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Network pharmacology and pharmacological evaluation of Wenzheng Jiedu Powder Modified Formula for neuropathic pain relief

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Abstract. This study aimed to elucidate the mechanism of Wenzheng Jiedu Powder Modified Formula (WJPMF) in treating neuropathic pain (NP). Network pharmacology and experimental verification were integrated to explore the therapeutic effects and key targets of WJPMF. Active components, corresponding target genes, and absorption, distribution, metabolism, and excretion (ADME) genes of WJPMF against NP were screened from public databases. Network analysis and molecular docking were conducted to identify key targets and verify binding abilities. *In vivo* experiments were performed on spared nerve injury (SNI) rats to assess the analgesic effects and regulatory mechanisms of WJPMF. WJPMF significantly improved pain behaviors in SNI rats by regulating ATP-binding cassette transporter A1 (ABCA1), peroxisome proliferator-activated receptor alpha (PPARA), peroxisome proliferator-activated receptor gamma (PPARG), and superoxide dismutase 2 (SOD2) expression, which were key targets involved in the peroxisome proliferator-activated receptor (PPAR) signaling pathway. WJPMF shows promising therapeutic potential for NP through the modulation of specific targets, offering a novel therapeutic strategy for managing NP.

Key words: Wenzheng Jiedu Powder Modified Formula — Neuropathic pain — Network pharmacology — Experimental verification — PPAR signaling pathway

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ADME, absorption, distribution, metabolism, and excretion; BSA, bovine serum albumin; DL, drug-like; GO, Gene Ontology; HIT, Herbal Ingredients' Targets; KEGG, Kyoto Encyclopedia of Genes and Genomes; NP, neuropathic pain; OB, oral bioavailability; PPI, protein-protein interaction; PPAR, peroxisome proliferator-activated receptor; PWT, Paw Withdrawal Threshold test; SNI, spared nerve injury; TCM, traditional Chinese medicine; TWL, Thermal Withdrawal Latency test; WJPMF, Wenzheng Jiedu Powder Modified Formula.

Highlights

- Wenzheng Jiedu Powder Modified Formula (WJPMF) relieves neuropathic pain in rats.
- ABCA1, PPARA, PPARG, and SOD2 might be the key targets of WJPMF in neuropathic pain.
- Resveratrol might be core ingredient of WJPMF in neuropathic pain.

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Introduction

Neuropathic pain (NP) is defined as a common chronic pain caused by damaged somatosensory nervous system (Finnerup et al. 2021). This worldwide socioeconomic health issue affects millions of individuals (Carrasco et al. 2018), and the symptoms include tactile allodynia and thermal hyperalgesia such as burning, pricking, squeezing quality, and evoked pain to light touch and cold (Huang et al. 2019). The mechanisms of NP include abnormal signaling in nociceptive nerves, the pathological activation of microglia, and compromised inhibitory modulation, and peripheral and central sensitization (Xu et al. 2016). The effectiveness of current treatments, including non-steroidal anti-inflammatory drugs and opioids, in relieving NP is limited, and present side effects that limit their use (Finnerup et al. 2015). Therefore, it is urgent to find novel drugs which exhibit high efficacy, safety, and little tolerability in NP treatment.

Traditional Chinese medicine (TCM) which was different from traditional analgesics shows the advantages of multiple components, abundant action targets, and few side effects for the treatment of NP (Li et al. 2020). Rhizoma Corydalis (Yanhusuo) and Angelicae Dahuricae Radix showing the analgesic effect are widely used to treat osteoarthritis pain, hypochondriac pain, stomach pain, and headache (Kaserer et al. 2020). Besides, Qi She Pill composed of six Chinese herbs, namely Caulis Sinomenii (Qing Feng Teng), Stephaniae Tetrandrae Radix (Fang Ji), Hedysarum Multijugum Maxim (Huang Qi), Calculus Bovis (Niu Huang), Chuanxiong Rhizoma (Chuan Xiong), and Moschus (She Xiang) could relieve pain and has obvious anti-inflammatory effects (Sun et al. 2015). Hence, Wenzheng Jiedu Powder Modified Formula (WJPMF) composed of seven Chinese herbs, namely Zombie silkworms (Jiangchan), Periostracum cicadae (Chantui), Turmeric (Jianghuang), Rhubarb (Dahuang), Rhizoma Corydalis (Yanhusuo), Angelicae Dahuricae Radix (Baizhi), and Salvia miltiorrhiza Burge (Danshen), has the potential effects of eliminating stagnation and relieving pain. Moreover, WJPMF has shown extensive application in the management of asthma and has demonstrated notable therapeutic effects in terms of anti-inflammatory actions, heat relief, and alleviating symptoms of depression (Wang et al. 2021). However, sufficient experimental verifications and the specific mechanism of WJPMF acting on NP have not been explored. Therefore, our work aims to clarify the biological mechanism of WJPMF acting on NP.

Network pharmacology is considered as a potent analysis tool for studying biological systems of TCM. It is a promising method to analyze drug targets and obtain a comprehensive insight into action mechanisms based on computational pharmacology from existed research results (Hopkins 2008). Moreover, molecular docking is considered as a novel technology to predict the binding affinity and predominant binding mode for the drug and selected targets (Saikia and Bordoloi 2019). For example, Wan et al. utilized network-based analysis integrated with experimental validation to verify the analgesic mechanism of TCM Yuanhu Zhitong Formula in treating NP (Liu et al. 2021). Therefore, network pharmacology integrated with molecular docking could effectively obtain the possible mechanism of WJPMF in the NP therapy.

In the current study, we performed a network pharmacologic analysis to find out the potential active ingredients, protein targets, and pathways of WJPMF acting on NP. Further screening and validation of active ingredients and targets were performed by molecule docking. Then, we conducted *in vivo* experiments to examine the impact of WJPMF on spared nerve injury (SNI) rats and the underlying mechanisms and provide novel views of the analgesic mechanism of WJPMF.

Materials and Methods

Network pharmacology prediction analysis

Screening of active compounds and targets of WJPMF and NP

The genes implicated in NP were gathered from the GeneCards database, accessible at https://www.genecards.org/ (Safran et al. 2010) and DisGeNET databases (Pinero et al. 2017) with the keywords "neuropathic pain". The main components of WJPMF are as follows: Zombie silkworms (Jiangchan), Periostracum cicadae (Chantui), Turmeric (Jianghuang), Rhubarb (Dahuang), Rhizoma Corydalis (Yanhusuo), Angelicae Dahuricae Radix (Baizhi), and Danshen. Active ingredients of all herbal compounds of WJPMF were screened using the Herbal Ingredients' Targets (HIT) (Yan et al. 2022), Traditional Chinese Medicine Systems Pharmacology (TCMSP) (Ru et al. 2014), and Traditional Chinese Medicine integrative (TCMID) (Huang et al. 2018) databases. The results were merged after removing duplicates and compounds without PubChem ID. Then, to obtain the target genes of active ingredients, we extracted data from the HIT database using the PubChem ID. Subsequently, we cross-referenced this information with the gene list obtained from the GeneCards database. Active ingredients that did not have any associated target genes were eliminated from further consideration.

