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Extracts from *Seseli mairei* Wolff attenuate imiquimod-induced psoriasis-like inflammation by inhibiting Th17 cells

Mengmeng Wang [1], Xunqing Yin [1], Yongcheng Zeng, Chunyan Hu, Yongmei Xue, Qionglian Fang, Xue Qiao, Xiujuan Zhao, Chenghong Du, Feng Huang [**], Yuping Lin [*]

School of Chinese Materia Medica and Yunnan Key Laboratory of Southern Medicinal Utilization, Yunnan University of Chinese Medicine, Kunming, China

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ABSTRACT

Objective: Seseli mairei Wolff extracts (SMWE) are widely used to treat psoriasis as a Chinese medicine, but their effect and mechanism are unclear. This study verified the effect of SMWE on psoriasis by regulating Th17 cells.

Methods: HaCaT cells were treated with IL-17A *in vitro* to evaluate the effect of SMWE on psoriasis. *In vivo*, the mice psoriasis model was established using imiquimod (IMQ, 62.5 mg/d), and intragastrically treated with the different drugs for six days. The severity of skin inflammation was evaluated with Psoriasis Area and Severity Index (PASI) scores and pathology. The levels of inflammation cytokines were assessed with immunofluorescence, immunochemistry, ELISA, and real-time PCR. The number of Th17 cells was determined with flows.

Results: SMWE inhibited the proliferation of HaCaT cells and reduced the IL-17A-induced IL-6 production *in vitro*. *In vivo*, SMWE deduced the levels of IL-1β, IL-6, IL-8, IL-17A, IL-17F, IL-22, IL-23, and TNF-α, while increasing the level of IL-10 compared to the model group. SMWE also inhibited the levels of NF-κB, JAK2, and STAT3 proteins, while declining the expressions of Gr-1, and MPO. Interestingly, SMWE significantly decreased the number of Th17 cells.

Conclusion: SMWE inhibited the proliferation of HaCaT cells and attenuated the development of psoriasis lesions by inhibiting Th17 cells to regulate the levels of inflammation cytokines.

1. Introduction

Psoriasis is an ancient, chronic, inflammatory, and recurring systemic disease induced by multiple factors, including the environment, immunity, drugs, and genetics [1,2]. With the change in environment and life, impaired quality of life, depression, and anxiety also affect the progress of psoriasis [3]. This disease is mainly characterized by erythema, papules, and scales on the skin. Psoriasis affects approximately 2–3% of the global population [4,5] and is unequally distributed across geographical regions, such as China (0.47%), Asia (1%), Central Europe (0.62%–5.32%), Western Europe (1.07%–3.46%), North America (0.63%–3.60%), and southern Latin America (0.36%–2.96%) [6,7]. It is categorized into four types: vulgaris, arthropathy, pustular, and erythrodermic [8,

* Corresponding author.
** Corresponding author.
E-mail addresses: 14030@ynutcm.edu.cn (F. Huang), linyuping@ynutcm.edu.cn (Y. Lin).
[1] These authors contributed equally.

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9]. The early stage of psoriasis mainly manifests on the limbs and trunk, characterized by flushing skin and a small number of scales on the surface of the cortex. The late stages are mainly characterized by hypertrophic plaques and scales [10]. There has been a relatively longstanding awareness of the involvement of the immune system in psoriasis [11], which has proven to be one of the important causes of psoriasis. Current immune-based intervention strategies have demonstrated excellent efficacy against psoriasis and thus identified as effective ways of treating the disease [12]. To date, there is no radical cure for psoriasis. Most clinical drugs used are immunosuppressants (MTX and cyclosporine), biological agents (IL-23 Guselkumab, Tildrakizumab, Risankizumab, and IL-17 Secukinumab, Ixekizumab, Brodalumab), vitamin D derivatives, and retinoids, among other drugs [13,14]. Immunosuppressive and biological agents are currently widely used to treat psoriasis. However, these drugs have limited clinical application because of various deficiencies, including gastrointestinal reactions, bone marrow suppression, cancer, serious liver and kidney damage, and expensive price et al. [15, 16]. It is thus particularly important to find safe and long-term effective drugs for psoriasis treatment.

In psoriasis pathogenesis, Th17 cells are now recognized as important cells [17]. Th17 cells can secrete many cytokines, such as IL-17A, IL-17F, IL-22, and TNF-α, and neutrophils were an abundant source of IL-17A in psoriasis [18]. Modern research has shown that the immune response of psoriasis included enhancing the activation of T cells and myeloid cells, while upregulating TNF-α and IL-23, IL-17, and IL-6 [19]. Therefore, psoriasis could be treated by regulating Th17, and then inhibiting the secretion of related inflammatory factors. MPO was used as a marker of neutrophils, so reducing the level of MPO in psoriatic skin may be an appropriate method to reduce skin inflammation [20]. Neutrophils and the IL-17-producing Th17 subset of CD4 T cells have also a relationship [21].

Previous studies postulate that *Seseli mairei* Wolff (SMW), also known as "Yun Fang Feng," has antibacterial, anti-inflammatory, non-specific immunity, anti-allergic, antipyretic, and analgesic effects [22]. SMW is mainly derived from the dried roots of *Seseli mairei* Wolff (Umbelliferae) and is distributed in most areas of Yunnan Province, especially in Dali, Lijiang, Simao, Chuxiong, Honghe, and Lincang. SMW has been used effectively and safely to treat psoriasis and is often used as the main medicinal material of traditional Chinese medicine formulas in the clinical treatment of psoriasis, including Fangfengshuangbao Decoction, Jingfangkeyin decoction, Fangfengtongsheng medicinal powder et al. [23–25]. Though SMWE has been used for skin and immune diseases, its effect and mechanism on psoriasis have not been fully clarified. In this study, we explored the effect and mechanism of SMW extracts (SMWE) on psoriasis by assessing the Psoriasis Area and Severity Index (PASI) Score, skin lesion changes, histopathology and staining, inflammatory cytokines, and protein expression.

2. Materials and methods

2.1. Materials

Imiquimod cream (IMQ) (batch number: 19070639) was provided by Sichuan MED-SHINE Pharmaceutical Co, Ltd. (Sichuan, China). Vaseline (batch number: 20151210) was purchased from Shandong Lircon Medical Technology Co. (Shandong, China). Methotrexate (MTX) (batch number: 180702) was provided by Tonghua Maoxiang Pharmaceutical Co. (Jilin, China). Mouse ELISA kits IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and IL-23 (batch numbers: No. 20112530M, No. 20112531M, No. 20112535M, No. 20112538M, No. 20110906M, No. 20110908M, and No. 20112526M) were acquired from Jiangsu Meimian Industrial Co, Ltd (Jiangsu, China). Anti-IL-1β Rabbit pAb (#GB11113), anti-IL-6 Rabbit pAb (#GB11117), anti-IL-10 Rabbit pAb (#GB11108), anti-IL-17A Rabbit pAb (#GB11110-1), anti-IL-22 Rabbit pAb (#BS-2623R), anti-IL-23 Rabbit pAb (#BS-1193R), anti-Myeloperoxidase Rabbit pAb (#GB11224, MPO), anti-Ly6g Rabbit pAb (#GB1129, Gr-1) were provided by servicebio (Wuhan, China). Anti-IL-8 Rabbit pAb (#ab106350) was provided by Abcam. IL-17A cytokine (#C021) was purchased from Novoprotein (Shanghai, China). Hematoxylin and Eosin staining (batch number: ZH200905, ZH202812) were obtained from Servicebio (Wuhan, China). Equipment used included an embedding machine (Wuhan Junjie Electronics Co, Ltd, Wuhan, China), pathology slicer (Shanghai Leica Instruments Co, Ltd, Shanghai, China), freezing table (Wuhan Junjie Electronics Co, Ltd, Wuhan, China), MCO-20AIC CO_2 cell culture tank (Sanyo Company, Japan), infinite M200 proenzyme micro-plate reader (Tecan Company, Switzerland), and an inverted microscope and imaging system (Nikon, Japan).

2.2. Extraction of SMW

SMW was collected from Dali (Yunnan, China) and was identified by Professor Chunxia Pu (Yunnan University of Chinese Medicine, China). The botanical specimens (L20190801) were preserved in the Pharmaceutical and Food Resources Development Laboratory, School of Chinese Materia Medica, Yunnan University of Chinese Medicine, Kunming, China. Dry roots of SMW (300 g) were extracted thrice with 2400 mL 95% ethanol through heat reflux (2 h per treatment) to obtain SMW extracts. The combined extractions were filtered and concentrated using a rotary evaporator (Shanghai Ailang Instrument Co, Ltd. Shanghai, China). The ethanol extracts were then obtained by freeze-drying.

