; increased SOD); and iii) promoting synovial cell apoptosis via Bcl-2 downregulation and AMPK-mediated mitochondrial fission (100). Bioinformatics integration of 5,369 RA-related targets with PF targets (Fig. S3E), followed by PPI network analysis (STRING) and MCC-based hub identification in Cytoscape, predicted 10 core targets: MAPK1, MAPK3,

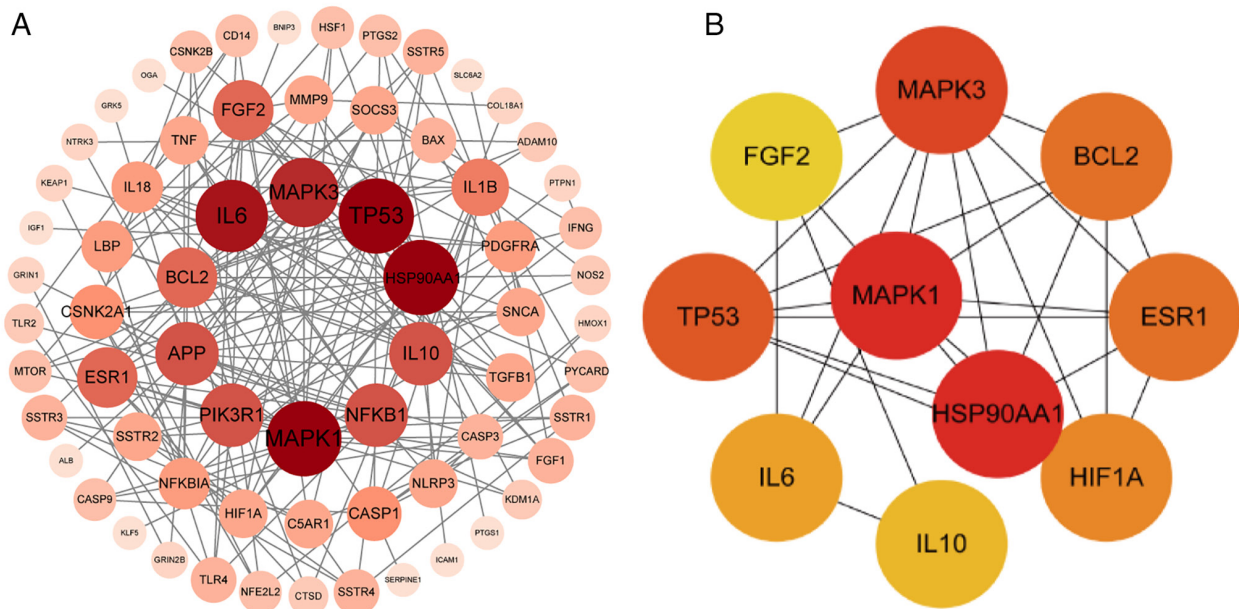


Figure 6. Network pharmacology analysis of paeoniflorin against OA. (A) Protein-protein interaction network of OA-associated targets. (B) Hub targets identified in OA. OA, osteoarthritis.

HSP90AA1, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 3B). Functional enrichment analysis implied profiles consistent with prior AS findings (Figs. 3C and S2), reinforcing PF's multi-disease modulatory potential.

Osteoarthritis (OA). OA, the most prevalent chronic joint disorder, is a leading cause of disability (101,102). A pivotal mechanism in OA pathogenesis involves disruption of cytokine homeostasis: Pro-inflammatory cytokines such as IL-1 β , TNF- α and IL-6 initiate a self-perpetuating cycle that ultimately damages articular cartilage and other intra-articular structures through activation of catabolic enzymes, notably matrix metalloproteinases (MMPs) (103,104). PF demonstrates chondroprotective effects on OA chondrocytes even at low concentrations. Flow cytometric analysis showed that PF reduced apoptosis to varying degrees by suppressing inflammatory cytokine production and modulating ferroptosis, elevating the proportion of normal cells to 82.7% (105). A total of 4,200 OA-associated targets were retrieved. Following intersection with PF targets, a PPI network of the shared targets was assembled via the STRING platform (Fig. 6A), yielding core targets: MAPK1, HSP90AA1, MAPK3, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2 (Fig. 6B). Molecular docking analysis further suggested stable binding between PF and all 10 hub gene-encoded proteins (Fig. S2).

