Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Huazhuo Decoction Contributes to Immune Homeostasis and Attenuates Neuronal Inflammation and Demyelination in a Mouse Model of Multiple Sclerosis

Jing-Ying Wu[1,*], Juan Zhang[1,*], Shan Xu[1,*], Shi-Yan Qian[1], Zheng Zha[1], Jia-Ling Hu[1], Jiang-Li Yang[1], Feng-Xia Zhang[2], Guo-Qing Zheng[1]

[1]Department of Neurology, The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine), Hangzhou, People's Republic of China; [2]Department of Neurology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, People's Republic of China

*These authors contributed equally to this work

Correspondence: Guo-Qing Zheng, Department of Neurology, The First Affiliated Hospital of Zhejiang Chinese Medical University, 54 Youdian Road, Shangcheng District, Hangzhou, 310006, People's Republic of China, Email gq_zheng@sohu.com; Feng-Xia Zhang, Department of Neurology, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, 16369 Jingshi Road, Jinan, 250011, People's Republic of China, Email fxzhang0987@163.com

Journal of Inflammation Research downloaded from https://www.dovepress.com/
For personal use only.

Background: Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS). Huazhuo decoction (HD) has been shown to ameliorate disease progression in MS patients, but the underlying mechanisms remain poorly understood.

Purpose: This study sought to assess the therapeutic efficacy of HD in an experimental autoimmune encephalomyelitis (EAE) model of MS, and to further investigate the underlying mechanisms involved.

Methods: We assessed the curative effects of HD on EAE mice via neurological function scoring and histological staining and identified the chemical composition of HD by UPLC-MS. RNA sequencing identified differentially expressed genes. Flow cytometry detected the proportions of splenic Th17, Th1, and Treg cells. Expression of key molecules was evaluated by Western blotting and quantitative PCR. Immunofluorescence was used to monitor microglia, astrocytes and oligodendrocytes.

Results: HD alleviated neurobehavioral impairment, inflammatory infiltration and demyelination in EAE mice. RNA sequencing revealed that HD may ameliorate EAE by modulating adaptive immunity and affecting NLRP3-related signaling pathways. Further studies demonstrated that HD suppressed the upregulation of NF-κB/NLRP3 pathway, balanced the proportions of peripheral Th17, Th1 and Treg cells and regulated inflammatory cytokines. HD influenced innate immunity by reducing microglia and astrocytes and increasing oligodendrocytes in the CNS. Notably, these protective effects of HD were attenuated by an NLRP3 agonist.

Conclusion: HD effectively alleviated the clinical symptoms of EAE mice and inhibited both peripheral and central immune inflammatory responses by modulating the NF-κB/NLRP3 pathway, providing a strong basis for its clinical application.

Keywords: multiple sclerosis, Huazhuo decoction, NLRP3, regulatory T cell, Th17 cell, neuroinflammation

Introduction

Multiple sclerosis (MS), an autoimmune disease characterized by central nervous system (CNS) demyelination, is the principal source of nontraumatic disability in young individuals (aged 18–40 years).[1] According to the MS Atlas,[2] there are approximately 2.8 million individuals affected by MS around the world. The symptoms of MS include muscle weakness, blurred vision, fatigue, balance difficulty and cognitive impairment,[3] which collectively impose a substantial burden on both individuals and society.

https://doi.org/10.2147/JIR.S567313
Received: 28 September 2025
Accepted: 25 February 2026
Published: 25 March 2026

© 2026 Wu et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

The etiology and pathogenesis of MS remain unclear. However, it is widely accepted that both innate and adaptive immune responses play significant roles in the onset and progression of this disease.[4–6] Recent studies have demonstrated that inflammasomes are expressed and activated in lymphocytes, particularly in T cells and B cells, playing a critical role in regulating the adaptive immune response.[7–9] T lymphocytes, the main effectors of the adaptive immune response, are represented in the pathogenesis of MS by a peripheral imbalance of proinflammatory Th1 and Th17 cells and functionally impaired regulatory T (Treg) cells.[10]

The homeostasis between Treg and Th17 cells orchestrates immune tolerance and host defense.[11] Within the CNS, T cells can initiate an inflammatory cascade that attracts additional leukocytes, resulting in direct T cell mediated damage to oligodendrocytes and neurons.[1] T cells can also interact with microglia to exacerbate inflammation and neurotoxicity.[12] Microglia, the innate immune cells of the CNS, are recognized as essential players in maintaining brain homeostasis.[13] In MS, microglia enhance phagocytosis, promote demyelination,[14] and exacerbate neuroinflammation. Reactive astrogliosis, part of the innate immune response of the brain to injury,[15] is common in MS plaques and leads to the continuous production of proinflammatory factors and neuroaxonal dysfunction.[16] Oligodendrocytes are myelin-making cells of the CNS.[17] In MS, remyelination of spared axons by oligodendrocytes restores saltatory conduction and even reverses loss of function.[18] Both innate and adaptive immune mechanisms have been identified as central to successful remyelination.[19,20]

In the acute phase of MS, current treatments involve mainly corticosteroid therapies, whereas the remission phase includes disease-modifying therapies (DMTs) and symptomatic treatment.[21] DMTs significantly curtail relapse rates and slow down disability progression.[22] However, significant side effects have been found to be closely related to their long-term use. Adverse events of DMTs include bradycardia, macular edema, heart block and secondary autoimmune adverse effects.[23] The existing clinical treatments for MS are insufficient, highlighting the urgent need for further research to explore more effective therapeutic strategies for MS patients.

Numerous studies have reported that natural compounds can ease MS symptoms, promote myelin repair, and offer significant therapeutic benefits for MS without serious side effects.[24,25] In 2006, Professor Xin-Lu Wang first discussed the relationship between turbid blood and encephalopathy.[26] Huazhuo decoction (HD), which is based on the blood turbidity theory of Chinese medicine proposed by Professor Xin-Lu Wang, has demonstrated effective clinical outcomes in MS patients. HD consists of five Chinese medicinal herbs: *Epimedium brevicornu* (Yinyanghuo), *Reynoutria multiflora* (Heshouwu), *Viscum coloratum* (Hujisheng), *Reynoutria japonica* (Huzhang) and *Hirudo* (Shuizhi). The total flavone of Epimedium, the main active component of *Epimedium brevicornu*, inhibited the astrocyte-derived inflammatory response, contributing to myelin protection and regeneration.[27] *Reynoutria multiflora* suppressed Th17 cells and restored Treg cells in experimental models of MS.[28] Polydatin, the major extracted component of *Reynoutria japonica*, inhibited the activation of NLRP3 inflammasome and the cleavage of caspase-1, thereby reducing the secretion of inflammatory cytokines.[29] Recently, multi-target and combinatorial drug therapies have become an important treatment modality in complex diseases, including traumatic brain injury, ischemic stroke, Alzheimer's and Parkinson's disease.[30–36] A Traditional Chinese Medicine (TCM) formula usually comprises several medicinal herbs,[37] which work together to achieve synergistic effects or reduce toxicity.[38] The aim of this study was to investigate the therapeutic effects and underlying mechanisms of HD as a multi-component herbal formulation in experimental autoimmune encephalomyelitis (EAE) mice, a recognized animal model of MS, thereby providing valuable insights into the clinical treatment of MS.

Materials and Methods

HD Preparation

All herbal components of HD were purchased commercially from the First Affiliated Hospital of Zhejiang Chinese Medical University, as detailed in Table 1. We confirmed the official herb names in The World Flora Online (https://www.worldfloraonline.org), MPNS (https://mpns.kew.org) and Chinese Herbal Medicine Database (https://herbaltcm.sn.polyu.edu.hk/).

The herbs were soaked for 30 minutes in distilled water. The mixture was boiled at high heat, and then decocted at low heat for 30 minutes, after which the liquid was filtered. Distilled water was added, and the above decoction process repeated. The two filtrates were subsequently combined and filtered.

Table 1 Detailed Information of Herbs in HD

Latin Name	Chinese Name	English Name	Part(s) Used	Weight (g)
Epimedium brevicornu	Yinyanghuo	Epimedium brevicornu	Leaf	30
Reynoutria multiflora	Heshouwu	Polygonum multiflorum	Root tuber	20
Viscum coloratum	Hujisheng	Viscum coloratum	Leaf and stem	30
Reynoutria japonica	Huzhang	Polygonum cuspidatum	Rhizome and root	30
Hirudo	Shuizhi	Leech	Dry whole	6

Detection of the Chemical Ingredients of HD

The constituents of HD were detected via a UPLC I-Class system (Waters Corporation, USA) linked to a quadrupole Orbitrap mass spectrometer featuring a HESI source (Thermo Fisher Scientific, USA). Separation was performed on an HSS T3 column (100 × 2.1 mm, 1.8 µm) maintained at 45°C with a 5 µL injection. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B), delivered at 0.35 mL/min. Mass spectrometry settings encompassed the utilization of the HESI ion source, with signal collection performed in positive/negative switching mode using DDA.

LC-MS data were processed using Progenesis QI V2.3 (Waters). Key steps included baseline filtering, peak detection, alignment, retention time adjustment, and standardization. The identification of compounds was achieved by matching precise mass values, secondary fragment patterns, and isotopic distributions against reference data. Furthermore, the characterization of these compounds was accomplished through consultation with the TCM database.

Mice

Animal studies were reported in compliance with the ARRIVE guidelines (Animal Research: Reporting of in vivo Experiments). C57BL/6 female mice were purchased from Sippr-BK Laboratory Animal Co. Ltd. (Shanghai, China). The mice were between 8–10 weeks of age, with an average body weight of 18–20 g. Mice were housed under a 12/12-h light/dark cycle in an SPF facility with ad libitum access to food and purified water. Prior to immunization, the mice were allowed to acclimate for 7 days to adapt to the experimental conditions. The animal study was reviewed and approved by the Laboratory Animal Management and Ethics Committee of Zhejiang Chinese Medical University (IACUC-20221212-14).

Establishment of the EAE Model and HD Treatment

Female C57BL/6 mice were immunized subcutaneously with 300 µg of MOG_{35-55} peptide (ChinaPeptides, China) emulsified in complete Freund's adjuvant (F5881, Sigma-Aldrich, USA) containing Mycobacterium tuberculosis H37Ra (231141, BD, USA). Pertussis toxin (300 ng; List Biological, 181) was injected intraperitoneally on day 0 and 2 post-immunization. The modelling method for EAE was the same as that used previously.[39] Neurological evaluations were performed daily via the EAE clinical scoring scale:[39] 0, no clinical disease; 1, complete tail paralysis; 2, tail paralysis and hind limb weakness; 3, severe hind limb weakness; 4, complete bilateral hind limb paralysis; 5, complete hindlimb paralysis and forelimb weakness; 5.5, complete paralysis (tetraplegia); and 6, death.

The mice were randomly separated into five groups of eight using a random number table: control, EAE model, EAE + HD 12.5 g/kg, EAE + HD 25 g/kg, and EAE + HD 50 g/kg. The medium dose of HD was derived from the clinical equivalent for humans, converted based on standard interspecies dosage translation guidelines.[40,41] The mouse equivalent dose was determined by multiplying the human dose by a factor of 12.3, based on body surface area conversion.[41] Therefore, the median therapeutic dose was set at 25 g/kg, with 12.5 g/kg (half the median dose) and 50 g/kg (double the median dose) used to assess dose-response linearity. After the optimal dose of HD was determined on the basis of our preliminary experiments, the new batch of mice was assigned to 4 groups: control, EAE model, EAE + HD, and EAE + HD + agonist. NLRP3 agonist 1 (HY-156413, MCE, USA) was injected intraperitoneally at a dose of 1.5 mg/kg every two days from day 1. HD was orally administered once daily by gavage, while EAE model and control mice were administered the same amount of saline (500 µL per mouse).

https://doi.org/10.2147/JIR.S567313

Histopathology

On day 21 after immunization, the mice were anaesthetized and perfused with saline and 4% paraformaldehyde (PFA). Spinal cord samples were collected, fixed in 4% PFA, embedded with OCT frozen section embedding agent, and sliced on a frozen section machine. Spinal cord sections were subjected to staining with hematoxylin and eosin (HE) to evaluate the degree of inflammatory infiltration, and with luxol fast blue (LFB) to evaluate the extent of demyelination. Inflammatory infiltration was evaluated in a blinded manner by two observers according to the following criteria:[42] 0, no inflammatory cell infiltration; 1, meningeal cells in the filtration; 2, up to four small areas of perivascular cell infiltration; 3, five or more than five small areas of perivascular cell infiltration, and/or more than one large scale cell infiltration; and 4, a large number of cells in filtrations involving more than 20% of the white matter. Demyelination was evaluated blindly by two investigators as follows:[42] 0, no myelin loss; 1, a small area of myelin loss; 2, two or three small areas of myelin loss; 3, up to two large areas of myelin loss; and 4, a large area of myelin loss involving more than 20% of the white matter.