2.3. Cell culture and treatment

HaCaT cells were obtained from the Kunming Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences (Kunming, China). The HaCaT cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin, and 89% streptomycin double-antibody at 37 °C in 5% carbon dioxide (CO_2) for 48 h. The HaCaT cells were then seeded in 96-well plates containing the above media and treated with SMWE at an increasing dose of 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, and 2 mg/mL. The IC10 and IC50 values

were calculated after 24 and 48 h. Based on the cell inhibition rates and cellular viability results, 0.25, 0.5, and 1 mg/mL were set as the low (SMWE-L), medium (SMWE-M), and high (SMWE-H) doses of SMWE.

2.4. Effects of SMWE on the secretion of inflammatory factors induced by IL-17 in HaCaT cells

The HaCaT cells were seeded in 96-well plates containing the above media and were pretreated for 6 h. The cells were then treated with IL-17A (100 ng/mL) and SMWE at an increasing dose of 10 μg/mL (SMWE-L), 25 μg/mL (SMWE-M), 50 μg/mL (SMWE-H) for 24 h. The cell culture medium was collected and centrifuged for 20 min (2000–3000 rpm/min), and the supernatant was carefully collected. The level of IL-6 in the supernatant was then determined using an ELISA kit.

2.5. Animals and experiment design

Male BALB/c mice (7–8 weeks old, 18–22 g) were obtained from Hunan SLAC Laboratory Animal Co, Ltd. (Hunan, China, Production License No. SCXK (Xiang) 2019-0004). All mice were bred and maintained under specific pathogen-free (SPF) conditions at the Animal Experiment Center, Yunnan University of Chinese Medicine (Kunming, China) at 24 ± 1 °C and 50 ± 10% relative humidity under a controlled 12/12-h light/dark cycle for seven days. A normal pellet diet and water were freely provided during the feeding period as recommended in the guidelines of the National Institutes of Health (National Research Council, 1996). All animal experiments were approved by the Animal Ethics Committee of Yunnan University of Chinese Medicine (R-062021134, Kunming, China) and were carried out following the National Guidelines for the Care and Use of Laboratory Animals.

Thirty BALB/c mice were randomly divided into 5 groups (n = 6): the normal group, model group, positive group (MTX), high-dose of *Seseli mairei* Wolff extracts (SMWE-H) group, and the low-dose of *Seseli mairei* Wolff extracts (SMWE-L) group. All mice were subjected to a daily topical dose of 62.5 mg IMQ cream on the shaved back skin (2 × 3 cm^2) for 6 consecutive days except for the normal group (vaseline 62.5 mg/d) [26,27]. Mice in the normal and model groups were given normal saline solution (1 mL/100 g), those in the MTX group were given 1 mg/kg of MTX (the MTX was dissolved in sterile saline), while those in the SMWE groups were given SMWE at a dose of 300 mg/kg (SMWE-H) and 150 mg/kg (SMWE-L) for 6 consecutive days, orally.

2.6. Evaluation of the severity of skin inflammation

PASI score was used to evaluate the severity of skin inflammation and lesions [28,29] and it involved skin erythema, scales, and thickness. Erythema, scales, and thickness were scored independently from 0 to 4, where 0 represents "none," 1 represents "slight," 2 represents "moderate," 3 represents "marked," and 4 represents "severe." The total severity of skin inflammation and lesion score was calculated as the sum of the three indexes (0–12). All mice in each group were scored daily for six consecutive days from the first day when IMQ was administered to evaluate the severity of the psoriasis-like skin condition.

2.7. Calculation of the spleen index

The body mass and spleen mass of all mice were measured on the seven days. This was used in the calculation of the spleen index using the formula: spleen index = spleen mass (mg)/body mass (g).

2.8. Histopathology, immunofluorescence, and immunohistochemistry studies

Fresh skin tissues collected from the backs of the mice were fixed in 4% paraformaldehyde for 24 h and then embedded in paraffin. The paraffin-embedded samples were then cut into 3–5 μm-thick sections and placed on slides for H&E staining [30]. Immunohistochemistry (IHC) was used to determine the expression of IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and IL-23. The levels of Gr-1 and MPO were measured by immunofluorescence (IF) to identify the intervention mechanism of *Seseli mairei* Wolff on psoriasis. The paraffin-embedded sections were then dewaxed using water and incubated in a 3% hydrogen peroxide solution containing 3% BSA for 30 min at room temperature. The blocking fluid was drained, and the primary antibody was added, followed by overnight incubation at 4 °C. The sections were then dried, and the secondary antibody (HRP-labeled) was added to cover the tissues. The sections were finally examined under a microscope, and images were captured for analysis.

2.9. ELISA experiment

The levels of IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and IL-23 in the sample skins were determined using enzyme-linked immunosorbent assay to ascertain their effect. The sample skins were first rinsed and quickly cut at low temperatures for tissue homogenization (tissue: PBS = 1: 9, pH = 7.2–7.4). The prepared tissue samples were then placed in a tissue homogenizer and ground at 5000 rpm for 15 min. The supernatant of 10% of the tissue homogenate was then used to determine the levels of IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and IL-23 based on the operational requirements of the kits.

2.10. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from skin tissue with TRIzol reagent (Cowin, Jiangsu, China) and reverse transcribed into cDNA using

SuperRT cDNA Synthesis Kit cDNA (Cowin). The obtained cDNA was subjected to real-time fluorescence quantitative analysis. The sequences of the PCR primers were provided in Table 1. The RT-qPCR analysis was performed using the real-time PCR kit following the manufacturer's instructions. The gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to that of GAPDH.

2.11. Flow cytometric assays

The spleen lymphocytes of mice were obtained by using the mouse spleen lymphocyte isolation kit according to the manufacturer's instructions (Beijing Solarbio Science & Technology Co., Ltd, China). FVS510 (Fixable Viability Stain 510), CD4-FITC, CD8a-PerCp, and IL-17A-AF647 anti-bodies (BD Biosciences, NJ, US) were added to lymphocytes for cell staining. For intracellular staining (such as IL-17A-AF647), cells were fixed and permeabilized by Cytofix/Cytoperm TM Plus (BD Biosciences, NJ, US). Flow cytometric analysis was performed on the BD FACSC celesta.

2.12. Western blot

Protein lysate (400 μL) was added to the tissue sample and lysed for 30 min (on ice), and then centrifuged at 10,000 g at 4 °C for 10 min. The protein concentration in the supernatant was determined by using BCA quantitative kit following the manufacturer's instructions (Biyuntian Biology Science and Technology Co., Ltd, China). Protein was electro-transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was incubated with primary antibodies NF-κB (Beijing Bioss biology technology Co., Ltd, China, 1:1000), STAT3 (Beijing Bioss biology technology Co., Ltd, China, 1:1000), JAK2 (Beijing Solarbio Science and Technology Co., Ltd, China, 1:1000), and β-actin (Proteintech Group, US, 1:1000) at 4 °C overnight. Then the membrane was washed three times using TBST and incubated with secondary antibodies HRP-Rabbit-anti-goat (Proteintech Group, US, 1:2000) for 1 h. β-actin served as an internal reference.

2.13. Statistical analysis

Data were analyzed using GraphPad Prism 8.0 software and presented as means ± standard deviation (M ± SD). One-way analysis of variance (ANOVA) was then used to analyze the levels of variance within the groups at a significance threshold of $P < 0.05$.

3. Results

3.1. SMWE inhibits in vitro proliferation of immortalized human keratinocytes (HaCaT cell line)

The physiological characteristics of HaCaT cells are highly similar to those of normal human keratinocytes and are thus widely used as a model to study the proliferation and differentiation of human epidermal cells and the pharmacological activity of psoriasis treatment [31]. The viability of HaCaT cells was detected on the different concentration gradients *in vitro* to determine the inhibitory effects of SMWE. The density of HaCaT cells gradually decreased with an increase in SMWE concentration and time (Table 2 and Fig. 1A–C). There was no notable reduction in cell viability upon treatment with 0.025–0.05 mg/ml SMWE. These concentrations were thus selected for subsequent experiments.

