Article

Discovery Medicine 2025; 37(192): 64–72
https://doi.org/10.24976/Discov.Med.202537192.6

Regulation of Concanavalin A-induced Immune Hepatitis in Mice by Dihydromyricetin at the M1/M2 Type Macrophage Level

Xinpeng Zhang[1,†], Yan Liu[2,†], Kaijin Yang[1], Jichao Tang[1], Kang Zhao[1], Yi Li[1,*]

[1]Department of General Surgery, Section for Day Surgery, The Third People's Hospital of Chengdu, Affiliated Hospital of Southwest Jiaotong University & The Second Affiliated Hospital of Chengdu, Chongqing Medical University, 610031 Chengdu, Sichuan, China
[2]Department of Pathology, Chengdu Women and Children's Central Hospital, 610031 Chengdu, Sichuan, China
*Correspondence: lyi1980@126.com (Yi Li)
[†]These authors contributed equally.
Published: 20 January 2025

Background: Autoimmune hepatitis (AIH) is an autoimmune disease accompanied by an autoimmune inflammatory response that often leads to severe liver damage. In addition, it may further lead to complications such as liver fibrosis, cirrhosis and liver failure. Dihydromyricetin (DHM) possesses various pharmacological properties, such as being anti-inflammatory, antioxidant, and antibacterial. In this experiment, we investigated the effect of DHM on autoimmune hepatitis mice based on the level of M1/M2 type macrophages.

Methods: An autoimmune hepatitis mouse model was established by the administration of DHM followed by tail vein injection of Concanavalin A (Con A). Liver tissues were examined for pathological and morphological changes. Interleukin-1β (IL-1β), interleukin-10 (IL-10), interleukin-6 (IL-6), and interleukin-4 (IL-4) levels in liver tissues were assessed. Serum hepatic function indexes were measured, including alanine aminotransferase (ALT), aspartate transaminase (AST), and lactatedehydrogenase (LDH). Oxidative stress indexes, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and Nitric oxide (NO), were quantified. Additionally, tumor necrosis factor-α (*TNF-*α) and nuclear factor kappa-B (*NF-*κ*B*) *p65* mRNA and protein expression polarization were determined. The presence of M1/M2-type macrophages was also investigated.

Results: Compared to the model group, mice in the DHM group exhibited a significant reduction in serum hepatic function indexes ($p < 0.05$). Liver tissues from the DHM group showed a noteworthy decrease in MDA and NO levels, along with a significant increase in SOD and GSH-Px levels ($p < 0.05$). Furthermore, in the liver tissues of mice from the DHM group, there was a notable reduction in the count of M1-type macrophages and a considerable elevation in the M2-type macrophages ($p < 0.05$). IL-1β and IL-6 expression levels exhibited a significant decrease, whereas IL-10 and IL-4 levels displayed a significant increase ($p < 0.05$). Additionally, both *TNF-*α and *NF-*κ*B p65* mRNA levels and protein expression experienced a noteworthy decrease ($p < 0.05$).

Conclusion: DHM mitigates the inflammatory response in AIH mice by reducing oxidative stress and modulating macrophage polarization and the TNF-α/NF-κB pathway.

Keywords: Dihydromyricetin; autoimmune hepatitis; macrophages; oxidative stress

Introduction

Autoimmune hepatitis (AIH) stands as an autoimmune disease stemming from an autoimmune response, manifesting as liver parenchymal damage. It is characterized by elevated aminotransferase levels, increased immunoglobulin G (IgG) levels, positive autoantibodies, interfacial hepatitis, and the infiltration of numerous plasma cells in the confluent zone [1]. AIH presents with diverse clinical manifestations, an insidious onset, and a tendency for recurrence, posing challenges in clinical management. Its progression may lead to complications such as liver fibrosis, cirrhosis, and hepatic failure. Current clinical approaches to treating autoimmune hepatitis primarily rely on glucocorticoids and immunosuppressants. These medications show effectiveness in reducing liver inflammation and improving liver injury. However, they have notable drawbacks, including high toxicity, significant side effects, susceptibility to drug resistance, and a pronounced risk of relapse upon discontinuation [2]. Therefore, the quest for novel, safer, and more effective treatments is pressing.

Originally derived from sword beans, Concanavalin A (Con A) is a crucial immunological research reagent extensively utilized to simulate human autoimmune liver diseases. Its primary function involves fostering T cell activation and proliferation, prominently characterized by CD4+ T lymphocytes and macrophage infiltration into the

liver. This infiltration triggers the production of inflammatory cytokines, ultimately culminating in acute liver injury [3]. The Con A-induced mouse hepatitis model can mimic the pathogenic mechanism and pathological change process of human autoimmune liver disease to a certain extent. It is the most commonly used autoimmune hepatitis research model because it is easy to generate, low-cost, convenient, and reproducible [4]. Therefore, in this study, Con A was selected to establish an autoimmune hepatitis model. One of the most critical elements of the innate immune response to hepatic damage is macrophages, essential immune cells in the liver. Macrophages can be categorized into two functional states based on their activation status: M1 macrophages, pro-inflammatory; and M2 macrophages, anti-inflammatory. The interplay between these two activation states is crucial in immune regulation and responses to inflammation. In both physiological and pathological scenarios, macrophages exhibit the ability to transition between M1 and M2 states through a polarization process dependent on microenvironmental cues [5]. The polarization process holds a pivotal regulatory function across all phases of the immune response and contributes significantly to tissue repair. Hepatic macrophages reside within the hepatic blood sinusoids. Recent investigations indicate an elevation in M1 macrophages and a reduction in M2 macrophages in individuals with AIH [6]. The prevalence of M1 macrophages in the early stages of the immune response, with hepatic inflammation intensifying post-depletion of liver-resident macrophages. As AIH progresses, M2 macrophages effectively delay the onset and eventually polarize towards M1 macrophages [7]. This emphasizes how important M1/M2 macrophage polarization is in the development of AIH.

