

The protective effects of Fuzhengzhiyanghefuzhiyang decoction (FZHFZY) on imiquimod-induced psoriasis in mice through suppression of P38/Erk/NF- κ B signaling

Haiming Chen

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Yue Lu

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Bin Tang

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Xiong Li

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Hongyu Zhang

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Junhong Zhang

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Jingjie Yu

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Yuhong Yan

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Hao Deng

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Shaoping Li

University of Macau

Lei Yang

Guangzhou Youcare Biopharmaceutics., LTD.

Ling Han (✉ linghan99@gzucm.edu.cn)

Guangdong Hospital of Traditional Chinese Medicine <https://orcid.org/0000-0002-6168-8828>

Chuanjian Lu

Guangzhou University of Traditional Chinese Medicine: Guangzhou University of Chinese Medicine

Keywords: Psoriasis, FZHFZY, P38/Erk/NF- κ B signaling, Imiquimod-induced, LPS-stimulated RAW264.7

Posted Date: March 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-342314/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Psoriasis is a chronic immune-mediated skin disease affecting approximately 2–3% of world's population. Fuzhenghefuzhiyang decoction (FZHFZY), a Chinese medicine formula created by Prof. Lu Chuanjian, has been shown to have remarkable anti-psoriasis effect in clinical practice. However, the mechanism of action of FZHFZY is unknown. The purpose of this study was to investigate the protective effects of FZHFZY in psoriasis-like skin inflammation both *in vitro* and *in vivo* and elucidate the mechanism of action of FZHFZY.

Methods

In *vivo* study, we evaluated the protective effect of FZHFZY in imiquimod-induced psoriasis-like mice model. Results indicated that FZHFZY can obviously decrease psoriasis area and severity index (PASI) scores. FZHFZY also suppressed the mRNA levels of IL-6, TNF- α , IL-23 and IL-8 in the skin and its anti-inflammatory activity may be related to its suppression of the P38/Erk/NF- κ B signaling. In addition, immunohistochemistry (IHC) data showed that FZHFZY can suppress the expression of F4/80 which is the marker of macrophages in the psoriasis skin. Therefore, we designed to investigate the roles and underlying mechanisms of FZHFZY in LPS-stimulated RAW264.7 macrophages *in vitro*.

Results

Our results revealed FZHFZY treatment could significantly inhibit inflammation by modulating the expression of mediators, such as IL-6, TNF- α , IL-23 and IL-8, which expression was increased remarkably in the activated RAW264.7 cells. Our results also showed that FZHFZY inhibited the P38/Erk/NF- κ B signaling pathways in RAW264.7 cells induced by LPS.

Conclusions

Taken together, our present study demonstrates that FZHFZY alleviated inflammatory response by suppressing the P38/Erk/NF- κ B signaling in imiquimod-induced psoriasis-like mice model and LPS-stimulated RAW264.7.

Background

Psoriasis is a chronic inflammatory skin disease. According to global epidemiological survey, the incidence of psoriasis ranges from 1–3% in the world's population [1]. In recent years, biological preparations have shown good safety and efficacy in the treatment of psoriasis, however, they have the disadvantage of recurrence in the short term after stopping the drug. Psoriasis is a chronic and recurrent

disease with a very long treatment cycle. Evidence of long-term use of the preparation is not sufficient, and the current high cost of drug purchase is not conducive to its promotion[2]. Traditional Chinese medicine has been used clinically for a long time due to its low price and good therapeutic effect. At present, researches on the pathogenesis of psoriasis mainly believe that it is an inflammatory response involving the immune system.

According to literature survey results, the nuclear factor κ B (NF- κ B) signaling pathway is involved in the pathological process of imiquimod-induced psoriasis-like dermatitis in mice [3]. And genome-wide association studies have shown that the activation of the NF- κ B pathway is closely related to psoriasis[4]. NF- κ B p65 is an important member of the NF- κ B/Rel family of proteins [5]. The phosphorylation of NF- κ B p65 protein can activate the NF- κ B signaling pathway and cause inflammation. Actually, in the skin lesions of imiquimod-induced psoriasis model mice, it was found that the expression of NF- κ B p65 phosphorylated protein was significantly increased [6].

Mitogen-activated protein kinase (MAPK) is a group of protein kinases that can be activated by different extracellular stimuli, such as cytokines, neurotransmitters and cell stress. Importantly, MAPKs have recently been associated with many autoimmune diseases (such as psoriasis), most prominently the p38 MAPK and Erk1/2 MAPK pathways [7]. The p38 MAPK signaling pathway is the key to regulating cellular and autoimmune responses [8]. It plays a critical role in the pathogenesis of psoriasis. The expression of phosphorylated p38 MAPK and Erk1/2 MAPK have been significantly increased in the epidermis of psoriasis patients [9].

Chinese medicine formula (CMF) is a personalized treatment carried out by clinicians based on the patient's individual situation, and it has attracted worldwide attention [10]. CMF is usually a combination of several Chinese herbal medicines, and its therapeutic effect is mainly the comprehensive activity of multiple active ingredients. In China, CMF is widely used in the clinical treatment of psoriasis, which is characterized by personalized treatment, low cost and low toxicity. Prof. Lu Chuanjian is a doctor of traditional Chinese medicine, and Fuzhenghefuzhiyang decoction (FZHFZY) is a combination of drugs that she summarized through clinical experience. Our previous study showed that the target cell fishing combined with LC-MS analysis is a useful tool for screening bioactive compounds from complicated FZHFZY and the active components would contribute to the anti-psoriasis effects[11]. In our research, we used LPS-stimulated RW264.7 cells as an *in vitro* model and imiquimod-modeled mice as an *in vivo* model to study the mechanism of FZHFZY in reducing inflammation symptoms in psoriasis model.

Materials And Methods

Animals

Male BALB/c mice (6–8 weeks old, weighing 20 ± 2 g) were purchased from the Center of Laboratory Animals of Southern Medical University (Guangzhou, China). Mice were housed in a standard housing room under controlled conditions (maintained at 22 ± 2 °C, 45 to 55% relative humidity) and provided free

access to food and water under a specific pathogen-free (SPF) environment. The animal protocols were approved by the Animal Experimental Ethics Committee of Guangdong Provincial Hospital of Chinese Medicine.

Chemicals and reagents

Compound dexamethasone acetate cream (DXA) was purchased from China Resources Sanjiu Medical & Pharmaceutical Co., Ltd. (Shenzhen, China). Imiquimod cream was obtained from Sichuan Mingxin Pharmaceutical Co., LTD (Sichuan, China).

Preparation of FZHFZY and LC-ESI-MSⁿ analysis

FZHFZY was provided by the Guangdong Provincial Hospital of Chinese Medicine and seven Chinese herbs involved were listed in Table 1. All these herbs had been cut into small pieces and soaked with water for 30 min, and then kept boiling for 1 h. After boiling twice, the water extracts was pooled together and then concentrated to the concentration of 0.5 g/mL by electric heating volatilization.

The main ingredients of FZHFZY were identified by LC-ESI-MSⁿ analysis and the ESI-MS spectra of samples and reference compounds were acquired in both negative and positive ionization mode.

Cell culture

RAW 264.7 cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). RAW 264.7 cells were cultured in DMEM medium containing 10% FBS, 100 µg/mL streptomycin and 100 IU/mL penicillin at 37 °C in a humidified 5% CO₂ atmosphere.

RAW 264.7 cell proliferation assay *in vitro*

RAW 264.7 cell proliferation was measured using MTT assay. Briefly, Raw 264.7 cells in logarithmic growth were seeded into a 96-well microplate in triplicate. After 24-h incubation, various concentrations (150, 300, 600, 1,200, 2,400 and 4,800 µg/mL, respectively) of FZHFZY was added to each well. After further incubation for 24 h, 10 mL MTT (5 mg/mL) was added to each well. After 4-h incubation at 37 °C, the supernatant then was removed and 100 µL of DMSO was added into each well. Then the supernatants were collected and the absorbance (A value) value at 490 nm was measured using a Microplate Reader and the cell viability was indicated.