3.2. SMWE reduces the level of IL-6 in HaCaT cells induced by IL-17A

IL-6 is a pleiotropic, proinflammatory cytokine that promotes the differentiation of B cells, T cells, and neutrophils. The mature neutrophils subsequently release proinflammatory cytokines, such as IL-23 and IL-17 [32]. The level of IL-6 induced by IL-17A in the supernatant of each group was detected using ELISA (Fig. 2). Notably, the level of IL-6 in the model group was significantly increased ($P < 0.001$) while the IL-6 content but significantly lower in the medium-dose ($P < 0.05$) and high-dose ($P < 0.01$) groups compared to

Table 1
Primer sequences of target genes.

Target gene	Primer sequence (5′ → 3′)
IL-17A (forward)	TCAATGCGGAGGGAAAG
IL-17A (reverse)	CTGCCTGGCGGACAAT
IL-17F (forward)	TGGGACTTGCCATTCTGA
IL-17F (reverse)	AACTGGAGCGGTTCTGG
IL-22 (forward)	CGTCAACCGCACCTTTAT
IL-22 (reverse)	TAGGGCTGGAACCTGTCTG
IL-23 (forward)	GACTCAGCCAACTCCTCC
IL-23 (reverse)	CTCCGTGGGCAAAGAC
TNF-α (forward)	TCTGCCTGGCTCATCTTTTC
TNF-α (reverse)	TTCCTCCTTGGTCCTCATTT
RORγt (forward)	AAGGCAAATACGGTGGTGT
RORγt (reverse)	GTGTAGAGGGCAATCTCATCC

Table 2
Effect of SMWE on HaCaT cell viability (n = 5).

Time	IC_{50} mg/mL	IC_{10} mg/mL
24h	1.92	0.32
48h	0.58	0.03

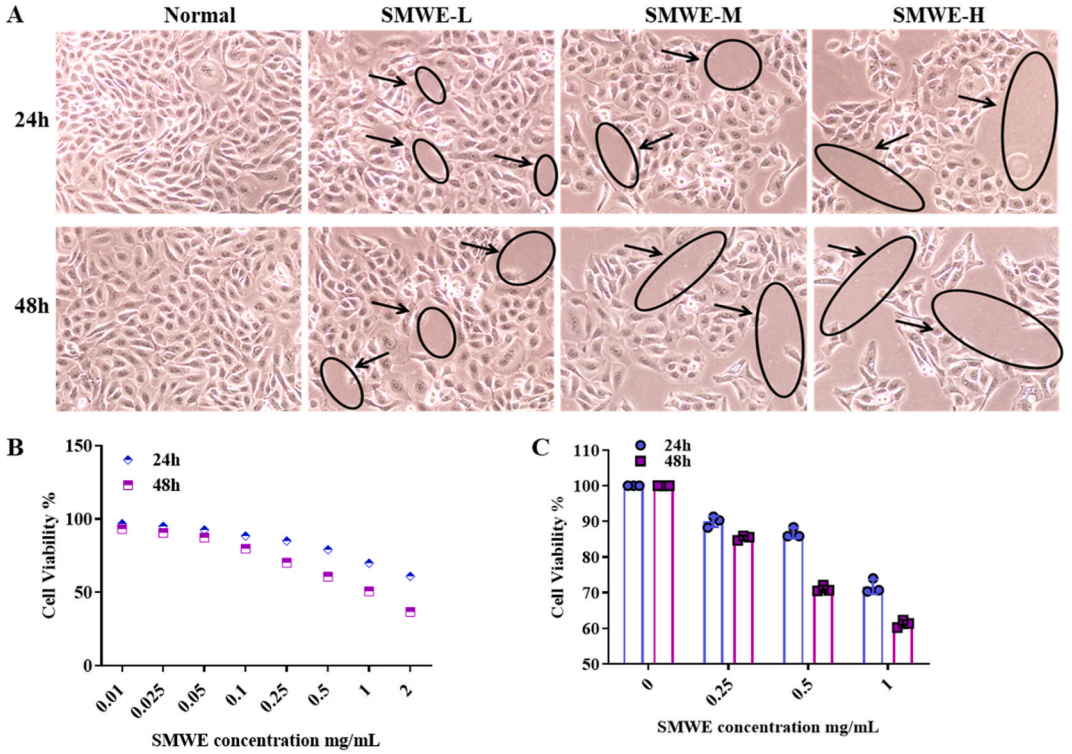

Fig. 1. Effect of SMWE on HaCaT Cells. (**A**) Morphology of HaCaT cells with different concentrations of SMWE. (**B**) Effects of SMWE on HaCaT cells with the different concentration gradients. (**C**) Effect of SMWE on HaCaT cell viability. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group.

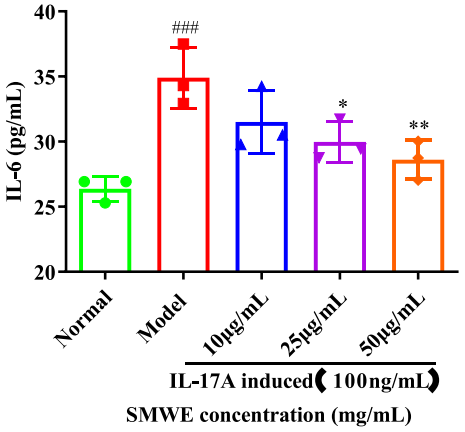

Fig. 2. Effect of SMWE on the level of IL-6 in HaCaT cells induced by IL-17 (Mean ± SD, n = 3). $^{###}P < 0.001$ versus the normal group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus the model group.

the model group. These results indicated that SMWE could inhibit the production of inflammatory cytokines (IL-6) in HaCaT cells stimulated by IL-17A.

3.3. SMWE attenuates the psoriatic skin lesions induced by IMQ

Imiquimod was applied to mimic psoriasis-like dermatitis in mice and was then treated with SMWE to evaluate the effect of SMWE on psoriasis. Fig. 3A and C shows the morphological observations and PASI scores. There was no change in the skin morphology of mice in the normal group, and they remained smooth, clean, and without any signs of inflammation. In contrast, there were notable symptoms of epidermal thickening, scales, and erythema on the back of mice in the model group. The PASI scores continually increased and were consistent with the clinical characteristics of psoriasis. Of note, the total PASI scores of mice treated with MTX and SMWE decreased significantly, and psoriasis symptoms, such as erythema and scales on the back's skin, were significantly attenuated on day 7 compared to scores of mice in the model group.

H&E staining further revealed the histopathological change. Compared to the Normal group, there showed increasing epidermal thickening (acanthosis), inflammatory cell infiltration, and hyperkeratosis in the back skin of the Model group. However, treatment of MTX and different doses of SMWE significantly reduced epidermal thickening, inflammatory cell infiltration, and hyperkeratosis in the dermis compared with the Model group (Fig. 3B and D) on day 7. These results suggested that SMWE could improve the symptoms of IMQ-induced psoriasis, including epidermal layer thickening, hyperkeratosis, and inflammatory cell infiltration.

3.4. SMWE ameliorates the splenic lesions induced by IMQ

The body weight and spleen mass of each group were determined and used to calculate the spleen index to evaluate the protective effect of SMWE on mouse splenic lesions induced by IMQ (Fig. 4A–B). The spleen index of mice in the model group significantly increased compared to that of mice in the normal group. In contrast, the spleen indexes of mice in the administration groups were significantly lower than that of mice in the model group ($P < 0.001$). These results demonstrated that SMWE ameliorated mouse splenic lesions induced by IMQ, highlighting its role in reducing the systemic inflammatory response.