Dihydromyricetin (DHM) is a dihydroflavonoid abundantly present in plants of the Serpentine genus within the Vitis vinifera family. Being the most prevalent natural flavonoid in Garcinia Cambogia, DHM boasts a diverse array of pharmacological effects. Beyond the typical properties of flavonoids, it demonstrates anti-inflammatory and antioxidant capabilities across various diseases [8]. Research indicates that DHM showcases antioxidant activity by curtailing malondialdehyde (MDA) levels while concurrently enhancing superoxide dismutase (SOD) activity [9]. By lowering interleukin-6 (*IL-6*) and tumor necrosis factor-α (*TNF-*α) mRNA expression in low-density lipoprotein (LDL) receptor-deficient animals fed with a high-fat diet, DHM has been shown to lessen hepatic and aortic inflammation [10]. According to a recent study, DHM inhibits M1 macrophage polarization in atherosclerosis by adjusting the SIRT1/nuclear factor kappa-B (NF-κB) signaling pathway mediated by miR-9 [11]. However, the mechanisms by which DHM affects macrophage polarization and thus improves AIH are still largely unknown.

Hence, to elucidate the impact of DHM in an AIH model, this study delved into potential mechanisms, specifically focusing on the equilibrium of macrophage subtypes. The aim was to uncover DHM's prospective role in treating AIH.

Materials and Methods

Reagents and Instruments

Reagents: Con A (C5275, Sigma-Aldrich, Shanghai, China); DHM (MB5834, MeilunBio®, Dalian, China); MDA, SOD, Nitric oxide (NO), Enzyme-linked immunosorbent assay (ELISA) kits (SP30131, SP12914, SP14504, Spbio, Wuhan, China); glutathione peroxidase (GSH-Px), alanine aminotransferase (ALT), aspartate transaminase (AST), lactatedehydrogenase (LDH), interleukin-1β (IL-1β), interleukin-10 (IL-10), IL-6, interleukin-4 (IL-4) ELISA kits (ml058194, ml063179, ml058659, ml002267, ml098416, mIC50274-1, ml098430, ml064310, mlbio, Shanghai, China); electrophoresis and membrane transfer solutions (D1060, Solarbio, Beijing, China); BCA kits (ml095490, mlbio, Shanghai, China); Hematoxylin-eosin (H&E) Staining kit (G1120, Solarbio, Beijing, China); RIPA lysis solution, DAPI solution (P0013B, C1006, Beyotime, Shanghai, China); BeyoECL Star (P0018AS, Beyotime, Shanghai, China).

Instruments and equipment: The MR-96A enzyme labeling instrument was purchased from Shenzhen Myriad Biomedical Electronics Co., Ltd. (Shenzhen, China); the FACSC Canto II flow cytometer was purchased from BD Company (Franklin Lakes, NJ, USA); and the 722 spectrophotometer was purchased from Shanghai Yidian Analyzer Co. (Shanghai, China).

Animal Modeling and Drug Administration

Thirty male SPF-grade BALB/c mice weighing 27–30 g were procured from Hunan Slake Jinda Laboratory Animal Co., Ltd. (Changsha, Hunan, China), with Production License No.: SCXK (Hunan) 2016-0002. The mice underwent a 3-day adaptation period in a controlled environment with constant temperature (20–26 °C) and humidity (40%–70% RH) before the experiment commenced. The study strictly adhered to the 3R principle and received approval from the Animal Ethics Committee of the Third People's Hospital of Chengdu, Affiliated Hospital of Southwest Jiaotong University & The Second Affiliated Hospital of Chengdu, Chongqing Medical University (No.20236735). The mice were randomly assigned to the Control group, Con A group, and DHM group. In the DHM group, a daily gavage of 75 mg·kg^{-1}·d^{-1} DHM (MB5834, MeilunBio®, Dalian, China) suspension was administered, while the Control group and Con A groups received an equal volume of saline orally at a fixed time point for 10 days. After the last administration for 3 hours, the mice were immobilized, and their tail veins were dilated by alcohol wiping. Except for the Control group, each mouse was given 15 mg·kg^{-1} Con A (C5275, Sigma-Aldrich, Shanghai, China)

Table 1. PCR primers list.

Gene	Direction	Sequence (5′-3′)
TNF-α	F	CCAGGTTCTCTTCAAGGGACAA
	R	GGTATGAAATGGCAAATCGGCT
NF-κB p65 (RELA)	F	CAGATACCACTAAGACGCACCC
	R	CTCCAGGTCTCGCTTCTTCACA
GAPDH	F	CTGGAGAAACCTGCCAAGTATG
	R	GGTGGAAGAATGGGAGTTGCT

TNF-α, tumor necrosis factor-α; *NF-κB*, nuclear factor kappa-B, *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

tail vein injection; the Control group was given with 0.15 mL PBS tail vein injection. The success of model replication could be assessed by H&E Staining to detect liver histopathology and test liver function indexes.

Sample Collection

After 10 hours of modeling, mice were deeply anesthetized with 2% sodium pentobarbital (45 mg/kg) and euthanized by removing the eyeballs and taking fresh blood in 1.5 mL EP tubes. The abdominal cavity was swiftly opened, and the liver tissue was extracted and divided into two portions. One part was fixed in 4% paraformaldehyde, while the other was stored at a low temperature of –80 °C.

H&E Staining

Standard operating protocols were followed in processing fixed liver tissues to create paraffin slices. Following H&E Staining, dehydration, and sealing of the slices, the tissue morphology was examined under a light microscope (CX33, Olympus Corporation, Tokyo, Japan).

ELISA

According to the manufacturer's instructions, ELISA kits were utilized to assess serum liver function indicators (ALT, AST, LDH). Measurement for the levels of oxidative markers (MDA, SOD, NO, GSH-Px) and inflammatory factors (IL-1β, IL-10, IL-6, IL-4) in liver homogenate were performed according to the provided instructions.