RNA Sequencing

On the basis of our preliminary study, the optimal HD dosage group, together with the model and control groups, was chosen for further RNA-seq analysis. Total RNA was extracted from spinal cord samples via a TRIzol-based Total RNA Extractor Kit (B511311, Sangon, China), followed by RNase-free DNase I treatment. RNA integrity was confirmed through 1.0% agarose gel electrophoresis. The RNA quality and quantity were further determined via spectrophotometer (NanoPhotometer, IMPLEN) and fluorometer (Qubit 2.0, Invitrogen). Qualified RNA samples were forwarded to Sangon Biotech (Shanghai) for subsequent library construction and sequencing.

Sequencing libraries were constructed via the VAHTSTM mRNA-seq V2 Library Prep Kit (Illumina, USA). Index codes were used to demultiplex sequencing reads to their corresponding samples. Specifically, mRNA was isolated from total RNA via poly-T oligo-attached magnetic beads. Library fragments were purified with the AMPure XP system (Beckman Coulter, USA). The quality of the libraries was evaluated via an Agilent Bioanalyzer 2100 system. The libraries were then subjected to paired-end sequencing on the NovaSeq platform.

The raw data were processed in the following steps. 1) The quality of the sequencing data was assessed by FastQC (version 0.11.2). Raw reads were filtered by Trimmomatic (version 0.36). 2) Clean reads were aligned to the reference genome using HISAT2 (version 2.0) with default parameters. 3) Gene expression values of the transcripts were computed by StringTie (version 1.3.3b). 4) DESeq2 (version 1.12.4) was used to identify differentially expressed genes (DEGs) between two samples. 5) The selected genes were subjected to further analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) to determine significantly enriched terms and pathways.

Flow Cytometry

Flow cytometry was used to measure the percentages of Th1 ($CD4^{+}IFN\text{-}\gamma^{+}$), Th17 ($CD4^{+}IL17A^{+}$), and Treg ($CD4^{+}CD25^{+}Foxp3^{+}$) cells in the spleens of the mice in each group. Mouse spleens were processed into single-cell suspensions according to the protocol in the instructions (7211011, DAKEWE, China). Cells were restimulated using a cell stimulation cocktail (423303, BioLegend, USA). CD4 and CD25 staining was performed with a CD4 antibody (11–0042-82, eBioscience, USA) and a CD25 antibody (101909, BioLegend, USA). Intracellular factors (IL17A and IFN-γ) were anchored to the Golgi using a transcription factor staining buffer kit (00–5523-00, eBioscience, USA). For intracellular and intranuclear staining, IL17A (506915, BioLegend, USA), IFN-γ (25–7311-41, eBioscience, USA) and Foxp3 (12–5773-80, eBioscience, USA) antibodies were used. Flow cytometry was conducted on NovoExpress with data analysis performed via FlowJo. The comprehensive figure illustrating the step-by-step gating hierarchy was shown in Figure S1.

Immunofluorescence

Frozen sections of the spinal cord were prepared as described in the histopathology section. Following several washes with phosphate-buffered saline (PBS), the sections were treated with 0.1% Triton X-100 for 15 minutes to enhance

permeability. The sections were subsequently blocked with 5% BSA for 1 hour to reduce nonspecific binding. The sections were then incubated with primary and secondary antibodies. Nuclear staining was performed with DAPI for 5 minutes, and the sections were mounted with anti-fluorescence quenching sealing tablets (0100–20, SouthernBiotech, USA). The detailed information of the antibodies used was as follows: anti-NLRP3 (AF06824, AiFang, China), anti-Olig2 (AB109186, Abcam, USA), anti-GFAP (SAF007, AiFang, China) and anti-Iba1 (AF301643, AiFang, China). A double-labelled trichrome multiple fluorescence staining kit (AFIHC023, AiFang, China) was used for secondary antibody incubation and fluorescent dye labelling. Images were captured with multichannel fluorescence scanner (KFBIO, China) and analyzed with ImageJ.

Quantitative PCR (qPCR)

Total RNA extraction reagent (9109, TAKARA, JPN) was used to isolate RNA from spinal cord tissue. The extracted RNA was reverse transcribed into cDNA using a reverse transcription kit (RR036A, TAKARA, JPN). Real-time PCR amplification was conducted using a qPCR master mix (RR420A, TAKARA, JPN) in accordance with the instructions. The relative expression level of the target gene was determined by the comparative $2^{-\Delta\Delta Ct}$ method using β-actin for normalization, with primers listed in Table S1.

Western Blotting

Total protein was isolated from spinal cord tissue with RIPA lysis buffer (P0013B, Beyotime) supplemented with a protease/phosphatase inhibitor cocktail (78441, Thermo). A BCA assay kit (P0010, Beyotime, China) was used to quantify protein content. Following separation on 10% SDS-PAGE gels, proteins were electrophoretically transferred to PVDF membranes (IPVH0010, Millipore, US). Membranes were blocked for 30 min at room temperature using a rapid blocking buffer (PS108P, Epizyme, China) and then incubated overnight at 4°C with anti-NLRP3 (ab270449, Abcam, USA), NF-κB (8242S, CST, USA), IL-1β (31202, CST, USA), IL-18 (ab207323, Abcam, USA) and caspase-1 (24232, CST, USA) antibodies. β-actin (66009-1-Ig, Proteintech, China) was utilized as internal control. Membranes were incubated with HRP-conjugated -goat anti-rabbit IgG (H+L) (111–035-003, Jackson, USA) or HRP-conjugated -goat anti-mouse IgG (H+L) (115–035-003, Jackson, USA) for 1 h at room temperature. Chemiluminescent substrate (FD8020, FDbio, China) was used for visualization, and ImageJ for quantitative analysis of the blots.

Statistical Analysis

Statistical analysis was conducted with SPSS 25.0 software. The data are expressed as the means ± standard deviations. Comparisons between groups were performed using the *t*-test for normally distributed data or the Mann–Whitney test for non-normal data. Comparisons of multiple groups were assessed by one-way ANOVA with LSD test or Kruskal–Wallis test for nonparametric tests. A P value <0.05 was considered statistically significant. GraphPad Prism 9.5 software was used to graph the data.

Results

HD Alleviated EAE Symptoms in Mice

Neurological scores were used to determine the efficacy of HD on clinical symptoms in EAE mice (Figure 1A). Neurological signs emerged in EAE mice on day 13 post-immunization, with clinical scores reaching a peak on day 17. Compared to the EAE group, HD treatment (12.5, 25, or 50 g/kg) significantly ameliorated the disease severity in EAE mice. The EAE mice in the HD (25 g/kg) group had the latest onset of disease and the lowest peak clinical scores. Inflammatory infiltration and demyelination of the CNS were assessed by HE and LFB staining, respectively. In the spinal cord, the EAE group presented severe inflammatory infiltration and demyelination (Figure 1B and C). The degree of inflammatory infiltration and demyelination in the spinal cords of HD treated EAE mice improved ($p < 0.05$, $p < 0.01$). These results indicated HD alleviated inflammation and demyelination in EAE mice.

https://doi.org/10.2147/JIR.S567313

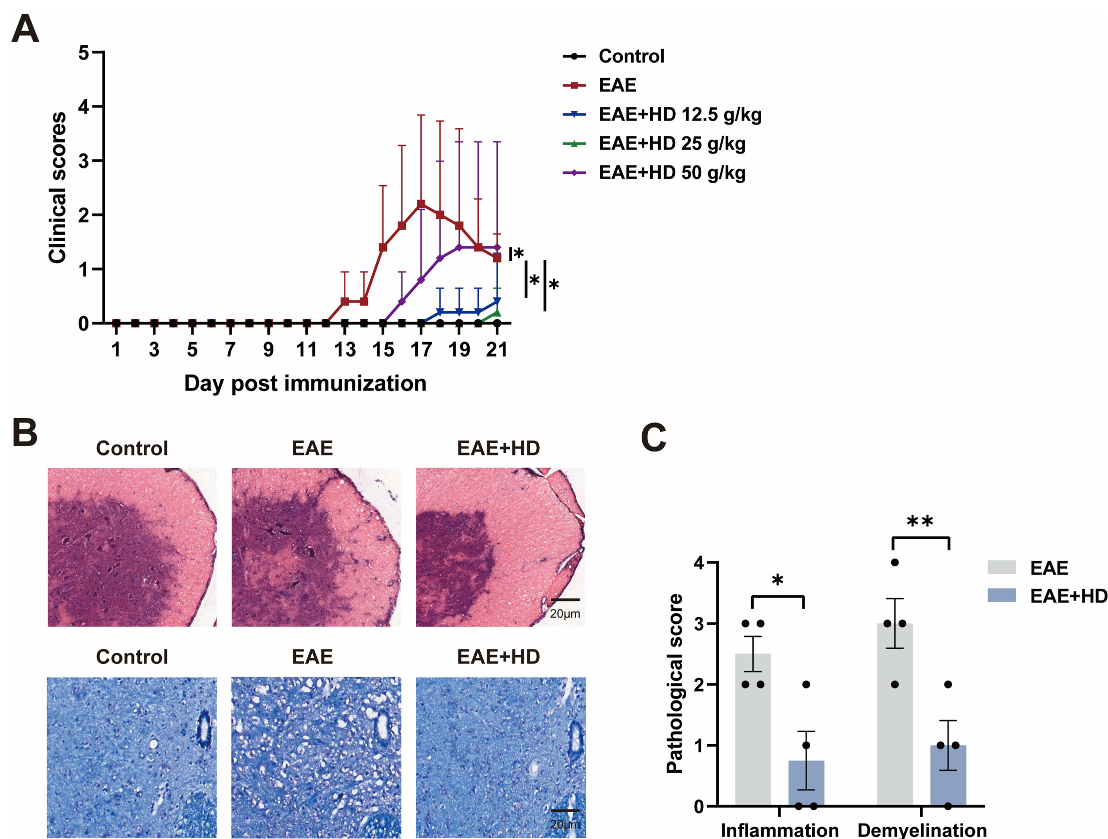

Figure 1 HD alleviated EAE symptoms in mice. (**A**) Line plots of neurological scores (n = 8). (**B**) Hematoxylin eosin (HE) and luxol fast blue (LFB) staining of spinal cords on day 21 after immunization. (**C**) Assessment of inflammatory infiltration and demyelination (n = 4). *p < 0.05 and **p < 0.01 compared to the EAE group.

Identification of the Chemical Constituents of HD

The UPLC-MS analysis identified 1154 compounds in the mid-dose HD. Compounds exhibiting higher scores and fragmentation scores were considered more stable. Applying thresholds of a score ≥58.0 and fragmentation score ≥94.0,[43] 13 compounds meeting these criteria were identified (Figure S2A). Chromatographic consistency across multiple extraction batches of HD confirmed its stability in both positive and negative ion modes (Figure S2B and C). The top 10 chemical components of HD such as baohuoside VI, afzelin, polydatin and icariin were summarized in Figure 2A. For all identified components, pie charts were constructed based on their quantity under each chemical classification (Figure 2B). HD mainly contained flavonoids, phenylpropanoids, terpenes, glycosides, alkaloids and phenols. The UV absorption at 254 nm was shown in Figure 2C. Components containing structures such as benzene rings and double bonds, had strong absorption at this wavelength. To explore the potential bioactive basis of HD's therapeutic effects, we cross-referenced the identified compounds with published literature on their pharmacological activities (Table S2).