3.5. SMWE reduces the expression of Gr-1 and MPO in IMQ-induced psoriasis mice

The important factors of neutrophils, which were involved in amplification feedback during the maintenance stage of psoriasis, were measured in the various treatment groups to determine the effect of SMWE on neutrophils. The expression of myeloperoxidase (MPO) (Fig. 5A–C) and lymphocyte antigen 6 complexes (Ly6C, Gr-1) (Fig. 6A–C) in the model mice was higher than in the normal

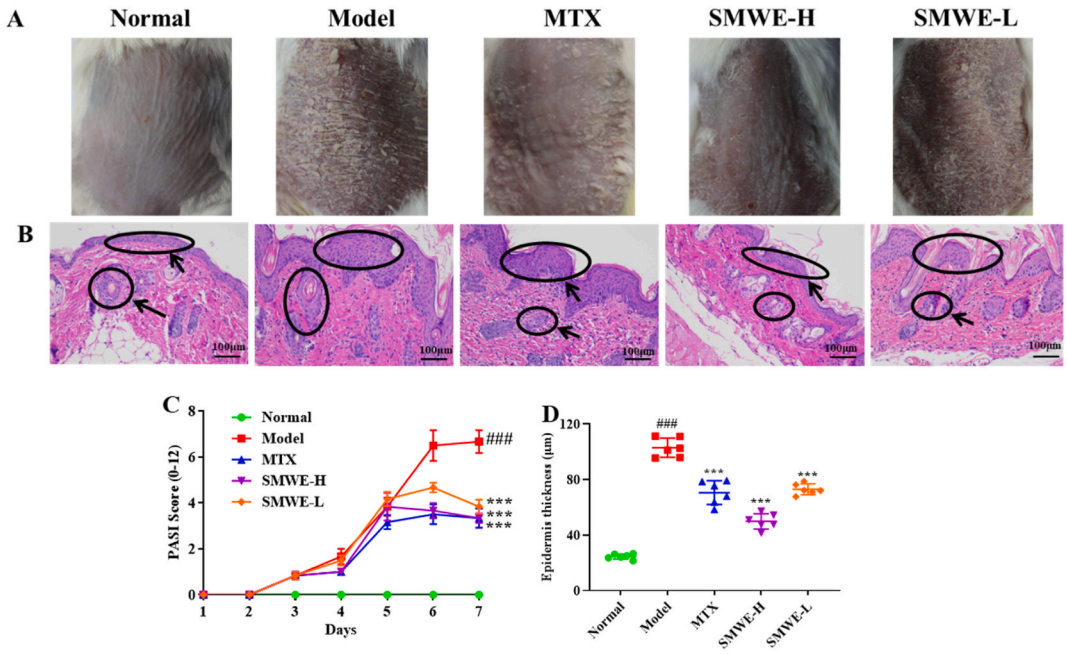

Fig. 3. Effect of SMWE on psoriasis mice induced by IMQ. (**A**) Skin morphology of mice in each group. (**B**) H&E staining of the dorsal skin in each group (200×). (**C**) The PASI score of the dorsal skin in each group. (**D**) Comparison of epidermis thickness of mice in each group on the 7th day (Mean ± SD, n = 6). $^{###}P < 0.001$ versus the normal group; $^{***}P < 0.001$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

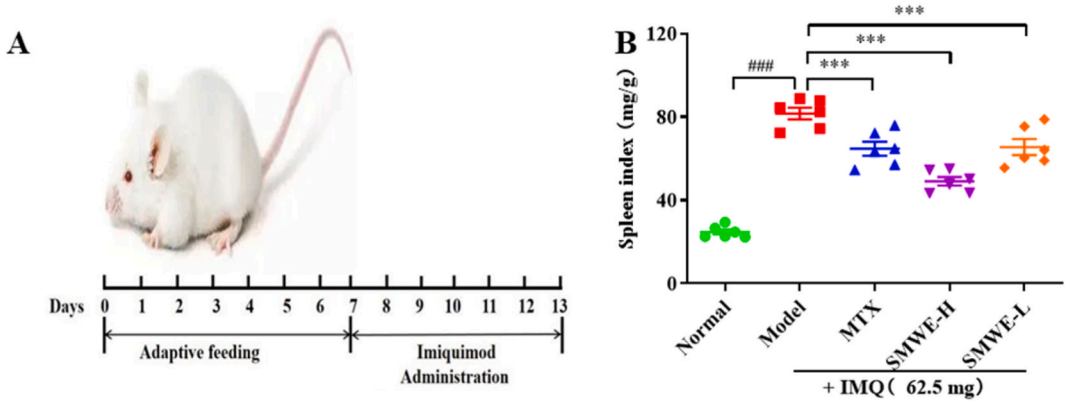

Fig. 4. Effect of SMWE on IMQ-induced psoriasis in mice. (**A**) Scheme of the IMQ-induced psoriasis mouse model and animal treatment. (**B**) Spleen index of mice in each group (Mean ± SD, n = 6). $^{###}P < 0.001$ versus the normal group; $^{***}P < 0.001$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

group. However, the expression of MPO and Gr-1 was significantly lower in the administration groups compared to the model group, indicating that SMWE could inhibit neutrophil activation.

3.6. SMWE reduces inflammatory cytokines in IMQ-induced psoriasis mice

The level of psoriasis-related inflammatory cytokines was detected by immunochemistry (Fig. 7A–G). IMQ reduced the level of IL-10 and increased the levels of IL-1β, IL-6, IL-8, IL-17A, IL-22, and IL-23 in the skin lesions of mice. However, treatment with MTX and SMWE decreased the expressions of IL-1β, IL-6, IL-8, IL-17A, IL-22, and IL-23 and significantly increased the expression of IL-10. These results confirmed the involvement of the aforementioned cytokines in the inflammatory process of psoriasis. The results further suggested that SMWE could inhibit the expression of these cytokines.

ELISA was further used to detect the inflammatory cytokines in the various treatment groups to verify the effect of SMWE on them (Fig. 8A–G). IL-1β, IL-6, IL-8, IL-17A, IL-22, and IL-23 increased, while IL-10 decreased in the model group compared to the control group. Consistent with the immunochemistry results, IL-1β, IL-6, IL-8, IL-17A, IL-22, and IL-23 decreased, while IL-10 increased significantly ($P < 0.001$) after MTX and SMWE administration. These results were consistent with the immunochemistry results and confirmed that SMWE could regulate psoriasis-related cytokines *in vivo*.

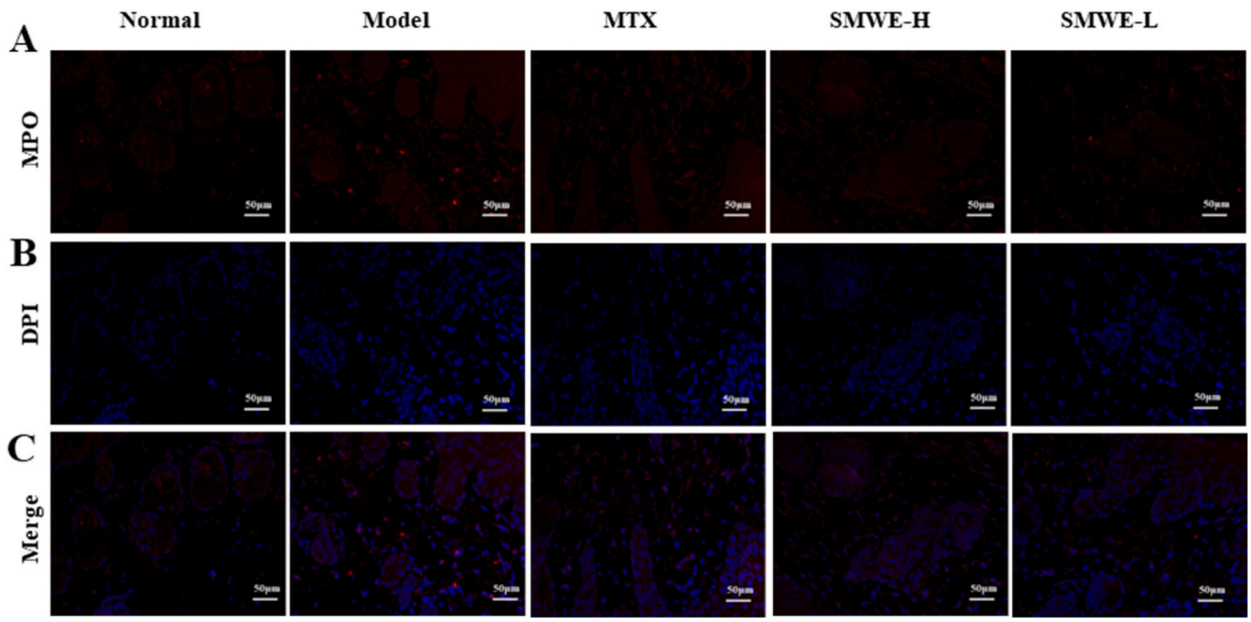

Fig. 5. Effect of SMWE on MPO (400×) (n = 3). (**A**) MPO; (**B**) DPI; (**C**) Merge. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