Administration of drugs

The BALB/c mice were randomly divided into the following 5 groups ($n = 6$): the control, vehicle, DXA (1 mg/kg/day) and FZHFZY (0.125 and 0.25 g/mL respectively). The control group was normal mice that were totally untreated. DXA and FZHFZY were topically administered to mice in the DXA and FZHFZY group respectively, while distilled water was given to the control and vehicle cream to induce psoriasis,

respectively. Moreover, FZHFZY group was treated with CMF three days before using imiquimod cream and the topical cream treatment administration was applied for 7 consecutive days.

Imiquimod-induced psoriasis-like mouse model

In accordance with our preliminary study [12], mice were topically administrated with a dose of 62.5 mg of 5% imiquimod cream applied to a shaved area (3 cm × 2.5 cm) on their back for 7 consecutive days.

The Psoriasis Area and Severity Index (PASI) had three parameters, namely skin erythema, scaling and thickness [13], which served as a measure to assess the severity of the psoriasis-like lesion severity. Parameters were scored independently on a scale ranging from 0 to 4 as shown in Table 2.

Histological analysis and immunohistochemistry

The skin lesions samples of the mice were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (5 µm) were then made and stained with hematoxylin and eosin (H&E) for histological analysis. For immunohistochemical staining, antigen retrieval was conducted with citrate buffer (pH 6.0) followed by treatment with 3.0% H₂O₂ to quench endogenous peroxidase activity. The sections were incubated overnight at 4 °C with specific primary antibodies against F4/80. The sections were then incubated with biotinylated secondary antibodies for 1 h at room temperature followed by diaminobenzidine staining and hematoxylin counter staining.

Measurements of mRNA expression of inflammatory cytokines via RT-PCR

The mRNA levels of TNF-α, IL-6, IL-8, IL-17, IL-23 and IL-1β were determined by RT-PCR. Total mRNA was isolated from mouse skin tissue or cells using Trizol reagents and mRNA was then reversed transcription to cDNA. The primer sequences were shown in Table 3. The relative mRNA expression levels of cytokines *versus* GAPDH were measured using an ABI 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA).

Western blotting analysis

Total protein from mouse skin samples was acquired with RIPA lysis buffer followed by centrifugation (12,000 rpm and 15 min) at 4 °C. Equal amounts of proteins from each treatment group were subjected to fractionation by SDS-PAGE and electro-transferred to PVD membranes. The membranes were then blocked with 5% (w/v) skim milk in TBS-T containing 0.1% Tween-20 at room temperature for 2 h and subsequently incubated with primary antibody at 4 °C overnight. Then, the membranes were washed with TBS-T and blotted with the appropriate secondary antibody for 1 h. Finally, the protein bands were detected using the enhanced chemiluminescence (ECL). The band intensity was quantified using Image J software (NIH Image, Bethesda, MD, USA), and GAPDH was used as the loading control.

Statistical analysis

The data were statistically evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test, and denoted as means \pm standard deviation (SD). Statistically significant differences were identified as either $P < 0.05$ or $P < 0.01$. All analyses were carried out by GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

Chemical analysis of FZHFZY

The FZHFZY sample and reference substances were analyzed by using the optimized LC-ESI-MSⁿ method. The TIC chromatogram of FZHFZY sample in the negative and positive ESI mode was shown in Fig. 1 and 18 main components were observed in the sample (Table 4).

In vivo evaluation

FZHFZY ameliorates imiquimod-induced psoriatic skin lesion in mice

In order to assess the anti-psoriatic effects of FZHFZY, we established the psoriasis-like mouse model which was topically treated with imiquimod. The marked phenotypic changes and PASI scores were observed in Fig. 2. After treatment with imiquimod, the vehicle group showed marked psoriasis-like lesions, including skin erythema, scaling and thickness, while topical administration with FZHFZY in BALB/c mice at 0.125 and 0.25 g/mL elicited a remarkable decrease in the phenotypic changes and PASI scores induced by imiquimod application on the skin.

Histological evaluations and immunohistochemistry of F4/80

The histological examinations *via* H&E staining (Fig. 3A) from the control group of normal mice showed normal smoother epidermis without any inflammation or lesion. While the vehicle group treated with imiquimod showed that increased epidermal hyperplasia, acanthosis, parakeratosis, elongated rete-like ridges and abundant inflammatory infiltrates were found in the skin. However, treatment with either FZHFZY or DXA treatment groups exhibited much smoother epidermis with less parakeratosis and reduced epidermal thickening.

The recruitment and activation of macrophages in psoriatic skin have been deemed to be important pathogenic factors [14, 15] and the use of F4/80 expression associated with markers can definitively distinguish macrophages from other cells [16]. Therefore, we detected the expression of F4/80 in the skin. As can be seen from Fig. 3B, the expression of F4/80 was significantly up-regulated in the vehicle group compared to the control group, and then down-regulated to different degrees by treatment with FZHFZY.

FZHFZY suppresses mRNA expressions of pro-inflammatory cytokines in imiquimod-treated psoriatic mice

To analyze the effect of FZHFZY on inflammation in the skin, the mRNA expressions of TNF- α , IL-17, IL-23 and IL-1 β in skin tissue were determined using RT-PCR. As shown in Fig. 4, the mRNA expressions of TNF- α , IL-17, IL-23 and IL-1 β after treatment with imiquimod were significantly enhanced as compared with those in other groups. While, the mRNA levels of these cytokines after administration with FZHFZY were obviously lowered in imiquimod-treated group.

FZHFZY inhibits P38/Erk/NF- κ B signaling in imiquimod-treated psoriatic mice

Previous data have shown that FZHFZY ameliorates psoriatic skin lesion and suppresses pro-inflammatory cytokines in imiquimod-induced mice. To investigate whether FZHFZY protected imiquimod-treated psoriatic mice through inhibiting P38/Erk/NF- κ B signaling, we determined the expression levels of P38, p-P38, Erk, p-Erk, NF- κ B and p-NF- κ B in imiquimod-induced mice skin by Western blot. As shown in Fig. 5, the results showed that FZHFZY treatment had no obvious effect on the expression of P38, Erk and NF- κ B, while remarkably reduced the proportion of p-P38/P38, p-Erk/Erk and p-NF- κ B/NF- κ B, respectively (all $P < 0.01$).

***In vitro* evaluation**

***In vitro* cytotoxicity of FZHFZY on RAW264.7 cells**

To evaluate the effect of FZHFZY on the viability of RAW 264.7 cells, RAW 264.7 cells treated with 0 to 4800 μ g/mL FZHFZY for 24 h and then MTT assay was performed. The results shown in Fig.6 suggested that FZHFZY (0-2400 μ g/mL) was not toxic towards RAW 264.7 cells. Therefore, four concentrations of FZHFZY (150, 300, 600 and 1200 μ g/mL) were selected for the next experiment.

FZHFZY suppresses the mRNA expressions of pro-inflammatory cytokines in LPS-induced RAW264.7 cells

To verify the effect of FZHFZY on LPS-induced RAW264.7 cells, RT-qPCR analysis was used to determine the mRNA expressions of pro-inflammatory cytokines. As shown in Fig.7, IL-6, TNF- α , IL-23 and IL-8 mRNA was significantly enhanced after LPS stimulation for 24 h. However, treatment with FZHFZY prior to the LPS challenge notably attenuated the enhancement of mRNA of these cytokines. The extent of the inhibition was likely to be dependent on the concentration of FZHFZY.

FZHFZY inhibits P38/Erk/NF- κ B signaling in LPS-induced RAW264.7 cells

Since FZHFZY restrained P38/Erk/NF- κ B signaling in imiquimod-induced psoriasis-like mice, we further explored the mechanisms underlying its anti-inflammatory effects in LPS-induced RAW264.7 cells. Therefore, Western blot analysis was performed to evaluate the effects of FZHFZY on P38/Erk/NF- κ B signaling pathway involving protein expression of phosphorylated- and total P38, Erk and NF- κ B. As shown in Fig. 8, the expression of p-P38, p-Erk and p-NF- κ B markedly increased in RAW 264.7 cells after treatment with LPS compared to the control group. Nevertheless, treatment with FZHFZY markedly suppressed the expression of p-P38, p-Erk and p-NF- κ B compared to the vehicle group in a dose-dependent manner.