Immunofluorescence

Liver tissue cryosections were treated separately with anti-F4/80 antibody (Ab100790, 1:50, Abcam, Shanghai, China), anti-CD206 antibody (Ab64693, 1:100, Abcam, Shanghai, China), and anti-iNOS antibody (Ab178945, 1:50, Abcam, Shanghai, China), the sections were processed with proteinase K (P9460, 1:200, Solarbio, Beijing, China) for 30 minutes, followed by permeabilization with Triton X-100 (P0096, 0.3%, Beyotime, Shanghai, China) for 20 minutes and blocking with normal goat serum (ZY640016RE, ZSGB-BIO, Tianjin, China) for 1 hour. Incubation with primary antibodies iNOS Monoclonal Antibody (CXNFT), CD206 (MMR) Recombinant Rabbit Monoclonal Antibody (JF0953) (53-5920-82, MA5-32498, 1:1000, Invitrogen, Waltham, MA, USA) overnight at 4 °C, followed by incubated with the coupled secondary antibody for 1 hour at room temperature. Finally, nuclei were visualized with DAPI solution (C1006, Beyotime, Shanghai, China) for 10 minutes at room temperature. A fluorescence quenching reagent was added, and the slides were observed under a fluorescence microscope. ImageJ software (V1.8.0.112, NIH, Madison, WI, USA) was used for data analysis.

qRT-PCR

According to the manufacturer's instructions, the collected liver tissue was homogenized with Trizol reagent (B511311, Sangon Biotech, Shanghai, China) to extract total RNA, and qualified RNA was selected for reverse transcription. After obtaining cDNA by reverse transcription, a PCR reaction was carried out in accordance with the provided instructions. The comparative threshold approach ($2^{-\Delta\Delta Ct}$ method) was utilized to analyze the target gene's relative expression. The PCR primer design is shown in Table 1.

Western Blot

120 mg of liver tissue was roughly cut, and RIPA lysis solution (Beyotime, Shanghai, China) was added. The mixture was then centrifuged at 12,000 rpm at 4 °C for 30 minutes to obtain the supernatant for protein concentration determination using the BCA method, and the sample volume was adjusted accordingly. Next, a 5× loading buffer was added and denatured at high temperatures. Gel preparation, electrophoresis, membrane transfer, and closure were then performed sequentially, incubated with TNF-α (YT4689, 1:2000, ImmunoWay, Suzhou, China), NF-κB p65 (YM3111, 1:2000, ImmunoWay, Suzhou, China), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (YM3215, 1:2000, ImmunoWay, Suzhou, China), HRP-conjugated secondary antibody (AS014, 1:2000, ABclonal, Wuhan, China), respectively. After incubation with the primary antibody, the samples were subsequently incubated with the secondary antibody at room temperature for 2 hours. And ECL luminescence development, gel imaging, and analysis of the relative grayscale values were performed using ImageJ (V1.8.0.112, NIH, Madison, WI, USA).

Flow Cytometry

A portion of the liver was minced in RPMI medium, ground, and centrifuged. The supernatant was discarded, and erythrocyte lysate was added before centrifugation at 5455 r·min^{-1} for 5 minutes with a centrifugation radius of 15 cm. The liver single-cell suspension was prepared by resuspending with Stain Buffer, adjusting the cell count to 1×10^6 cells per milliliter. A 100 µL aliquot of the cell suspension was taken and stained with anti-mouse CD11b (101207, 1:100, Biolegend, San Diego, CA, USA), anti-

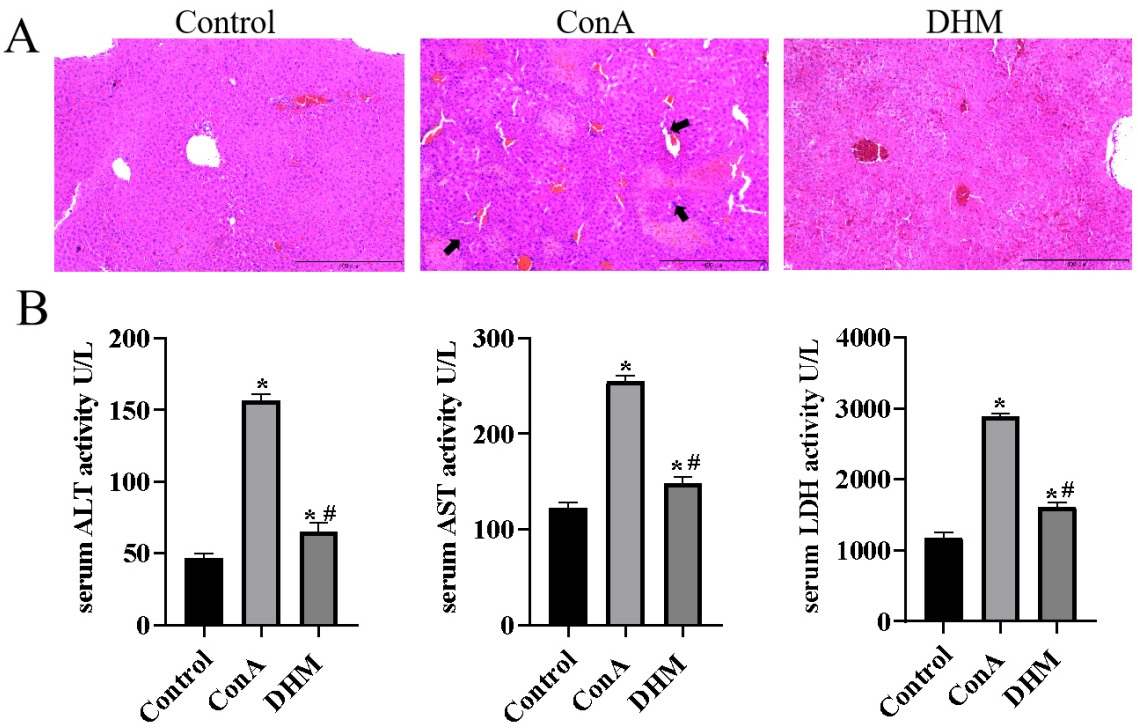

Fig. 1. Effect of Dihydromyricetin (DHM) on liver injury in autoimmune hepatitis (AIH) mice. (A) Hematoxylin-eosin (H&E) Staining, Arrows (↑) show inflammatory cells (neutrophils and lymphocytes) and areas of necrosis (100×, Scale = 400 μm). (B) Enzyme-linked immunosorbent assay (ELISA) to detect the level of Liver function index. *$p < 0.05$ vs Control, $^{\#}p < 0.05$ vs Con A. n = 3. Con A, Concanavalin A.