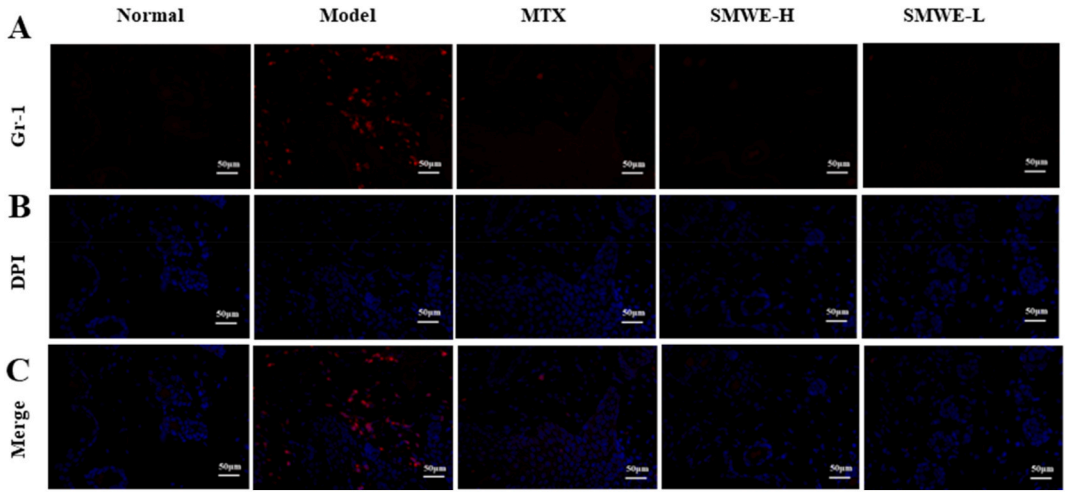

Fig. 6. Effect of SMWE on Gr-1 (400×) (n = 3). (**A**) Gr-1; (**B**) DPI; (**C**) Merge. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

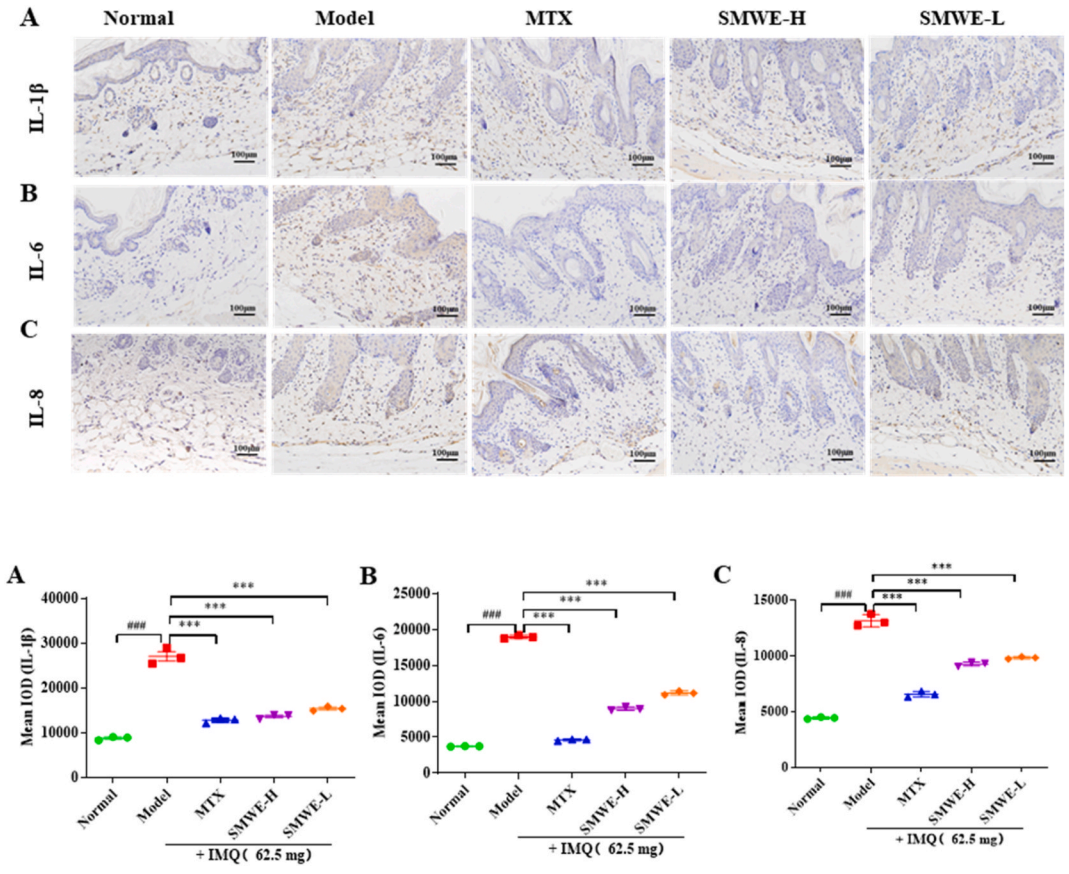

Fig. 7. The expressions and semi-quantitative analysis of IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22 and IL-23 immunohistochemistry (200×), Mean ± SD, n = 3. (**A**) IL-6; (**B**) IL-1β; (**C**) IL-8; (**D**) IL-17A; (**E**) IL-22; (**F**) IL-23; (**G**) IL-10. $^{###}P < 0.001$ versus the normal group; $^{***}P < 0.001$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

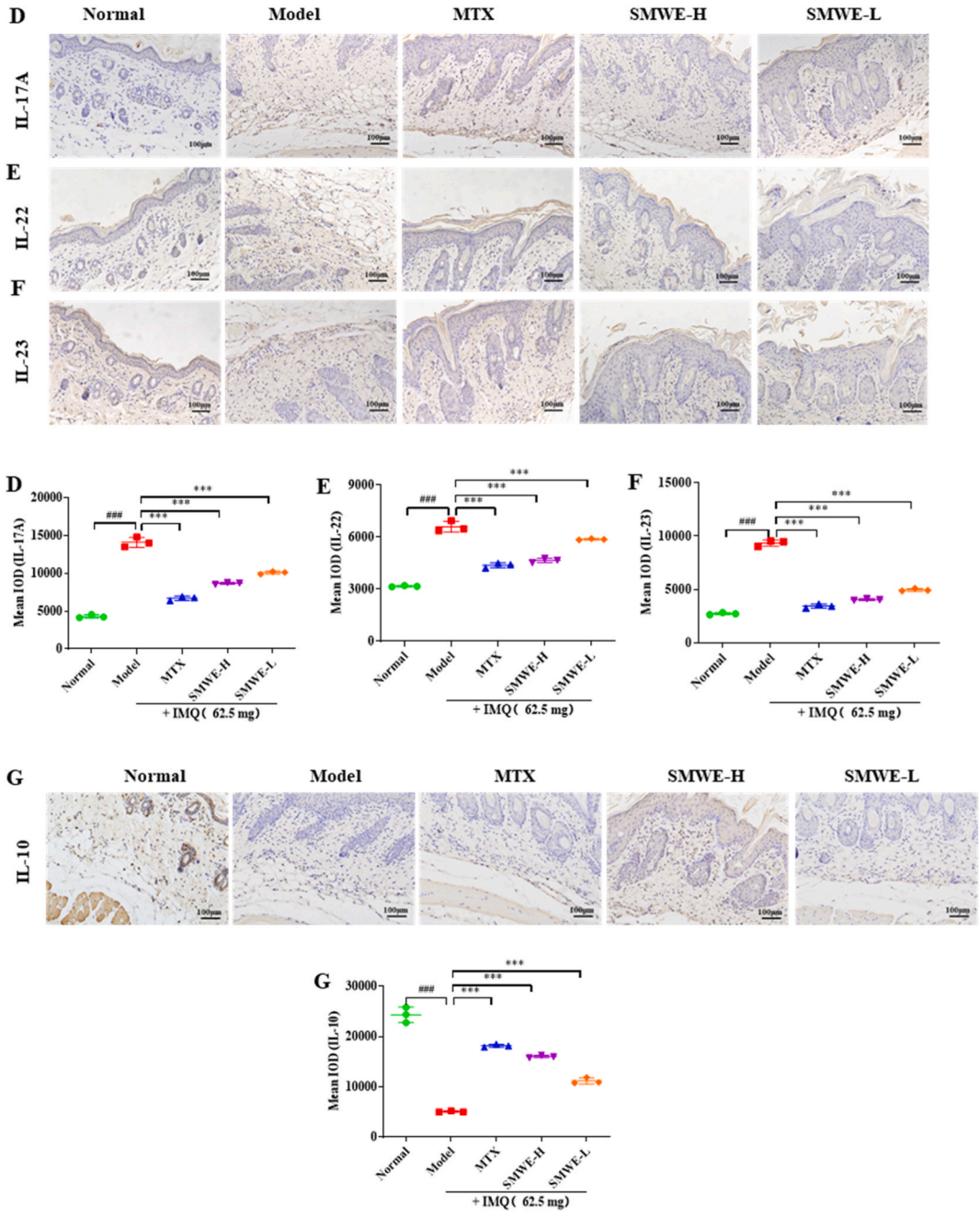

Fig. 7. (*continued*).

