Hindawi
Journal of Chemistry
Volume 2023, Article ID 5224921, 10 pages
https://doi.org/10.1155/2023/5224921

Research Article

Emodin Alleviates Lupus Nephritis in Rats by Regulating M1/M2 Macrophage Polarization

Weijian Xiong, Ying Li, Ling Zhang, Yanying Xiong, Jin Zhong, Xunjia Li, Yan Luo, Hong Liu, Renhong Kang, and Yunjie Chen

Department of Nephrology, Chongqing Traditional Chinese Medicine Hospital, Chongqing 400000, China

Correspondence should be addressed to Ying Li; sabrina_pipi@cdutcm.edu.cn and Ling Zhang; 1648770473@qq.com

Received 26 August 2022; Revised 23 September 2022; Accepted 28 September 2022; Published 4 February 2023

Academic Editor: Muhammad Zia-Ul-Haq

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Lupus nephritis (LN) is one of the most common clinical manifestations of systemic lupus erythematosus (SLE), causing death and disability. The current research study explored whether there was any improvement effect on LN after emodin administration. Network pharmacology was used to screen the target genes of emodin for the treatment of LN. LPS and IL-4 were employed for RAW264.7 macrophage M1/M2 polarization induction, and 0.1% $HgCl_2$ was used for the LN rat model's establishment. Flow cytometry was performed to detect the effect of 20, 40, and 80 μM emodin on RAW264.7 macrophage polarization. HE and PAS staining were subsequently conducted to detect 70 mg/kg emodin action on renal injury in LN rats. The effect of emodin on the content of urinary proteins and dsDNA antibodies was also determined. The results indicated that peroxisome proliferators-activated receptors gamma (PPARG) may be a target gene of emodin in LN, and emodin had no significant toxicity to macrophages at different concentrations. Compared with the control, emodin significantly inhibited LPS-induced polarization in M1 macrophages and improved that of IL-4-induced M2 macrophages. Besides, emodin alleviated kidney injury and markedly reduced the levels of urinary protein and dsDNA antibodies in rats. Moreover, after targeting interference with the PPARG expression, the improvement effect of emodin on LN is significantly reduced, indicating that emodin may relieve the symptoms of LN by activating the PPARG expression. Our study revealed that PPARG may be applied as a new therapy for LN.

1. Introduction

Systemic lupus erythematosus (SLE), a serious systemic autoimmune disease, affects most females of childbearing age, especially pregnant women [1]. During the disease progression of SLE patients, there are often severe inflammatory responses, immune dysfunction, abnormal recognition of autoantibodies and nonautoantibodies, and immune tolerance disorders during the progression of the disease. Clinically, more than half of SLE patients present with symptoms of kidney injury, which eventually progress to lupus nephritis (LN) if left untreated. The clinical symptoms of LN include hematuria, proteinuria, elevated markers of renal injury, as well as serum creatinine, antidouble-stranded DNA titers, a progressive decline in renal function, and eventual death due to renal failure [2–4]. Hematuria, proteinuria, renal injury, elevated markers of renal failure, and renal failure are also present.

The pathological mechanism of LN has not been clarified, but some studies have confirmed that abnormal activation and differentiation of immunocytes play key biological roles in LN pathogenesis [5, 6]. The infiltrating cells and macrophages in the kidney of LN patients can be roughly polarized into two phenotypes, namely M1-type macrophages secreting inducible nitric oxide synthase (iNOS) and M2-type macrophages secreting arginase-1 (Arg-1). In LN, M1 macrophages exert a proinflammatory and profibrotic role, while M2 macrophages are responsible for antiinflammation, which promotes the reconstruction and repair of injured body sites [7]. The most ideal goal of the treatment of autoimmune diseases is immune reconstruction to restore the body's immune tolerance to autoantigens, but it has not been achieved so far. Immunosuppressive therapy is still the main treatment for autoimmune diseases in clinical practice. Most of the

mechanisms of existing immunosuppressive agents are cytotoxicity, antimetabolism, and inhibition of signal transduction, which often have inhibitory or even killing effects on immune cells and cells of other systems, causing a wide range of adverse reactions [8].

Emodin, known as 1,3,8-trihydroxy-6-methylanthraquinone, is a free anthraquinone derivative and a major effective monomer of rhubarb. Emodin is one of the most common traditional Chinese medicines and has abundant resources and broad research prospects. The main effect of emodin is to treat dry stools and hot knot constipation. Emodin has recently indicated multiple pharmacological effects, such as antiallergy, antitumor, antiviral, antibacterial, antiosteoporosis, antidiabetes, immunity inhibition, nerve protection, and liver protection [9–11]. Additional studies have also demonstrated the anti-inflammation action of emodin. Emodin can dose-dependently hinder IκB degradation and NF-κB activation after LPS induction, which reduces concentrations of inflammatory cytokines IL-6 and IL-1β and the expressions of chemokines CCL2 and IL-8 [12]. Ha et al. have indicated the roles of emodin dose-dependently in inhibiting the secretion of inflammatory mediators IL-6, IL-8, IL-1β, and TNF-α and downregulating VEGF, MMP-1, MMP-13, and PGE2 levels in a rheumatoid arthritis cell model. It is suggested that emodin inhibits the production of inflammatory cytokines and VEGF due to its anti-inflammation function [13]. The study by Alisi et al. has shown that emodin restricts the increase of TNF-α induced by hyper glucose and high-fat diets in rats, thus hiding steatohepatitis [14]. The results of Song et al. have indicated that emodin can enhance the left ventricular function of rats with experimental autoimmune myocarditis by gavage, thereby reducing the severity of myocarditis [15].