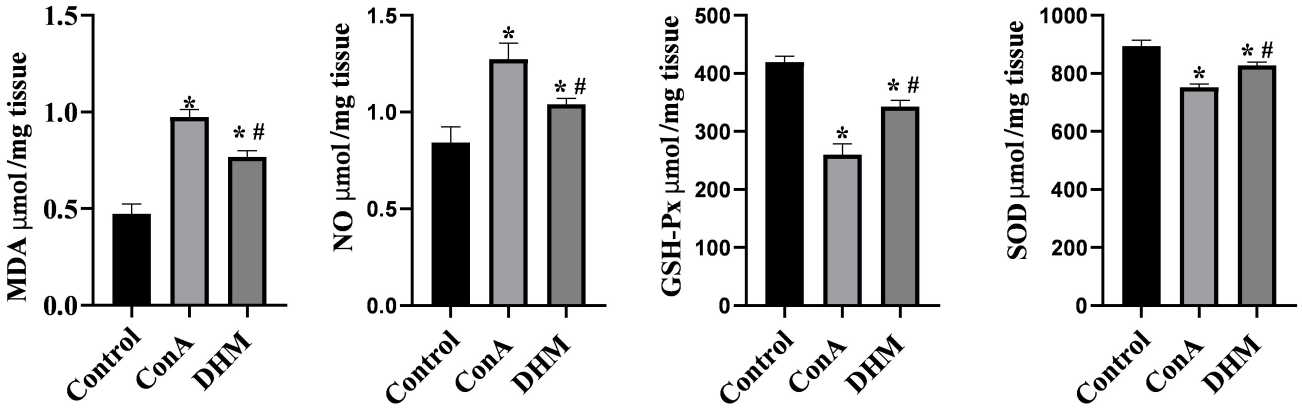

Fig. 2. Effect of DHM on oxidative stress in AIH mice. ELISA to detect the level of oxidative stress factor. *$p < 0.05$ vs Control, $^{\#}p < 0.05$ vs Con A. n = 3.

mouse F4/80 (123108, 1:100, Biolegend, San Diego, CA, USA), anti-mouse CD206 (141720, 1:100, Biolegend, San Diego, CA, USA), and iNOS Monoclonal antibody (17-5920-82, 1:100, Thermo Fisher, Shanghai, China). They were then tested using flow cytometry.

Statistical Analysis

The software SPSS 22.0 (IBM, Corp., Armonk, NY, USA) was applied for data analysis. The data is shown as mean ± standard deviation. The normality of the data in each group was tested by the S-W test ($p > 0.05$ indicates that the data satisfy normal distribution), and the variance homogeneity of the variance between the data in each group was tested by the F-test ($p > 0.05$ indicates that the variance satisfies homoscedasticity). One-way ANOVA was utilized for multiple-group comparisons, while the LSD-*t* test was employed for two-group comparisons. A statistically significant difference was defined as $p < 0.05$.

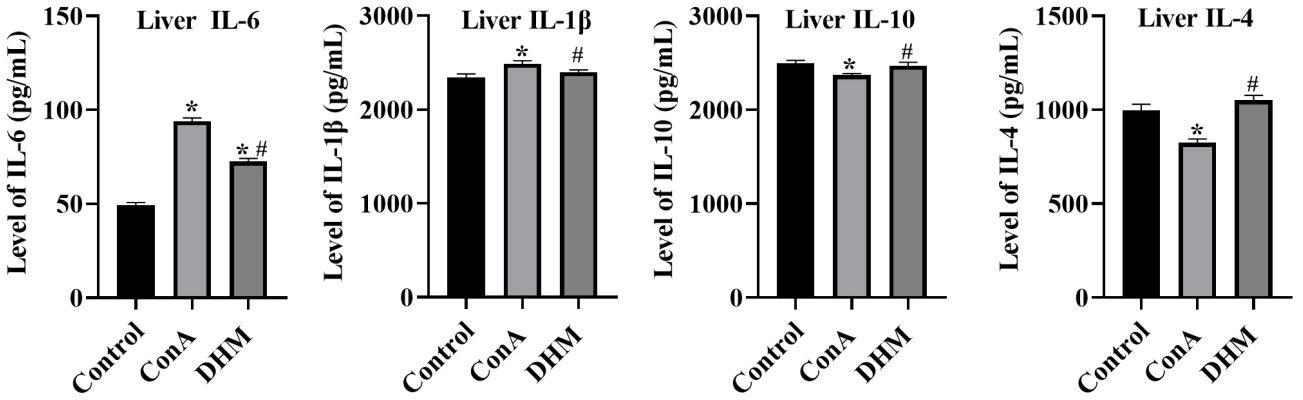

Fig. 3. Effect of DHM on inflammatory factors in the liver of AIH mice. ELISA to detect the level of inflammatory factors. $^{*}p < 0.05$ vs Control, $^{\#}p < 0.05$ vs Con A. n = 3.

Results

Effect of DHM on Liver Injury in AIH Mice

The pathological section results revealed a large infiltration of inflammatory cells (neutrophils and lymphocytes) and destruction of the hepatic sinusoidal structure in the Con A group. Additionally, there was evident disorganization of hepatic lobules and instances of localized hepatocellular swelling and cellular necrosis (Fig. 1A). Serum hepatic function index activities in the Con A group's supernatant were considerably higher than in the Control group; however, following DHM administration, the Con A group's results were reversed ($p < 0.05$) (Fig. 1B). These results suggest that DHM effectively mitigates the liver damage induced by Con A in AIH mice.