3.7. SMWE reduces mRNA expression of inflammatory cytokines in IMQ-induced psoriasis mice

According to the immunochemistry and ELISA results, the inflammatory factors IL-17A, IL-17F, IL-23, IL-22, TNF-α, and RORγt mRNA in mouse skin were detected by qRT-PCR. The results showed that the mRNA levels of IL-17A, IL-17F, IL-23, IL-22, TNF-α, and RORγt in model mice were significantly increased. After treatment of MTX and SMWE, the mRNA expression levels of IL-17A, IL-17F, IL-23, IL-22, TNF-α, and RORγt decreased significantly (Fig. 9A–F). These results suggested that SMWE could inhibit these cytokines to improve psoriasis.

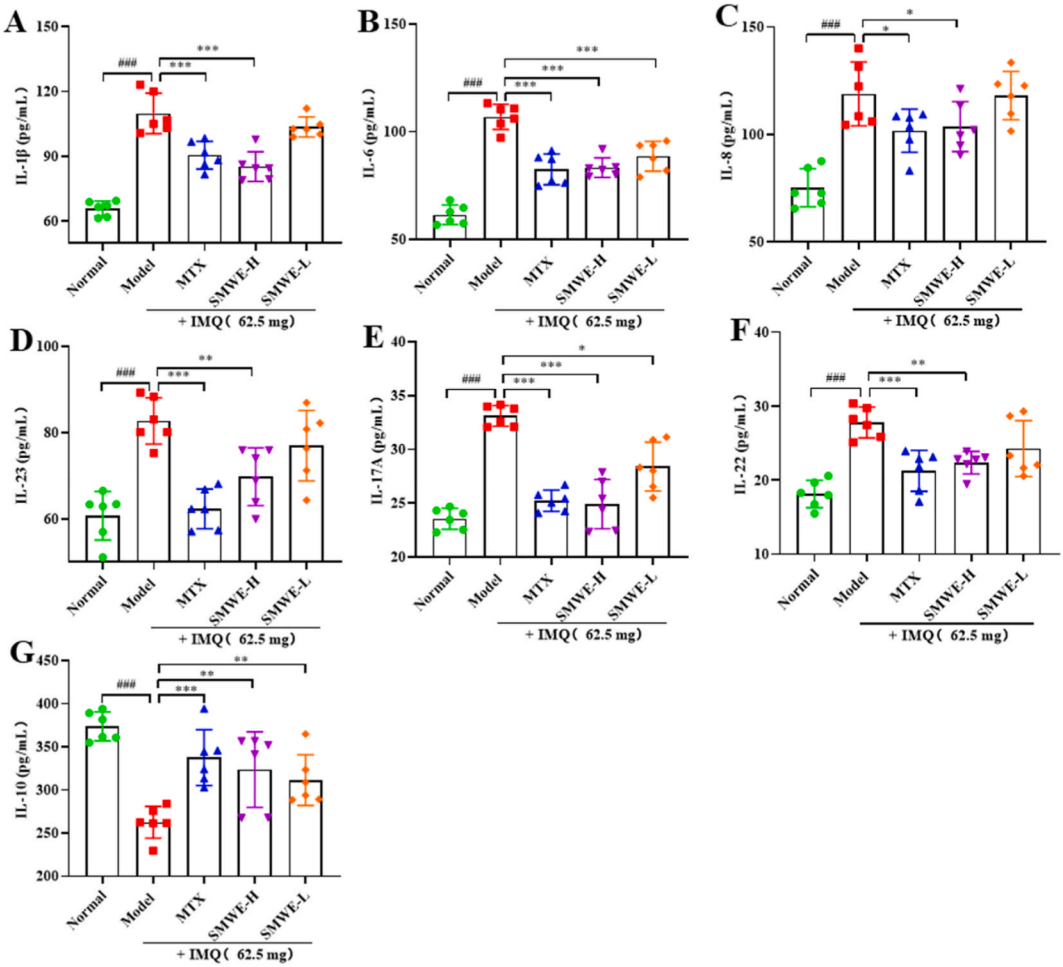

Fig. 8. Effect of SMWE on cytokines. (**A**) IL-1β, (**B**) IL-6, (**C**) IL-8, (**D**) IL-23, (**E**) IL-17A, (**F**) IL-22, (**G**) IL-10, (Mean ± SD, n = 6). $^{###}P < 0.001$ versus the normal group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

3.8. SMWE reduces $CD4^{+}IL\text{-}17A^{+}$ in IMQ-induced psoriasis mice

The Th17 cells play an essential role in the pathogenesis of psoriasis. The Th17 subset was one of the subsets discovered as a lineage of $CD4^{+}$ T cells and IL-17A was mainly produced by Th17 cells [33]. Therefore, this study analyzed the proportion of IL-17A in CD4 by flow cytometry and found that the proportion of CD4+IL-17A+ in the spleen of mice with psoriasis induced by IMQ was more than that in the normal group. Compared with the model group, the proportion of CD4+IL-17A+ in the spleen of the SMWE high-dose group and MTX mice decreased significantly. However, compared with the normal group, the CD4+/CD8 + ratio in the model group decreased. Compared with the model group, the ratio of CD4+/CD8+ in the MTX group and SMWE group increased (Fig. 10A–D). These results suggested that SMWE could inhibit Th17 cells to secret cytokines insulting in psoriasis.

3.9. SMWE inhibits NF-κB, STAT3, and JAK2 in IMQ-induced psoriasis mice

NF-κB pathways, STAT3, and JAK play an important role in psoriasis [34]. To study whether SMWE could affect NF-κB pathways, STAT3, and JAK in IMQ-induced psoriasis mice, the expression of NF-κB, STAT3, and JAK2 in the skin tissue of mice was detected by Western blotting. Results showed that the expression of NF-κB, STAT3, and JAK2 in the skin of mice in the model group increased significantly. Compared with the model group, the expressions of NF-κB, STAT3, and JAK2 in the SMWE group decreased (Fig. 11A–D), indicating that SMWE may intervene in psoriasis by decreasing the expression of NF-κB, STAT3, and JAK2.

4. Discussion

The Th17 cells play a major role in the pathogenesis of IMQ-induced psoriasis-like skin inflammation [18]. Our study demonstrated