Muscle atrophy. Muscle breakdown, characterized by loss of skeletal muscle mass and locomotive ability, is categorized as primary or secondary and arises from an imbalance between protein anabolism and catabolism. The effective treatments remain scarce due to incomplete understanding of the underlying mechanisms (106,107), underscoring the need for deeper mechanistic insight and novel therapeutic targets. Cisplatin, a highly effective broad-spectrum chemotherapeutic agent, frequently induces skeletal muscle atrophy as a side effect, highlighting the need for protective strategies (108). Oral administration of PF markedly attenuated cisplatin-induced skeletal muscle loss and functional

impairment. Mechanistically, PF suppressed activation of the NF- κ B signaling pathway in skeletal muscle tissue and reduced the expression of associated inflammatory cytokines. Concurrently, PF downregulated muscle-specific ubiquitin E3 ligases and restored expression of related proteins, indicating that PF reverses cisplatin-induced atrophy by inhibiting NF- κ B-mediated inflammation and subsequently attenuating key atrophy-associated ubiquitin ligases (109). A total of 8,660 candidate genes were intersected with PF targets (Fig. S3F), yielding core targets: MAPK1, MAPK3, TP53, HSP90AA1, ESR1, BCL2, HIF1A, IL1B, IL6 and IL18 (Fig. S6A). Molecular docking analysis further confirmed consistent binding characteristics (interaction sites, binding free energy) between PF and the top three targets, with interaction patterns comparable to those observed in prior disease models and the ALI model (Figs. 3C, 4C and S6B-K).

Cross-disease mechanisms of PF. Across the diseases examined, PF consistently suppresses pro-inflammatory mediators (TNF- α , IL-6 and IL-1 β) and engages NF- κ B signaling in multiple models (ALI, cholestatic liver injury, muscle wasting). However, disease-specific regulatory modules are equally prominent: in AS, PF modulates I κ B- α /NF- κ B/p38/JNK to counter vascular inflammation; in HIRI, it uniquely drives macrophage polarization via the THBS1-CD47 and SPP1-CD44 axes; in cholestatic liver injury, PF activates Nrf2 while inhibiting the NLRP3 inflammasome; in colitis, it regulates Wnt/ β -catenin; in UC, it engages PI3K-AKT-mTOR for epithelial repair; in AKI, it targets Slc7a11/GPX4 to counteract ferroptosis; and in RA, it modulates AMPK-Drp1. This spectrum of pathway engagement illustrates that PF's therapeutic actions comprise a conserved anti-inflammatory core complemented by tissue-specific regulatory adaptations dictated by the dominant pathology of each disease.

NP analysis integrating PF across representative inflammatory diseases, including AS, points to its broad-spectrum, multi-target therapeutic potential (Table I). Comprehensive

evaluation of molecular docking results for the 20 non-redundant core targets (Table SI) further suggests high-affinity binding of PF to critical pathogenic proteins across diverse inflammatory conditions. To assess the stability of the hub genes identified by the MCC algorithm, Degree and Betweenness centrality for each disease were additionally calculated. The rankings were largely consistent across the three methods (Table SII), confirming the reliability of the hub gene selection and providing a preliminary mechanistic framework for its clinical application in managing inflammatory diseases.

5. Multi-target mechanisms of PF in inflammatory regulation

Poly-pharmacological network of PF in inflammatory cascade regulation. PF, a naturally derived active compound with low toxicity and multi-target capacity, exerts its pharmacological actions through diverse mechanisms. Central among these is its modulation of key inflammatory signaling cascades, particularly the PI3K/AKT and NF- κ B axes, both of which play a pivotal role in the initiation and perpetuation of inflammatory responses (110). By engaging these pathways, PF fine-tunes immune activity (for example, impeding B lymphocyte migration), stimulates mitophagy, and reduces the secretion of pro-inflammatory cytokines such as IL-6 and IL-22, thereby containing the onset and propagation of inflammation. This broad regulatory profile positions PF as a promising candidate for treating inflammatory conditions. Current studies draw on NP, integrated with PPI analysis and molecular docking, to achieve coordinated modulation of inflammatory cascades. Through high-throughput data integration, including the screening and molecular docking of thousands of candidate targets, these studies demonstrate that PF delivers comprehensive, system-level anti-inflammatory effects via simultaneous engagement of multiple pathways.