Effect of DHM on Oxidative Stress in Hepatitis in AIH Mice

The liver tissues of the Con A group showed a substantial decrease in the levels of antioxidant factors and a notable elevation of oxidative factors compared to the Control group. Nevertheless, following DHM administration, the Con A group's results were reversed ($p < 0.05$) (Fig. 2). DHM reduces oxidative stress, which lessens the liver damage caused by Con A in AIH mice.

Effect of DHM on Inflammatory Factors in the Liver of AIH Mice

The mice in the Con A group exhibited a noticeable reduction in anti-inflammatory factor levels and a significant increase in pro-inflammatory factor levels in their liver tissue compared to the Control group. Nevertheless, after receiving DHM, this pattern was reversed ($p < 0.05$) (Fig. 3). Our findings demonstrate that DHM effectively promotes the release of pro-inflammatory factors while inhibiting the release of anti-inflammatory factors.

Effect of DHM on the Polarization of Hepatic Macrophages in AIH Mice

To investigate the effects of DHM on liver macrophages, we examined the expression of specific markers in M1 and M2-type macrophages using immunofluorescence. The results of co-localization of F4/80 and iNOS showed that the fluorescence intensity was significantly decreased after DHM administration ($p < 0.05$) (Fig. 4A). The results of co-localization of F4/80 and CD206 showed that the fluorescence intensity was significantly increased after DHM administration ($p < 0.05$) (Fig. 4B), i.e., DHM administration significantly increased the level of M2-type macrophages and down-regulated the M1-type macrophages. This result was verified by flow cytometry assay, which examined M1-type (CD11b F4/80 iNOS) and M2-type (CD11b F4/80 CD206) macrophages and showed that DHM administration significantly decreased the percentage of M1-type macrophages out of the total macrophages activated by Con A ($p < 0.05$) (Fig. 4C), while increasing the percentage of M2-type macrophages ($p < 0.05$) (Fig. 4D). These findings imply that DHM treatment promotes a shift in inflammatory M1-type macrophages towards the anti-inflammatory M2 subtype in the liver.

Effect of DHM on TNF-α, NF-κB P65 Expression in Liver Homogenates of AIH Mice

In comparison to the Control group, the Con A group's liver tissues exhibited significantly higher levels of TNF-α and NF-κB P65 protein and mRNA expression ($p < 0.05$) (Fig. 5A–C). In contrast to the Con A group, this increase was reversed by the administration of DHM ($p < 0.05$) (Fig. 5D,E). These findings imply that DHM reduces inflammation by inhibiting TNF-α and NF-κB P65 expression.