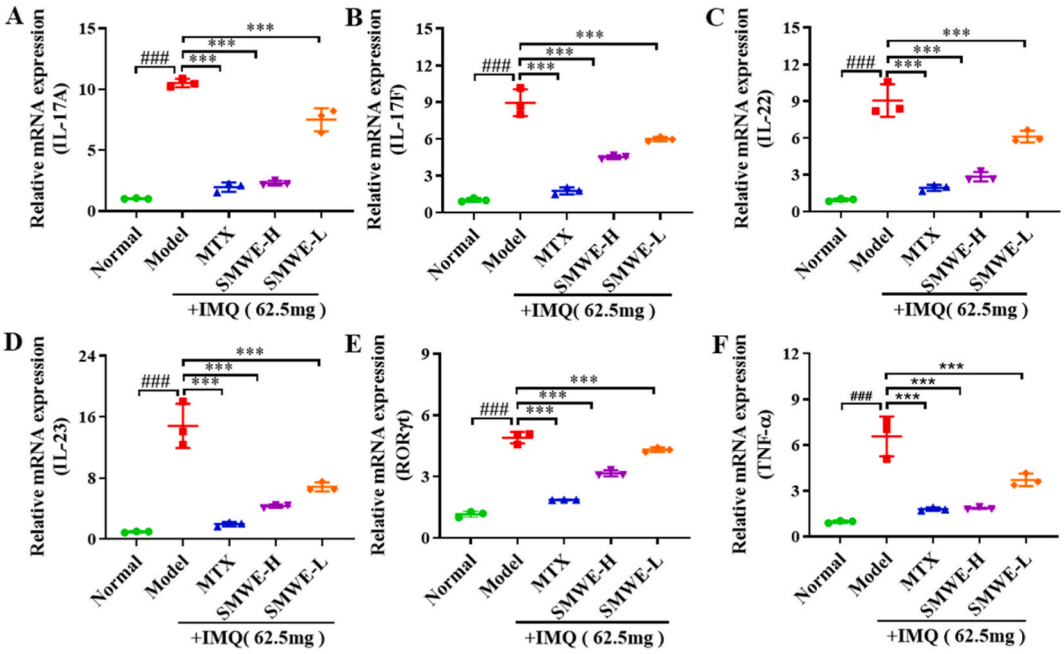

Fig. 9. qRT-PCR was performed to mRNA measure the expression of IL-17A (**A**), IL-17F (**B**), IL-22 (**C**), IL-23 (**D**), RORγt (**E**), and TNF-α (**F**) in skin lesions (Mean ± SD, n = 3). GAPDH served as an internal reference. $^{###}P < 0.001$ versus the normal group, $^{***}P < 0.001$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group. These results showed one representative of three repeated experiments.

that SMWE inhibited the increasing release of Th17 cells and the inflammation cytokines, including IL-23, IL17A, and IL-22 in the skin of IMQ-induced mice. SMWE attenuated IMQ-induced skin inflammation by suppressing the factors, STAT3, JAK2, and RORγt. These data suggested that SMWE could improve the pathogenesis of psoriasis-like skin inflammation by regulating Th17 cells.

Psoriasis is a chronic recurrent disease characterized by epidermal hyperplasia, erythema, and scales, affecting patients physically and psychologically [35]. Numerous studies postulated that Traditional Chinese Medicines (TCM) play a role in preventing and treating psoriasis. *Seseli mairei* Wolff is an ethical medicine used to treat psoriasis. In this study, we report the therapeutic effect and the possible anti-inflammatory mechanism of the ethanol extract of *Seseli mairei* Wolff on imiquimod-induced psoriasis mouse lesions and in HaCaT cells *in vitro*.

Imiquimod is a compound of the imidazoline-quinoline family and a toll-like receptor agonist [36], which promotes the secretion of various cytokines, including IL-17, IL-1β, IL-6, and IL-8 [37,38]. IMQ application can rapidly cause dermatitis similar to human psoriasis, thereby inducing systemic inflammatory responses [39]. Animal IMQ-induction models are thus widely used as a preclinical tool for the research of psoriasis because it well imitates the histopathological changes of psoriasis [40,41]. In this study, 5% IMQ was used to induce psoriasis in BALB/c mice, causing skin erythema, scales, and skin thickening. Notably, histopathological changes, including hyperkeratosis, parakeratosis, and lymphocyte infiltration, increased with time in the IMQ-induced mice model and corresponded to the clinicopathological manifestations of psoriasis. These results suggested that SMWE could attenuate the psoriasis symptoms, including erythema, scaling, thickening, and lymphocyte infiltration of the skin.

Psoriasis research mainly focuses on the immune regulation of $CD4^+$ T lymphocyte 17 cells ($CD4^+$ Thelper17, Th17) and Treg cells [42], because of their vital function in the development of many anti-immune diseases, such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease. Th17 cells enhance inflammatory processes and produce numerous cytokines, including IL-17A, IL-22, and IL-23, among other cytokines [43,44]. These cytokines participate in abnormal follicular proliferation, infiltration of lymphocytes, and other inflammation immune-mediated processes [45,46]. In addition, the increased pro-inflammatory factors can migrate to the skin tissues and recruit or activate inflammatory cells, such as neutrophils and macrophages, through the blood cycle, thereby exhibiting or aggravating psoriasis-like pathologies [47]. Cytokines are thus considered the master regulators that mediate the pathogenesis of psoriasis and are effective targets for treating psoriasis [48,49]. Drugs targeting IL-17 (Secukinumab and Ixekizumab) or IL-17R (Brodalumab) are effective in treating psoriasis among the recently developed biologic drugs [50,51]. In this study, the expression of IL-1β, IL-6, IL-8, IL-10, IL-17A, IL-22, and IL-23 among the various treatment groups with psoriasis were thus examined by immunochemistry and ELISA, while the mRNA levels of these inflammatory factors were examined by qRT-PCR. Notably, SMWE reduced the expression of IL-1β, IL-6, IL-8, IL-17A, IL-17F, IL-22, IL-23 and TNF-α, while increased the expression of IL-10. Flow cytometry results showed that SMWE decreased the expression of CD4+IL-17A + Th17 and the ratio of CD4+/CD8+ T cells in mice spleen lymphocytes.

RORγt has been demonstrated to be involved in several physiological and pathological processes in recent studies. RORγt has an essential role in developing Th17 cells, which are commonly expressed in the liver, thymus, and skin. Recent research advances have

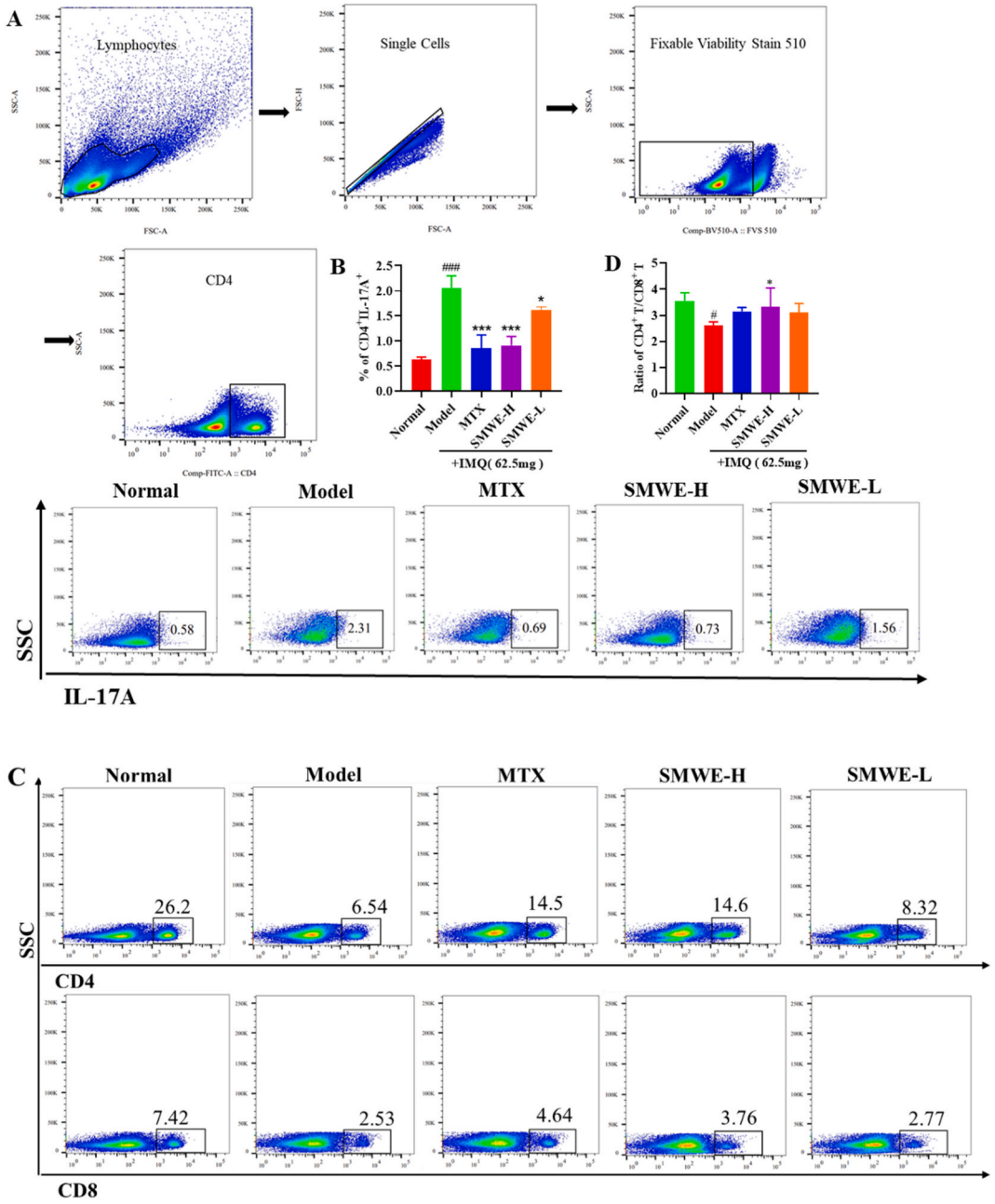

Fig. 10. The ratio of CD4+IL-17A+ (**A-B**) and ratio of CD4+/CD8+ T cells (**C-D**) in mice spleen lymphocytes were analyzed by flow cytometry. Histograms circled lymphocytes, single cells, FVS510- (viable cells), $CD4^+$, $CD8^+$, and IL-17A+, respectively. Data of the column graph are shown as mean ± SD (n = 3). $^{###}P < 0.001$, $^{#}P < 0.05$ versus the normal group; $^{***}P < 0.001$, $^{*}P < 0.05$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group.