Unluckily, few scientific reports have been found investigating the ameliorative effects of emodin on LN. To the best of our knowledge, only Liu et al. found that emodin inhibits proliferation and promotes apoptosis in LN patients' kidney fibroblasts, which may improve the prognosis of LN [16]. Recently, emodin has been reported to inhibit T-lymphocyte-like receptor 4 expression in renal tubular epithelial cells induced by lipopolysaccharide, and down-regulate the synthesis of IL-6 and TNF-α [17]. Meanwhile, the number of regulatory T cells can be increased, and dendritic cells can be mediated by emodin during the processes of differentiation and maturation [18]. The findings demonstrated the role of emodin in the regulation of immune responses. Therefore, the present work attempted to investigate whether emodin can improve LN symptoms by affecting macrophages, and hopefully offer a theoretical basis for the application of emodin again LN.

2. Methods

2.1. Network Pharmacology. The predicted targets of emodin were exported using the websites PubChem (https://pubchem.ncbi.nlm.nih.gov) and SwissTargetPrediction (https://www.swisstargetprediction.ch/), and the disease targets of LN were obtained from GeneCards (https://www.genecards.org). Overlapped targets of emodin and LN were uploaded to STRING and information on protein-protein interaction (PPI) was collected for the construction of a PPI network diagram. To illustrate the role of emodin targets in gene function and signaling pathways, ClueGo, a plug-in of Cytoscape 3.6.1, was employed for GO enrichment analysis and KEGG pathway annotation to identify possible targets of emodin in LN treatment, and the analysis results were visualized.

2.2. Cell Culture. The macrophage RAW264.7 cell line (SCSP-5036) was obtained from the Cell Bank of the Chinese Academy of Sciences. DMEM containing 10% FBS (Gibco, USA), 1% Glutamax (35050061, Invitrogen, USA), and 1% sodium pyruvate 100 mM Solution (11360070, Invitrogen, USA) was used for culture with 5% CO_2 at 37°C.

2.3. Preparation of Emodin. Emodin (HY-14393, CAS No. 518-82-1) was purchased from MedChemExpress, USA. For introduction experiments, emodin was dissolved in dimethyl sulfoxide at 20, 40, and 80 mM and diluted 1000 times to 20, 40, and 80 μM when used. For in vivo experiments, emodin was dissolved in 0.5% carboxymethylcellulose at 4 mg/mL.

2.4. MTT Assay. We employed the MTT cell proliferation and cytotoxicity assay kit (M1020, Solarbio, Beijing, China) to determine cell viability. Following cell collection in the logarithmic phase, the cells were inoculated into 96-well plates at a density of 1×10^6/mL, or 100 μL per well. Culture at 37°C and 5% CO_2 for 12 hours. The 96-well plates were removed and cultured with low, medium, and high concentrations (20, 40, and 80 μM) of emodin for 6 and 12 h, respectively. After the supernatant was carefully sucked away, 90 μL DMEM and 10 μL MTT solutions were added for culture for another 4 h. The supernatant was discarded again. Of 110 μL formazan solution was supplied to each well and mixed well by vibrating 10 min mildly to fully dissolve the crystals. The absorbance of each well was detected at 490 nm by ELISA. Cell viability was computed using the formula as follows:

$$\text{Cell viability} = \frac{\left(A_{\text{test}} - A_{\text{blank}}\right)}{\left(A_{\text{control}} - A_{\text{blank}}\right)} \times 100\%. \tag{1}$$

2.5. Flow Cytometry. RAW264.7 cells at a density of 1×10^6/mL were inoculated into 6-well plates, 2 mL for each well, and placed in the cell incubator for further culture overnight until they adhered to the wall. Then we discarded the medium and washed it twice with PBS. The polarization of RAW264.7 M1 was induced by 1 μg/mL LPS. The control group was cultured with a complete medium. The LPS group was cultured with a complete medium containing 1 μg/mL LPS. Low, medium, and high dose emodin groups were cultured with a complete medium containing 1 μg/mL LPS + 20 μM emodin, 1 μg/mL LPS + 40 μM emodin, and 1 μg/mL LPS + 80 μM emodin, respectively. Raw264.7 M2 polarization was induced by 20 ng/mL IL-4. The grouping

and dosage of emodin in M2 polarization detection were the same as in M1 polarization detection. After the cells were cultured for 48 h, the M1 marker F4/80$^+$CD86$^+$ and the M2 marker F4/80$^+$CD206$^+$ were detected by flow cytometry.

2.6. Lentivirus Transfection. The targeted interference of the PPARG lentivirus vector was constructed by Chongqing Biomedicine Biotechnology Co. Ltd. RAW264.7 cells were inoculated onto plates, cultured for 12 h, and divided into 3 groups. The blank group had no virus infection, the vector group was given a negative control virus, and the interference group had a lentivirus containing the PPARG interference sequence. 48 h after infection, the culture solution was replaced with DMEM medium containing 4 mg/L puromycin for 2 days to screen for resistant cells. The cells were then used for flow cytometry detection.

2.7. Animal Modeling and Grouping. Twenty SPF SD rats (male, weighing 180–220 g) were provided by Chongqing Enswell Biotechnology Co., Ltd. After 7 days of adaptive feeding in the animal room, the laboratory animals were grouped into control, model, emodin, and PPARG lentivirus interference groups. The experiment lasted for 14 days. Except for the control group, which was injected with an identical volume of pH = 3.8 water for injection (prepared with 0.1 M hydrochloric acid), the LN model was established by subcutaneous injection of 0.1% $HgCl_2$ (1 mg/kg) into a foot pad, once every 2 days. The emodin treatment group received an intraperitoneal injection of 70 mg/kg of emodin per day. The lentivirus group was first injected in situ with PPARG lentivirus at 5 injection sites (10 μL for each site) and then injected with emodin at 70 mg/kg daily. Animal room temperature was maintained at 23–25°C with 12 h/12 h light and dark alternately. Eating and drinking were provided ad libitum.

2.8. Detection of Urinary Protein and Double-Stranded DNA Antibodies in Rats. Following 24 h of the last administration, urine and blood samples of rats were collected using a sterile centrifugal tube, and urine protein and serum dsDNA antibody levels of rats were detected as per the instructions of ELISA kits. Rat urine protein detection kits (RX302447R) and dsDNA antibody detection kits (RX302544R) were from Quanzhou Ruixin Biotechnology Co., Ltd.