Discussion

Psoriasis is a common chronic inflammatory skin disorder, characterized by clearly delineated erythematous plaques which may be painful and itching with an overall prevalence of 2–3% of population worldwide and a substantial negative impact on the quality of patients' life [17–19]. The etiology and pathogenesis are thought to involve a hereditary component and environmental factors which trigger an inflammatory response, leading to hyperproliferation of keratinocytes [19, 20]. It is widely accepted that the etiology of psoriasis involves genetic susceptibility, environmental, as well as sex and age-related factors [21]. Although conventional treatments are effective, unwanted side effects can impact on the long-term management of psoriasis [22]. Thus, there is an urgent need to investigate and develop novel strategies for psoriasis with minimal side effects. Chinese herbal medicine has long history of treating psoriasis in China, and numerous herbs have been used for psoriasis [22, 23].

FZHFZY is a novel formulated Chinese medicine formula containing Dictamni Cortex, Angelica sinensis Radix, Rehmanniae radix Preparata, Cnidii Fructus, Granati Pericarpium, Smilax glabra Rhizoma and Cynanchum paniculatum Radix et Rhizoma. FZHFZY, a Chinese medicine formula proposed by Prof. Lu Chuanjian, has been shown to have remarkable anti-psoriasis effect in clinical practice with the effect of strengthening the body resistance and dispelling dampness, easing the skin and relieving itching. However, the mechanism of action of FZHFZY is unknown. In our research, we investigated the protective effects and the mechanism of action of FZHFZY in psoriasis-like skin inflammation both *in vitro* and *in vivo*. It is observed that FZHFZY markedly decreased the PASI scores, decreased the epidermal hyperplasia and epidermal thickening, which revealed that FZHFZY effectively ameliorated imiquimod-induced murine psoriasis.

The psoriasis biologics recommended by *The Lancet* mainly target TNF- α , IL-12, IL-23 and IL-17, and have achieved high clinical effects [2]. Inflammatory cytokines are small molecular peptides or glycoproteins synthesized and secreted by lymphocytes and monocytes, which participate in and mediate inflammation. Small molecule targeted drugs, such as methotrexate, apremilast and dimethyl fumarate, are accelerating the development. These small molecule drugs mainly target some inflammatory pathways and cytokine factors, such as TNF- α , IL-23 and NF- κ B [24]. Similarly, our research results show that FZHFZY can inhibit the expression of NF- κ B p65 protein (Figs. 5 & 8) and reduce the mRNA levels of inflammatory mediators TNF- α and IL-23 (Figs. 4 & 7).

Furthermore, studies have found that P38 MAPK and ERK1/2 MAPK are widely involved in the pathogenesis of psoriasis [25], especially the role of P38 MAPK in psoriasis is well documented [26]. Phosphorylation of P38 MAPK in human keratinocytes cells stimulated by particular matters is critically important for the increase in the expression of inflammatory factors such as IL-1 α and IL-1 β [27]. Moreover, the mRNA and protein expression of IL-6 and IL-8 induced by TNF- α is dependent on p38 MAPK [28]. Similarly, our research results show that FZHFZY can inhibit the expression of p38 MAPK and Erk1/2 MAPK and reduce the mRNA levels of inflammatory factors IL-1 α , IL-1 β , IL-6 and IL-8.

Conclusions

Taken together, our data demonstrated FZHFZYF alleviated inflammatory response by suppressing of the P38/Erk/NF- κ B signaling in imiquimod-induced psoriasis-like mice model and LPS-stimulated RAW264.7.

Abbreviations

PASI, Psoriasis area and severity index; FZHFZY, Fuzhenghefuzhiyang decoction; IHC, Immunohistochemistry; NF- κ B, Nuclear factor κ B; MAPK, Mitogen-activated protein kinase; CMF, Chinese medicine formula; DXA, Compound dexamethasone acetate cream; H&E, Hematoxylin and eosin;

Declarations

Acknowledgments

Not applicable.

Authors' contributions

All authors have participated extensively in the study and had proofread the final manuscript. CJL and LH conceived and designed the research. HMC and YL conducted the experiments. BT, XL, JHZ, HYZ, JJY and YHY collected data. SPL, HD and LY helped analyzed the data. HMC and YL wrote the manuscript. CJL and LH approved and reviewed the final manuscript. All authors have read and agreed with the final manuscript.

Funding

The research was partially supported by grants from the National Natural Science Foundation of China (No. 81803804), the Natural Science Foundation of Guangdong Province (No. 2017B030314166 & 2020A1515010607 & 2019A1515111094 & 2018A030310530) the Science and Technology Planning Project of Guangdong Province (No. 2017B030314166 & 2017A020213011 & 2017A050506041), the Science and Technology Planning Project of Guangzhou (No. 201807010051), Technology Research Projects of Guangdong Provincial Hospital of Chinese Medicine (No. YN2019QJ08 & YN2019QL11), National Administration of Traditional Chinese Medicine Project (No. JDZX2015196).

Ethics approval and consent to participate

This study was carried out in accordance with the recommendations of Chinese national guidelines and institutional review board of Guangdong Provincial Academy of Chinese Medical Sciences. The protocol was approved by the Institutional Animal Care and Use Committee of Guangdong Provincial Academy of Chinese Medical Sciences.

Consent for publication

We declare that the Publisher has the Author's permission to publish the relevant Contribution.

Availability of data and materials

The datasets used and/or analyzed during the current study would be available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ State Key laboratory of Dampness Syndrome of Chinese Medicine, The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, P. R. China.

² Guangdong Provincial Key Laboratory of Clinical Research on Traditional Chinese Medicine Syndrome, Guangzhou, P. R. China.

³ Guangdong Clinical Research Center for Dermatoses in Chinese Medicine, Guangzhou, P. R. China.

⁴ Guangdong-Hong Kong-Macau Joint Lab on Chinese Medicine and Immune Disease Research, Guangzhou University of Chinese Medicine, Guangzhou, P. R. China.

⁵ State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China

⁶ Guangzhou Youcare Biopharmaceuticals Co., Ltd, Guangzhou, P. R. China

Data Availability Statement

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

References

1. Michalek IM, Loring B, John SM. A systematic review of worldwide epidemiology of psoriasis. *Journal of the European Academy of Dermatology Venereology: JEADV*. 2017;31(2):205–12.
2. Mahil SK, Smith CH. Psoriasis biologics: a new era of choice. *Lancet*. 2019;394(10201):807–8.
3. Xu J, Duan X, Hu F, Poorun D, Liu X, Wang X, Zhang S, Gan L, He M, Zhu K, et al. Resolvin D1 attenuates imiquimod-induced mice psoriasiform dermatitis through MAPKs and NF- κ B pathways. *J Dermatol Sci*. 2018;89(2):127–35.
4. Boehncke WH, Schön MP: **Psoriasis**. *Lancet (London, England)* 2015, **386**(9997):983–994.