RNA Sequencing Identified Genes Associated with the Effect of HD for EAE

To further explore the underlying mechanisms of HD treatment in EAE mice, RNA sequencing was performed to identify the differentially expressed transcripts (DETs) in spinal cord tissue across groups. GO enrichment analysis of DETs enables the identification of distinct biological characteristics across three categories: biological processes, cellular composition, and molecular function. The top 30 enriched GO terms were visualized in a bubble diagram. Biological process terms were enriched in immune system process, inflammatory response and leukocyte activation (Figure 3A and B). Cellular component analysis indicated that DETs were involved mainly in MHC protein complex, T cell receptor complex and inflammasome complex (Figure S3A and B). Molecular function was enriched in chemokine activity, cytokine binding and T cell receptor binding (Figure S3C and D).

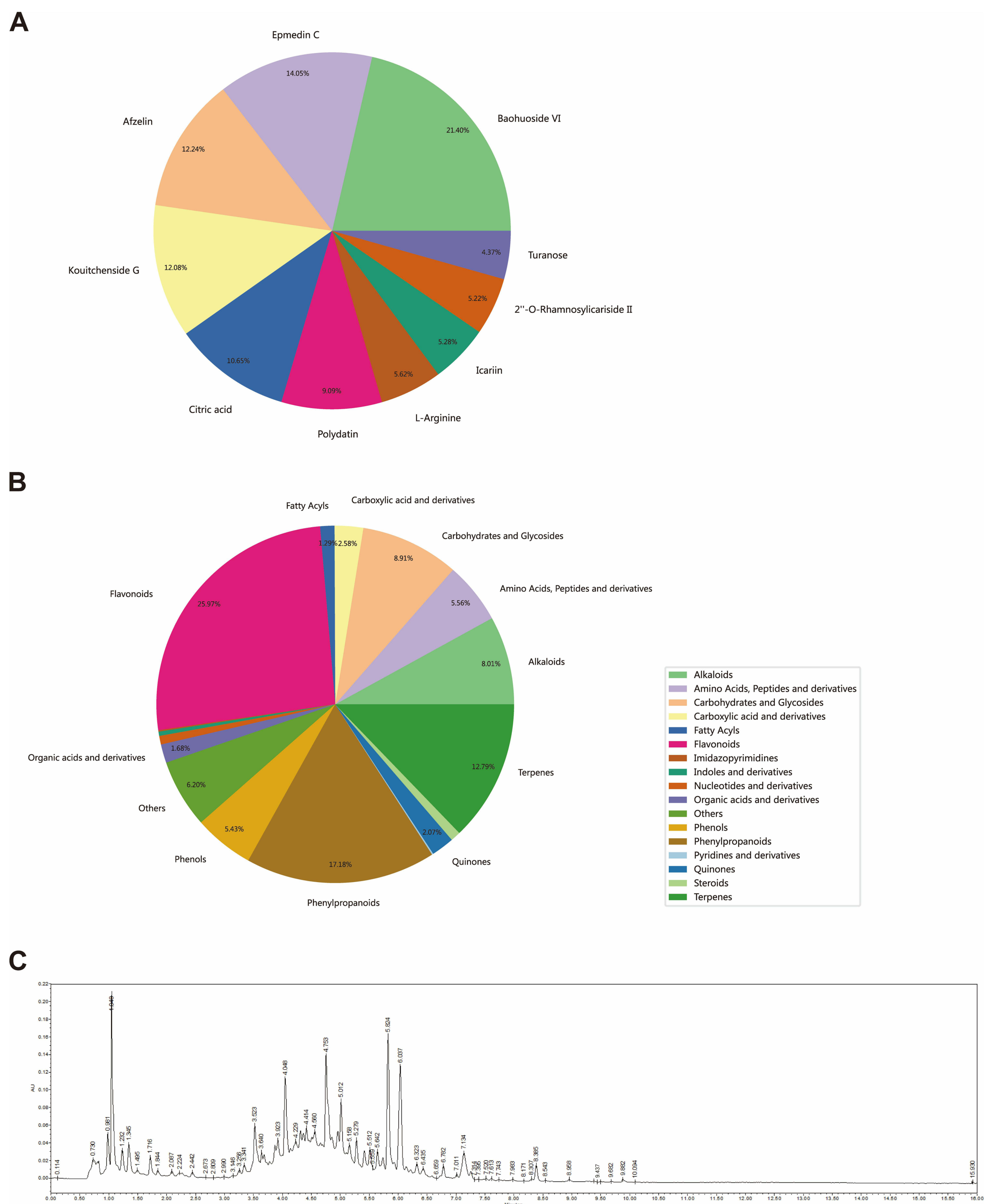

Figure 2 Identification of the chemical constituents of HD. (**A**) The top 10 chemical components of HD. (**B**) Quantitative distribution of all components in each chemical classification. (**C**) The UV absorption at 254 nm.

https://doi.org/10.2147/JIR.S567313

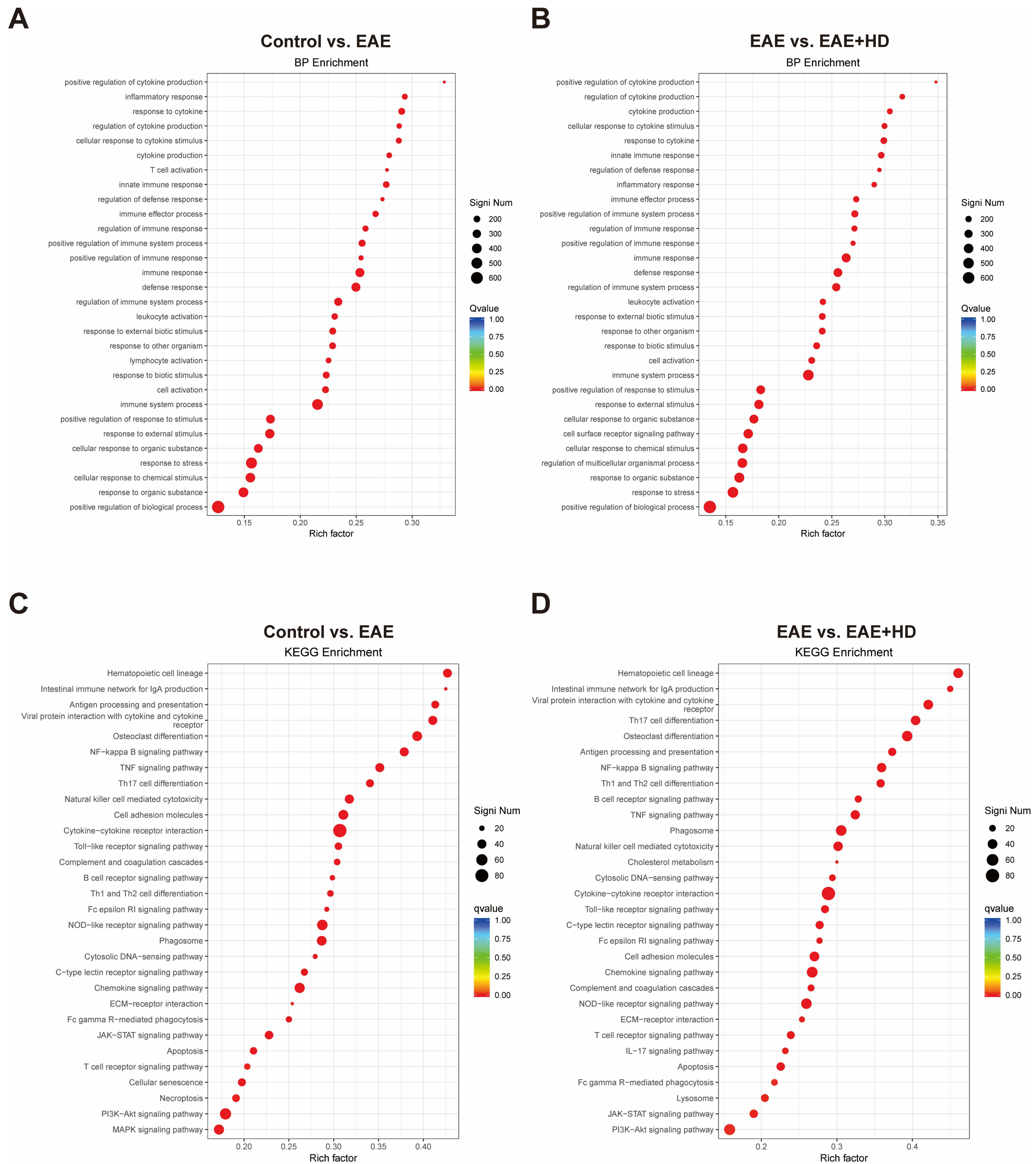

Figure 3 RNA sequencing identified genes associated with the effect of HD for EAE. (**A** and **B**) GO enrichment analysis in biological processes (BP) of Control vs. EAE and EAE vs. EAE+HD. (**C** and **D**) KEGG pathway enrichment analysis of Control vs. EAE and EAE vs. EAE+HD.

KEGG pathway analysis revealed multiple enriched signaling pathways, and the top 30 pathways were visualized with a bubble diagram (Figure 3C and D). The enriched signaling pathways in Control vs. EAE and EAE vs. HD were both involved in NF-kappa B signaling pathway, Th17 cell differentiation, NOD-like receptor signaling pathway and T cell receptor signaling pathway. In summary, the DETs and enriched pathways suggested that HD exerted its therapeutic effects on MS by modulating

adaptive immunity, and highlighted the underlying mechanisms, such as the NF-κB and NLRP3 signaling pathways. In combination with recent studies,[44–46] targeting the NLRP3 inflammasome could be a potential therapeutic strategy for MS.

HD Ameliorated Symptoms in EAE Mice Through the NF-κB/NLRP3 Signaling Pathway

A new batch of mice were assigned to 4 groups: control, EAE model, EAE + HD (25 g/kg), and EAE + HD + agonist and their neurological function were scored (Figure 4A). Compared to the EAE group, HD treatment significantly reduced the peak and cumulative scores of EAE mice (Figure 4B and C, $p < 0.01$, $p < 0.001$). The agonist group had higher peak and cumulative scores than the HD group ($p < 0.01$). Inflammatory infiltration and demyelination were less severe in the spinal cord of mice in the HD group compared to the agonist group (Figure 4D and E, $p < 0.01$). These results indicated that the agonist partially diminished the effects of HD in ameliorating symptoms and reducing inflammation and demyelination in EAE mice.

On day 21 after immunization, the protein levels of NF-κB, NLRP3, IL-18, IL-1β and caspase-1 were significantly increased in the EAE mice ($p < 0.05$, $p < 0.01$ and $p < 0.05$, Figure 5A and B). After HD treatment, the expression of NF-κB, NLRP3, caspase-1, IL-18 and IL-1β was remarkably decreased in EAE mice ($p < 0.05$, $p < 0.01$ and $p < 0.05$). To determine whether HD exerts its effects through the NF-κB/NLRP3 signaling pathway, an NLRP3 agonist was administered. The results showed a significant increase in NF-κB, NLRP3, caspase-1, IL-18 and IL-1β levels in the HD + agonist group compared to the HD group ($p < 0.01$, $p < 0.001$, $p < 0.05$ and $p < 0.01$). These findings suggested that the NLRP3 agonist partially inhibited the regulatory effect of HD on the NF-κB/NLRP3 pathway. In terms of mRNA expression, NF-κB and NLRP3 levels were greater in EAE mice than in control mice ($p < 0.05$, $p < 0.001$, Figure 5C and D), whereas their expression was remarkably reduced by HD. The NLRP3 agonist significantly reduced the inhibitory effect of HD on the mRNA expression of NF-κB and NLRP3 ($p < 0.01$). These results suggest that HD may alleviate EAE through the NF-κB/NLRP3 signaling pathway. Moreover, the protective effects of HD were diminished by an NLRP3 agonist, further supporting the involvement of the NF-κB/NLRP3 pathway.