Screening of ADME genes for WJPMF in NP

The ADME gene group comprises genes that play a crucial role in drug absorption, distribution, metabolism, and excretion processes. The PharmaADME database, at present, contains a comprehensive compilation of 298 ADME genes.

These genes encompass encoding phase I (functionalization) (Hu et al. 2019) and phase II (binding) drug metabolizer enzymes, transporters, and modifiers (Rabbani et al. 2018), providing a valuable resource for studying the ADME properties of drugs. PharmaADME database was used to seek for ADME genes of WJPMF acting on NP by the screening conditions: oral bioavailability (OB) ≥ 30%, drug-like (DL) ≥ 0.18, and half-life (HL) ≥ 4 h. Therefore, the higher the OB amount and the lower the HL value, indicating this component as a promising drug candidate. Additionally, the screened "active ingredient targets", "disease targets", and "ADME genes" were subjected to mapping by VennDiagram 1.7.3 and the common targets were obtained (Chen and Boutros 2011).

Construction of the protein-protein interaction (PPI) network and selection of hub-targets and active ingredients

To better visualize the drug-target interaction, the STRING platform was used to build PPI network and cytoscape software was for visualization of results (Szklarczyk et al. 2021). Then, the target genes were screened according to the condition of score_cutoff > 0.4 and size_cutoff < 20. The target genes with high degree in the network were screened out and were likely key targets of drug acting on NP. In order to further find hub-genes in PPI network, MCODE algorithm was used to compute core genes. Next, intersection of hub-genes in PPI network and absorption, distribution, metabolism, and excretion (ADME) genes were used as candidate genes for molecular docking. Then, the target genes corresponding to each active ingredient in WJPMF were intersected with ADME genes. The ingredient which has the largest number of overlapping targets was considered as the core active ingredient of drug acting on NP.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

GO and KEGG pathway enrichment analysis was performed on WJPMF targets acting on NP in the PPI network using the clusterProfiler 4.4.2 (Yu et al. 2012). p-value < 0.05 was set to identify significantly enriched in specific GO and biological pathways.

Molecular docking

Molecular docking provides valuable insights into the binding affinity between the core active ingredients of WJPMF and selected key targets. First, the crystal structures of the target proteins in pdb format were obtained from RCSB Protein database (http://www.pdb.org/), and the sdf structure of the active ingredients was obtained by PubChem. To prepare the files for molecular docking, we converted the downloaded crystal structure files and the active ingredient SDF file into PDBQT format by Open Babel GUI software.

Then, the configurations of protein structures were modified by Pymol and Autodock1.5.6 software containing elimination of the original ligand and water, addition of hydrogen, as well as the optimization of amino acids and calculation of charge (Seeliger et al. 2010). We utilized Autodock 1.5.6 software for the docking simulations and Pymol software for visualization of docking results. The binding mode and affinity of active ingredient of WJPMF and the targets in docking simulation were evaluated.

Experimental verification of network analysis results

Animals care

Adult male Sprague-Dawley (SD) rats (200–250 g) aged 6–7 weeks were housed at a standard temperature (24 ± 1°C). The rats were fed under a 12 h light-dark cycle with free access to water and food. The animal experiments were approved by the Institutional Animal Care and Use Committee of Xiamen University (XMULAC20220034-14). Because estrogen can influence the sensory and pain systems (Chen et al. 2021), and the pain threshold in female rats varies with the estrous cycle (Ibironke and Aji 2011), to reduce the resulting variability caused by the estrous cycle stages in female rats, we chose male rats to investigate the effects of WJPMF on neuropathic pain.

Animal grouping and SNI model establishment

Eighteen male SD rats were randomly assigned into three groups, with six rats in each group. The groups were named Sham, Model, and WJPMF group. To investigate the mechanisms underlying NP and assess the effectiveness of new pharmacological drugs, we employed the SNI model following established procedures (Decosterd and Wolf 2000). Briefly, rats in the Sham group were anesthetized by intraperitoneal injection of 1% pentobarbital sodium (30 mg/kg), and a small incision was made in the lateral skin of the left thigh to expose the sciatic nerve and its three branches: the common peroneal, sural, and tibial nerve. In the SNI group, the experimental procedure involved exposing the sciatic nerve is similar to before, but common peroneal and tibial nerves were specifically ligated, while the sural nerve remained intact. Then, muscle layers were sutured by 6.0 suture silk and skins was sutured by 5.0 suture silk. On the 7^{th} day after SNI surgery, WJPMF group was given 10.8 g/kg WJPMF solutions *per* oral gavage dose twice a day. Rats in the Sham and Model groups received gastric administration of an equal volume of normal saline for 14 days. On postoperative day 21, the rats were euthanized under pentobarbital anesthesia for subsequent experiments.

Behavioral tests

Paw Withdrawal Threshold (PWT) and Thermal Withdrawal Latency (TWL) tests were to evaluate mechanical allodynia and thermal hyperalgesia at baseline and 1, 3, 7, 14, and 21 days after SNI or sham surgery. The PWT was assessed by stimulating the median plantar of paw with the Von Frey filament as preciously described (Chaplan et al. 1994). Briefly, rats were introduced into a transparent plexiglass box and provided a 30-min acclimation period before testing commenced. Starting from the middle strength of the defined range (0.07 to 26.0 g) of filaments, a probe was gently placed perpendicular to the mid-plantar surface of the hind paw, gradually increasing in strength to bend filaments for 6 s. To ensure accuracy and reliability, a minimum of three measurements were taken for each hind paw, with a 2-min interval between each stimulation. PWT was assessed at 2 h after drug administration.

The TWL was tested using a thermal radiometer. Rats were placed in a clear plexiglass box, and thermal radiation was applied to median hind paws of rats (intensity: 20%). When the hind paw of the rat was raised quickly or the foot licking occurred, the thermal radiometer would automatically stop and record the latency time. Three reflex responses were taken on each rat with a 5-min interval between each test, and the average was recorded.