5. Li CC, Korner M, Ferris DK, Chen E, Dai RM, Longo DL. NF-kappa B/Rel family members are physically associated phosphoproteins. *Biochem J.* 1994;303(Pt 2):(Pt 2):499–506.
6. Li Y, Zhang G, Chen M, Tong M, Zhao M, Tang F, Xiao R, Wen H. Rutaecarpine inhibited imiquimod-induced psoriasis-like dermatitis via inhibiting the NF-κB and TLR7 pathways in mice. *Biomedicine pharmacotherapy = Biomedecine pharmacotherapie.* 2019;109:1876–83.
7. Xu F, Xu J, Xiong X, Deng Y. Salidroside inhibits MAPK, NF-κB, and STAT3 pathways in psoriasis-associated oxidative stress via SIRT1 activation. *Redox report: communications in free radical research.* 2019;24(1):70–4.
8. Mavropoulos A, Orfanidou T, Liaskos C, Smyk DS, Billinis C, Blank M, Rigopoulou EI, Bogdanos DP. p38 mitogen-activated protein kinase (p38 MAPK)-mediated autoimmunity: lessons to learn from ANCA vasculitis and pemphigus vulgaris. *Autoimmun rev.* 2013;12(5):580–90.
9. Johansen C, Kragballe K, Westergaard M, Henningsen J, Kristiansen K, Iversen L. The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br J Dermatol.* 2005;152(1):37–42.
10. Sham TT, Chan CO, Wang YH, Yang JM, Mok DK, Chan SW. A review on the traditional Chinese medicinal herbs and formulae with hypolipidemic effect. *BioMed research international.* 2014;2014:925302.
11. Chen L, Chen H, Lu Y, Han L, Wang S, Liu M, Li X, Zhao J, Lu C, Li S. Decoding active components in a formulation of multiple herbs for treatment of psoriasis based on three cell lines fishing and liquid chromatography-mass spectrometry analysis. *J Pharm Biomed Anal.* 2020;186:113331.
12. Chen H, Liu H, Tang B, Chen Y, Han L, Yu J, Yan Y, Lu C: **The Protective Effects of 18β-Glycyrrhetic Acid on Imiquimod-Induced Psoriasis in Mice via Suppression of mTOR/STAT3 Signaling.** 2020, **2020**:1980456.
13. van der Fits L, Mourits S, Voerman JS, Kant M, Boon L, Laman JD, Cornelissen F, Mus AM, Florencia E, Prens EP, et al: **Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis.** *Journal of immunology (Baltimore, Md: 1950)* 2009, **182**(9):5836–5845.
14. Schön MP, Erpenbeck L. The Interleukin-23/Interleukin-17 Axis Links Adaptive and Innate Immunity in Psoriasis. *Frontiers in immunology.* 2018;9:1323.
15. Stratis A, Pasparakis M, Rupec RA, Markur D, Hartmann K, Scharffetter-Kochanek K, Peters T, van Rooijen N, Krieg T, Haase I. Pathogenic role for skin macrophages in a mouse model of keratinocyte-induced psoriasis-like skin inflammation. *J Clin Investig.* 2006;116(8):2094–104.
16. Dos Anjos Cassado A. F4/80 as a Major Macrophage Marker: The Case of the Peritoneum and Spleen. *Result Probl Cell Differ.* 2017;62:161–79.
17. Zhao YK, Zhang YQ, Wang F, Wu HH, Luo ZY, Luo DQ, Chen WN. Developing Shingles-Induced Koebner Phenomenon in a Patient With Psoriasis: A Case Report. *Medicine.* 2015;94(26):e1009.
18. Sonkoly E, Wei T, Janson PC, Sääf A, Lundeborg L, Tengvall-Linder M, Norstedt G, Alenius H, Homey B, Scheynius A, et al. MicroRNAs: novel regulators involved in the pathogenesis of psoriasis? *PloS one.* 2007;2(7):e610.

19. Menter A, Gottlieb A, Feldman SR, Van Voorhees AS, Leonardi CL, Gordon KB, Lebwohl M, Koo JY, Elmets CA, Korman NJ, et al. Guidelines of care for the management of psoriasis and psoriatic arthritis: Sect. 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics. *J Am Acad Dermatol*. 2008;58(5):826–50.
20. Pathirana D, Ormerod AD, Saiag P, Smith C, Spuls PI, Nast A, Barker J, Bos JD, Burmester GR, Chimenti S, et al. European S3-guidelines on the systemic treatment of psoriasis vulgaris. *Journal of the European Academy of Dermatology Venereology: JEADV*. 2009;23(Suppl 2):1–70.
21. Chandra A, Ray A, Senapati S, Chatterjee R. Genetic and epigenetic basis of psoriasis pathogenesis. *Molecular immunology*. 2015;64(2):313–23.
22. Traub M, Marshall K. Psoriasis–pathophysiology, conventional, and alternative approaches to treatment. *Alternative medicine review: a journal of clinical therapeutic*. 2007;12(4):319–30.
23. Yu JJ, Zhang CS, Zhang AL, May B, Xue CC, Lu C. Add-on effect of chinese herbal medicine bath to phototherapy for psoriasis vulgaris: a systematic review. *Evidence-based complementary alternative medicine: eCAM*. 2013;2013:673078.
24. Rendon A, Schäkel K. **Psoriasis Pathogenesis and Treatment**. *International journal of molecular sciences* 2019, 20(6).
25. Yu XJ, Li CY, Dai HY, Cai DX, Wang KY, Xu YH, Chen LM, Zhou CL. Expression and localization of the activated mitogen-activated protein kinase in lesional psoriatic skin. *Exp Mol Pathol*. 2007;83(3):413–8.
26. Soegaard-Madsen L, Johansen C, Iversen L, Kragballe K. Adalimumab therapy rapidly inhibits p38 mitogen-activated protein kinase activity in lesional psoriatic skin preceding clinical improvement. *Br J Dermatol*. 2010;162(6):1216–23.
27. Yeung YT, Bryce NS, Adams S, Braidy N, Konayagi M, McDonald KL, Teo C, Guillemin GJ, Grewal T, Munoz L. p38 MAPK inhibitors attenuate pro-inflammatory cytokine production and the invasiveness of human U251 glioblastoma cells. *Journal of neuro-oncology*. 2012;109(1):35–44.
28. Munoz L, Ramsay EE, Manetsch M, Ge Q, Peifer C, Laufer S, Ammit AJ. Novel p38 MAPK inhibitor ML3403 has potent anti-inflammatory activity in airway smooth muscle. *Eur J Pharmacol*. 2010;635(1–3):212–8.

Tables

Table 1 The composition and ratio of seven herbs used in the preparation of FZHFZY

Linnean classification	Chinese name	Part used	Ratio
<i>Dictamnus dasycarpus</i> Turcz.	Baixianpi	Root	3
<i>Angelica sinensis</i> Oliv. Diels	Danggui	Root	2
<i>Rehmannia glutinosa</i> Libosch.	Shudihuang	Root	3
<i>Cnidium monnieri</i> L. Cuss.	Shechuangzi	Fruit	2
<i>Punica granatum</i> L.	Shiliupi	Peel	3
<i>Smilax glabra</i> Roxb.	Tufuling	Rhizome	3
<i>Cynanchum paniculatum</i> Bge. Kitag.	Xuchangqing	Root	3

Table 2 The Psoriasis Area and Severity Index (PASI) for mice psoriasis

Parameter	Scores
Erythema	0 = none
	1 = slight
	2 = moderate
	3 = marked
	4 = very marked
Scaling	0 = none
	1 = slight
	2 = moderate
	3 = marked
	4 = very marked
Thickness	0 = none
	1 = slight
	2 = moderate
	3 = marked
	4 = very marked

Table 3 Primer sequences of target genes

Target gene	Primer sequence (5'→3')
TNF- α Forward	ACTGATGAGAGGGAGGCCAT
Reverse	CCGTGGGTTGGACAGATGAA
IL-6 Forward	TTCTTGGGACTGATGCTGGT
Reverse	CCTCCGACTTGTGAAGTGGT
IL-8 (Forward)	CTAGGCATCTTCGTCCGTCC
Reverse	CAGAAGCTTCATTGCCGGTG
IL-17 (Forward)	TCAAAGCTCAGCGTGTCCAA
Reverse	TCTTCATTGCGGTGGAGAGTC
IL-23 Forward	CAAAGGATCCGCCAAGGTCT
Reverse	GGAGGTGTGAAGTTGCTCCA
IL-1 β (Forward)	TGCCACCTTTTGACAGTGATG
Reverse	AAGGTCCACGGGAAAGACAC
GAPDH (Forward)	CAGGTTGTCTCCTGCGACTT
Reverse	TATGGGGGTCTGGGATGGAA