2.9. HE Staining. Twenty-four hours following the last administration, laboratory animals were anesthetized by intraperitoneal injection of 2% pentobarbital sodium (0.2 mL/100 g) and then sacrificed. Kidney tissue was collected for 24 h of fixation using 4% paraformaldehyde. The tissue was paraffin-embedded as usual and sectioned subsequently. Xylene was used for dewaxing twice, 5 min each. anhydrous ethanol was used for immersion twice, 5 min each. 95%, 85%, and 75% ethanol were treated for 2 min at each concentration. Distilled water was used for immersion for 2 min, and then the samples were stained with hematoxylin staining solution for 10 min followed by a 10-second rinse using running water. The differentiation solution was differentiated for 5 seconds, and the water was washed for 30 seconds. The eosin staining solution was dyed for 2 min and then washed with running water for 5 s. The samples were soaked with ethanol at 75%, 85%, 95%, and 100% (I) for 3 s and 100% ethanol (II) for 1 min, followed by xylene transparency twice, each time for 1 min. Finally, the section was sealed with neutral gum and photographed under a microscope (CX43, Olympus, Japan).

2.10. PAS Staining. Paraffin sections of kidney tissue were taken and rinsed with running water for 2 min, then rinsed with distilled water twice. The slices were placed and kept in an oxidizer at room temperature for 5 min, rinsed once with tap water, and then immersed twice in distilled water. Slices were immersed in Schiff staining solution and dyed in dark at room temperature for 10 min following a 10-min cycle of rinsing with running water. The slices were kept in the hematoxylin staining solution for 2 min. An acidic differentiation solution was used for differentiation for 5 s. After rinsing with tap water for 10 min, step-by-step ethanol dehydration and transparent xylene and neutral gum sealing were performed according to the HE staining steps.

2.11. Data Analysis. GraphPad 8.0 was employed for data analysis, which was then expressed as mean ± standard deviation. A one-way ANOVA was used for multiple group comparisons and $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. PPAGR may be the Target Gene for Emodin in Treating LN. Through network pharmacology analysis, 70 genes were predicted to intersect with LN (Figure 1(a)), and the top five connected nodes by degree were HSP90AA1, EGFR, ESR1, BCL2L1, and RRARG. According to the analysis results and literature review, PPARG played an essential role in macrophage polarization, and it was speculated that it also played an important role in the emodin improvement of LN. GO functional enrichment analysis was also carried out to analyze overlapped genes, and the top 10 terms were subsequently visualized. As shown in Figure 1(b), among the first 10 items, one of them belongs to molecular function, heat shock protein binding. The other 9 are biological processes, which are related to inflammatory response regulation and macrophage differentiation and activation. It is suggested that emodin may be associated with macrophages in alleviating LN symptoms. In KEGG enrichment analysis results, the PI3K-Akt signaling pathway enrichment index was the highest (Figure 1(c)), indicating that emodin regulation of the disease may be achieved by regulating PPARG activation of this signaling pathway.

3.2. Emodin Has No Significant Toxicity to Macrophages. To verify whether there is any influence of emodin at different concentrations on RAW264.7 cell viability,