Table 1. The 25 intersection genes of disease-related targets, active ingredients-related genes, and ADME genes of WJPMF

Gene name	Source	Type	Class
ABCB1	DisGeNET	Core ADME Gene	Transporter
CYP2D6	GeneCards	Core ADME Gene	Phase I
CYP3A4	GeneCards	Core ADME Gene	Phase I
CYP1A2	GeneCards	Core ADME Gene	Phase I
SLC22A2	GeneCards	Core ADME Gene	Transporter
ABCG2	GeneCards	Core ADME Gene	Transporter
CYP2C19	GeneCards	Core ADME Gene	Phase I
CYP17A1	DisGeNET	Extended ADME Gene	Phase I
NOS1	DisGeNET	Extended ADME Gene	Phase I
PPARG	DisGeNET	Extended ADME Gene	Modifier
NR1I2	DisGeNET	Extended ADME Gene	Modifier
CYP19A1	DisGeNET	Extended ADME Gene	Phase I
NOS3	DisGeNET	Extended ADME Gene	Phase I
SOD1	DisGeNET	Extended ADME Gene	Modifier
CFTR	GeneCards	Extended ADME Gene	Modifier
PPARA	GeneCards	Extended ADME Gene	Modifier
CAT	GeneCards	Extended ADME Gene	Modifier
CES1	GeneCards	Extended ADME Gene	Phase I
MPO	GeneCards	Extended ADME Gene	Modifier
SOD2	GeneCards	Extended ADME Gene	Modifier
ABCA1	GeneCards	Extended ADME Gene	Transporter
ALDH7A1	GeneCards	Extended ADME Gene	Phase I
PON1	GeneCards	Extended ADME Gene	Phase I
SOD3	GeneCards	Extended ADME Gene	Modifier
XDH	GeneCards	Extended ADME Gene	Phase I

Enzyme-linked immunosorbent assay (ELISA)

To obtain the supernatants, the harvested left L4–6 spinal cord tissues of injured rats in each group (100 mg) were homogenized and centrifuged (10,000 rpm, 10 min). The levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in the spinal cord were measured by commercial ELISA kits (Mlbio, Shanghai, China) according to the instructions of the manufacturer.

Immunofluorescence staining

By utilizing immunofluorescent techniques, the specific localization and levels of Iba-1 and GFAP proteins were visualized. These proteins serve as markers for activated microglia (Iba-1) and astrocytes (GFAP) respectively, and their expression patterns in the dorsal horn can provide valuable insights into the inflammatory and glial activation response in the injured spinal cord (Wang et al. 2014). The left L4–6 spinal cord tissues of injured rats in each group were fixed in 10% formaldehyde for 48 h. The collected samples were cut at a thickness of 4 μm using a paraffin slicer (Leica, BW, Germany). After blocking with a 5% solution of bovine serum albumin (BSA) for a duration of 1 h at a temperature of 37°C, the sections were subjected to incubation with an anti-GFAP primary antibody (rabbit, 1:100, ab68428, Abcam), anti-Iba-1 (rabbit, 0.5 μg/ml, ab153696, Abcam) overnight at 4°C, followed by incubating with secondary antibodies Cy3 Goat Anti-Rabbit IgG (YT107, Baiaolaibo) or FITC Goat Anti-Rabbit IgG (AS011, ABclonal Technology) for 2 h at room temperature. Finally, DAPI was used to stain the sections, and laser scanning confocal microscopy was used to examine all the sections.

Western blotting

The protein expression of Iba-1, GFAP, ABCA1, PPARA, PPARG, and SOD2 was measured *via* Western blotting. In brief, the obtained tissues (60 mg) were homogenized in 200 μl of RIPA buffer and then centrifuged at 12,000 rpm for 10 min at 4°C to extract total protein. The proteins were quantified by a bicinchoninic acid assay. Equal amounts of protein (25 μg/well) were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membrane. The above membranes were blocked in 5% skim milk for 1 h and incubated with the primary antibodies against Iba-1 (1: 500, ab153696, Abcam, UK), GFAP (1: 10,000, ab68428,

Abcam, UK), ABCA1 (1 μg/ml, ab66217, Abcam, UK), PPARA (1: 500, 15540-1-AP, Proteintech, China), PPARG (1: 2,000, 16643-1-AP, Proteintech, China), and SOD2 (1: 5,000, ab13533, Abcam, UK) overnight at 4°C. Nest, membranes were washed with TBST buffer, followed by incubation with secondary HRP-conjugated goat anti-rabbit IgG antibody (1:2,000, ab6721, Abcam, UK) for 1 h at 37°C. The protein bands were made visible using an electrochemiluminescence reagent (Applygen Co., Ltd. Beijing, China).

Statistical analysis

Statistical analysis was conducted using GraphPad 7.0 software for all data processed in this study. The results were presented as the mean value ± standard deviation. To evaluate differences within groups, a one-way analysis of variance (ANOVA) was performed. For the analysis of statistical significance between two treated groups, Student's *t*-test was employed. *p*-values less than 0.05 were considered statistically significant, indicating significant differences between groups.

Results

Network pharmacology prediction analysis

Putative common targets of WJPMF treating NP

A pharmacological network analysis was performed to investigate the role of WJPMF in NP treatment. WJPMF is composed of Jiangchan, Chantui, Jianghuang, Dahuang, Yanhusuo, Baizhi, and Danshen. As a result, a sum of 631 potential components from WJPMF were retrieved, containing 221, 189, 103, 98, 56, 30, and 9 from Baizhi, Danshen, Dahuang, Jianghuang, Yanhusuo, Jiangchan, and Chantui, respectively (Fig. 1A). Additionally, there are 907 target genes related to 173 active ingredients. The 298 ADME genes were defined by the PharmaADME Consortium, and a total of 2532 NP-related targets were screened from GeneCards and DisGeNET databases. Then "2532 disease-related targets", "907 targets of active ingredients", and "298 ADME genes" were mapped in the Venn diagram (Fig. 1B).