Table 4 Chemical profiles of FZHFZY

No.	Time (min)	Compounds	ESI mode
1	2.4	Gallic acid	Negative
2	8.6	Catechin	Negative
3	11.7	Chlorogenic acid	Negative
4	15.7	Epicatechin	Negative
5	16.9	3-O-caffeoylshikimic acid	Negative
6	18.2	4-O-caffeoylshikimic acid	Negative
7	20.9	5-O-caffeoylshikimic acid	Negative
8	32.4	Neoastilbin	Negative
9	34.6	Astilbin	Negative
10	37.1	Neoisoastilbin	Negative
11	37.7	Ellagic acid	Negative
12	38.5	Isoastilbin	Negative
13	41.8	Engeletin	Negative
14	54.5	Dictamnine	Positive
16	56.9	Obakunone	Positive
17	57.0	Fraxinellone	Positive
18	59.5	Osthole	Positive

Figures

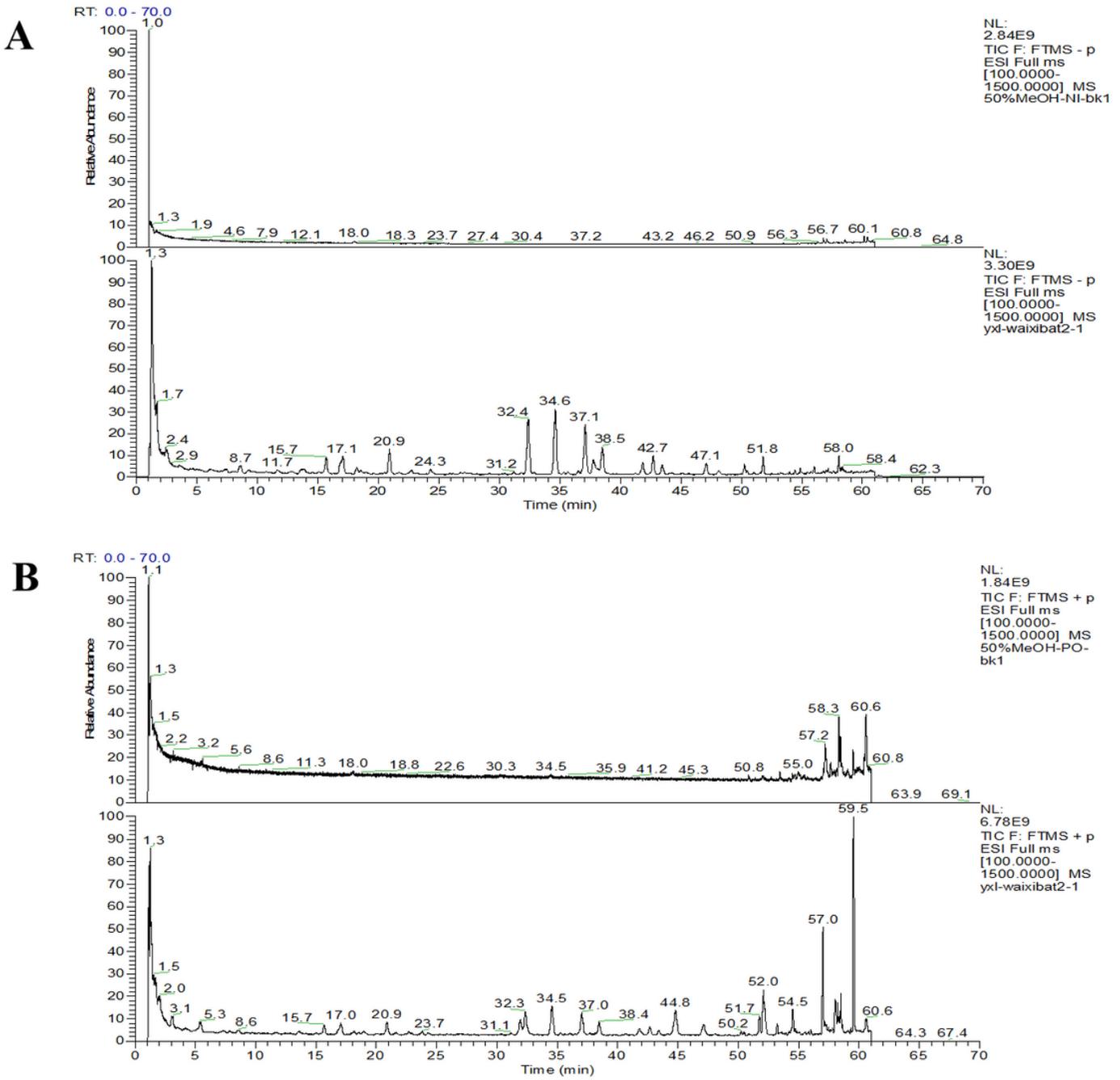


Figure 1

TIC chromatograms of FZHFZY extract in negative (A) and positive (B) ionization modes.

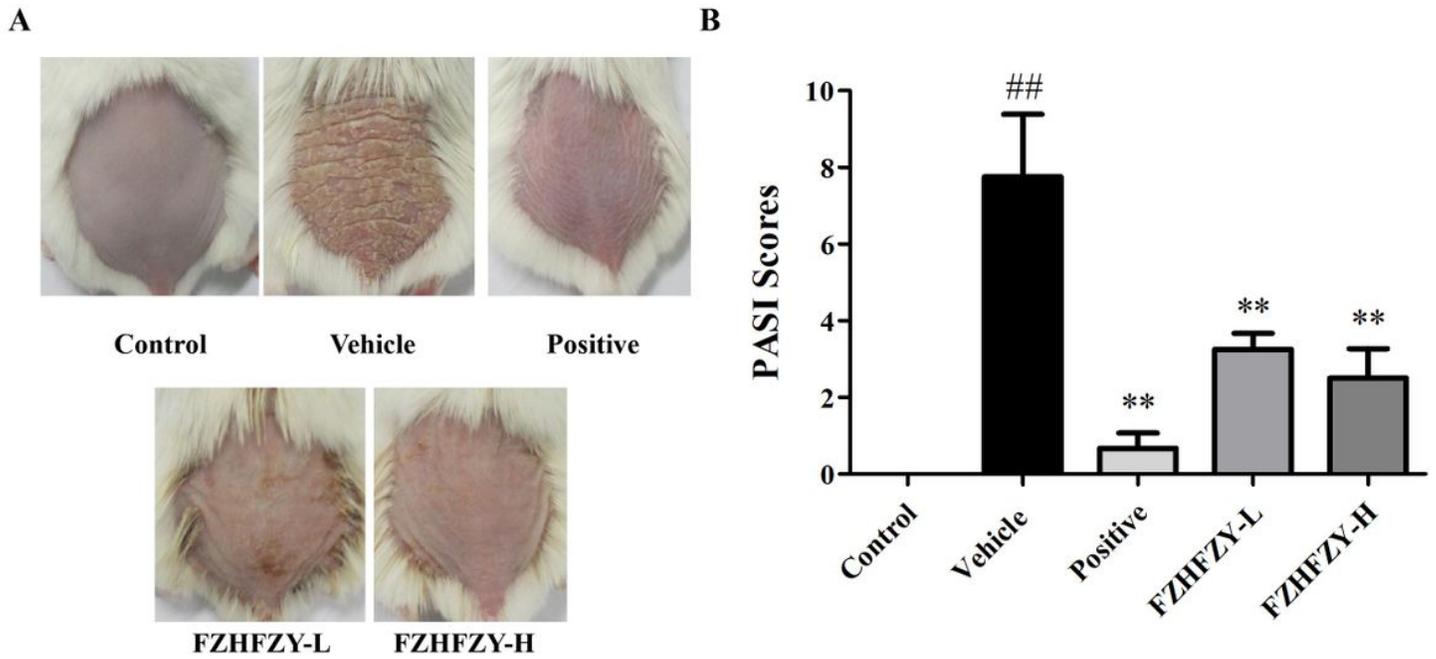


Figure 2

FZHFZY reduces the PASI score and ameliorates the skin lesion of imiquimod-induced psoriasis-like mice. (A) The representatives of photos of dorsal skin in imiquimod-induced psoriasis-like mice 7 days after imiquimod-treatment with or without FZHFZY. (B) The PASI scores of the skin lesion in imiquimod-induced psoriasis-like mice after related treatment. Data are presented as the means \pm SD ($n = 6$, $\#P < 0.05$ and $\#\#P < 0.01$ vs. control group, $*P < 0.05$ and $**P < 0.01$ vs. vehicle group). (FZHFZY-L: low-dose of FZHFZY and FZHFZY-H: high-dose of FZHFZY).