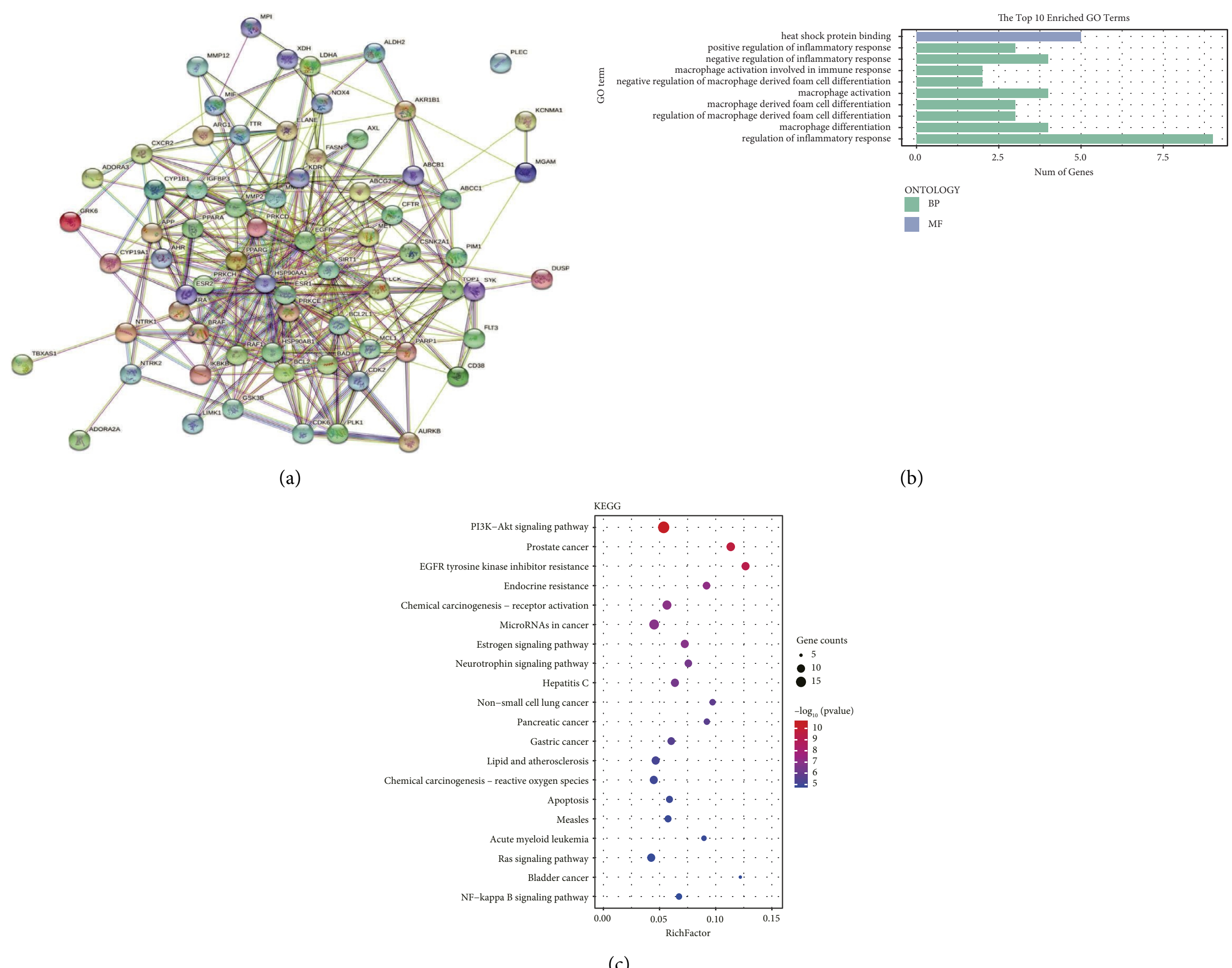


Figure 1: Results of network pharmacology analysis. (a) GO enrichment analysis; (b) PPI network diagram; and (c) KEGG enrichment analysis.

RAW264.7 cells were treated with low, medium, and high concentrations (20, 40, and 80 μM) of emodin for 6 h and 12 h, respectively. MTT results showed that no significant decrease in cell viability was observed regardless of whether emodin was used for 6 h (Figure 2(a)) or 12 h (Figure 2(b)), indicating that emodin had no significant toxicity to cells at 20, 40, and 80 μM.

3.3. Emodin Drives Macrophage Polarization Toward Anti-Inflammatory M2 Phenotypes. The polarization direction of macrophages is positively associated with the inflammatory response during persistent tissue injury, and network pharmacological analysis suggested that macrophages play a role in the improvement of LN. Therefore, M1-type polarization markers F4/80$^+$CD86$^+$ and M2-type polarization markers F4/80$^+$CD206$^+$ were detected by flow cytometry for evaluating emodin's action on cell polarization, implying that the detection rates of F4/80$^+$CD86$^+$ and F4/80$^+$CD206$^+$ were markedly higher than control after LPS and IL-4 induction, respectively (Figures 3(a) and 3(b)) ($P < 0.01$). Compared with the LPS group, emodin at low, medium, and high concentrations significantly reduced the macrophages polarized towards M1 phenotypes ($P < 0.01$). However, only medium- and high-concentration emodin greatly promoted the macrophages polarized towards M2 phenotypes ($P < 0.01$) compared with IL-4 group.

3.4. Interference with PPARG Expression Decreases M2 Phenotypes Polarization. To verify if PPARG can affect the therapeutic effect of LN, this study used lentiviral vectors to target macrophages infected by PPARG for 2 d and induced macrophage polarization with IL-4 and LPS, respectively. The detection rate of M1 macrophage marker F4/80$^+$CD86$^+$ indicated a marked increase after lentivirus interfered with the PPARG gene versus LPS + vector group (Figure 4(a)) ($P < 0.01$). The detection rate of M2 macrophage marker F4/80$^+$CD206$^+$ was also much higher than that of the IL-4 + vector group (Figure 4(b)) ($P < 0.01$), suggesting that PPARG gene exerts a decisive role in macrophage polarization.

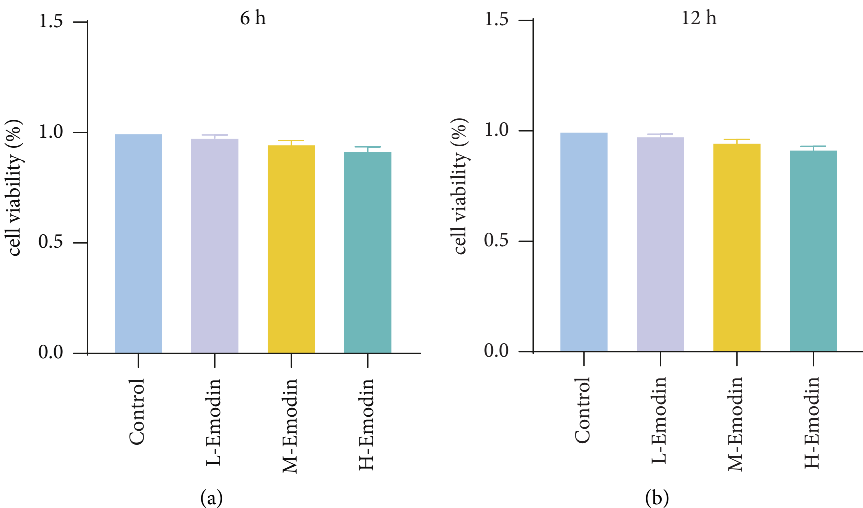


FIGURE 2: Emodin effects on RAW264.7 cell viability ($n = 3$). (a) Cell viability after 6 h of emodin treatment; (b) cell viability after 12 h treatment with emodin.