HD Regulated the Proportions of Th17, Th1 and Treg Cells

The ratio of Th17 and Th1 cells in the spleens of EAE mice was significantly elevated (Figure 6A–F, $p < 0.01$, $p < 0.001$), whereas the proportion of Treg cells did not change significantly. HD treatment markedly reduced the proportions of Th17 and Th1 cells ($p < 0.05$, $p < 0.01$). Conversely, there was a noticeable increase in the proportion of Treg cells ($p < 0.01$), while agonist administration weakened this effect. Gene expression of $CD4^+$ T cell subtype specific cytokines in EAE mice was further examined. As shown in Figure 6G, the expression of the Th17 cell specific cytokine IL-17A was greater in the EAE mice than in the control mice ($p < 0.01$). HD treatment significantly decreased the expression of IL-17A ($p < 0.01$), whereas the expression of Treg cell specific cytokines IL-10 and TGF-β were increased (Figure 6H and I, $p < 0.01$). These changes were more pronounced in the HD group than in the HD + agonist group. These results suggested that HD could reduce the proportion of Th17/Treg cells and regulate the adaptive immune response in EAE mice.

HD Suppressed Excessive Activation of Microglia and Astrocytes

We performed double labeling for NLRP3, IBA1 (microglia marker), and GFAP (astrocyte marker) to detect microglia and astrocyte activation (Figures 7A and 8A). We detected a significant increase in the number of IBA1, GFAP, NLRP3/IBA1 and NLRP3/GFAP double-positive cells in the EAE mice ($p < 0.001$, Figures 7B, C and 8B, C). HD treatment reduced the number of IBA1 cells, GFAP cells and corresponding cells double-labelled with NLRP3 ($p < 0.001$). Reactive microglia and astrocytes also produce inflammatory cytokines such as TNF-α and IL-1β, leading to further immune activation.[47,48] Consistent with the fluorescence results, the mRNA expression of TNF-α and IL-1β in EAE mice was reduced by HD treatment ($p < 0.05$, $p < 0.001$, Figure 8D and E), while the presence of an agonist partially attenuated this effect. These results suggested that HD treatment inhibited excessive activation of microglia and astrocytes in EAE mice.

HD Increased Oligodendrocyte Number

Oligodendrocytes are myelin-forming cells in the CNS.[49] We performed double labelling for NLRP3 and Olig2 (oligodendrocyte marker) to detect oligodendrocytes (Figure 9A). HD treatment significantly increased the number of Olig2-labelled cells compared to the EAE group ($p < 0.001$, Figure 9B). Conversely, the number of NLRP3-labelled cells

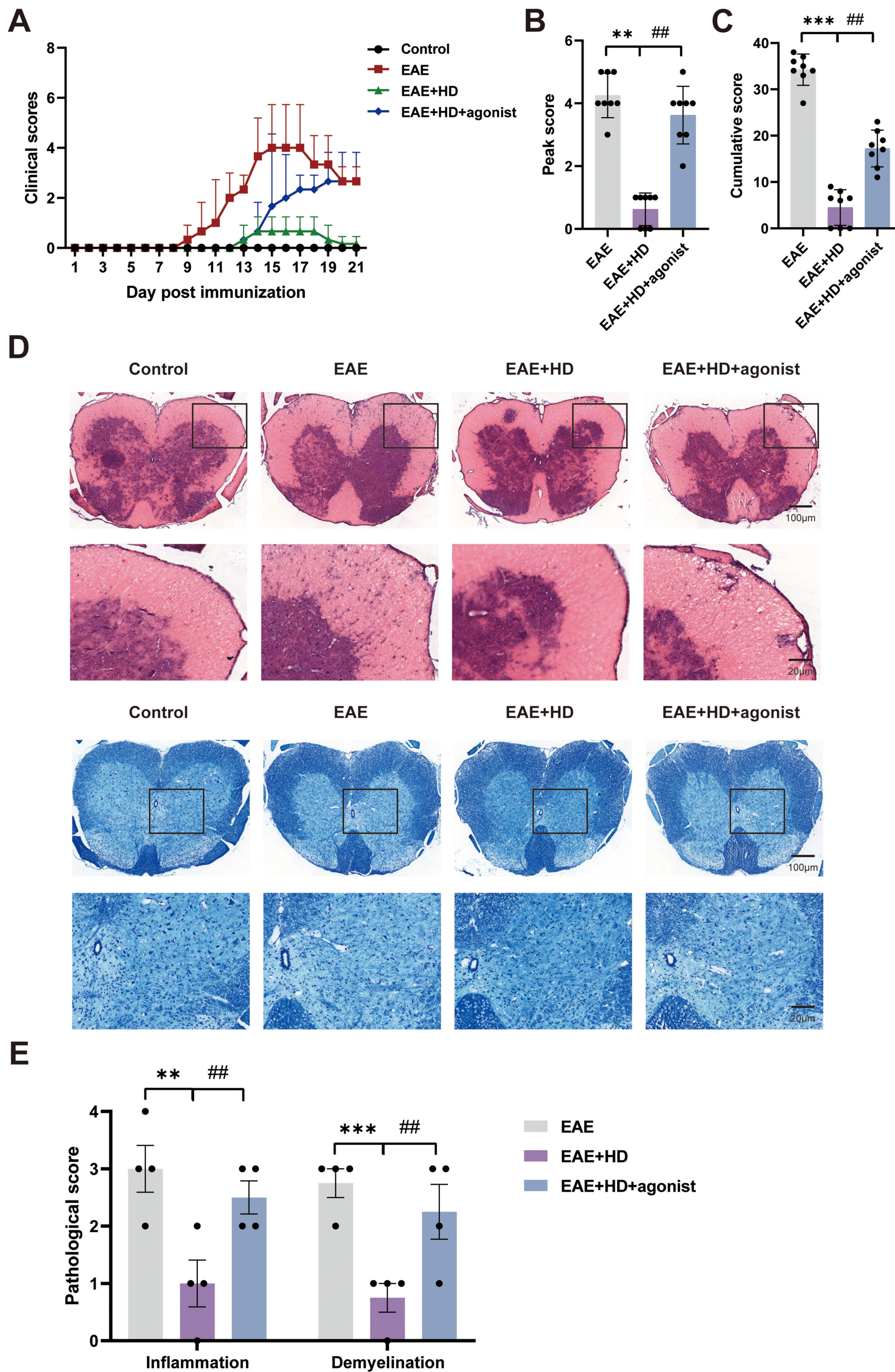

Figure 4 HD ameliorated symptoms in EAE mice through the NF-κB/NLRP3 signaling pathway. (**A**) Line plots of neurological function scores. (**B** and **C**) Peak and cumulative scores (n = 8). (**D**) Hematoxylin eosin (HE) and luxol fast blue (LFB) staining of spinal cords on day 21 after immunization. (**E**) Assessment of inflammatory infiltration and demyelination (n = 4). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the EAE group; $^{\#\#}p < 0.01$ compared to the EAE + HD group.

https://doi.org/10.2147/JIR.S567313

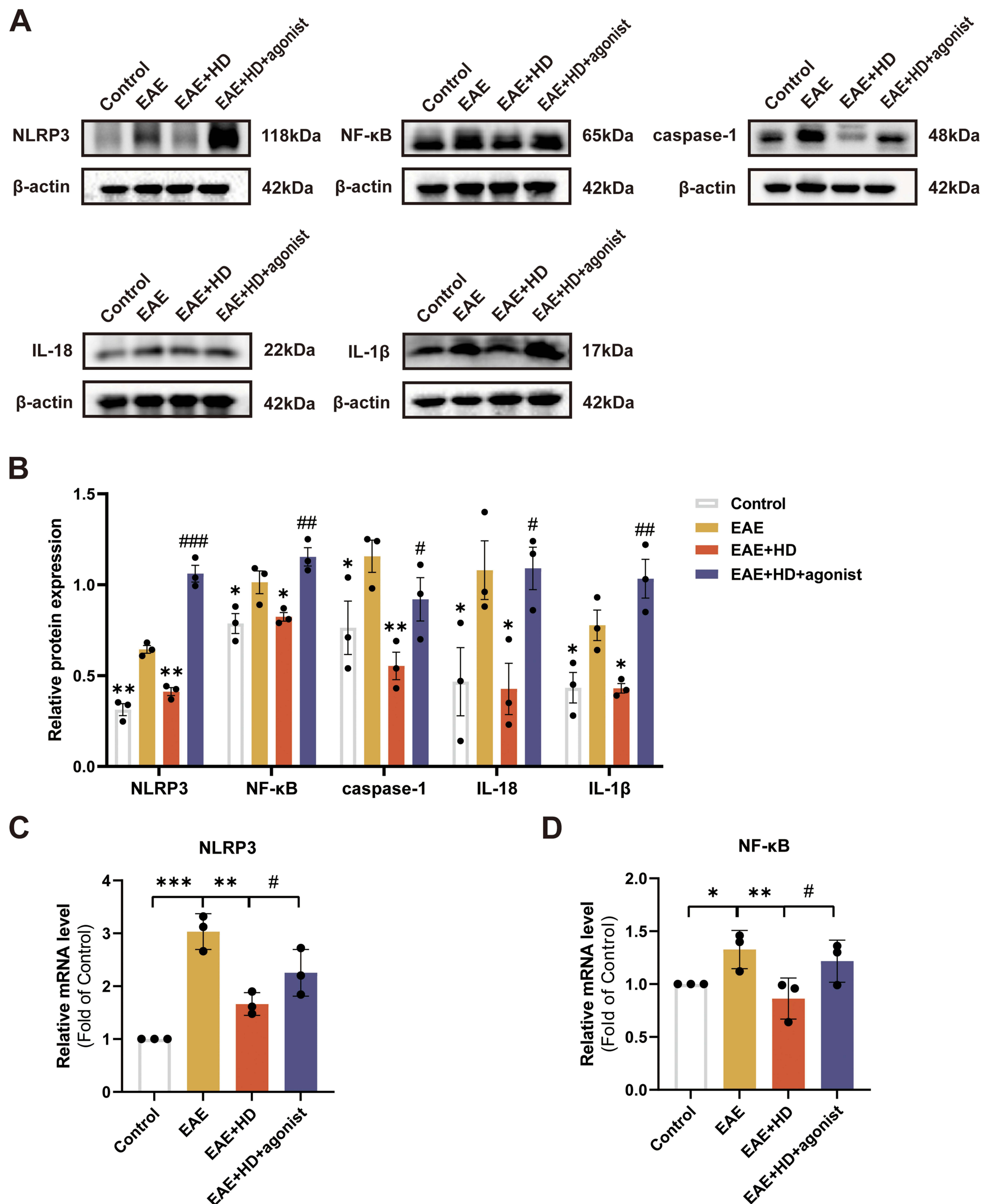

Figure 5 HD inhibited the upregulation of NF-κB/NLRP3 signaling pathway. (**A** and **B**) Level of each protein and statistical analysis of NF-κB, NLRP3, caspase-1, IL-18 and IL-1β in the spinal cord on day 21 after immunization (n = 3). (**C** and **D**) The mRNA level of NLRP3 and NF-κB in the spinal cord (n = 3). *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ compared to the EAE group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared to the EAE + HD group.