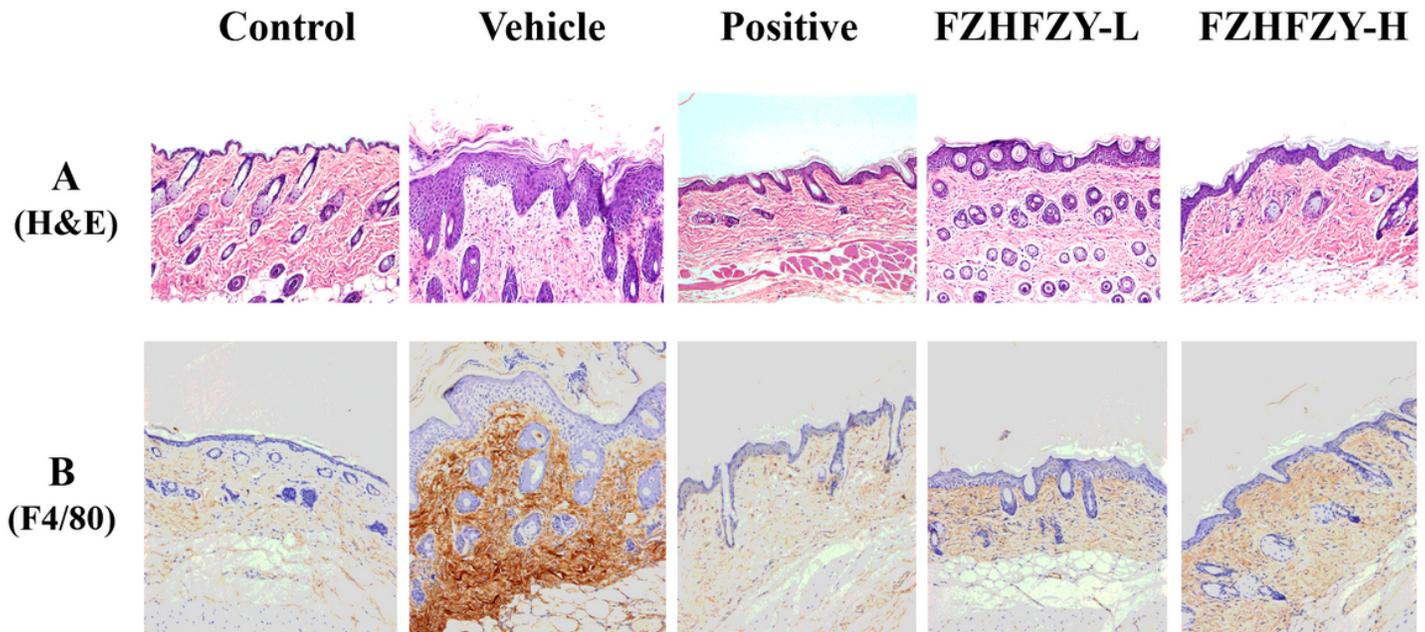


Figure 3

H&E analysis and immunohistochemistry of inhibitory effects of FZHFZY on imiquimod-induced psoriasis-like skin inflammation. H&E staining of the dorsal skin lesion in different treatment groups (A). Immunohistochemical images of F4/80 (B) staining (magnification: 100×) of dorsal skin in control or imiquimod-induced psoriasis-like mice after the treatment.

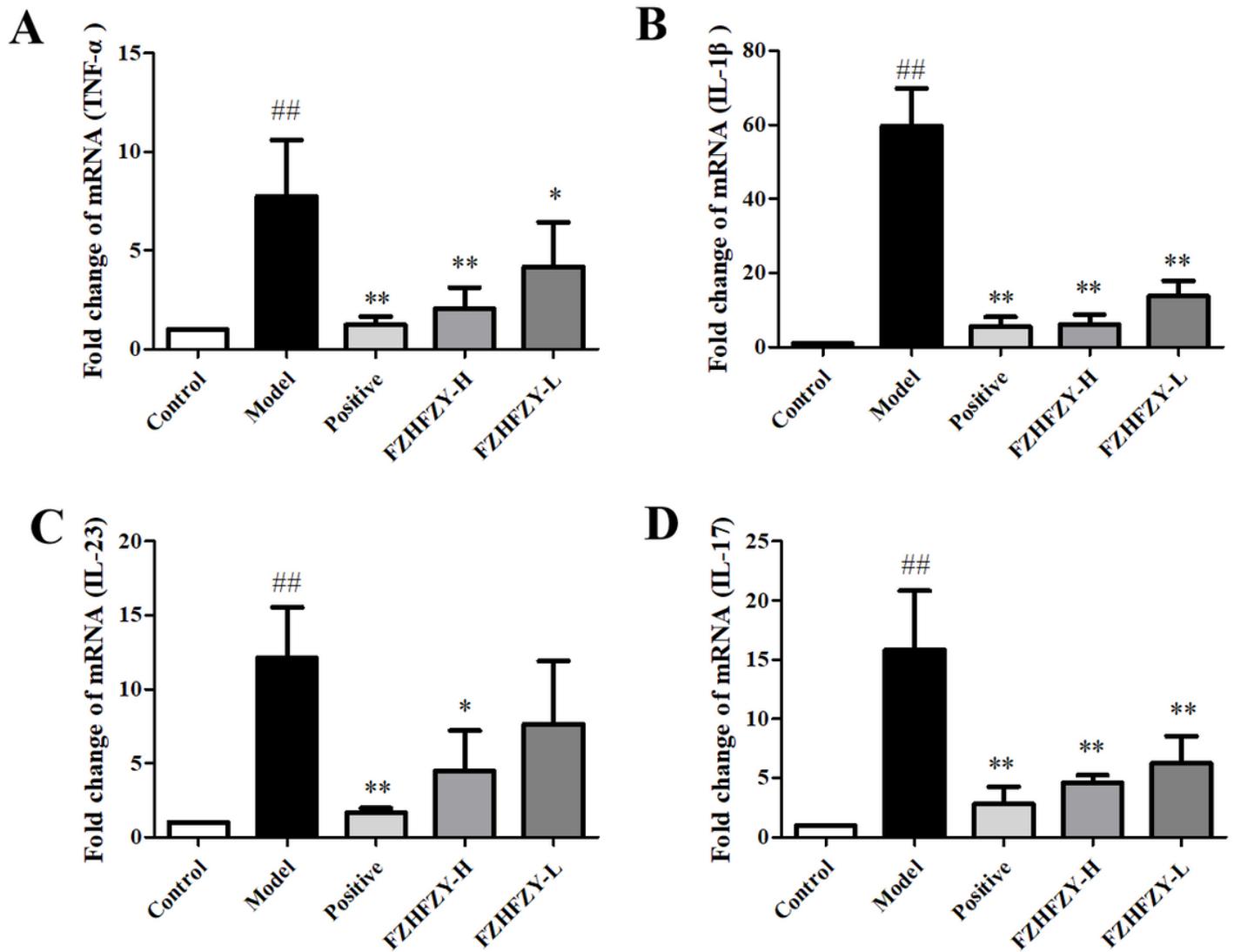


Figure 4

Effect of FZHFZY on inflammatory cytokine expression in imiquimod-induced psoriasis. Mice were administrated with FZHFZY, and topically treated with imiquimod as well. The mRNA levels of TNF- α (A), IL-1 β (B), IL-23 (C) and IL-17 (D) in the skin were determined using RT-PCR. Data shown are the means \pm SD (n = 3), #P < 0.05 and ##P < 0.01 compared with control group, *P < 0.05 and **P < 0.01 compared with vehicle group.