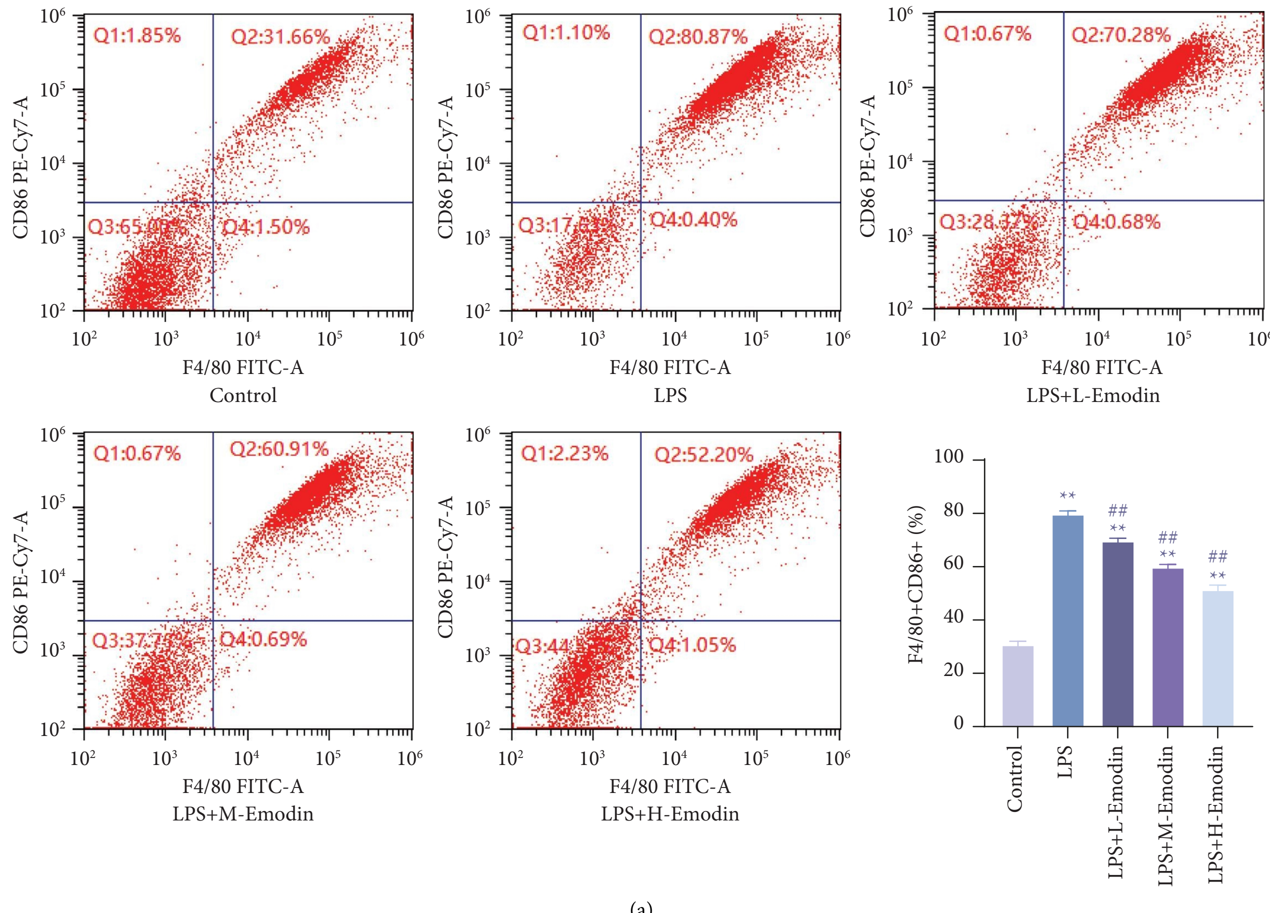
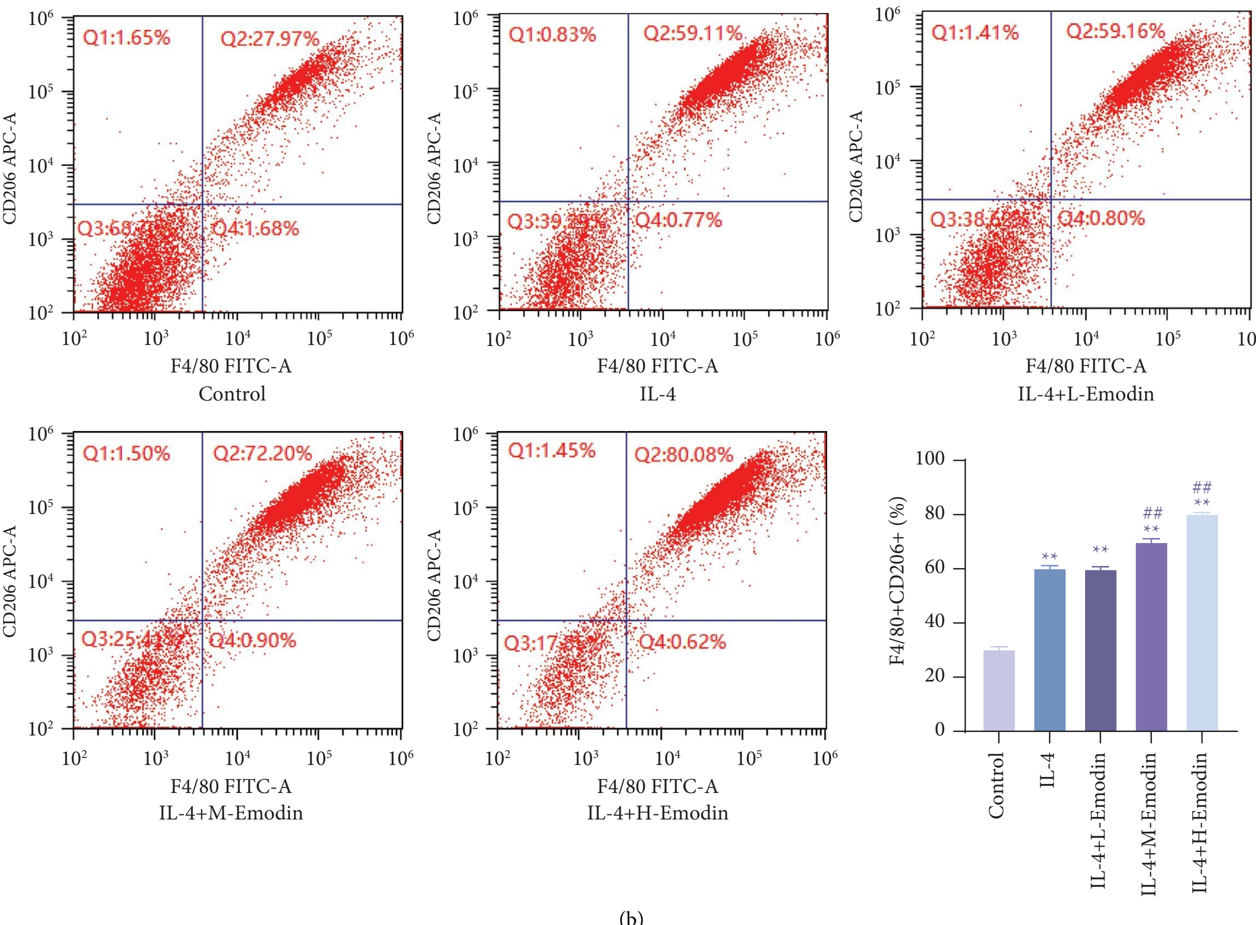


FIGURE 3: Continued.