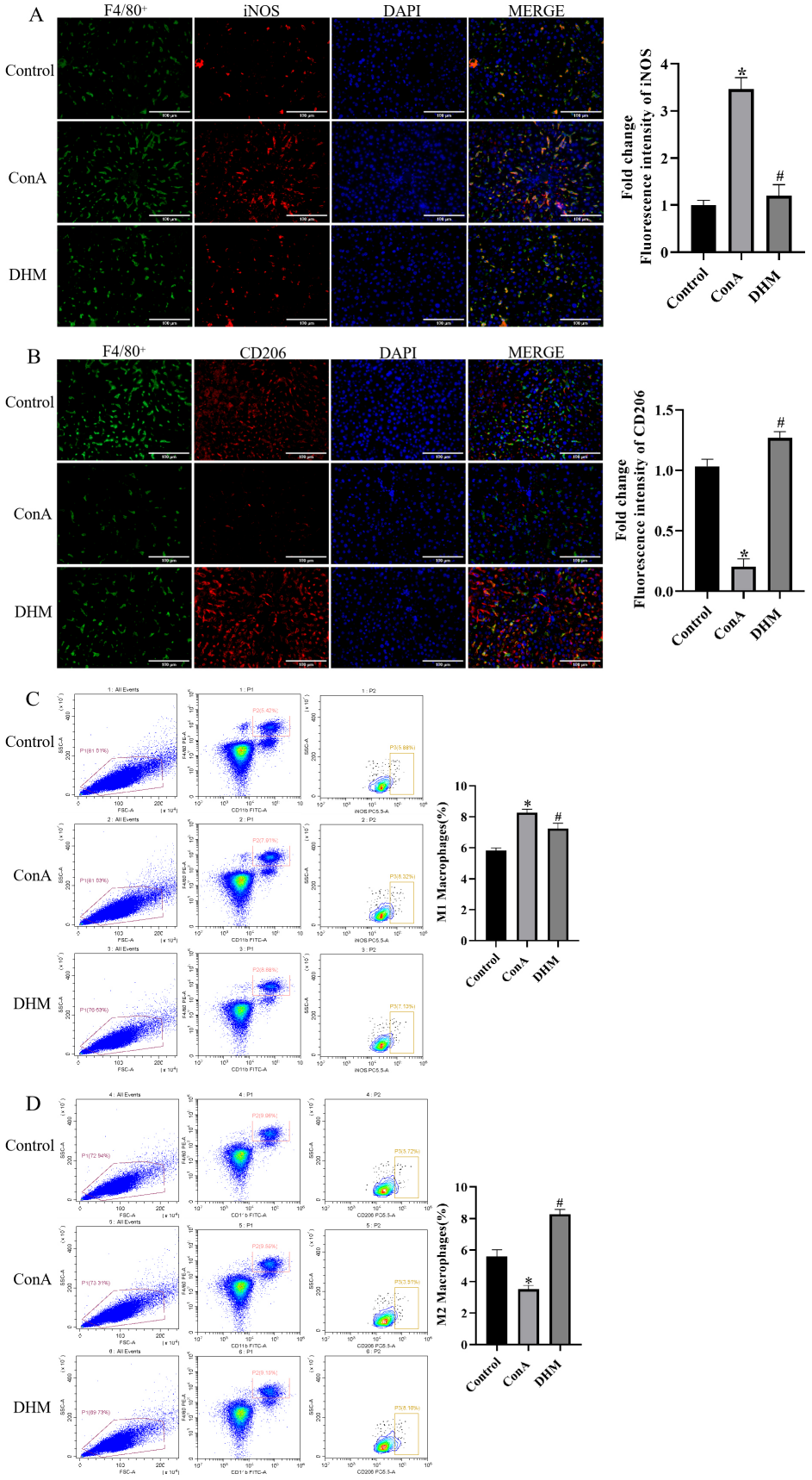

Fig. 4. Effect of DHM on the polarization of hepatic macrophages in AIH mice. (A,B) Immunofluorescence to examine the expression of specific markers in M1 and M2-type macrophages (400×, Scale = 100 μm). (C,D) Flow cytometry to examine the expression of specific markers in M1 and M2-type macrophages. *$p < 0.05$ vs Control, $^{\#}p < 0.05$ vs Con A. n = 3.

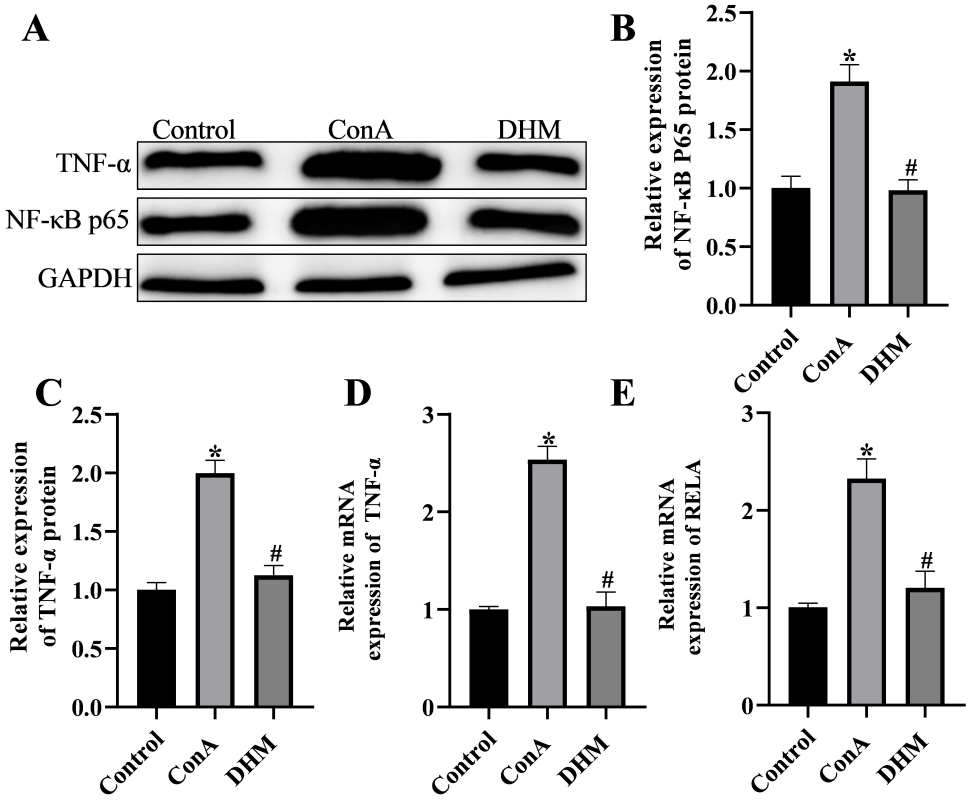

Fig. 5. Effect of DHM on the expression of TNF-α, NF-κB P65 in liver homogenates of AIH mice. (A–C) Western blot to detect the expression of TNF-α and NF-κB P65 protein. (D,E) RT-qPCR to detect the expression of *TNF-α* and *NF-κB P65* (*RELA*) mRNA. *$p <$ 0.05 vs Control, $^{\#}p < 0.05$ vs Con A. n = 3.

Discussion

AIH is a self-destructive liver disease mediated by an autoimmune system and induced by environmental factors. It is found in people of all ages and is highly prevalent in females, accounting for 80%–85% of the total incidence of the disease [12]. Currently, autoimmune hepatitis is mainly treated with nonspecific immunosuppressive drugs to relieve symptoms and improve liver function, hepatic pathology, and tissue damage. The current therapies have limited effects and more adverse reactions include myelosuppression, reduced body resistance to infection, increased incidence of tumors, and risk of teratogenicity and sterility [13]. Numerous recent studies have indicated that DHM possesses anti-inflammatory and anti-tumor properties [14,15]. Previous research has demonstrated that DHM pretreatment in a liver ischemia/reperfusion injury model significantly reduces serum transaminase activity and inhibits apoptosis, providing protective effects for the liver [16]. Nevertheless, its anti-inflammatory mechanism in AIH remains unclear. The current study's findings revealed that following Con A treatment, mice in the Con A group exhibited a substantial increase in serum hepatic function index activities. H&E Staining further revealed extensive infiltration of immune cells and significant pathological changes. The above-mentioned blood indices considerably decreased with DHM treatment and liver histological damage was also improved. These findings imply that DHM may be able to lessen liver damage in mice with autoimmune hepatitis.

DHM has demonstrated robust antioxidant properties both *in vitro* and *in vivo*. In studies involving ischemia-reperfusion, DHM exhibited the ability to mitigate disease progression through its antioxidant effects, reducing infarct size and decreasing apoptosis [17]. Corresponding with previous findings, our study revealed that the significant increase in oxidizing factor levels and the notable decrease in antioxidant factor levels observed in the hepatic tissues of AIH mice were reversed following prophylactic administration of DHM. The study demonstrated the potent anti-inflammatory effects of DHM in CCl4-induced liver injury in mice, resulting in a significant reduction in blood transaminases and pro-inflammatory levels, as well as a marked decrease in apoptotic cell numbers [18]. Our investigation revealed a substantial reduction in anti-inflammatory factor levels and a significant increase in pro-inflammatory factor levels in the liver tissues of the Con A group. DHM treatment effectively reversed these changes. All of these findings point to the possibility that DHM reduces AIH by reducing oxidative damage and encouraging the production of anti-inflammatory molecules. Additionally, it inhibits pro-inflammatory factors from being secreted in the livers of AIH animals.