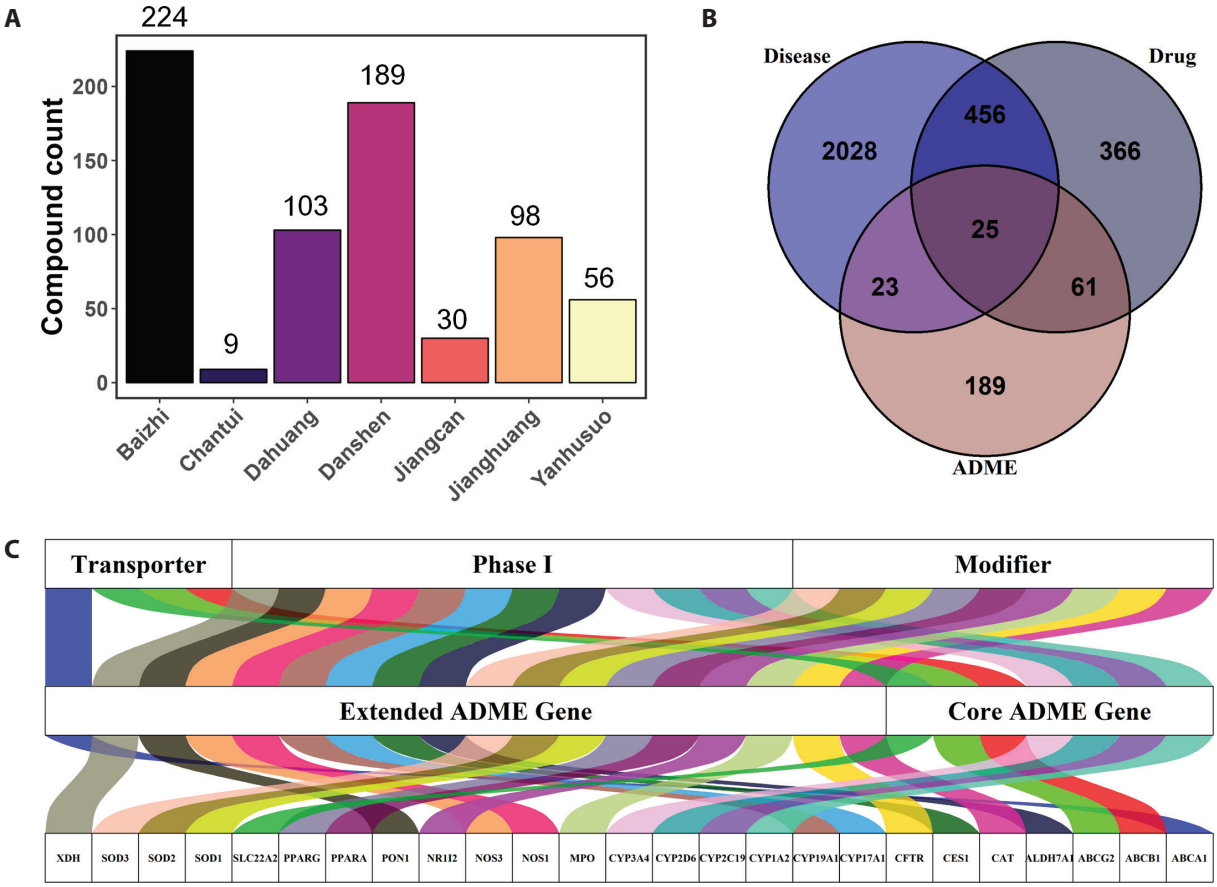

Figure 1. Screening of ADME Genes of WJPMF in the treatment of neurogenic pain. **A.** Screening of active ingredients of WJPMF in NP. **B.** The Venn diagram illustrating target gene intersections of NP-related targets, active ingredients-related genes, and ADME genes. **C.** Functional classification of AMDE in 25 intersection genes.

As a result, 25 combined targets were screened out, detailing in the Table 1. Functional classification of AMDE in 25 intersection genes included the transporter, phase I, and modifier (Fig. 1C).

PPI network

We constructed the network of six compounds, 25 target genes, and 67 active ingredients of WJPMF by Cytoscape, illustrated in the Figure 2A. An analysis of protein-protein interactions was performed on the 25 predictive common targets (Fig. 2B) using STRING database. Then, cytoHubba software was used to obtain a total of 14 core genes (Fig. 2C) using MCODE algorithm. Among them, ABCA1, PPARA, PPARG, and SOD2 were ADME genes by Venn diagram (Fig. 2D). Therefore, the main core targets (ABCA1, PPARA, PPARG, and SOD2) might be promising targets of WJPMF in NP.