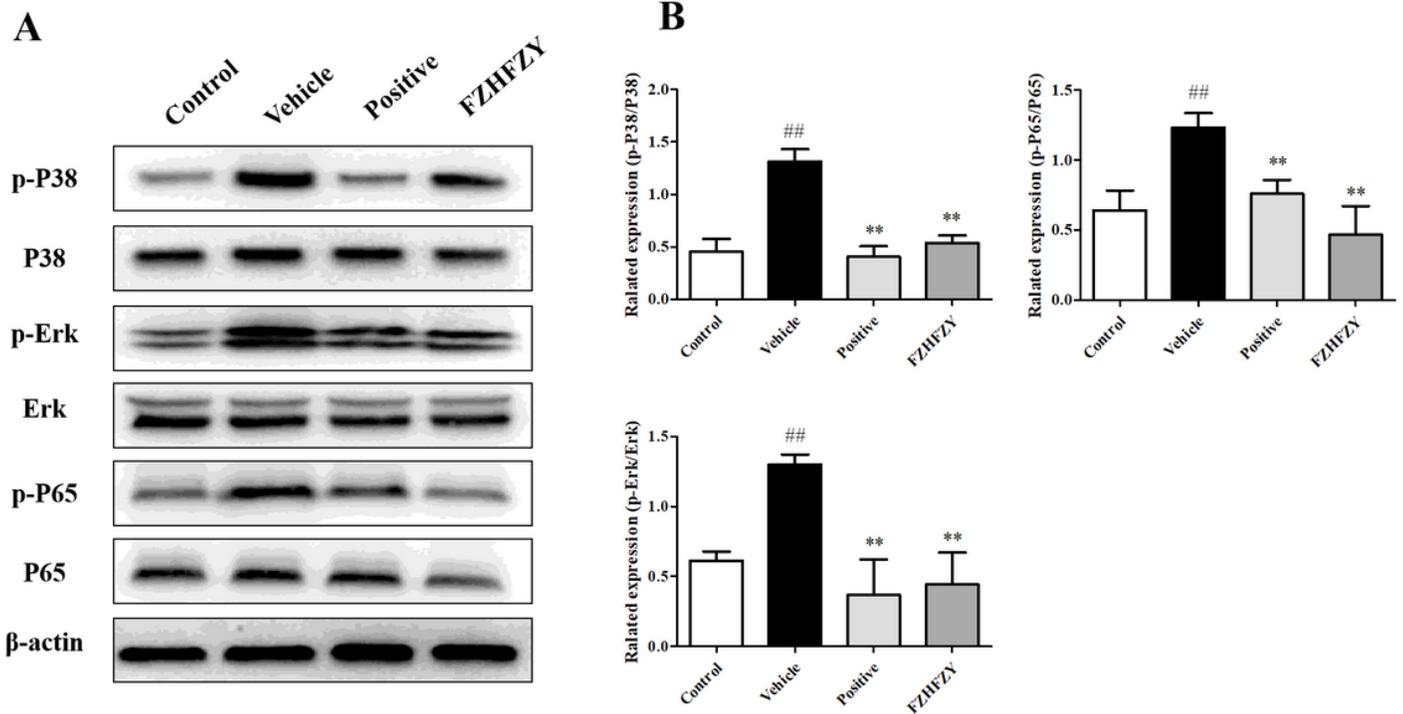


Figure 5

Effect of FZHFZY on the P38/Erk/NF- κ B signaling pathway in mice with imiquimod-induced psoriasis. (A) Representative Western blot of P38/Erk/NF- κ B pathway protein expression in the skin of mice with imiquimod-induced psoriasis. (C) Quantification of the levels of p-P38, p-Erk and p-P65 relative to P38, Erk and P65 in mice skin. Data shown are the means \pm SD (n = 3). #P < 0.05 and ##P < 0.01 vs. control group, *P < 0.05 and **P < 0.01 vs. vehicle group.

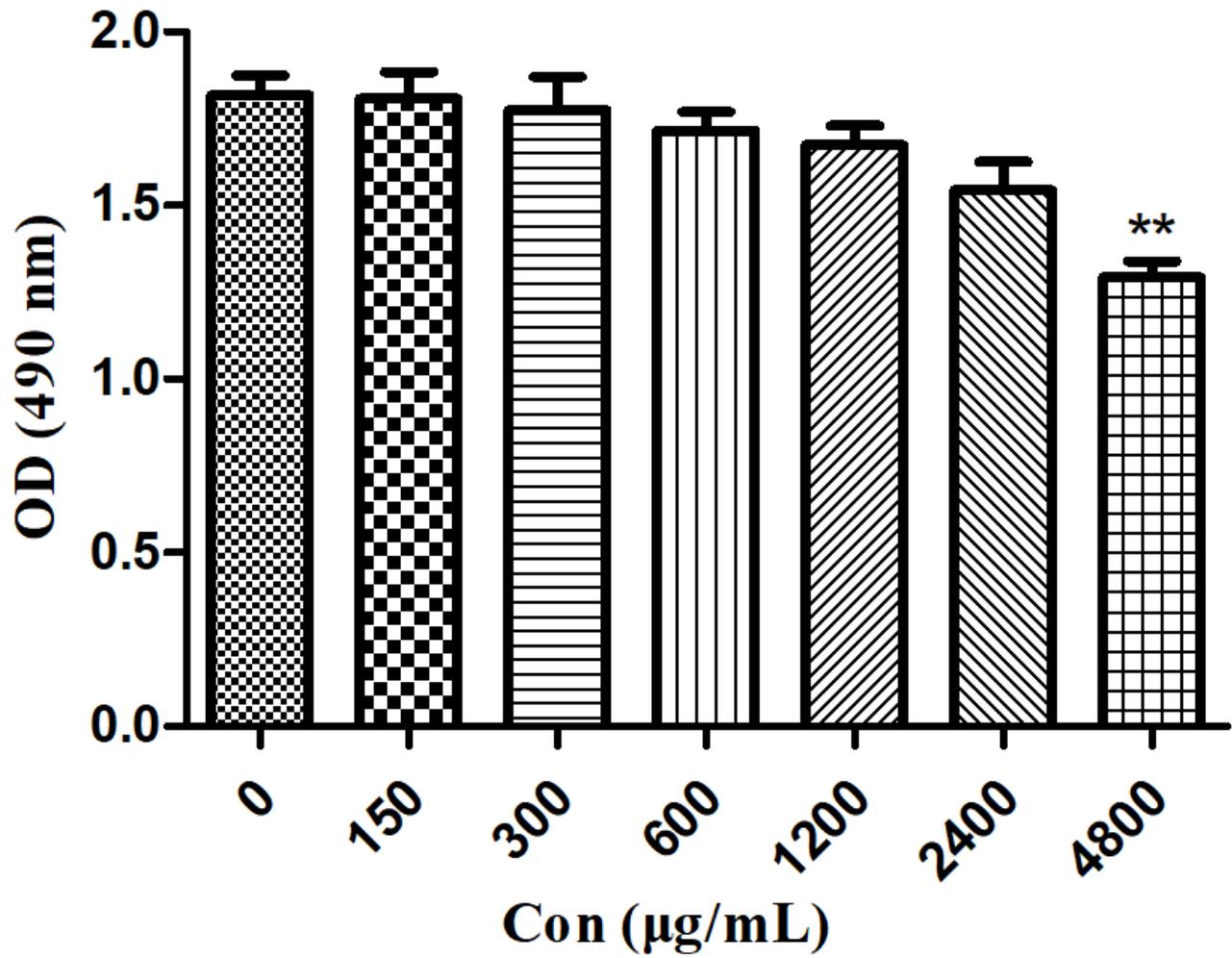


Figure 6

Effect of FZHFZY on the viability of RAW264.7 cells. MTT assay was performed to determine cell viability after treatment with 4800, 2400, 1200, 600, 300, 150, or 0 µg/mL FZHFZY for 24 h.