 https://doi.org/10.2147/JIR.S567313

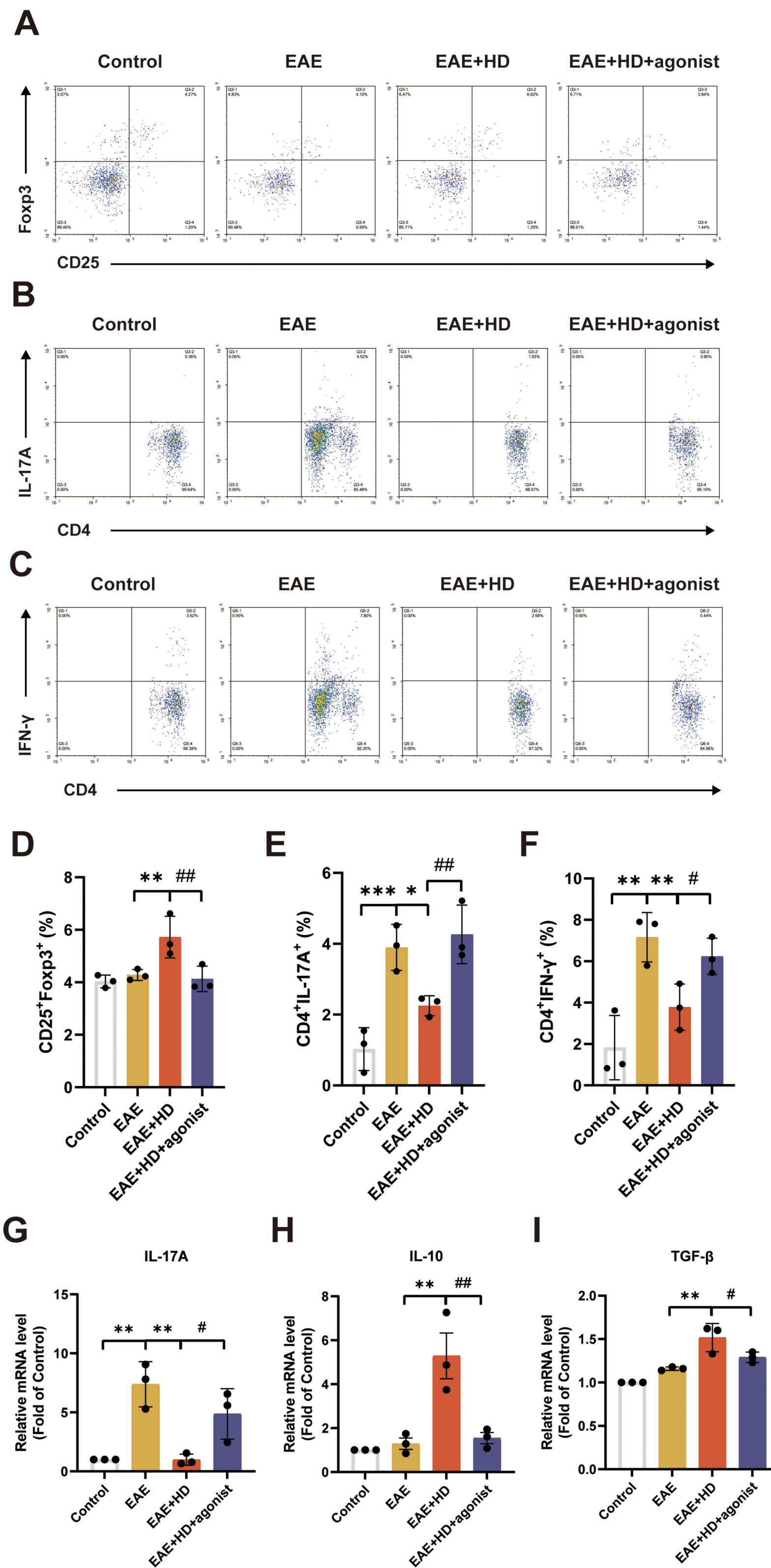

Figure 6 HD regulated the proportions of Th17, Th1 and Treg cells. (**A-C**) Flow cytometry analysis of Th1 ($CD4^{+}IFN\text{-}\gamma^{+}$), Th17 ($CD4^{+}IL17A^{+}$) and Treg ($CD4^{+}CD25^{+}Foxp3^{+}$) cells in the spleen on day 21 after immunization. (**D-F**) Quantification of Th1, Th17 and Treg cell proportions (n = 3). (**G-I**) The mRNA expression of IL-17A, IL-10 and TGF-β (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the EAE group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the EAE + HD group.

 https://doi.org/10.2147/JIR.S567313

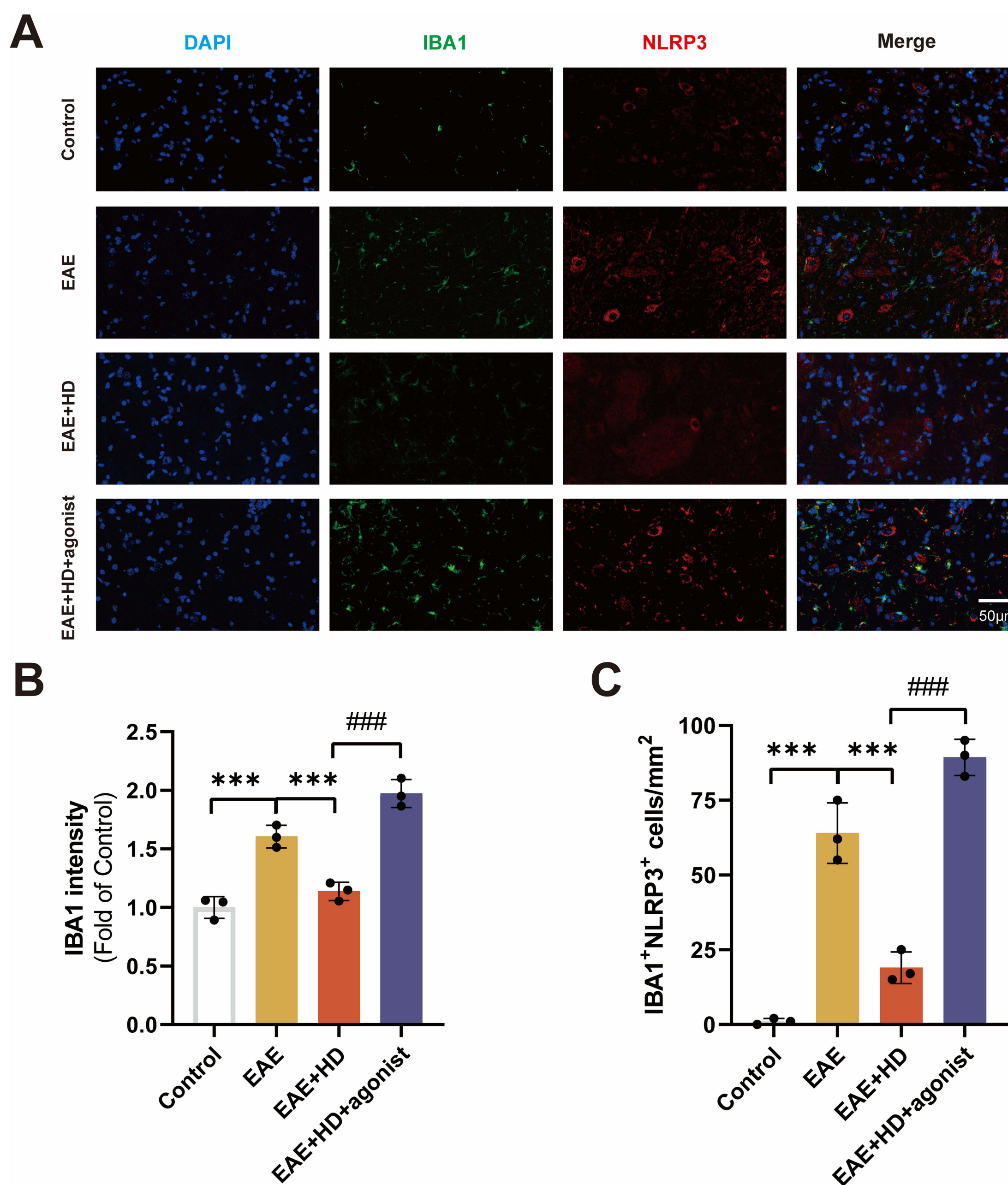

Figure 7 HD suppressed excessive activation of microglia. (**A**) Representative immunofluorescent staining images of IBA1 (green), NLRP3 (red), and DAPI (blue) in the spinal cord on day 21 after immunization. (**B**) Quantification of IBA1 expression level. (**C**) Quantification of the number of $IBA1^+$ $NLRP3^+$ cells. ***p < 0.001 compared to the EAE group; ###p < 0.001 compared to the EAE + HD group.

in the HD group was much lower than that in the EAE group ($p < 0.01$, Figure 9C). These changes were more pronounced in the HD group than in the HD + agonist group. The results showed that HD treatment increased the number of oligodendrocytes, which in combination with LFB staining showed that HD attenuated demyelination, which could indicate a neuroprotective effect of HD.

Discussion

MS is considered to be one of the most common causes of non-traumatic neurological disability in young adults.[1] Recent evidence proved that both the innate and adaptive immune systems played crucial roles in the onset and progression of MS.[4–6,19,20] Additionally, a substantial body of research had established that the NLRP3 inflammasome, as a key

https://doi.org/10.2147/JIR.S567313

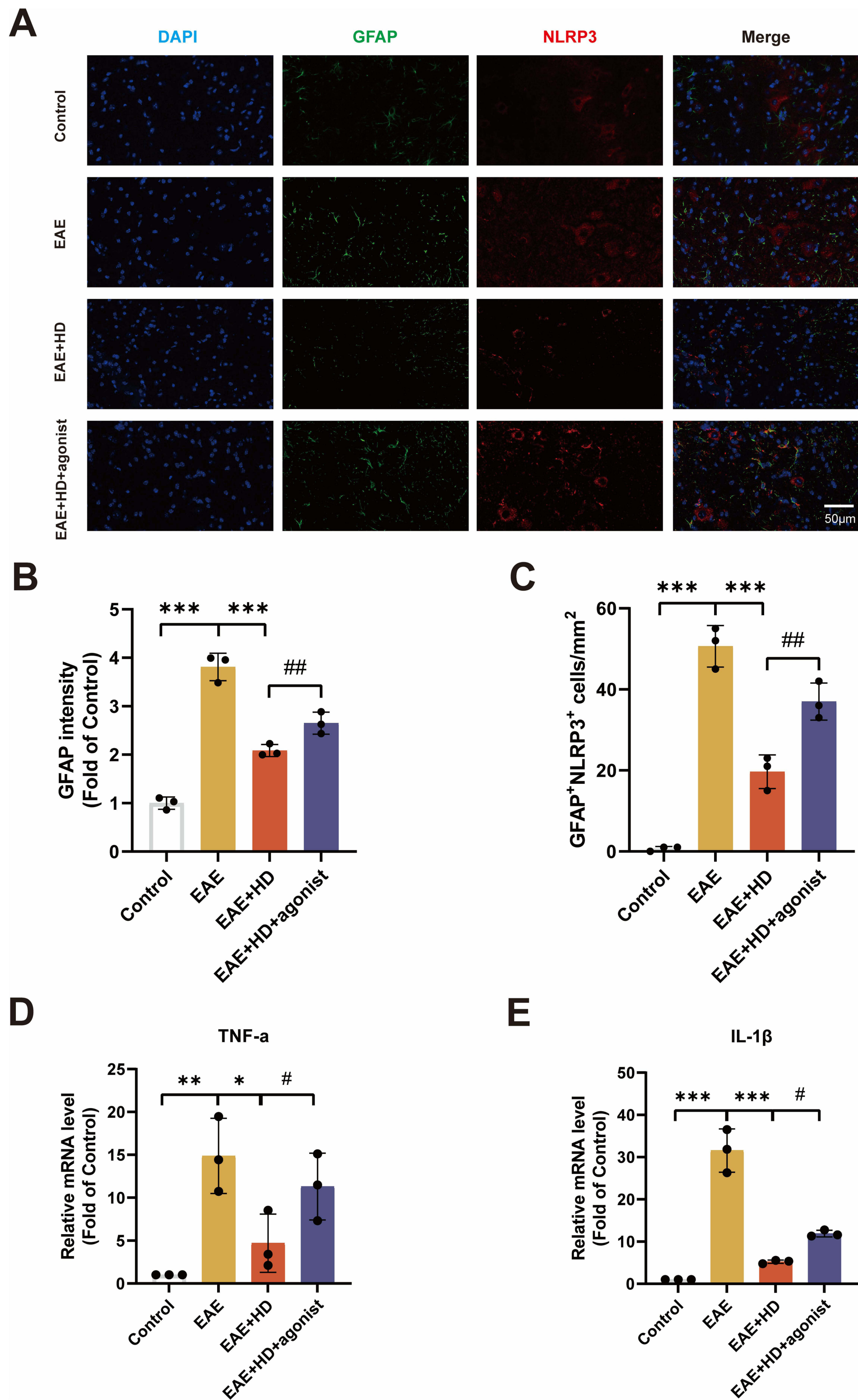

Figure 8 HD suppressed excessive activation of astrocytes. (**A**) Representative immunofluorescent staining images of GFAP (green), NLRP3 (red), and DAPI (blue). (**B**) Quantification of GFAP expression level. (**C**) Quantification of the number of GFAP$^+$ NLRP3$^+$ cells (n = 3). (**D** and **E**) The mRNA expression of TNF-α and IL-1β in the spinal cord (n = 3). $^*p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the EAE group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to the EAE + HD group.