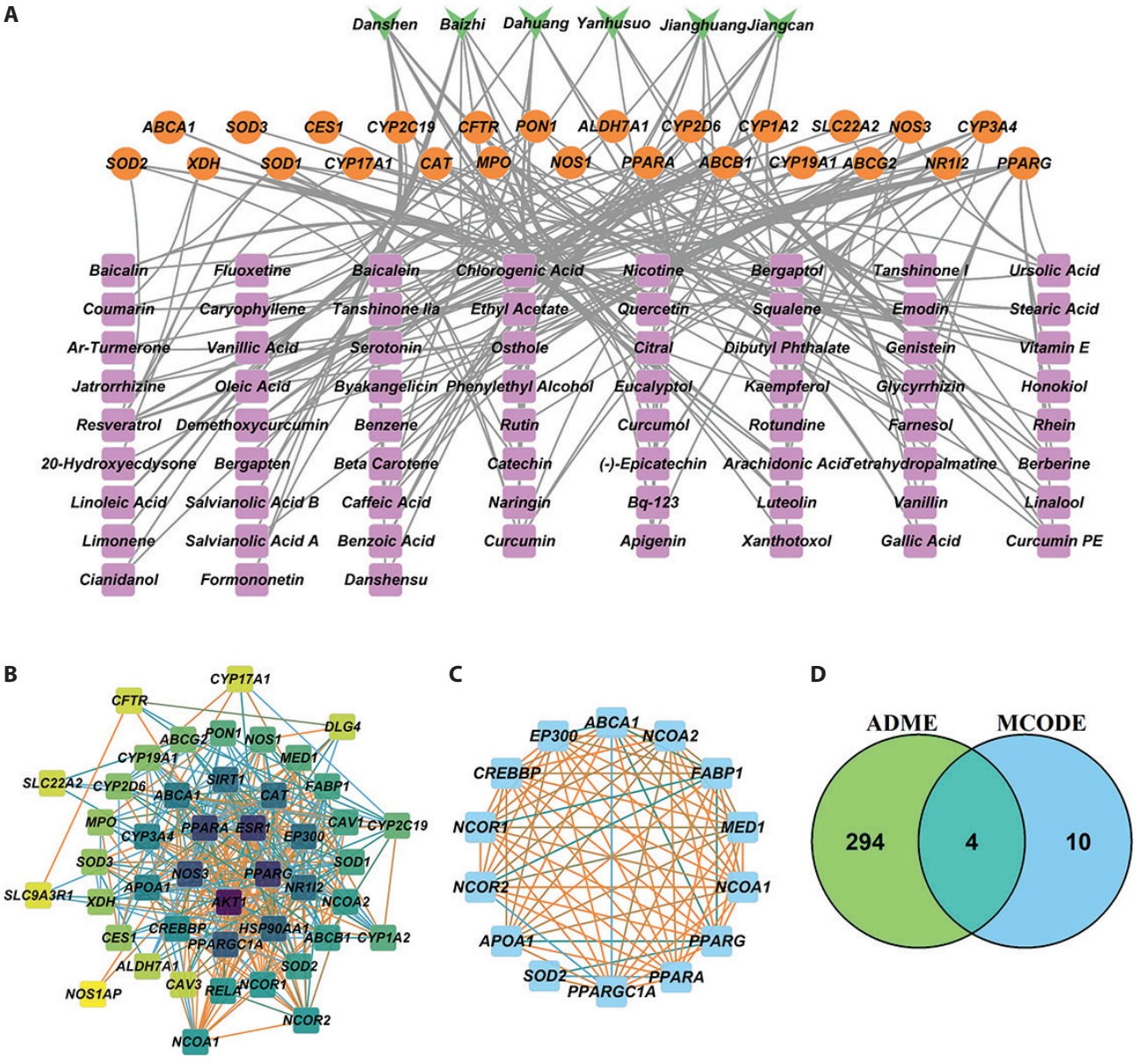

Figure 2. Core targets of WJPMF acting on neurogenic pain. **A.** The network of drug, target genes, and active ingredients of WJPMF. Purple modules represent the active compounds of WJPMF. Green modules are drugs, and orange nodes are target genes. **B.** Protein-protein interaction (PPI) network of the 25 common targets. The darker degree of the dots indicates higher correlation. **C.** Hub-genes were screened by MCODE algorithm in PPI network. **D.** Venn diagram of ADME genes and hub-genes screened by MCODE algorithm in PPI network. (See online version for color figure.)

GO and pathway enrichment analysis

To gain a deeper understanding of mechanisms underlying the effects of WJPMF on NP, further investigations were carried out. GO and pathway analysis of 25 candidate genes in the PPI network was done by clusterProfiler 4.4.2. These targets were involved in the 1090 biological process and 64 signaling pathways in total. Figure 3A exhibited the top 10 categories, which showed that these target genes may be involved in steroid metabolic process, regulation of small molecule metabolic process, and fatty acid metabolic process related to NP. The KEGG analysis suggested that target genes of WJPMF might affect NP *via* these regulatory pathways such as the thyroid hormone, estrogen, and longevity regulating signaling pathways (Fig. 3B). KEGG signaling pathways which core targets (ABCA1, PPARA, PPARG, and SOD2) involved in was also showed in the Figure 3C. By modulating these targets, WJPMF had potential to regulate the intricate physiological pathways associated with NP, like longevity regulating pathway, glucagon, and PPAR signaling pathway.

Molecular docking

Resveratrol, an active compound of WJPMF acting on NP, has the largest number of overlapping ADME targets used for molecular docking. The binding mode of resveratrol and the core targets (ABCA1, PPARA, PPARG, and SOD2) were investigated by molecular docking. The results showed that ABCA1, PPARA, PPARG, and SOD2 fitted well to resveratrol. The binding energy was all less than −5.0 kcal/mol (Table 2) and hydrogen bonds can be formed between receptors and ligands, shown in Figure 4. Among them, PPARA had the highest binding energy with resveratrol (−7.1 kcal/mol), followed by PPARG, ABCA1, and SOD2. The molecular docking results indicated that ABCA1, PPARA, PPARG, and SOD2 may be the promising treatment targets of WJPMF affecting the NP (Feng et al. 2021).

Experimental verification of network analysis results

WJPMF improved the pain behaviors and relieved inflammatory response in SNI rats

To establish the preinjury thresholds, baseline assessments were conducted to measure mechanical allodynia and thermal hyperalgesia. NP behaviors were evaluated on 0, 1, 3, 7, 14, 21 days following SNI. Compared to the sham rats, the SNI rats exhibited a significant decrease in both PWT and TWL on postoperative day 1, 3, 7, 14, and 21 ($p < 0.01$; Fig. 5A, B). Following the treatment with WJPMF, there was a significant increase in the pain threshold of SNI rats compared to the model rats ($p < 0.01$) at day 14 and 21. Inflammatory cytokine TNF-α, IL-1β, and IL-6 in SNI rats were detected *via* ELISA. Significantly, expression levels of TNF-α, IL-1β, and IL-6 in SNI rats were obviously upregulated compared to the sham group ($p < 0.01$; Fig. 5C). WJPMF treatment obviously ameliorated the inflammatory response in SNI rats with the decreased levels of TNF-α, IL-1β, and IL-6 ($p < 0.01$).

Table 2. Free energy information of resveratrol with core target molecules

Target	Pubchem_ID	Compound	Free binding energy (kcal/mol)
ABCA1	445154	Resveratrol	−6.2
PPARA			−7.1
PPARG			−7
SOD2			−5.5

WJPMF improved the spinal microglia damage in SNI rats by upregulating GFAP, ABCA1, PPARA, PPARG, and SOD2

The distribution and expression of makers of microglial activation (Iba-1) and astrocyte damage (GFAP) correlated with NP were further detected. As shown in the Figure 6A and B, after modeling, compared to the sham group, the SNI rats demonstrated increased immunoreactivity of Iba-1 and GFAP in the spinal cord, particularly in the cell membrane for Iba-1 and cytoplasm for GFAP ($p < 0.01$). Notably, in contrast to the model group, the significant decrease of Iba-1 and GFAP levels were seen in the ipsilateral spinal dorsal horn of WJPMF treated rats ($p < 0.05$). Furthermore, Western blotting analysis further verified the network pharmacological results. The outcomes indicated that the lower expression levels of ABCA1, PPARA, PPARG, and SOD2 were seen in the spinal cord of model than sham groups ($p < 0.01$; Fig. 6C). Compared to the model group, the administration of WJPMF led to a notable upregulation of ABCA1, PPARA, PPARG, and SOD2 in the spinal cord of the treated rats ($p < 0.05$; Fig. 6C). Meanwhile, *in vivo* studies demonstrated that WJPMF could improve the development of NP in SNI rats by regulating the expression of ABCA1, PPARA, PPARG, and SOD2, consistent with the results of network pharmacological analysis.