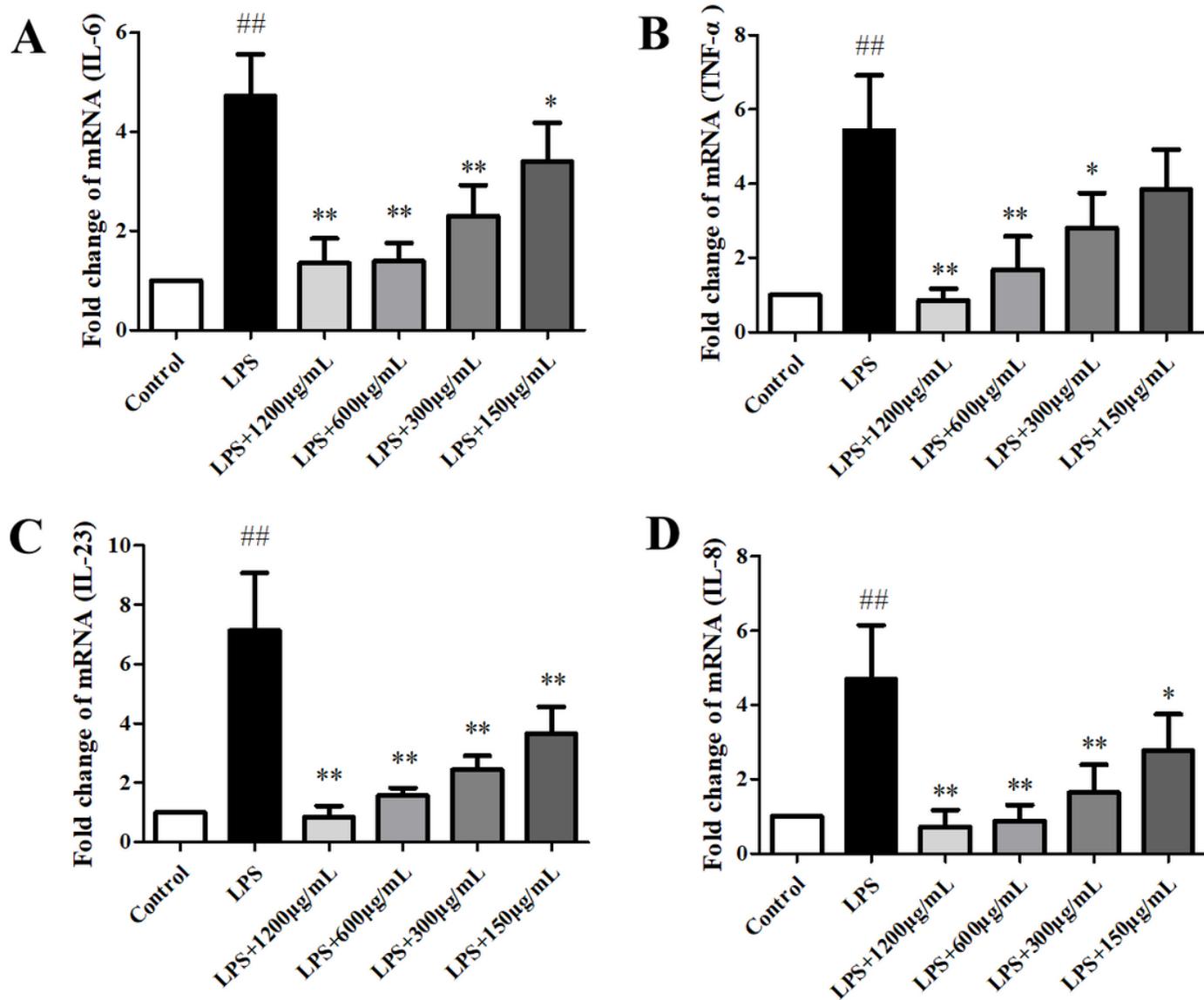


Figure 7

Effects of FZHFZY on the mRNA expression of inflammatory cytokine IL-6, TNF- α , IL-23 and IL-8 in LPS-induced RAW 264.7 cells. The mRNA levels of IL-6 (A), TNF- α (B), IL-23 (C) and IL-8 (D) in RAW 264.7 cells were determined using RT-PCR. Data shown are the means \pm SD (n = 6). #P < 0.05 and ##P < 0.01 compared with control group, *P < 0.05 and **P < 0.01 compared with vehicle group.

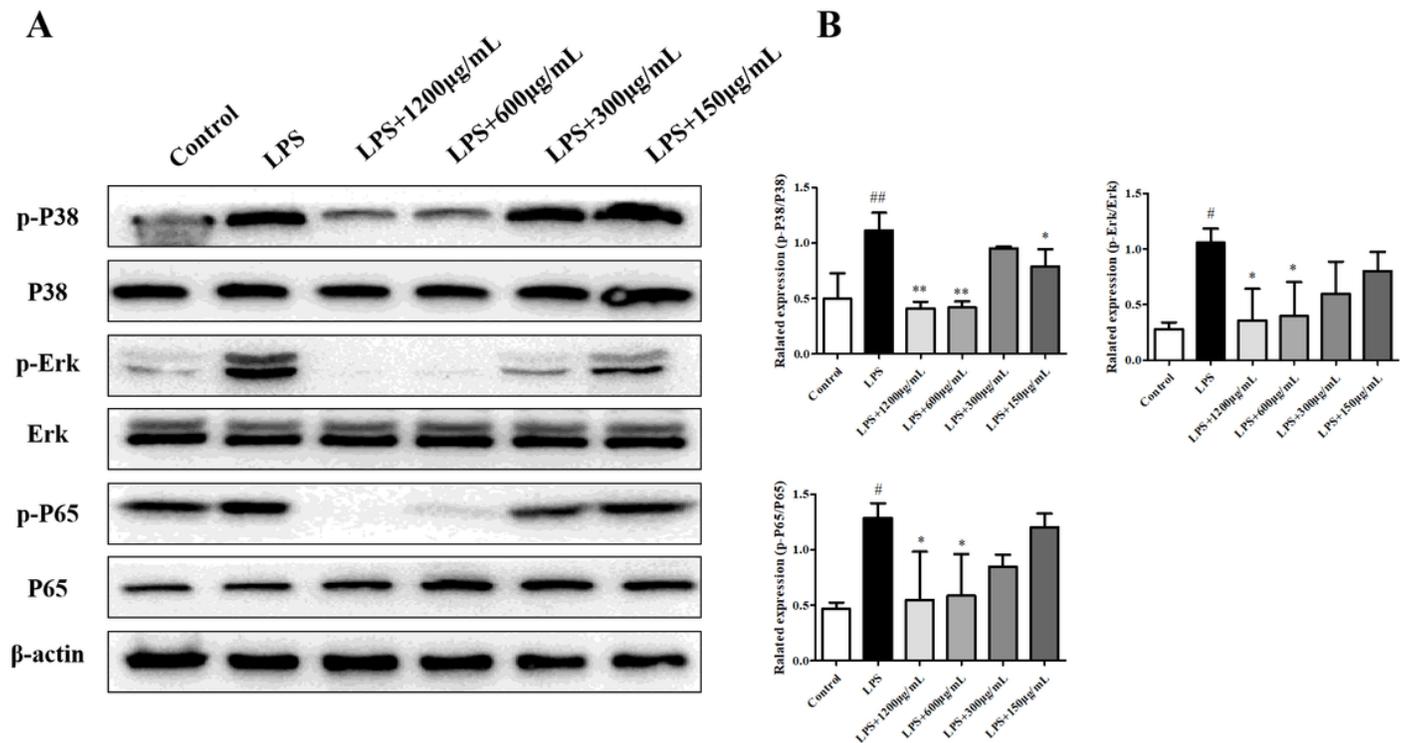


Figure 8

FZHFZY suppresses P38/Erk/NF- κ B signaling in LPS-induced RAW 264.7 cells. (A) Representative Western blot of P38/Erk/NF- κ B protein expression in LPS-induced RAW264.7 cells treated with 150, 300, 600 or 1200 μ g/mL FZHFZY for 24 h. (B) Quantification of the expression levels of p-P38, p-Erk and p-P65 relative to p38, ERK and P65 at 24 h. Data shown are the means \pm SD (n = 3), #P < 0.05 and ##P < 0.01 vs. control group, *P < 0.05 and **P < 0.01 vs. vehicle group.