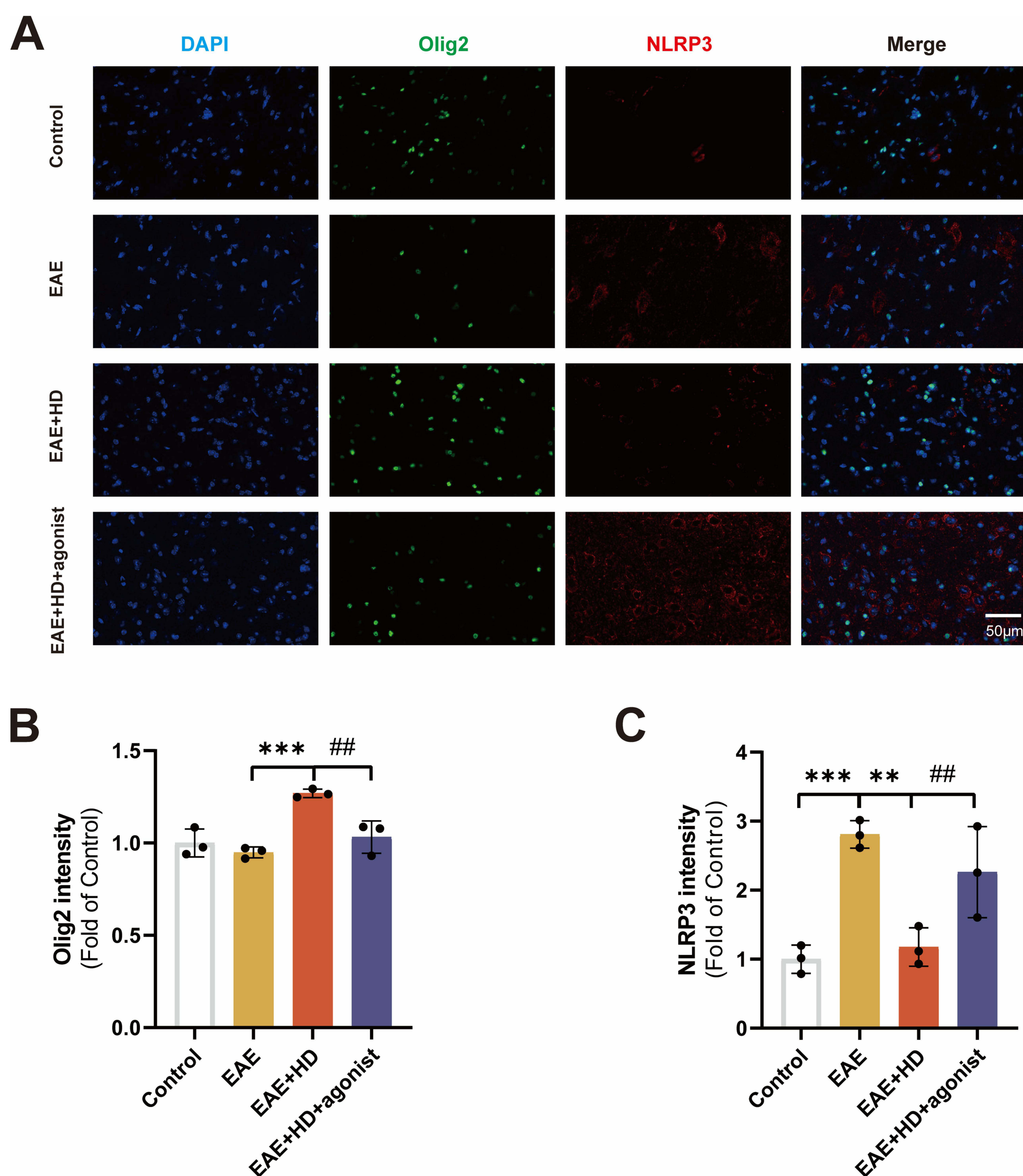

Figure 9 HD increased oligodendrocyte number. (**A**) Representative immunofluorescent staining images of Olig2 (green), NLRP3 (red), and DAPI (blue) in the spinal cord on day 21 after immunization. (**B**) Quantification of Olig2 expression level. (**C**) Quantification of NLRP3 expression level (n = 3). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to the EAE group; $^{\#\#}p < 0.01$ compared to the EAE + HD group.

component of the innate and adaptive immune response, engaged in various pathways in the pathogenesis of MS.[7–9,44–46] In this study, we investigated the therapeutic effects of HD in MS with EAE model. Our findings demonstrated that HD significantly alleviated neurobehavioral impairments and reduced inflammatory cell infiltration and demyelination in EAE mice. Our further experiments revealed that HD exerted its effects through the NF-κB/NLRP3 inflammasome pathway, modulating the balance of Th1/Th17/Treg cells in the periphery, while reducing the levels of microglia and astrocytes in the CNS. These findings established a pharmacological foundation for the clinical use of HD in the treatment of MS, emphasizing its potential to modulate immune responses and enhance neuroprotection.

The maintenance of immune homeostasis is of vital importance in the prevention of autoimmunity, suppression of excessive effector immune responses and host tissue injury.[50] Th17 cells mediate proinflammatory responses to invading pathogens by secreting IL-17. Treg cells suppress self and microbiota antigens and prevent Th17 and Th1 cell

differentiation by releasing mainly IL-10 and TGF-β.[51] Treg cells also promote oligodendrocyte differentiation and remyelination.[52] The transdifferentiation of Th17 cells into Treg cells may facilitate the resolution of inflammation, offering a potential therapeutic approach for MS.[53] B cells secrete autoantibodies involved in antibody-dependent cytotoxicity damage. Antibody oligoclonal bands are detected in more than 90% of MS patients.[54] B cells in the CNS could act in MS by secreting chemokines/cytokines and by presenting antigen to T cells.[55] Peripheral B cells of MS patients exhibit aberrant pro-inflammatory cytokine responses, including increased TNF-α[56] and decreased IL-10 secretion.[57] Furthermore, Th1 and Th17 cells secrete pro-inflammatory cytokines that activate macrophages, dendritic cells, and recruit more B cells, thereby promoting CNS pathology.[58] Our results confirmed that HD maintained immune homeostasis by balancing the proportion of Th17/Treg cells, thereby further ameliorating the progression of EAE.

Microglia secrete inflammatory mediators which can sustain chronic inflammation in the CNS[59] and further damage myelin and disrupt synaptic activity.[60] Inflammatory microglia, by releasing cytokines such as IL-1β and TNF-α, can induce astrocytes to transform into highly reactive and neurotoxic cells.[61,62] These reactive astrocytes lose the ability to promote neuronal synaptogenesis and phagocytosis and induce the death of oligodendrocytes.[61] Oligodendrocyte depletion is an important determinant of remyelination failure,[63] which has been suggested as a pathological substrate for disease progression in MS.[64] Studies have confirmed the pro-regenerative myelin effect of an anti-inflammatory environment.[14,65] Treatment of progressive MS should be based on a combination of anti-inflammatory, myelin regenerative, and neuroprotective strategies.[66,67] Our results demonstrated that HD suppressed excessive activation of microglia and astrocytes and increased oligodendrocyte numbers, thereby attenuating inflammatory infiltration and demyelination. Whether HD promotes myelin regeneration can be further explored in the future with additional experiments, such as electron microscopy of myelin thickness or lineage tracing of oligodendrocytes. Th17 cells can interact with microglia to exacerbate inflammation and neurotoxicity,[12] such as secretion of IL-17A to activate microglia. Treg cells promote oligodendrocyte differentiation and remyelination.[52] Therefore, HD not only maintained T cells immune homeostasis but also reduced the interaction between T cells and microglia, and promoted the increase of oligodendrocytes, further reducing inflammation and neuronal damage.

The NLRP3 inflammasome plays a crucial role in the expansion of Th1/Th17 cells and its inhibition results in an increased frequency of Treg cells.[68] A study had shown that T cells need to be primed in NLRP3 inflammasome-sufficient mice to migrate into the CNS and induce EAE, which the involvement of IL-1β and IL-18 was demonstrated.[69] The NF-κB/NLRP3 axis in activated microglia is essential in neuroinflammation.[70,71] Our results confirmed that HD significantly decreased the expression of NF-κB, NLRP3, caspase-1, IL-18 and IL-1β in EAE mice. We further showed that an NLRP3 agonist significantly weakened the effects of HD on regulating the Th17/Treg ratio, suppressing excessive activation of microglia and astrocytes, and attenuating demyelination. These findings indicated that HD alleviated EAE through the NF-κB/NLRP3 pathway.

Based on the recent research,[25] plenty of natural compounds exhibit therapeutic and preventive effects against MS. Small molecule compounds, particularly polyphenols, flavones and alkaloids, generally have great therapeutic potential for MS without causing serious adverse effects. The chemical components of HD analyzed by UPLC-MS included flavonoids, phenylpropanoids, terpenes, alkaloids and phenols, with flavonoids accounting for the highest percentage. The top 10 chemical components of HD included baohuoside VI, afzelin, polydatin and icariin. Icariin and afzelin also belonged to the flavonoids. Previous studies have shown that icariin, the main active compound of *Epimedium*, can effectively penetrate the blood-brain barrier and has potent anti-neuroinflammatory and antioxidant activity against multiple diseases.[72–74] Icariin also enhanced remyelination and axons rewrapped in CPZ-induced mice.[75] Molecular docking revealed that Icariin could stably bind to the pocket of NLRP3 with relatively high docking score of 9.3 kcal/mol.[76] Icariin decreased microglial activation by inhibiting the NLRP3 inflammasome and the secretion of IL-1β and TNF-α. Afzelin, a flavonoid-rich compound in *Reynoutria multiflora* (Heshouwu) extract, can mitigate oxidative stress, modulate inflammation, and promote cellular regeneration in neurodegenerative and cancer diseases.[77,78] Polydatin, also known as piceid, is the most abundant form of resveratrol in nature[79] and belongs to the polyphenols. Polydatin has been shown to be neuroprotective,[80,81] downregulate the expression of NF-κB, and ameliorate blood-brain barrier permeability.[80,82] Evidence suggests that polydatin has potent antioxidant activity,[83] ameliorates mitochondrial dysfunction, promotes remyelination and improves motor coordination in a cuprizone model of MS.[84] Polydatin can also attenuate neuronal damage of the optic nerves and spinal cord,[85] and prevent neuronal loss

in a chronic relapsing EAE model.[86] Herbs are frequently combined to enhance efficacy and minimize toxicity. The HD compound decoction is relatively concise, with each herb contributing its unique function within the prescription. Our research demonstrated that HD ameliorated clinical symptoms and reduced inflammatory infiltration and demyelination in EAE mice. Further molecular experiments revealed that HD exerted effects through the NF-κB/NLRP3 inflammasome pathway, regulating the balance of Th1/Th17/Treg, reducing microglia and astrocytes and increasing oligodendrocytes.