(b)

Figure 3: Emodin effects on the polarization of macrophages were detected by flow cytometry ($n = 3$). (a) Emodin inhibits LPS-induced polarization of M1 macrophages; (b) Emodin promotes IL-4-induced polarization of M2 macrophages. $^{**}P < 0.01$ vs. control; $^{\#\#}P < 0.01$ vs. IL-4 or LPS.

3.5. Emodin Ameliorates Kidney Injury in LN Rats. In order to better reveal the action of emodin, the present study constructed the rat LN model using 0.1% mercury chloride, collected the kidney, and observed pathological alternations in the renal structure through HE and PAS staining. HE staining indicated normal glomeruli in the control group in both size and shape, with clear boundaries, no inflammatory cell infiltration in the renal interstitium, and complete brush border margin structure inside the renal tubules without obvious abnormalities (Figure 5(a)). In the model group, the glomerular cavity was shrunk, part of the cyst wall adhered to the glomerulus, and a large number of red blood cells were infiltrated between the glomeruli. Inflammatory cell infiltration and obvious bleeding were seen in the renal interstitium. The tubule epithelial cells were swollen and protruded into the lumen, which occluded the lumen. The tubule epithelial cells were lytic necrotic and some cells vacuolated. In the interference group, some renal tubules were swollen, the epithelial cell structure was destroyed, and cell fragments and inflammatory cells were found in the lumen. Inflammatory cells are occasionally seen in the renal interstitium. In the emodin group, some renal tubular epithelial cells were atrophied and decreased, the renal tubular structure was destroyed, and intertubular space increased but decreased inflammatory cells. PAS findings revealed that the glomerular vascular loops in the control group were thin and clear with normal structure (Figure 5(b)). In the model group, glomerular pagination atrophy, a large number of PAS-positive substances in the mesangium, and positive deposits in the lumen of renal tubular epithelial cells were observed. The volume of the basal glomerulus was reduced, and PAS-positive substances were accumulated in the mesangium in the interference group vs. the control group. Glomerular atrophy was slight in the emodin group. These results suggest that emodin can effectively reduce the renal inflammatory response in LN rats, but the inflammatory response is aggravated after interfering with PPARG.

3.6. Emodin Decreases Urinary Protein and dsDNA Antibody Levels in LN Rats. Proteinuria is the main clinical urine index used to detect nephritis, and the dsDNA antibody is a serum marker of the autoimmune disease SLE. The detection of these two indicators can predict kidney inflammation and immune tolerance. Proteinuria and dsDNA antibody levels of experimental group rats were found markedly higher than in the control (Figures 6(a) and 6(b))