Discussion

NP is a common chronic pain, which is a consequence of disease of the central or peripheral somatosensory nervous system with major impact on quality of life (Finnerup et al. 2021). Although currently recommended pharmaco-

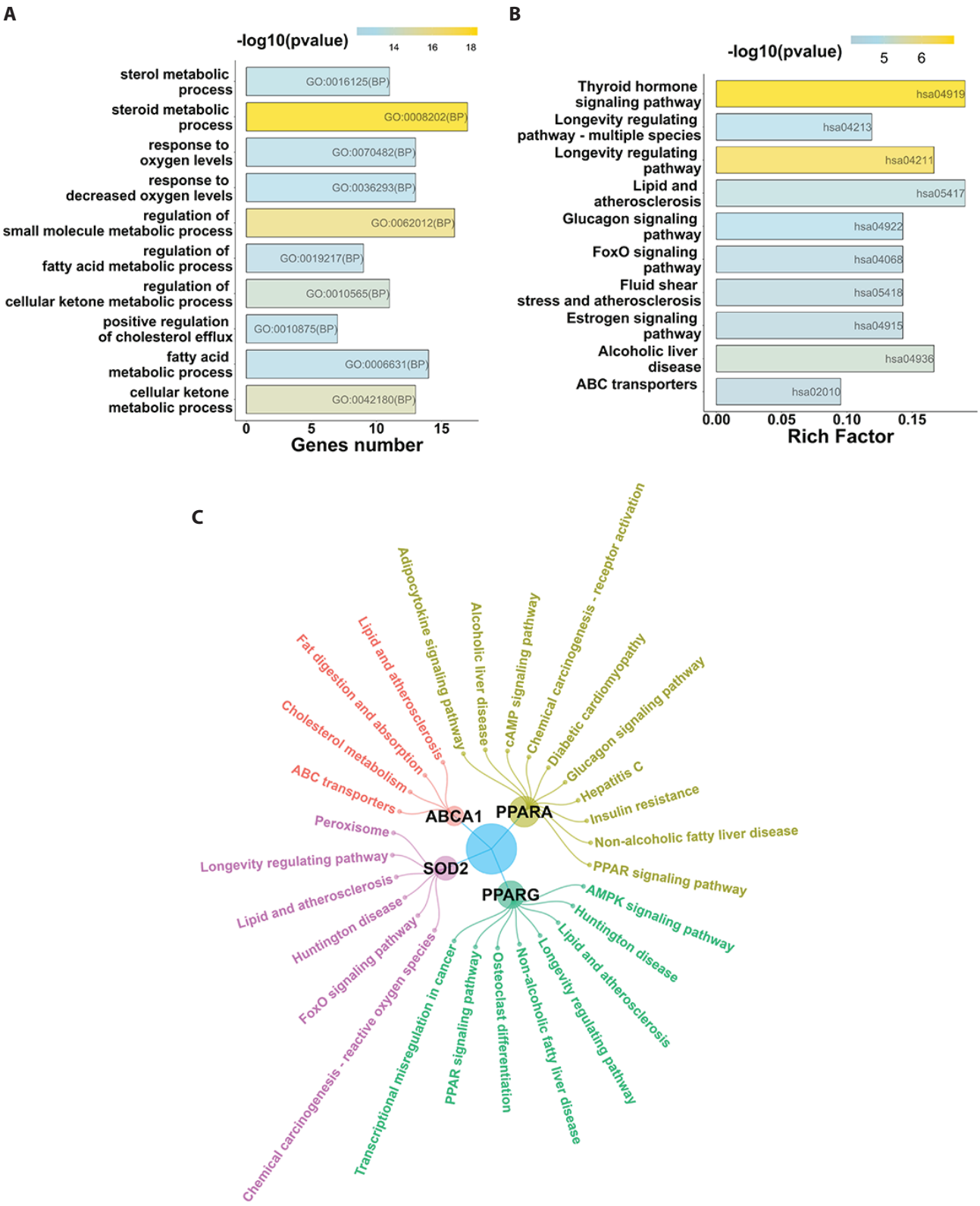

Figure 3. Gene ontology (GO) and pathway enrichment analysis of key targets of WJPMF acting on neurogenic pain (NP). The 10 most significance of GO (**A**) and pathway enrichment (**B**) analysis of 25 targets in the protein-protein interaction (PPI) network. **C.** Cycle tree of KEGG signaling pathways which core targets (ABCA1, PPARA, PPARG, and SOD2) involved.

logical treatments such as antidepressants and anticonvulsants (gabapentin and pregabalin) have good therapeutic outcomes, traditional drugs fail to give adequate control of the chronic pain and show badly side effects (Xu and Zhang 2016). It is urgent to seek for effective and few side effects drugs in NP treatment. TCM treatments have been proved to exhibit ameliorative effects on NP through multiple targets (Li et al. 2020). WJPMF, a classical Chinese medicinal formula, is believed to show the effect of relieving depression, reducing fever, and relieving heat (Yilmaz et al. 2018). Previous studies demonstrated that WJPMF possess anti-inflammation, anti-virus, anti-asthma, and anti-tumor properties (Li et al. 2017). Herbal formulations from WJPMF are widely used for treatment of pain-related diseases (Li et al. 2020), but the exact effect of WJPMF was undefined.

In our work, a network pharmacology analysis integrated with experimental validation was utilized to investigate the possible mechanisms of WJPMF acting on NP. Firstly, there are 25 key ADME targets of WJPMF in NP according to our network analysis results. According to pathway enrichment analysis of the core targets, these targets are mainly involved in the thyroid hormone, estrogen, longevity regulating, and PPAR signaling pathways, which are related to the NP development. The study of Suda et al. (2022) found that thyroid hormones are vital for central and peripheral nervous system functions. About 50% of adults with adult-onset hypothyroidism experience sensory symptoms, including pain, potentially due to peripheral neuropathy (Suda et al. 2022). Moreover, gender differences in the manifestation of pain are well-documented (Bartley and Fillingin 2013), with a pivotal role attributed to estrogen (Qu et al. 2015). Deng et al. (2017) discovered that the influence of female hormones involves the upregulation of N-methyl-D-aspartate receptor 1 in dorsal root ganglia, affecting neuropathic pain and subsequently increasing sensitivity. Meanwhile, we further obtained key targets of WJPMF (ABCA1, PPARA, PPARG, and SOD2) by topological analysis and molecular docking. Increasing evidence has shown that PPAR signaling

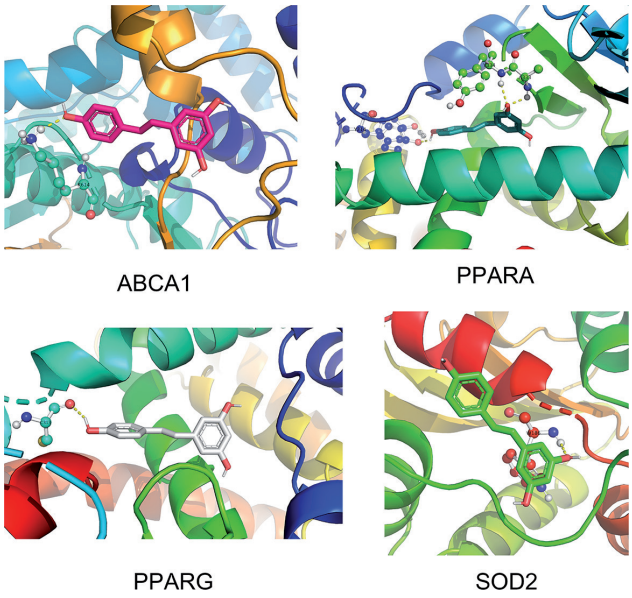

Figure 4. Molecular docking of resveratrol with ABCA1, PPARA, PPARG, and SOD2 molecule.