Among the 10 disease models examined, 10 targets, MAPK1, MAPK3, HSP90AA1, TP53, ESR1, BCL2, HIF1A, IL6, IL10 and FGF2, emerged as recurrent core hubs. Their consistent co-occurrence across diverse pathological settings supports designation as a 'Highly Conserved Core Target Module' within PF's anti-inflammatory network. The repeated identification of this module across distinct disease contexts can be traced to three interrelated factors: i) These targets predominantly converge within central inflammatory signaling cascades, including the MAPK, PI3K-AKT and p53 pathways, which function as universal regulatory hubs for inflammation irrespective of the triggering etiology; ii) PF interacts with these shared signaling nodes to elicit broad-spectrum anti-inflammatory outcomes that extend beyond individual disease boundaries; and iii) the module reflects a hierarchical regulatory logic in which PF operates through a 'multi-target $\rightarrow$ common pathway $\rightarrow$ disease-specific downstream effector' framework. This pattern likely originates from PF's recognition of a shared conformational state among these targets, thereby activating common anti-inflammatory mechanisms across diverse pathologies, including ankylosing spondylitis (AS), RA and IR injury (8,100). Cross-model comparison of degree centrality across the 10 inflammatory disease models further stratified the recurrent hubs into three functional categories. MAPK1, MAPK3, IL6, IL10 and BCL2 ranked among

the top ten in ≥ 9 models with little inter-disease fluctuation, qualifying them as generic inflammatory relays, indispensable nodes essential for PF's broad-spectrum anti-inflammatory activity.

Conversely, HSP90AA1 and ESR1 exhibited marked disease-selective enrichment. HSP90AA1 ranked 10th in AS, 4th in ALI, and 3rd in HIRI, yet it emerged as the top-ranked hub in RA (degree=1) and remained highly ranked in OA (3rd) and MA (2nd), consistent with its recognized function as a stress-responsive chaperone in synovial tissues. ESR1, while broadly detectable, showed its highest betweenness centrality specifically in RA and CHOL, where it co-occurred with disease-selective partners such as LGALS3 and SSTR2, pointing to context-dependent roles. These two targets are therefore classified as disease-enriched effectors.

TP53 presented an intermediate pattern, highly conserved across models (top three in 8 of 10 models) yet displaying context-dependent partner switching (for example, switching from NLRP3/APP in CHOL to CASP3/PTGS2 in colitis). Importantly, although the core target module remains highly conserved, distinct ancillary targets and downstream effector molecules operate in different diseases, a feature that explains why PF is effective across a wide range of inflammatory conditions while exhibiting differential efficacy spectra across specific disorders.

Within each disease context, PF markedly lowers the levels of pro-inflammatory cytokines, thereby attenuating tissue injury and dampening inflammatory cell infiltration. These actions involve the modulation of canonical inflammatory axes, NF- κ B, MAPK, JAK/STAT and TLR4/NLRP3, underscoring the broad regulatory reach of PF. In parallel, PF simultaneously engages multiple protein targets (for example, IL-1 β , NLRP3, STAT1, STAT3 and TLR4), shaping downstream signals to enable multi-tiered inflammatory control (46,48,111). Going forward, research efforts should center on these shared targets to accelerate the development of PF-based broad-spectrum anti-inflammatory therapeutics for related inflammatory disorders.

At the mechanistic level, PF achieves precise regulation of inflammation through interconnected synergistic routes, including the modulation of macrophage polarization, blockade of NLRP3 inflammasome assembly, interference with the TLR4/NF- κ B signaling cascade, and upregulation of anti-inflammatory effectors such as IL-10 and Arg-1 (112-114). In addition, PF indirectly restrains the perpetuation and advancement of inflammation by reprogramming energy metabolism, counteracting oxidative stress, and promoting tissue repair. NP combined with molecular docking further confirms PF's high binding affinity toward multiple key inflammatory targets, providing a molecular rationale for its multi-target synergistic anti-inflammatory actions. Collectively, evidence from both cellular models and animal studies shows that the anti-inflammatory effects of PF do not hinge on a single target but emerge from network-based regulation spanning multiple targets and pathways, an attribute that confers distinct therapeutic advantages in managing complex inflammatory diseases.