This study had several limitations. First, this study focused primarily on the immune responses of T cells and glial cells, yet the mechanisms underlying neurodegeneration in MS are multifaceted, involving mitochondrial dysfunction, iron toxicity and oxidative stress.[15] Future research should explore HD's role in these interconnected pathways to offer a more comprehensive understanding of its therapeutic potential. Second, our study pays more attention to the responses of T cells and glial cells. Crosstalk among T cells, glial cells and neurons can be studied in depth. Third, MS is a highly heterogeneous disease characterized by various subtypes and diverse clinical manifestations. The unpredictability of MS progression poses significant challenges in determining the optimal timing for natural compound intervention.[25]

Conclusion

HD presents a promising therapeutic strategy for MS by improving clinical symptoms, regulating immune responses and reducing neuroinflammation and demyelination. The mechanisms of action of HD, especially its modulation of the NF-κB/NLRP3 pathway, establish a robust foundation for its clinical application.

Abbreviations

BP, biological processes; BPC, base peak chromatogram; CC, cellular component; CNS, central nervous system; DETs, differentially expressed transcripts; DMTs, disease-modifying therapies; EAE, experimental autoimmune encephalomyelitis; GO, Gene Ontology; HD, Huazhuo decoction; HE, hematoxylin eosin; KEGG, Kyoto Encyclopedia of Genes and Genomes; LFB, luxol fast blue; MF, molecular function; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PBS, phosphate-buffered saline; PFA, paraformaldehyde; Treg, regulatory T.

Data Sharing Statement

All data involved in this research can be accessed by submitting a valid request to the corresponding author.

Ethics Approval and Consent to Participate

The experiments were approved by Laboratory animal management and ethics committee of Zhejiang Chinese Medical University (IACUC-20221212-14) and adhered to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health of the United States.

Acknowledgments

Special thanks to the First Central Laboratory of Zhejiang Chinese Medical University for providing the experimental equipment.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. Jing-Ying Wu: Investigation, Methodology, Data curation, Writing – original draft, Writing – review and editing; Juan Zhang: Methodology, Conceptualization, Writing – original draft, Writing – review and editing; Shan Xu: Data curation, Methodology, Writing – original draft, Writing – review and editing; Shi-Yan Qian: Methodology, Investigation, Writing – review and editing; Zheng Zha: Methodology, Writing – review and editing; Jia-Ling Hu: Investigation, Writing – review and editing; Jiang-Li Yang: Data curation, Writing – review and editing; Feng-Xia Zhang: Methodology, Writing – original draft, Writing – review and editing; Guo-Qing Zheng: Conceptualization, Methodology, Writing – original draft, Writing – review and editing, Funding acquisition, Supervision.

Funding

This study was funded by the preponderant discipline (2023) of the First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Chinese Medicine).

Disclosure

The authors declare that they have no known competing interests.

References

1. Jakimovski D, Bittner S, Zivadinov R, et al. Multiple sclerosis. *Lancet*. 2024;403(10422):183–202. doi:10.1016/s0140-6736(23)01473-3
2. Walton C, King R, Rechtman L, et al. Rising prevalence of multiple sclerosis worldwide: insights from the Atlas of MS, third edition. *Mult Scler*. 2020;26(14):1816–1821. doi:10.1177/1352458520970841
3. Marcus R. What Is Multiple Sclerosis? *JAMA*. 2022;328(20):2078. doi:10.1001/jama.2022.14236
4. Hemmer B, Kerschensteiner M, Korn T. Role of the innate and adaptive immune responses in the course of multiple sclerosis. *Lancet Neurol*. 2015;14(4):406–419. doi:10.1016/s1474-4422(14)70305-9
5. Van Kaer L, Postoak JL, Wang C, Yang G, Wu L. Innate, innate-like and adaptive lymphocytes in the pathogenesis of MS and EAE. *Cell Mol Immunol*. 2019;16(6):531–539. doi:10.1038/s41423-019-0221-5
6. Lassmann H. Multiple Sclerosis Pathology. *Cold Spring Harb Perspect Med*. 2018;8(3):a028936. doi:10.1101/cshperspect.a028936
7. Zhang H, Gao J, Tang Y, Jin T, Tao J. Inflammasomes cross-talk with lymphocytes to connect the innate and adaptive immune response. *J Adv Res*. 2023;54:181–193. doi:10.1016/j.jare.2023.01.012
8. Wu J, Sun X, Jiang P. Metabolism-inflammasome crosstalk shapes innate and adaptive immunity. *Cell Chem Biol*. 2024;31(5):884–903. doi:10.1016/j.chembiol.2024.04.006
9. Deets KA, Vance RE. Inflammasomes and adaptive immune responses. *Nat Immunol*. 2021;22(4):412–422. doi:10.1038/s41590-021-00869-6
10. Ingelfinger F, Gerdes LA, Kavaka V, et al. Twin study reveals non-heritable immune perturbations in multiple sclerosis. *Nature*. 2022;603 (7899):152–158. doi:10.1038/s41586-022-04419-4
11. DiToro D, Harbour SN, Bando JK, et al. Insulin-like growth factors are key regulators of T helper 17 regulatory T cell balance in autoimmunity. *Immunity*. 2020;52(4):650–667.e10. doi:10.1016/j.immuni.2020.03.013
12. Dong Y, Yong VW. When encephalitogenic T cells collaborate with microglia in multiple sclerosis. *Nat Rev Neurol*. 2019;15(12):704–717. doi:10.1038/s41582-019-0253-6
13. Li Q, Barres BA. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol*. 2018;18(4):225–242. doi:10.1038/nri.2017.125
14. Miron VE, Boyd A, Zhao JW, et al. M2 microglia and macrophages drive oligodendrocyte differentiation during CNS remyelination. *Nat Neurosci*. 2013;16(9):1211–1218. doi:10.1038/nn.3469
15. Yong HYF, Yong VW. Mechanism-based criteria to improve therapeutic outcomes in progressive multiple sclerosis. *Nat Rev Neurol*. 2022;18 (1):40–55. doi:10.1038/s41582-021-00581-x
16. Linnerbauer M, Wheeler MA, Quintana FJ. Astrocyte crosstalk in CNS inflammation. *Neuron*. 2020;108(4):608–622. doi:10.1016/j.neuron.2020.08.012
17. Kalafatakis I, Karagogeos D. Oligodendrocytes and microglia: key players in myelin development, damage and repair. *Biomolecules*. 2021;11 (7):1058. doi:10.3390/biom11071058
18. Plemel JR, Liu WQ, Yong VW. Remyelination therapies: a new direction and challenge in multiple sclerosis. *Nat Rev Drug Discov*. 2017;16 (9):617–634. doi:10.1038/nrd.2017.115
19. Franklin RJM, Simons M. CNS remyelination and inflammation: from basic mechanisms to therapeutic opportunities. *Neuron*. 2022;110 (21):3549–3565. doi:10.1016/j.neuron.2022.09.023
20. Creswell R, Dombrowski Y. Innate and adaptive immune mechanisms regulating central nervous system remyelination. *Curr Opin Pharmacol*. 2022;63:102175. doi:10.1016/j.coph.2021.12.002
21. Dobson R, Giovannoni G. Multiple sclerosis - a review. *Eur J Neurol*. 2019;26(1):27–40. doi:10.1111/ene.13819
22. Olek MJ. Multiple sclerosis. *Ann Intern Med*. 2021;174(6):Itc81–itc96. doi:10.7326/aitc202106150
23. McGinley MP, Goldschmidt CH, Rae-Grant AD. Diagnosis and treatment of multiple sclerosis: a review. *JAMA*. 2021;325(8):765–779. doi:10.1001/jama.2020.26858
24. Moudgil KD, Venkatesha SH. The anti-inflammatory and immunomodulatory activities of natural products to control autoimmune inflammation. *Int J Mol Sci*. 2022;24(1):95. doi:10.3390/ijms24010095
25. Yuan J, Tao Y, Wang M, Huang F, Wu X. Natural compounds as potential therapeutic candidates for multiple sclerosis: emerging preclinical evidence. *Phytomedicine*. 2024;123:155248. doi:10.1016/j.phymed.2023.155248
26. Wang X-L. Discussion on the relation of turbid blood and encephalopathy. *Shandong J Tradit Chin Med*. 2006;09:579–582. doi:10.16295/j.cnki.0257-358x.2006.09.001
27. Meng-Ru Z, Ruo-Xuan S, Ming-Yang Y, et al. Antagonizing astrocytic platelet activating factor receptor-neuroinflammation for total flavone of epimedium in response to cuprizone demyelination. *Int Immunopharmacol*. 2021;101(Pt A):108181. doi:10.1016/j.intimp.2021.108181
28. Jun Z, Junhua W, Xiaobing LI, et al. Efficacy of Heshouwu () on gut mircobiota in mice with autoimmune encephalomyelitis. *J Tradit Chin Med*. 2023;43(4):676–685. doi:10.19852/j.cnki.jtcm.2023.04.001
29. Zhang X, Wang Z, Li X, et al. Polydatin protects against atherosclerosis by activating autophagy and inhibiting pyroptosis mediated by the NLRP3 inflammasome. *J Ethnopharmacol*. 2023;309:116304. doi:10.1016/j.jep.2023.116304
30. Athauda D, Foltynie T. Drug repurposing in parkinson's disease. *CNS Drugs*. 2018;32(8):747–761. doi:10.1007/s40263-018-0548-y
31. Bordet R, Ihl R, Korczyn AD, et al. Towards the concept of disease-modifier in post-stroke or vascular cognitive impairment: a consensus report. *BMC Med*. 2017;15(1):107. doi:10.1186/s12916-017-0869-6