Macrophages, intrinsic immune cells in the liver, play a crucial role in hepatic homeostasis. Responding to environmental stimuli, macrophages can differentiate into pro-inflammatory M1-type or inflammation-suppressive M2-type. The imbalance of M1/M2-type macrophages can significantly impact the progression of inflammatory diseases, such as autoimmune hepatitis [19]. Previous research has shown that DHM-encapsulated liposomes successfully decreased the inflammation of the liver brought on by intense exercise. This decrease was ascribed to the stimulation of the Sirtuin-3/Hypoxia-inducible factor 1α (SIRT3/HIF-1α) signaling pathway, which led to the polarization of macrophages from the M1 to the M2 subtype [20]. Our investigation found that prophylactic DHM treatment in AIH mice effectively reduced M1-type macrophage expression while elevating M2-type macrophage expression. This implies that M2-type macrophage polarization is a mechanism of DHM-reducing inflammation.

A key signals transducer that starts inflammation, NF-κB's activation is essential for the recruitment and development of macrophages and the subsequent generation of chemokines and cytokines that promote inflammation [21]. TNF-α, a crucial inflammatory mediator, induces an inflammatory response upon overexpression, contributing to immune cell infiltration into the liver and tissue damage. TNF-α stimulates NF-κB activation in the nucleus, which, in turn, activates numerous target genes, including TNF-α itself, establishing a positive cycle of pro-inflammatory responses [22]. The inflammatory factor TNF-α and the transcription factor NF-κB were markedly upregulated in rats with hepatitis caused by Con A [23]. Aligning with prior research, our study demonstrated a significant reduction in the TNF-α and NF-κB P65 expression in the liver tissues of AIH mice following DHM pretreatment administration. This suggests that DHM exerts anti-inflammatory effects by inhibiting the activation of the TNF-α/NF-κB signaling pathway.

Macrophage polarization is regulated by a complex network comprising transcription factors, epigenetic processes, signaling pathways, and post-transcriptional regulators. The NF-κB pathway is generally recognized as a classical pro-inflammatory signaling route due to its function in upregulating the expression of pro-inflammatory genes [24]. In M1 macrophages, NF-κB signaling is typically activated, releasing high quantities of pro-inflammatory cytokines [25]. In a tumor angiogenesis mouse model, pyrrolidine-dithiocarbamate (PDTC) was reported to diminish retinal neovascularization by inhibiting NF-κB, which causes a change in macrophage orientation towards M1 to M2 [26]. According to another study, lean mice's M1 polarization and inflammation decreased when the NF-κB subunit p65 was knocked down in adipocytes and macrophages [27]. A recent study on rheumatoid arthritis found that by inhibiting the NF-κB and ERK pathways, MTX@Tapt-tFNAs exert an excellent anti-inflammatory effect by blocking TNF-α-NF-κB signaling and promoting M2 macrophage polarization [28]. This suggests that the TNF-α/NF-κB pathway may be involved in the macrophage activation process, which exerts an anti-inflammatory effect in AIH mice by regulating macrophage polarization toward the M2 type.

Conclusion

Collectively, the findings from this study strongly indicate that DHM mitigates the inflammatory response in AIH mice by reducing oxidative stress and modulating the TNF-α/NF-κB pathway and macrophage polarization. These findings suggest DHM's potential as a therapeutic agent for AIH. However, additional studies are warranted to corroborate these findings, providing a more comprehensive understanding of its mechanism of action and potential adverse effects before considering its application in clinical therapy.

Availability of Data and Materials

The data used and/or analyzed during the current study are available from the corresponding author.

Author Contributions

XZ, YLiu and KY designed the research study. YLiu, KY, JT and KZ performed the research. XZ, JT and YLi provided help and advice on the experiments. KZ and YLi analyzed the data. All authors contributed to the drafting and critical revision of the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study received approval from the ethics committee of The Third People's Hospital of Chengdu, Affiliated Hospital of Southwest Jiaotong University & The Second Affiliated Hospital of Chengdu, Chongqing Medical University (No.20236735).

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Takahashi A, Ohira H. Autoimmune hepatitis, fatty liver, and Fukushima. Fukushima Journal of Medical Science. 2019; 65: 25–29.

[2] Chaiteerakij R, Sanpawat A, Avihingsanon A, Treeprasertsuk S. Autoimmune hepatitis in human immunodeficiency virus-infected patients: A case series and review of the literature. World Journal of Gastroenterology. 2019; 25: 5388–5402.

[3] Wu L, Wang C, Li J, Li S, Feng J, Liu T, *et al*. Hepatoprotective effect of quercetin via TRAF6/JNK pathway in acute hepatitis. Biomedicine & Pharmacotherapy. 2017; 96: 1137–1146.

[4] Liu Y, Hao H, Hou T. Concanavalin A-induced autoimmune hepatitis model in mice: Mechanisms and future outlook. Open Life Sciences. 2022; 17: 91–101.

[5] Yunna C, Mengru H, Lei W, Weidong C. Macrophage M1/M2 polarization. European Journal of Pharmacology. 2020; 877: 173090.

[6] Peng Y, Zhou M, Yang H, Qu R, Qiu Y, Hao J, *et al*. Regulatory Mechanism of M1/M2 Macrophage Polarization in the Development of Autoimmune Diseases. Mediators of Inflammation. 2023; 2023: 8821610.

[7] Chi G, Pei J, Li X. The imbalance of liver resident macrophages polarization promotes chronic autoimmune hepatitis development in mice. PeerJ. 2023; 11: e14871.