PF combinatorial therapy and structural modification. Harnessing drug synergy offers a forward-looking route to

Table I. The models and mechanisms of inflammatory diseases treated by PF.

Authors, year	Body system	Disease	Cell/animal model	Inflammatory mediators	Signaling pathways	(Refs.)
Yu <i>et al.</i> , 2022	Cardiovascular system	Atherosclerosis	Apoe ^{-/-} mice fed high-fat diet	TNF- α $\downarrow$, IL-6 $\downarrow$, IL-1 β $\downarrow$, CRP $\downarrow$	IkB- α /NF- κ B/p38/JNK	(8)
Liu <i>et al.</i> , 2024	Respiratory system	Acute lung injury	LPS-induced BALB/c mice	TNF- α $\downarrow$, IL-6 $\downarrow$, IL-1 β $\downarrow$	NF- κ B, MAPK	(69)
Tang <i>et al.</i> , 2025	Hepatobiliary system	Hepatic ischemia-reperfusion injury	C57BL/6 mice with hepatic I/R surgery	-	THBS1-CD47, SPPI-CD44	(76)
Chen <i>et al.</i> , 2021	Hepatobiliary system	ANIT-induced cholestatic liver injury	ANIT-induced ICR or C57BL/6 mice	TNF- α $\downarrow$, IL-1 β $\downarrow$, IL-6 $\downarrow$	Nrf2, NF- κ B, NLRP3	(80)
Wang <i>et al.</i> , 2022	Digestive system	Chronic colitis	IL-10 ^{-/-} mice	TNF- α $\downarrow$, IL-17A $\downarrow$, IFN- γ $\downarrow$, IL-23 $\downarrow$, IL-1 β $\downarrow$, IL-6 $\downarrow$	Wnt/ β -catenin	(86)
Ma <i>et al.</i> , 2023	Digestive system	Ulcerative colitis	DSS-induced C57BL/6 mice	TNF- α $\downarrow$, IL-1 β $\downarrow$	PI3K-AKT-mTOR	(91)
Ma <i>et al.</i> , 2023	Urinary system	Ischemia/reperfusion-induced acute kidney injury	C57BL/6 mice with bilateral renal ischemia-reperfusion	TNF- α $\downarrow$, IL-1 β $\downarrow$, IL-6 $\downarrow$	Slc7a11/GPX4	(96)
Huang <i>et al.</i> , 2021	Musculoskeletal system	Rheumatoid arthritis	Collagen-induced arthritis rats	TNF- α $\downarrow$, IL-1 β $\downarrow$, IL-6 $\downarrow$	AMPK-Drp1	(100)
He <i>et al.</i> , 2019	Musculoskeletal system	Osteoarthritis	IL-1 β -induced rabbitosteoarthritis chondrocytes	-	-	(105)
Jang <i>et al.</i> , 2021	Musculoskeletal system	Cisplatin-induced muscle wasting	Cisplatin-treated C57BL/6 mice	TNF- α $\downarrow$, IL-6 $\downarrow$, IL-1 β $\downarrow$	NF- κ B, MuRF1/MAFbx	(109)

broaden therapeutic impact. Pairing agents that act through unrelated mechanisms frequently sharpens both efficacy and the overall safety margin (115). When PF is paired with mainstream anti-inflammatory drugs, whether non-steroidal anti-inflammatory drugs, immunosuppressants, or biologics, the two can reinforce one another, lifting treatment response while softening the liability of adverse events through cooperative interaction. As one illustration, giving PF alongside other botanicals (for example, *Polygonatum odoratum*, *Cornus officinalis*) or their isolates outperforms standalone therapy in correcting the inflammatory-immune disequilibrium and metabolic derangement seen in RA models (100,116). PF additionally tempers the hepatotoxic burden imposed by standard RA regimens such as methotrexate and leflunomide (117).