established that RORγt is important target for treating multiple sclerosis, rheumatoid arthritis, and psoriasis [52,53]. It is thus a promising therapeutic approach to inhibit RORγt to treat immune diseases. Herein, SMWE inhibited the expression of RORγt in IMQ-induced psoriasis mice.

Although the underlying mechanisms of SMWE psoriasis resistance remain unclear, this study demonstrated that SMWE played an important role in anti-inflammatory effect by suppressing the expression of IL-1β, IL-6, IL-8, IL-17A, IL-22, and IL-23. SMWE also inhibited the vitality of HaCaT cells in a dose- and time-dependent manner and further suppressed IL-6 production, which was stimulated by IL-17A, in the HaCaT cells.

Psoriasis has a complex pathogenesis that is dependent on the interplay of multiple factors [54,55]. Studies postulate that multiple signal transduction pathways, such as MAPK, JAK/STAT, PI3K/mTOR, NF-κB, and WNT passages, among others, interact during the

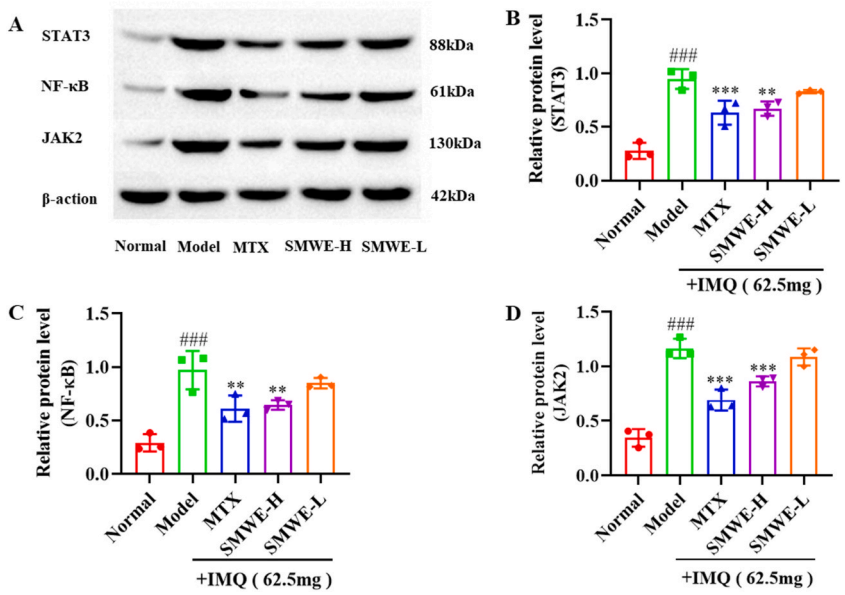

Fig. 11. SMWE inhibits STAT3 (**A, B**), NF-κB (**A, C**), and JAK2 (**A, D**) in IMQ-induced psoriatic skin lesions. The back skin of mice was taken for Western blot analysis (Mean ± SD, n = 3). β-actin served as an internal reference. $^{###}P < 0.001$ versus the normal group; $^{***}P < 0.001$, $^{**}P < 0.01$ versus the model group. MTX, Methotrexate; SMWE-H, high-dose of *Seseli mairei* Wolff extracts group; SMWE-L, low-dose of *Seseli mairei* Wolff extracts group.

pathogenesis of psoriasis [56–58]. The results showed that SMWE decreased the expression of NF-κB, JAK2, and STAT3 in the skin of psoriasis mice. Neutrophils participate in amplification feedback, leading to the release of many chemokines from keratinocytes in psoriasis. Neutrophil depletion has been proven to significantly improve intractable psoriasis in humans. Notably, neutrophils and their associated ROS and NETs play important roles in psoriasis [59]. NETs are web-like structures composed of decondensed chromatin coated with numerous neutrophilic components like Gr-1 and MPO. In this study, SMWE inhibited the expression of MPO and

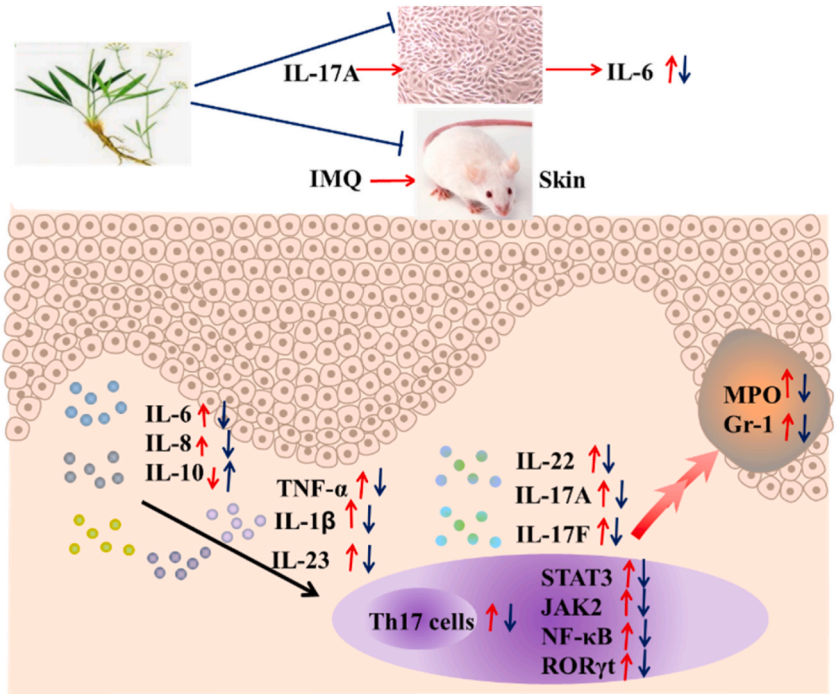

Fig. 12. SMWE attenuates IMQ-Induced Psoriasis Mice by inhibiting key inflammatory cytokines related to Th17 cells. Up arrows show increases and down arrows show decreases.

Gr-1, thereby ameliorating skin inflammation in mice by suppressing neutrophil activation. These results indicated SMWE improved psoriasis relating to the Th17 cells and neutrophils.

5. Conclusions

SMWE treatment attenuated the pathogenesis of psoriasis skin lesions, including scales, erythema, and skin thickening *in vivo* and inhibited the proliferation of HaCaT cells *in vitro*. Moreover, MTX and SMWE treatment down-regulated interleukins, RORγt, MPO, Gr-1, NF-κB, JAK2, and STAT3, while upregulating IL-10. SMWE interfered with psoriasis by regulating immune factors, Th17 cells activation, neutrophil activation, and inhibiting the differentiation and proliferation of HaCaT cells (Fig. 12). Nonetheless, further studies are still needed to better elucidate the mechanisms of SMWE on neutrophils.

Ethics approval and consent to participate

All procedures involving animals were approved by the Institutional Ethical Committee on Animal Care and the animal experiment program was approved by the Animal Ethics Committee of Yunnan University of Chinese Medicine (program number: R-062021134).

Author contribution statement

Mengmeng Wang: Xunqing Yin: Conceived and designed the experiments; Wrote the paper.
Yongcheng Zeng: Xue Qiao: Qionglian Fang: Contributed reagents, materials, analysis tools or data.
Chunyan Hu: Yongmei Xue: Xiujuan Zhao: Chenghong Du: Performed the experiments; Analyzed and interpreted the data.
Feng Huang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
Yuping Lin: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17315.

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