[8] Zhang J, Chen Y, Luo H, Sun L, Xu M, Yu J, *et al*. Recent Update on the Pharmacological Effects and Mechanisms of Dihydromyricetin. Frontiers in Pharmacology. 2018; 9: 1204.

[9] Xie C, Chen Z, Zhang C, Xu X, Jin J, Zhan W, *et al*. Dihydromyricetin ameliorates oleic acid-induced lipid accumulation in L02 and HepG2 cells by inhibiting lipogenesis and oxidative stress. Life Sciences. 2016; 157: 131–139.

[10] Liu TT, Zeng Y, Tang K, Chen X, Zhang W, Xu XL. Dihydromyricetin ameliorates atherosclerosis in LDL receptor deficient mice. Atherosclerosis. 2017; 262: 39–50.

[11] Yang Z, Li T, Wang C, Meng M, Tan S, Chen L. Dihydromyricetin Inhibits M1 Macrophage Polarization in Atherosclerosis by Modulating miR-9-Mediated SIRT1/NF-κB Signaling Pathway. Mediators of Inflammation. 2023; 2023: 2547588.

[12] Que W, Lin H, Li X, Zhang B, Liu M, Hu X, *et al*. Koumine ameliorates concanavalin A-induced autoimmune hepatitis in mice: involvement of the Nrf2, NF-κB pathways, and gut microbiota. International Immunopharmacology. 2023; 114: 109573.

[13] Pape S, Schramm C, Gevers TJ. Clinical management of autoimmune hepatitis. United European Gastroenterology Journal. 2019; 7: 1156–1163.

[14] Hu Q, Zhang T, Yi L, Zhou X, Mi M. Dihydromyricetin inhibits NLRP3 inflammasome-dependent pyroptosis by activating the Nrf2 signaling pathway in vascular endothelial cells. BioFactors. 2018; 44: 123–136.

[15] Fu CG, Liu S, Deng T. Inhibitory effect of dihydromyricetin combined with radiotherapy on tumor growth in breast cancer-bearing mice. Chinese Journal of Clinical Pharmacology. 2020; 36: 2455–2458.

[16] Chen Y, Lv L, Pi H, Qin W, Chen J, Guo D, *et al*. Dihydromyricetin protects against liver ischemia/reperfusion induced apoptosis via activation of FOXO3a-mediated autophagy. Oncotarget. 2016; 7: 76508–76522.

[17] Wasan H, Singh D, Joshi B, Upadhyay D, Sharma U, Dinda AK, *et al*. Dihydromyricetin alleviates cerebral ischemia-reperfusion injury by attenuating apoptosis and astrogliosis in peri-infarct cortex. Neurological Research. 2022; 44: 403–414.

[18] Xie J, Liu J, Chen TM, Lan Q, Zhang QY, Liu B, *et al*. Dihydromyricetin alleviates carbon tetrachloride-induced acute liver injury via JNK-dependent mechanism in mice. World Journal of Gastroenterology. 2015; 21: 5473–5481.

[19] Wang Y, Guo X, Jiao G, Luo L, Zhou L, Zhang J, *et al*. Splenectomy Promotes Macrophage Polarization in a Mouse Model of Concanavalin A- (ConA-) Induced Liver Fibrosis. BioMed Research International. 2019; 2019: 5756189.

[20] Zhou X, Yi L, Lang H, Zhang J, Zhang Q, Yu L, *et al*. Dihydromyricetin-Encapsulated Liposomes Inhibit Exhaustive Exercise-Induced Liver Inflammation by Orchestrating M1/M2 Macrophage Polarization. Frontiers in Pharmacology. 2022; 13: 887263.

[21] Pagliari LJ, Perlman H, Liu H, Pope RM. Macrophages require constitutive NF-kappaB activation to maintain A1 expression and mitochondrial homeostasis. Molecular and Cellular Biology. 2000; 20: 8855–8865.

[22] Mussbacher M, Derler M, Basílio J, Schmid JA. NF-κB in monocytes and macrophages - an inflammatory master regulator in multitalented immune cells. Frontiers in Immunology. 2023; 14: 1134661.

[23] Ibrahim KM, Ahmed HI, Ramadan L, Balah A. A low dose of naloxone mitigates autoimmune hepatitis by regulating TLR4/NF-κB and Nrf2/HO-1 signaling pathways. Inflammopharmacology. 2023; 31: 2467–2478.

[24] Jia S, Si R, Liu G, Zhong Q. Diosgenin protects against cationic bovine serum albumin-induced membranous glomerulonephritis by attenuating oxidative stress and renal inflammation via the NF-κB pathway. Pharmaceutical Biology. 2024; 62: 285–295.

[25] Li GL, Tang JF, Tan WL, Zhang T, Zeng D, Zhao S, *et al*. The anti-hepatocellular carcinoma effects of polysaccharides from *Ganoderma lucidum* by regulating macrophage polarization *via* the MAPK/NF-κB signaling pathway. Food & Function. 2023; 14: 3155–3168.

[26] Sui A, Chen X, Demetriades AM, Shen J, Cai Y, Yao Y, *et al*. Inhibiting NF-κB Signaling Activation Reduces Retinal Neovascularization by Promoting a Polarization Shift in Macrophages. Investigative Ophthalmology & Visual Science. 2020; 61: 4.

[27] Gao Z, Zhang J, Henagan TM, Lee JH, Ye X, Wang H, *et al*. P65 inactivation in adipocytes and macrophages attenuates adipose inflammatory response in lean but not in obese mice. American Journal of Physiology. Endocrinology and Metabolism. 2015; 308: E496–E505.

[28] Zhang M, Wen Y, Huang Z, Qin X, Zhou M, Xiao D, *et al*. Targeted therapy for autoimmune diseases based on multifunctional frame nucleic acid system: blocking TNF-α-NF-κB signaling and mediating macrophage polarization. Chemical Engineering Journal. 2023; 454: 140399.