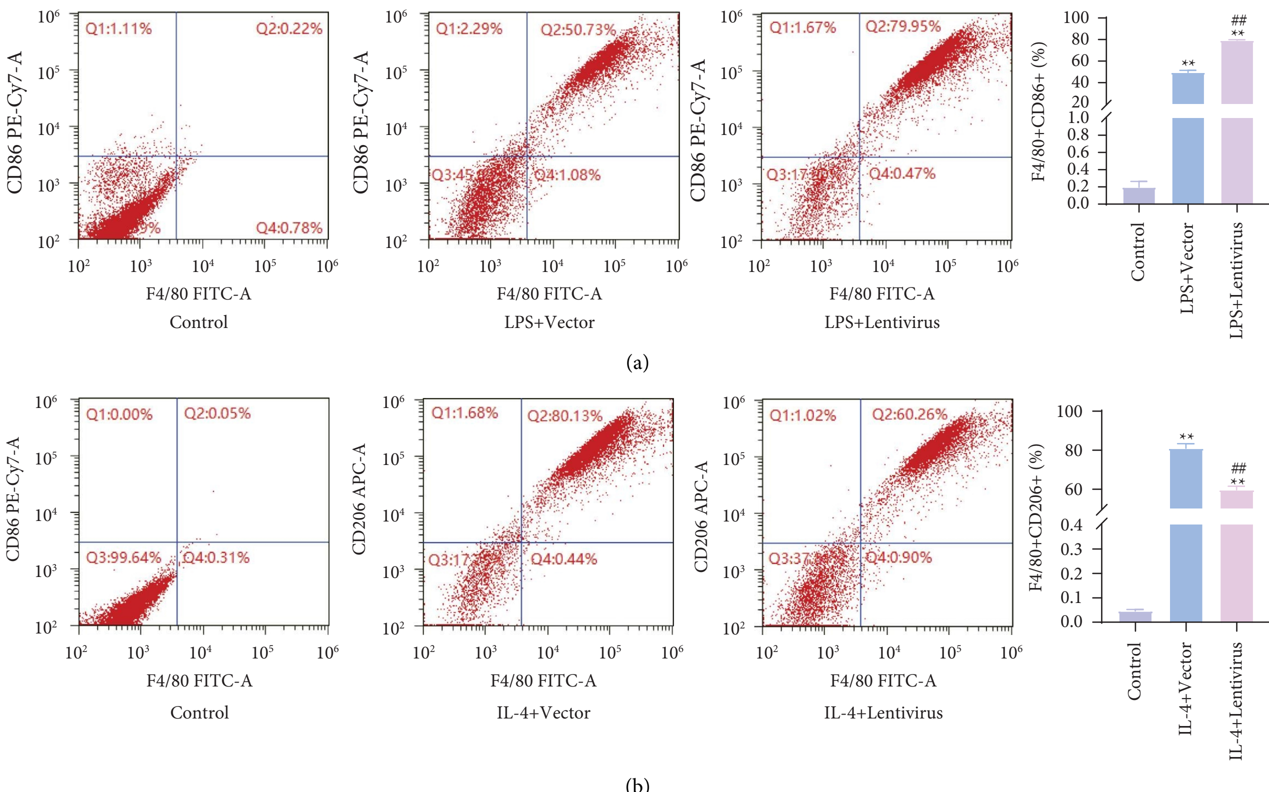


Figure 4: Effect of the PPARG interference on polarization of macrophages ($n = 3$). (a) Interference with PPARG promoted LPS-induced polarization of M1 macrophages; (b) interference with PPARG inhibits IL-4 induced polarization of M2 macrophages. $^{**}P < 0.01$ vs. Control; $^{\#\#}P < 0.01$ vs. Model.

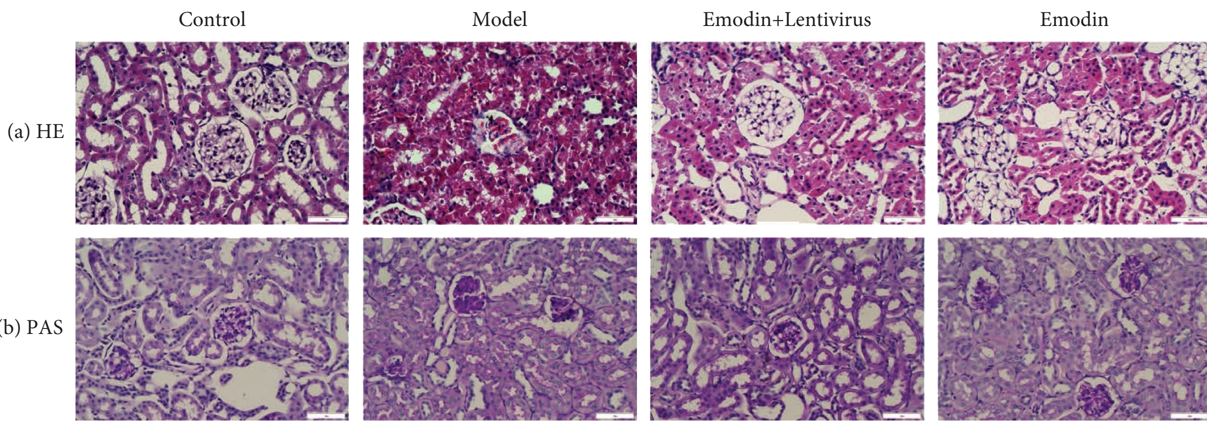


Figure 5: Effect of emodin on renal tissue injury in LN rats. (a) HE staining; (b) PAS staining. In PAS staining, the nucleus is blue, and the mesangial matrix, collagen fiber, basement membrane, fibrin, vascular hyaline, amyloidosis, and other positive reactions can be bright pink. Scale bar, 50 μm.

($P < 0.01$), indicating the presence of impairment in glomerular filtration function in experimental rats. After 14 d of administration of 70 mg/kg emodin by intraperitoneal injection, levels of urinary protein and dsDNA in rats were greatly lower than in the model ($P < 0.01$), indicating that emodin had a role in alleviating the damage to glomerular function caused by LN.

4. Discussion

In this study, 70 targets related to emodin therapy for LN were first screened through network pharmacology, and PPARG was predicted to play a vital role in the process. Then, through GO analysis, most of the targets were identified as being enriched in heat shock protein binding,

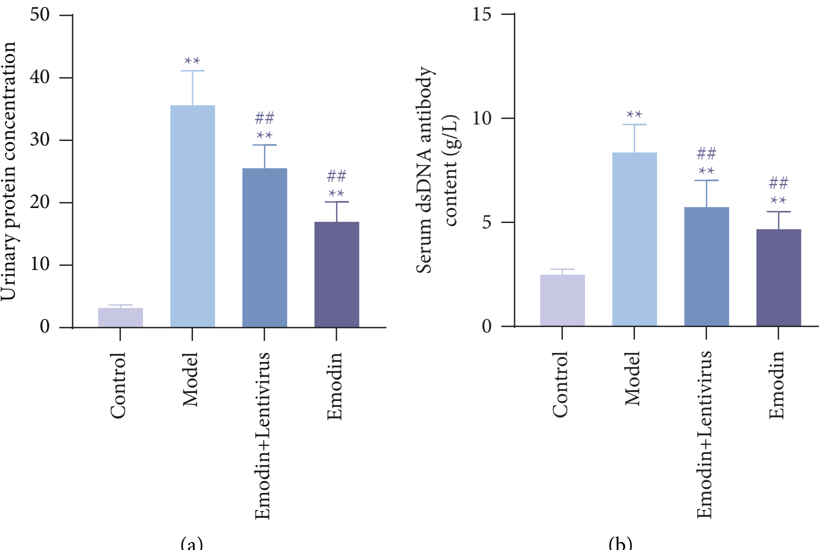


FIGURE 6: Effect of emodin on urinary proteins and serum dsDNA antibodies in LN rats ($n = 3$). (a) Urinary protein levels; (b) dsDNA antibody levels. $^{**}P < 0.01$ vs. Control; $^{\#\#}P < 0.01$ vs. Model.

immune response regulation, macrophage differentiation, etc. KEGG analysis found that the most related targets were involved in the PI3K-Akt signaling pathway. Therefore, based on network pharmacology analysis, we carried out subsequent related tests using cells and animals, respectively. First, the MTT assay was used to verify that emodin at 20, 40, and 80 μM had no significant toxicity to rat macrophages. Emodin inhibited polarization of the M1 phenotype while promoting polarization of the M2 phenotype. Then, after the targeted interference of the PPARG gene with lentivirus, the polarization direction of macrophages was significantly affected, namely, M1-type polarization increased while M2-type polarization decreased.