Plant metabolites have long furnished chemists with structurally diverse lead scaffolds. Judicious chemical remodeling then opens a practical route to build new entities with stronger pharmacological behavior (118). Derivatizing PF, through esterification or glycosylation, for example, or wrapping it in nano-formulations can raise bioavailability, sharpen target engagement, stabilize the molecule, and ease its passage across membranes. Close scrutiny of highly active analogues such as CP-25, with attention to anti-inflammatory potency, clinical tolerability, and pharmacokinetic behavior, stands to surface preferable drug candidates (119). Such analogues further bring distinct strengths to bear on inflammatory-cell fate, blunting T helper 17 (Th17) differentiation, for instance, and on reshaping the immune milieu, thereby opening fresh angles for combined immunomodulatory and anti-inflammatory intervention. Along similar lines, the PF metabolite albiflorin shows tighter docking to sites such as MMP-9 and stronger cellular anti-inflammatory activity than the parent compound, making clear how minor structural tweaks can sharply raise potency (120). Cooperative effects between PF and other bio-actives (flavonoids among them) also deepen the dampening of inflammatory cascades, chiefly NF- κ B and MAPK, through structurally complementary action (69).

Because HSP90AA1 and ESR1 stand out as disease-enriched effectors in RA and OA, future analogue design that centers on these nodes should give rise to agents that are both more selective and better matched to their disease context. Taken together, edits to the sugar moiety, swaps in aglycone substituents, and deliberate compound pairing all bear directly on how well PF binds its targets and what it does biologically. Progress in docking and structural engineering now places PF and its derivatives at the center of molecular templates for building anti-inflammatory drugs that hit multiple targets, work robustly, and stay safe.

Advancing NP for PF mechanisms and translational prospects. NP has operated as a central framework for dissecting the anti-inflammatory machinery of PF. By systematically broadening target discovery, this discipline enables integrated analysis of the intricate interaction networks underpinning PF's activity. Such work lays the conceptual groundwork for PF-centered drug development and clinical advancement, while affirming the field's value in modernizing traditional Chinese medicine and expediting novel therapeutic discovery. Prospective convergence of multi-omics, artificial intelligence and refined computational modeling should sharpen dynamic,

high-resolution insight into PF's multi-target actions. The specificity-index stratification proposed here can function as a quantitative compass for biomarker exploration, guiding, for example, the prioritization of HSP90AA1 in RA/OA and ESR1 in RA/CHOL as disease-tailored pharmacodynamic readouts for future clinical profiling. This merged strategy should accelerate the deployment of PF across a wider range of inflammation-linked disorders.

Although these network-pharmacology interrogations yield informative system-level views of PF's candidate multi-target mechanisms, their computational basis calls for candid treatment of methodological limitations. The interactome datasets drawn upon originate from public repositories (ChEMBL, GeneCards and STRING, among others) that capture static knowledge states and therefore miss the temporal evolution of drug-target engagement. These same resources carry selection bias: Heavily studied canonical nodes dominate, whereas poorly characterized or recently identified targets risk omission. Computational inference also invites false-positive links spawned by indirect connectivity and incomplete or flawed annotation. Off-target engagement and poly-pharmacology likewise escape full resolution through topological measures alone, given the absence of thermodynamic and kinetic binding terms. Accordingly, the candidate targets surfaced here, MAPK1, MAPK3 and HSP90AA1 included, merit interpretation as computational hypotheses rather than validated pharmacological entities, and demand orthogonal experimental corroboration. Useful corroboration routes include surface plasmon resonance for affinity determination, cellular thermal shift assay and drug-affinity responsive target stability for engagement verification, and RNA interference-based knockdown for functional readout. Independent of these *in silico* predictions, the clinical advancement of PF still hinges on more rigorous pharmacokinetic characterization and controlled clinical assessment.