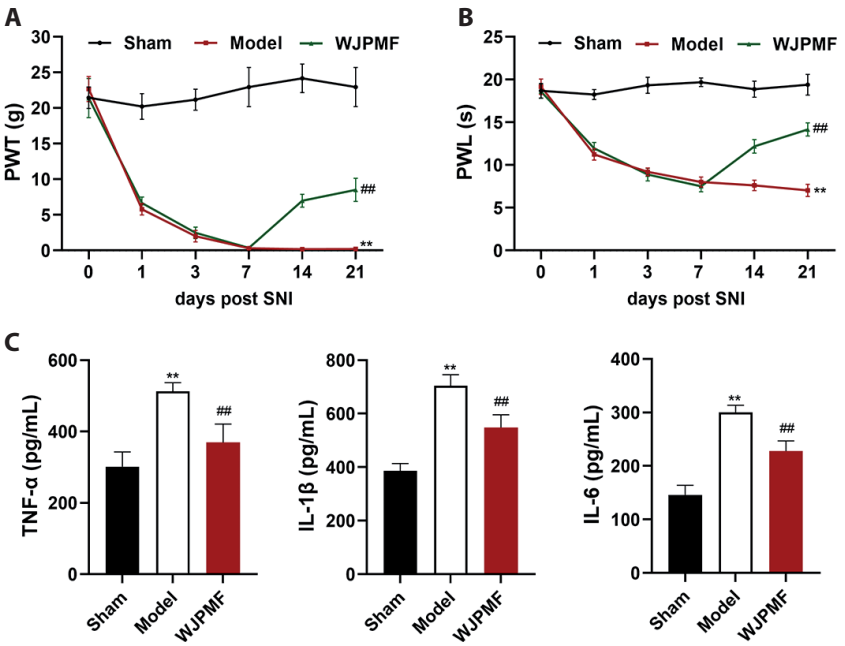

Figure 5. WJPMF improved the pain behaviors and relieved inflammatory response in spared nerve injury (SNI) rats. Paw withdrawal threshold (PWT) to mechanical stimulation (**A**) and paw withdrawal latency (PWL) to thermal stimulation (**B**) were detected after SNI or sham operation following treatment of WJPMF or not. **C.** The levels of TNF-α, IL-1β, and IL-6 in rats were assayed by ELISA. ** $p < 0.01$ *vs.* Sham group; ## $p < 0.01$ *vs.* Model group.

pathway plays a role in alleviating NP. Increased expression of chemokines leads to chronic inflammation, which is important to the development of NP (White et al. 2005). The PPAR signaling can up-regulate the inflammation chemokine like CCL2/CCR2 expression to affect the progression of NP (Gao et al. 2021).

Then, we utilized ADME parameters coupled with OB, DL, and HL characteristics as a meaningful standard for drug candidates screening. Our findings suggested that resveratrol was more appropriate as the drug candidate for the therapeutic potential of NP. Moreover, molecular docking validation of the target proteins with the resveratrol by Autodock software revealed that resveratrol had stable binding energy to ABCA1, PPARA, PPARG, and SOD2. Previous study showed that neuroinflammation is critical to the transition to and perpetuation of NP states (Lees et al. 2017). Meanwhile, the pathogenesis of NP is closely linked to the development of inflammation in both the peripheral and central nervous systems (Moalem and Tracey 2006). Miller et al. suggested that the progression of NP could be regulated by the TLR4 inflammarafts and gene expression in microglia, leading to blocking the perpetuation of neuroinflammation (Navia-Pelaez et al. 2021). Resveratrol is a natural polyphenolic compound with many beneficial properties, including anti-inflammatory, anti-oxidative stress, and neuroprotective effects which could relieve the NP (Xu et al. 2018). Meanwhile, Gao et al. suggested that resveratrol may ameliorate NP though down-regulating