Macrophages are derived from monocytes in the blood circulation system (M0 macrophages) and differentiate into peripheral tissues, which are highly heterogeneous. Its role includes participating in immune response, regulating metabolism, maintaining homeostasis of the internal environment, and participating in angiogenesis, especially playing an important role in the inflammatory response [18, 19]. Mononuclear macrophages can develop into different subpopulations according to different activation stimuli, such as the proinflammatory macrophage population (M1) formed by the classical activation pathway or the anti-inflammatory macrophage population (M2) formed by substituted activation [20]. M0 macrophages are activated to M1 macrophages after being stimulated by lipopolysaccharide (LPS), tumor necrosis factor, interferon γ, and granulocyte-macrophage colony-stimulating factor, which highly express IL-12 and IL-23. Reactive oxygen species, TNF-α, nitric oxide, inducible nitric oxide synthase, monocyte chemoattractant protein 1 (MCP- 1), and other proinflammatory factors, participate in the immune process of antigen presentation and promote the inflammatory immune response [21]. M0 macrophages are activated into M2-type macrophages after induced stimulation by IL-1, IL-4, TGF-β, and glucocorticoid, which highly express anti-inflammatory factors TGF-β, IL-L0, IL-6, IL-10, and arginase-1 to inhibit immune response and inflammatory immune response promote angiogenesis, tissue repair, and remodeling [22]. M1- and M2-type macrophages can undergo phenotypic transformation under certain in vivo conditions or under the action of certain endogenous substances. For example, M1-type macrophages can transform into M2-type macrophages during renal repair to provide nutrients and promote renal tubule repair [23]. In LN, M1-type macrophages mainly promote inflammation and fibrosis, while M2-type macrophages mainly inhibit inflammation but facilitate the repair and reconstruction of injured tissues. It has been reported that the mouse model of nephritis has been greatly improved following M1-type macrophage depletion, recruitment inhibition, and polarization interruption of inflammatory macrophages [24]. Studies have suggested that LN renal damage is associated with M1-type macrophage infiltration and lymphocyte infiltration. Meanwhile, being an inflammatory factor IFN-γ, secreted by M1-type macrophages, promotes macrophage recruitment and up-regulates inflammatory immune response [25]. In addition, M1-type macrophages in LN have high expression of the proinflammatory factor MCP-1. Studies have used gene knockout mice or inhibitors to block MCP-1 or its receptors to minimize M1-type macrophage infiltration, thereby reducing inflammatory response [25, 26]. There are few studies on M2-type macrophages in LN, but it has been proven that M2 macrophages can secrete nutritional factors to promote angiogenesis through endocytosis, promote the remodeling of extracellular matrix, mediate tissue healing, and inhibit a variety of proinflammatory factors for anti-inflammation [27]. Thus, polarization and proliferation of macrophages are of great importance in LN pathogenesis.

Peroxisome proliferator-activated receptors (PPARs), recognized as a class of ligand-activated transcription factors, can realize macrophage polarization, regulate macrophage metabolism, inhibit proinflammatory genes, and promote the transformation of the M2 phenotype [27, 28]. Studies have shown that peroxisome proliferators-activated receptor gamma (PPARG) overbinds with other transcription factors to Arg1, Fizz1, and Ym1 gene promoter regions, thereby promoting Arg1 and Fizz1 gene expression and regulating the M2 polarization level of macrophages, and Arg1 expression is significantly down-regulated when PPARG macrophages are knocked out [29, 30]. PPARG can enhance LPL expression by interacting with PPARG response elements in the LPL gene promoter region and mediating TNF-α expression [31]. The regulation of NF-κB, signal transducers, and transcriptional activators inhibit the expression and secretion of cytokines and inflammatory factors IL-6 and IL-1β, thus playing an important anti-inflammatory role [32]. This suggests that PPARG drives macrophages into an anti-inflammatory state.

The results of cell and animal level tests showed that emodin promoted the transformation from M1 to M2 macrophages, and after interfering with PPARG, M2 macrophage markers were reduced, kidney inflammation was aggravated in rats, and urinary protein levels and serum dsDNA antibody levels were increased. Emodin indicated a role in promoting the transformation of M2 macrophages by activating PPARG, thus improving LN. The limitation of this study is that emodin action on improving LN was only preliminarily detected. Although it serves as an application reference against LN, the effect of emodin on the marker genes of both M1 and M2 macrophages was not further proven at the molecular level. The related pathway of PPARG (PI3K-Akt signaling pathway) has not been studied, so the mechanism of emodin is still unclear. In the future research plan, we will continue to improve the research at the molecular level based on the results of this experiment so as to more clearly explain the mechanism of emodin's action on LN and provide more favorable proof for the development and utilization of emodin in LN.

5. Conclusion

In the current work, emodin was demonstrated to inhibit polarization of the RAW 264.7 cell line towards the M1 phenotype and facilitate its polarization towards the M2 phenotype. Emodin improved the inflammatory injury of the kidneys in LN rats and reduced the levels of urinary protein and dsDNA antibodies. The therapeutic effect of emodin on the treatment of LN may be realized through the activation of PPARG. These findings offer a novel drug candidate for LN management.

Data Availability

The data used to support the finding of this study are available from the corresponding author upon request.

Ethical Approval

All animal experiments were performed as per the Guidelines for the Use of Experimental Animals issued by the Chongqing Hospital of Traditional Chinese Medicine and approved by the Ethical Management Committee of Experimental Animal Welfare of the Chongqing Hospital of Traditional Chinese Medicine.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The research was supported by the National Natural Science Foundation of China (No. 81904012) and Chongqing Science and Technology Bureau Project (cstc2018jxjl130048, cstc2018jcyjAX0818, cstc2021jxjl130003).

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