6. Challenges in the clinical translation of PF

PF is a natural bioactive compound that has attracted considerable attention for its anti-inflammatory effects. Current evidence indicates that PF can regulate multiple inflammatory signaling pathways, including the NF- κ B, MAPK, JAK/STAT and PI3K/AKT pathways, leading to changes in the production and release of proinflammatory mediators and attenuation of inflammatory responses. This multi-target activity may contribute to the broad effects of PF across different inflammatory conditions, while also creating challenges for predicting its effects across disease contexts. Unlike agents developed to act primarily on a defined molecular target, PF may influence multiple signaling cascades simultaneously. Such poly-pharmacology may be therapeutically advantageous in complex inflammatory diseases but can also result in context-dependent effects, dose-dependent responses, and difficulties in identifying the contribution of individual targets to clinical outcomes. Despite its therapeutic potential, several challenges must be addressed before PF can be translated into a well-defined clinical intervention:

i) Uncertainty of effects: PF's poly-pharmacological activity can produce different effects across disease models, depending on the biological context and the pathways involved.

For example, activation of the PI3K pathway may promote mucosal repair in colitis (91), whereas activation of the same pathway may contribute to disease progression in acne (121).

ii) Dose-dependent adverse effects: Dose dependency also warrants careful consideration. Although higher PF doses have shown greater efficacy in some models of polycystic ovary syndrome, they may also be associated with adverse events such as Grade 3 diarrhea (91,122). Extensive clinical evaluation is therefore required to define the therapeutic window and establish the dose-response relationship of PF. PF can also exert protective effects at relatively low concentrations. In OA chondrocytes, for example, low concentrations of PF inhibited inflammatory factor production and modulated ferroptosis (105). These findings suggest that improving the delivery and tissue distribution of PF may allow therapeutic effects to be achieved at lower systemic doses. Recent studies of PF nano-formulations provide a potential strategy for addressing this issue. Glycyrrhiza protein-based PF nanoparticles, ~150 nm in size, significantly increased oral bioavailability, with a 2.6-fold increase in C_{max} and a reported 0.95-fold change in AUC, and enhanced analgesic efficacy through caveolin-mediated endocytosis (123). Targeted delivery systems may thus help increase PF exposure at the intended site of action while limiting unnecessary systemic exposure. The contrasting effects observed at different doses and in different disease models highlight the need to define an appropriate therapeutic window rather than assuming that increased PF exposure will uniformly improve efficacy.

iii) Clinical evidence and translational challenges of TGP: Total glucosides of peony (TGP), a standardized preparation containing PF as a major component together with other constituents, has been widely investigated and applied in autoimmune and inflammatory diseases (124) evaluate the clinical evidence for TGP and its relevance to the translation of PF, English-language clinical studies published after 2020 were examined. In RA, a systematic review and meta-analysis of 63 randomized controlled trials involving 5,293 participants reported that TGP may improve disease activity, as measured by DAS28, and reduce ESR, CRP, TNF- α and IL-6 levels in patients with inflammatory arthritis, including RA, AS, OA, JIA and PsA (125). In psoriasis, a meta-analysis of 36 RCTs involving 3,140 participants showed that TGP combined with conventional therapy was superior to conventional therapy alone in achieving PASI 60 (RR=1.32; 95% CI: 1.25-1.39) and PASI 50 (RR=1.44; 95% CI: 1.13-1.84) (126). In autoimmune hepatitis, TGP treatment was associated with improved liver function, reflected by reductions in serum ALT, AST, GGT and total bilirubin levels in patients with autoimmune liver disease (127). However, before PF can be advanced as a single-agent drug, the translational obstacles inherent to TGP-derived products must be critically examined. These clinical findings suggest that TGPMAPK has beneficial effects in inflammatory diseases, providing a proof-of-concept validation for the anti-inflammatory potential of PF, as the latter has been shown to modulate multiple core signaling pathways including NF- κ B and JAK/STAT to balance immune cell function. However, TGP is a multi-component extract rather than purified PF, and the specific contribution of PF to TGP's overall efficacy cannot be fully determined from TGP clinical data alone. Thus, while TGP's clinical efficacy supports the

rationale for investigating PF as an active anti-inflammatory agent, dedicated studies on purified PF, such as the present review, remain essential to establish its direct pharmacological effects and to bridge the gap between TGP-based clinical evidence and PF-based drug development.