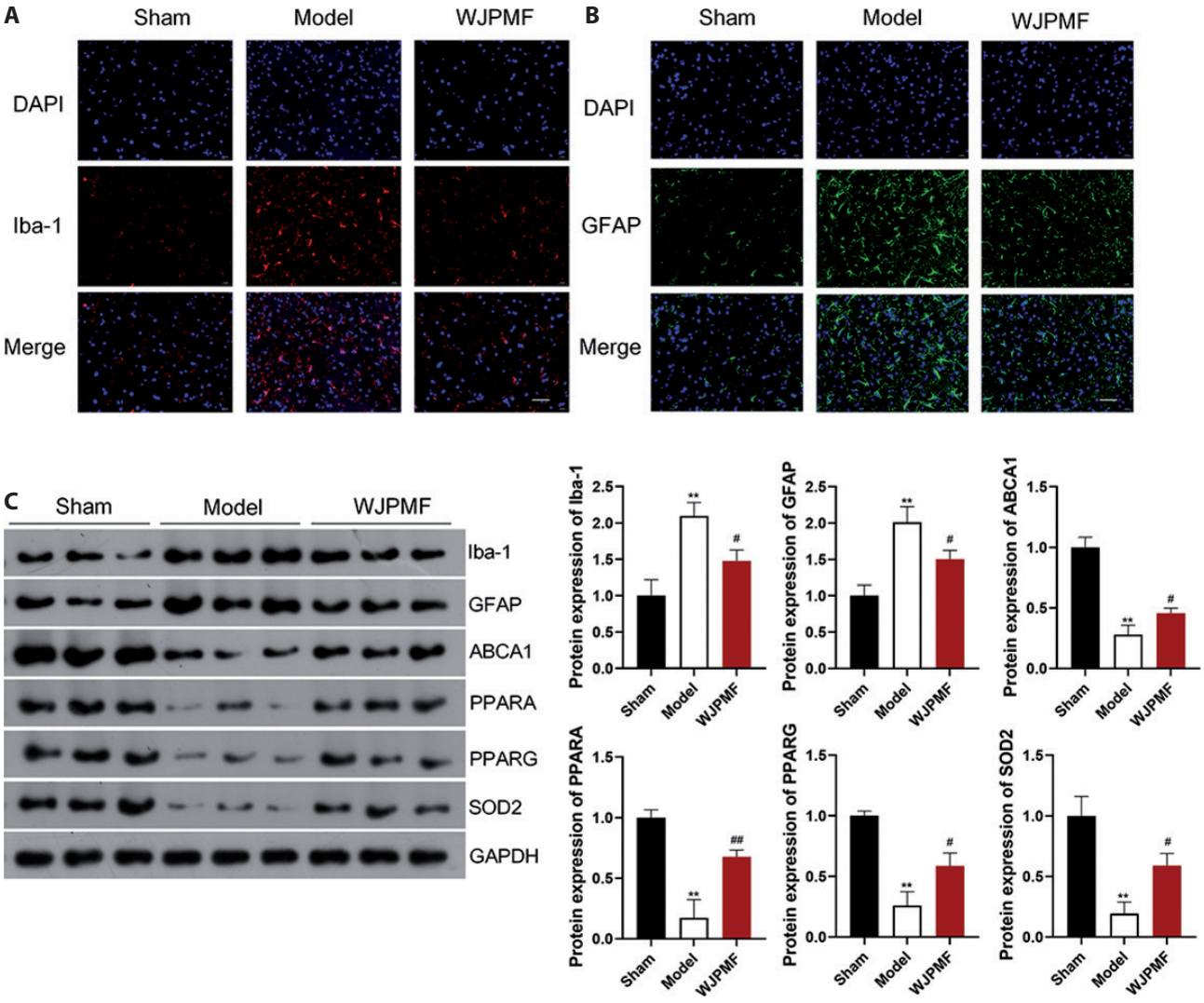

Figure 6. WJPMF improved the spinal microglia damage in SNI rats by upregulating GFAP, ABCA1, PPARA, PPARG, and SOD2. Immunofluorescence staining of Iba-1 (**A**) and GFAP (**B**) in the ipsilateral spinal dorsal horn of rats on days 21 after repeated gavage administration of WJPMF or not. Scale bar = 40 μm, $n = 6$/group. **C.** The expression of Iba-1, GFAP, ABCA1, PPARA, PPARG, and SOD2 in the spinal cord of rats was determined by Western blotting. $^{**}p < 0.01$ *vs*. Sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs*. Model group.

P2X3 expression and ERK phosphorylation in dorsal root ganglion of SNI rats (Guo et al. 2021). Furthermore, extensive research has confirmed the impact of resveratrol on thyroid hormones, estrogen, longevity regulation, and the PPAR signaling pathways. Giuliani and others found that resveratrol possesses anti-thyroid properties both *in vitro* and *in vivo* (Giuliani et al. 2017). Ahmed et al. (2023) verified resveratrol's specific regulatory ability on estrogen receptors. Resveratrol is also considered one of the natural approaches to promote longevity (Pulakat and Chen 2020). Moreover, it acts as a natural agonist of PPAR (Enayati et al. 2022). Thus, the active compound (resveratrol) of WJPMF might exert the analgesia effect by regulating neuroinflammation in the nervous system and relative signaling pathways.

To further verify the prediction, we detected the actions of WJPMF in SNI rats. The analysis demonstrated that WJPMF treatment notably exerted a pain relief effect, and improved nociceptive behavior with decreased inflammatory response in SNI rats. Besides, it was also reported that TCM could alleviated NP in mice by suppressing pro-inflammatory pathways (Xu et al. 2021). In our experimental verification, the level of TNF-α, IL-1β, and IL-6 was both down-regulated, indicating that WJPMF could relive NP by decreasing inflammation. Especially, the expression levels of the ABCA1, PPARA, PPARG, and SOD2 were up-regulated after the treatment of WJPMF in SNI rats. ATP-binding cassette transporter A1 (ABCA1) is an important cholesterol transport gene which mediates neuroinflammation and pathology in neurodegenerative animal models (Karasinska et al. 2013). The increased level of ABCA1 has recognized anti-inflammatory properties (Tang et al. 2009). Additionally, manganese superoxide dismutase (SOD2) serves as a crucial enzyme responsible for the detoxification of oxygen radicals within mitochondria. It acts as a cellular modifier, contributing to neuronal oxidative defense mechanisms (Vincent et al. 2007). The overexpressing of SOD2 reduced neuropathic pain sensitivity in mice (Kotulska et al. 2011). Furthermore, peroxisome proliferator-activated receptors (PPARs) are part of the nuclear receptor subfamily, consisting of PPARA, PPARD, and PPARG. These receptors play a crucial role in regulating the expression of target genes and modulating a range of biological processes, such as cellular differentiation and cell proliferation (Dubois et al. 2017). PPAR-γ activation has beneficial effects in anti-inflammatory activities and animal models of nerve diseases (Esposito et al. 2011). Previous studies using experimental models have verified that administration of PPAR ligands could relieve inflammatory pain and NP by repressing expression of pro-inflammatory cytokines (Maeda et al. 2009).

The above results indicated that WJPMF could reduce the inflammation in SNI rats and up-regulate the expression of ABCA1, PPARA, PPARG, and SOD2 protein. Moreover, WJPMF attenuates chronic pain partially through suppressing pro-inflammatory pathways by PPAR pathway. Meanwhile, it is important to acknowledge and address some limitations of this study. One limitation of this study is that it only focused on analyzing the effects of a single compound within WJPMF. As a result, the findings may not fully capture the collective effects of all components present in the herbal formula. Second, dose-response studies using a wider range of concentrations are needed to provide valuable insights into the concentration-dependent effects of WJPMF in the NP treatment. Indeed, the analgesic effect of WJPMF may be attributed to its ability to act on multiple pathways, targets, and biological processes.

Conclusions

To sum up, the pharmacological mechanism of WJPMF regulation on NP was conducted with the integration of network pharmacology and *in vivo* experiments. Our findings showed that resveratrol is main active ingredient of WJPMF. WJPMF could alleviate inflammatory response by regulating ABCA1, PPARA, PPARG, and SOD2 protein expression. The pro-inflammatory pathways may be the key pathways of WJPMF acting on NP. Further investigation is warranted to conduct clinical research on WJPMF as a potential novel medication for managing NP. These findings further offer a research strategy for gaining a comprehensive insight into the mechanism of WJPMF in treating NP.

Conflict of interest. The Authors declare that there is no conflict of interest.

Author contributions. YL: Conceptualization, writing – original draft, formal analysis, funding acquisition. FY: Resources, funding acquisition, investigation. WL: Data curation, validation, funding acquisition. QA: Validation, funding acquisition, methodology. ZP: Formal analysis, funding acquisition, validation. WS: Writing – review and editing, funding acquisition, visualization. XS: Conceptualization, writing – review and editing. All authors have read and approved the final manuscript.

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