32. Casas AI, Hassan AA, Larsen SJ, et al. From single drug targets to synergistic network pharmacology in ischemic stroke. *Proc Natl Acad Sci U S A*. 2019;116(14):7129–7136. doi:10.1073/pnas.1820799116
33. Cummings JL, Tong G, Ballard C. Treatment combinations for alzheimer's disease: current and future pharmacotherapy options. *J Alzheimers Dis*. 2019;67(3):779–794. doi:10.3233/jad-180766
34. Kabir MT, Uddin MS, Mamun AA, et al. Combination drug therapy for the management of alzheimer's disease. *Int J Mol Sci*. 2020;21(9):3272. doi:10.3390/ijms21093272
35. Somayaji MR, Przekwas AJ, Gupta RK. Combination therapy for multi-target manipulation of secondary brain injury mechanisms. *Curr Neuropharmacol*. 2018;16(4):484–504. doi:10.2174/1570159x15666170828165711
36. Löscher W, Klein P. New approaches for developing multi-targeted drug combinations for disease modification of complex brain disorders. Does epilepsy prevention become a realistic goal? *Pharmacol Ther*. 2022;229:107934. doi:10.1016/j.pharmthera.2021.107934
37. Xutian S, Cao D, Wozniak J, Junion J, Boisvert J. Comprehension of the unique characteristics of traditional Chinese medicine. *Am J Chin Med*. 2012;40(2):231–244. doi:10.1142/s0192415x12500188
38. Li M, Wang Y, Chen Y, et al. A comprehensive review on pharmacokinetic mechanism of herb-herb/drug interactions in Chinese herbal formula. *Pharmacol Ther*. 2024;264:108728. doi:10.1016/j.pharmthera.2024.108728
39. Chen S, Zhang J, Yu WB, et al. Eomesodermin in CD4(+)T cells is essential for Ginkgolide K ameliorating disease progression in experimental autoimmune encephalomyelitis. *Int J Biol Sci*. 2021;17(1):50–61. doi:10.7150/ijbs.50041
40. Yeh CL, Wu JM, Chen KY, et al. Effects of different routes and forms of vitamin D administration on mesenteric lymph node CD4+ T cell polarization and intestinal injury in obese mice complicated with polymicrobial sepsis. *Nutrients*. 2022;14(17):3557. doi:10.3390/nu14173557
41. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*. 2016;7(2):27–31. doi:10.4103/0976-0105.177703
42. Zhao Z, Bao XQ, Zhang Z, Li F, Liu H, Zhang D. Novel phloroglucinol derivative Compound 21 protects experimental autoimmune encephalomyelitis rats via inhibiting Th1/Th17 cell infiltration. *Brain Behav Immun*. 2020;87:751–764. doi:10.1016/j.bbi.2020.03.009
43. Wei X, Liang J, Liu J, et al. Anchang Yuyang Decoction inhibits experimental colitis-related carcinogenesis by regulating PPAR signaling pathway and affecting metabolic homeostasis of host and microbiota. *J Ethnopharmacol*. 2024;326:117995. doi:10.1016/j.jep.2024.117995
44. Yao J, Wang Z, Song W, Zhang Y. Targeting NLRP3 inflammasome for neurodegenerative disorders. *Mol Psychiatry*. 2023;28(11):4512–4527. doi:10.1038/s41380-023-02239-0
45. Shao S, Chen C, Shi G, et al. Therapeutic potential of the target on NLRP3 inflammasome in multiple sclerosis. *Pharmacol Ther*. 2021;227:107880. doi:10.1016/j.pharmthera.2021.107880
46. Shen HH, Yang YX, Meng X, et al. NLRP3: a promising therapeutic target for autoimmune diseases. *Autoimmun Rev*. 2018;17(7):694–702. doi:10.1016/j.autrev.2018.01.020
47. Kamma E, Lasisi W, Libner C, Ng HS, Plemel JR. Central nervous system macrophages in progressive multiple sclerosis: relationship to neurodegeneration and therapeutics. *J Neuroinflammation*. 2022;19(1):45. doi:10.1186/s12974-022-02408-y
48. Guadagno J, Swan P, Shaikh R, Cregan SP. Microglia-derived IL-1β triggers p53-mediated cell cycle arrest and apoptosis in neural precursor cells. *Cell Death Dis*. 2015;6(6):e1779. doi:10.1038/cddis.2015.151
49. Ai RS, Xing K, Deng X, et al. Baicalin promotes CNS remyelination via PPARγ signal pathway. *Neurol Neuroimmunol Neuroinflamm*. 2022;9(2). doi:10.1212/nxi.0000000000001142
50. Liston A, Dooley J, Yshii L. Brain-resident regulatory T cells and their role in health and disease. *Immunol Lett*. 2022;248:26–30. doi:10.1016/j.imlet.2022.06.005
51. Weaver CT, Hatton RD. Interplay between the TH17 and TReg cell lineages: a (co-)evolutionary perspective. *Nat Rev Immunol*. 2009;9(12):883–889. doi:10.1038/nri2660
52. Dombrowski Y, O'Hagan T, Dittmer M, et al. Regulatory T cells promote myelin regeneration in the central nervous system. *Nat Neurosci*. 2017;20(5):674–680. doi:10.1038/nn.4528
53. Shi C, Zhang J, Wang H, et al. Trojan horse nanocapsule enabled in situ modulation of the phenotypic conversion of Th17 cells to treg cells for the treatment of multiple sclerosis in mice. *Adv Mater*. 2023;35(11):e2210262. doi:10.1002/adma.202210262
54. Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol*. 2018;17(2):162–173. doi:10.1016/s1474-4422(17)30470-2
55. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple sclerosis: mechanisms and immunotherapy. *Neuron*. 2018;97(4):742–768. doi:10.1016/j.neuron.2018.01.021
56. Comi G, Bar-Or A, Lassmann H, et al. Role of B cells in multiple sclerosis and related disorders. *Ann Neurol*. 2021;89(1):13–23. doi:10.1002/ana.25927
57. Li R, Rezk A, Miyazaki Y, et al. Proinflammatory GM-CSF–producing B cells in multiple sclerosis and B cell depletion therapy. *Sci Transl Med*. 2015;7(310):310ra166. doi:10.1126/scitranslmed.aab4176
58. van Langelaar J, Rijvers L, Smolders J, van Luijn MM. B and T cells driving multiple sclerosis: identity, mechanisms and potential triggers. *Front Immunol*. 2020;11:760. doi:10.3389/fimmu.2020.00760
59. Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science*. 2016;353(6301):777–783. doi:10.1126/science.aag2590
60. Distéfano-Gagné F, Bitarafan S, Lacroix S, Gosselin D. Roles and regulation of microglia activity in multiple sclerosis: insights from animal models. *Nat Rev Neurosci*. 2023;24(7):397–415. doi:10.1038/s41583-023-00709-6
61. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541(7638):481–487. doi:10.1038/nature21029
62. Clark IC, Gutiérrez-Vázquez C, Wheeler MA, et al. Barcoded viral tracing of single-cell interactions in central nervous system inflammation. *Science*. 2021;372(6540). doi:10.1126/science.abf1230
63. Tepavčević V, Lubetzki C. Oligodendrocyte progenitor cell recruitment and remyelination in multiple sclerosis: the more, the merrier? *Brain*. 2022;145(12):4178–4192. doi:10.1093/brain/awac307
64. Franklin RJ, Ffrench-Constant C. Remyelination in the CNS: from biology to therapy. *Nat Rev Neurosci*. 2008;9(11):839–855. doi:10.1038/nrn2480
65. Vanherle S, Guns J, Loix M, et al. Extracellular vesicle-associated cholesterol supports the regenerative functions of macrophages in the brain. *J Extracell Vesicles*. 2023;12(12):e12394. doi:10.1002/jev2.12394

66. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol*. 2015;14(2):183–193. doi:10.1016/s1474-4422(14)70256-x
67. Allanach JR, Farrell JW 3rd, Mésidor M, Karimi-Abdolrezaee S. Current status of neuroprotective and neuroregenerative strategies in multiple sclerosis: a systematic review. *Mult Scler*. 2022;28(1):29–48. doi:10.1177/13524585211008760
68. de Castro LF, Longhi LNA, Paião MR, et al. NLRP3 inflammasome is involved in the recognition of Paracoccidioides brasiliensis by human dendritic cells and in the induction of Th17 cells. *J Infect*. 2018;77(2):137–144. doi:10.1016/j.jinf.2018.03.004
69. Inoue M, Williams KL, Gunn MD, Shinohara ML. NLRP3 inflammasome induces chemotactic immune cell migration to the CNS in experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A*. 2012;109(26):10480–10485. doi:10.1073/pnas.1201836109
70. Li Y, Xia Y, Yin S, et al. Targeting Microglial α-Synuclein/TLRs/NF-kappaB/NLRP3 Inflammasome Axis in Parkinson's Disease. *Front Immunol*. 2021;12:719807. doi:10.3389/fimmu.2021.719807
71. Afonina IS, Zhong Z, Karin M, Beyaert R. Limiting inflammation-the negative regulation of NF-κB and the NLRP3 inflammasome. *Nat Immunol*. 2017;18(8):861–869. doi:10.1038/ni.3772
72. Gao J, Long L, Xu F, et al. Icariside II, a phosphodiesterase 5 inhibitor, attenuates cerebral ischaemia/reperfusion injury by inhibiting glycogen synthase kinase-3β-mediated activation of autophagy. *Br J Pharmacol*. 2020;177(6):1434–1452. doi:10.1111/bph.14912
73. Liu MB, Wang W, Gao JM, Li F, Shi JS, Gong QH. Icariside II attenuates cerebral ischemia/reperfusion-induced blood-brain barrier dysfunction in rats via regulating the balance of MMP9/TIMP1. *Acta Pharmacol Sin*. 2020;41(12):1547–1556. doi:10.1038/s41401-020-0409-3
74. Gao J, Ma C, Xia D, et al. Icariside II preconditioning evokes robust neuroprotection against ischaemic stroke, by targeting Nrf2 and the OXPHOS/NF-κB/ferroptosis pathway. *Br J Pharmacol*. 2023;180(3):308–329. doi:10.1111/bph.15961
75. Zhang Y, Yin L, Zheng N, et al. Icariin enhances remyelination process after acute demyelination induced by cuprizone exposure. *Brain Res Bull*. 2017;130:180–187. doi:10.1016/j.brainresbull.2017.01.025
76. Gao D, Zheng CC, Hao JP, Yang CC, Hu CY. Icariin ameliorates behavioral deficits and neuropathology in a mouse model of multiple sclerosis. *Brain Res*. 2023;1804:148267. doi:10.1016/j.brainres.2023.148267
77. Kciuk M, Garg N, Dhankhar S, et al. Exploring the comprehensive neuroprotective and anticancer potential of afzelin. *Pharmaceuticals*. 2024;17(6):701. doi:10.3390/ph17060701
78. Sayuti NH, Zulkefli N, Tan JK, et al. Ethanolic extract of polygonum minus protects differentiated human neuroblastoma cells (SH-SY5Y) against H(2)O(2)-induced oxidative stress. *Molecules*. 2023;28(18):6726. doi:10.3390/molecules28186726
79. Romero-Pérez AI, Ibern-Gómez M, Lamuela-Raventós RM, de La Torre-Boronat MC. Piceid, the major resveratrol derivative in grape juices. *J Agric Food Chem*. 1999;47(4):1533–1536. doi:10.1021/jf981024g
80. Ji H, Zhang X, Du Y, Liu H, Li S, Li L. Polydatin modulates inflammation by decreasing NF-κB activation and oxidative stress by increasing Gli1, Ptch1, SOD1 expression and ameliorates blood-brain barrier permeability for its neuroprotective effect in pMCAO rat brain. *Brain Res Bull*. 2012;87(1):50–59. doi:10.1016/j.brainresbull.2011.09.021
81. Cheng Y, Zhang HT, Sun L, et al. Involvement of cell adhesion molecules in polydatin protection of brain tissues from ischemia-reperfusion injury. *Brain Res*. 2006;1110(1):193–200. doi:10.1016/j.brainres.2006.06.065
82. Huang B, Liu J, Meng T, et al. Polydatin Prevents Lipopolysaccharide (LPS)-induced parkinson's disease via regulation of the AKT/GSK3β-Nrf2/NF-κB signaling axis. *Front Immunol*. 2018;9:2527. doi:10.3389/fimmu.2018.02527
83. Chen Y, Zhang DQ, Liao Z, et al. Anti-oxidant polydatin (piceid) protects against substantia nigral motor degeneration in multiple rodent models of parkinson's disease. *Mol Neurodegener*. 2015;10:4. doi:10.1186/1750-1326-10-4
84. Ghaiad HR, Nooh MM, El-Sawalhi MM, Shaheen AA. Resveratrol promotes remyelination in cuprizone model of multiple sclerosis: biochemical and histological study. *Mol Neurobiol*. 2017;54(5):3219–3229. doi:10.1007/s12035-016-9891-5
85. Shindler KS, Ventura E, Dutt M, Elliott P, Fitzgerald DC, Rostami A. Oral resveratrol reduces neuronal damage in a model of multiple sclerosis. *J Neuroophthalmol*. 2010;30(4):328–339. doi:10.1097/WNO.0b013e3181f7f833
86. Fonseca-Kelly Z, Nassrallah M, Uribe J, et al. Resveratrol neuroprotection in a chronic mouse model of multiple sclerosis. *Front Neurol*. 2012;3:84. doi:10.3389/fneur.2012.00084

Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/journal-of-inflammation-research-journal