For safety profile, recent reviews have consistently demonstrated that PF exhibits favorable safety characteristics, including low acute toxicity, minimal subacute and chronic toxicity, and no genotoxic or mutational toxic effects (128). TGP has been clinically applied in multiple autoimmune diseases with satisfactory therapeutic outcomes and minor side effects (124). However, it should be noted that the clinical safety data derive primarily from TGP rather than purified PF as a single agent; systematic human safety evaluations of purified PF remain to be conducted.

Notably, the core anti-inflammatory target network of PF established in the present study, comprising highly conserved hub modules such as MAPK1, MAPK3 and HSP90AA1, can serve as a computational screening framework for evaluating novel PF derivatives or compound combinations. Through molecular docking and NP comparisons, this framework enables the rapid prioritization of lead candidates by assessing their target coverage and pathway perturbation profiles prior to experimental validation. First, to mitigate dose-dependent toxicity, priority should be assigned to developing PF-derived agents that offer improved efficacy alongside reduced adverse profiles. In light of PF's protective action on osteoarthritic chondrocytes at low concentrations contrasted with grade 3 diarrhea at high doses secondary to systemic exposure, specific carrier systems is proposed, such as nanocarriers, can be used to enhance drug delivery efficiency and reduce side effects while improving efficacy (129). These methods aim to enrich PFs at the site of disease, allowing for successful treatment at lower doses while limiting systemic off-target effects. Notably, the context-dependent regulation of JNK by PF, switching between inhibition in chronic inflammation and activation in acute oxidative stress, further underscores the need for cell-type-specific and dose-response profiling when optimizing delivery strategies. Second, to address efficacy uncertainty originating from PF's poly-pharmacological properties, biomarker discovery initiatives should leverage PF as a molecular probe alongside multi-omics technologies. There have been successful studies on developing *Corydalis decumbens* as molecular probes for targeting FosL2 to treat RA and inflammation. For PF, similar methods can also be adopted (130). Such efforts aim to stratify patient subpopulations and disease phases according to PF responsiveness, thereby translating population-level TGP clinical data into individualized therapeutic strategies. Third, future work should move beyond single-constituent optimization and adopt the rational engineering of pharmaceutical systems inspired by foundational TCM therapeutic concepts. This involves developing evidence-based, adjustable multi-agent therapeutic modules, for example, fixed-ratio formulations containing PF and a partner component (termed 'Component X'), paired with adaptive release technologies that afford spatiotemporally programmed liberation matching distinct treatment phases (for example, acute anti-inflammatory vs. immune regulatory stages). During the COVID-19 pandemic, a successful graph artificial intelligence framework has been established

for quantifying multi-drug treatment modules in Chinese herbal formulas, increasing target coverage by 86.7% (131). This indicates that paeonol's future development will likely achieve significant success following this approach. In closing, the strategic emphasis should shift from iterative refinement of individual molecules to the intelligent engineering of drug systems anchored in core TCM therapeutic principles.

In summary, PF is a plant-derived natural compound with broad anti-inflammatory activity and multiple molecular targets. Its ability to regulate interconnected inflammatory pathways is consistent with the multi-component and multi-target characteristics emphasized in TCM. NP combined with molecular and cellular approaches has expanded understanding of the signaling pathways potentially involved in PF activity. These approaches also provide mechanistic hypotheses for further experimental investigation. Translation into clinical applications and development of PF-based therapeutics still require systematic evaluation of efficacy, safety, pharmacokinetics, bioavailability, tissue distribution, and dose-response relationships. Preclinical findings should be validated using well-controlled studies, followed by adequately designed clinical trials that distinguish the effects of purified PF from those of PF-containing preparations. Rigorous evaluation of these factors will be necessary to determine whether the mechanistic activity observed in experimental models can be translated into clinically meaningful therapeutic effects. The findings of the present review should therefore be interpreted as mechanistic evidence and hypotheses rather than direct predictions of clinical efficacy. Further pharmacokinetic and clinical investigations are required to establish the therapeutic potential and safety profile of PF.

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Authors' contributions

JZ wrote the original draft. JY and KJ conducted investigation and wrote, reviewed and edited the manuscript. XS and XY developed methodology and wrote, reviewed and edited the manuscript. YZ and NZ acquired funding and wrote, reviewed

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Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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