



Tryptanthrin ameliorates imiquimod-induced psoriasis in mice by suppressing inflammation and oxidative stress via NF- κ B/MAPK/Nrf2 pathways

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Abstract

Nowadays, approximately 3% of the world's population suffers from psoriasis, an inflammatory dermatosis with high recurrence. Tryptanthrin (TRYP) is a natural alkaloid that possesses anti-inflammatory activities on multiple diseases. The present study aimed to unravel whether TRYP could relieve psoriasis and how it works. Imiquimod (IMQ)-induced psoriatic mouse models were administered saline (model), TRYP (25 and 100 mg/kg), or methotrexate (MTX, 1 mg/kg) and considered as the positive control. TNF- α -induced keratinocytes (HaCaT cells) with TRYP (0, 10, 20 and 50 nM) were used for in vitro verification. Psoriasis area severity index (PASI) and spleen index were evaluated. Th17 cell infiltration in both spleens and lymph nodes was detected by flow cytometry. The expression levels of inflammatory cytokines, glutathione (GSH), malondialdehyde (MDA) and catalase (CAT), as well as superoxide dismutase (SOD), were examined by ELISA, while the NF- κ B/MAPK/Nrf2 pathways-related proteins were determined by western blot. TRYP significantly attenuated psoriatic skin lesions, increased GSH, SOD, and CAT levels, reduced spleen index, accumulation of MDA, the abundance of Th17 cells in both the spleen and lymph nodes, and secretion of inflammatory cytokines in IMQ-induced psoriatic mouse models. Mechanically, TRYP suppressed IMQ-activated NF- κ B (I κ B and p65), MAPK (JNK, ERK1/2, and p38), and activated Nrf2 signaling pathways. Similar alterations for inflammation and oxidative stress parameters and NF- κ B/MAPK/Nrf2 pathways were also observed in TNF- α -treated HaCaT cells upon TRYP treatment. Our findings suggested TRYP is effective in protecting against inflammation and oxidative stress in psoriasis-like pathogenesis by modulating the NF- κ B/MAPK/Nrf2 pathways.

Keywords Psoriasis · Tryptanthrin · Inflammation · IL-17A · Oxidative stress

Introduction

Psoriasis is a skin disorder due to systemic inflammation, the prevalence rate of which is approximately 3% in the world [1]. Psoriasis is described by scaly erythematous plaques caused by dysregulated keratinocyte proliferation and differentiation, as well as excessive inflammatory response, which has a profound influence on patient quality of life [2, 3]. Until now, psoriasis is incurable but can be controlled with medications. For the treatment of psoriasis, topical therapies with corticosteroid agents are widely used in mild psoriasis, while systemic agents are often used in severe conditions [4]. Some patients with psoriasis will develop resistance to topical therapy, and systemic therapies usually lead to unwanted side effects, such as nausea and gastrointestinal intolerance [5].

Even though the pathogenesis of psoriasis remains unclear, the essential role of inflammatory response and

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oxidative stress in the occurrence, development, and progression of psoriasis has been gradually uncovered in the past decades [6, 7]. It has been reported that CD4⁺T cell-mediated autoimmune response plays a central role in the abnormal activation of keratinocytes during psoriasis occurrence [8]. As one of the subtypes of CD4⁺T cell, the T helper 17 (Th 17) cell has been demonstrated as a key regulator in the pathogenesis of psoriasis in recent years [9, 10]. Cytokines secreted by Th17, especially IL-17A, accelerate the recruitment of inflammatory cells via antimicrobial protein, thereby enhancing the proliferation and suppressing the differentiation of keratinocytes [11]. Notably, anti-IL-17A has been approved by FDA for psoriasis therapy in 2015 [12], proving targeting IL-17A is a credible direction for exploring effective novel agents for psoriasis. Besides, increasing evidence supports that the dysfunction of the antioxidant system accompanied by increased reactive oxidative species (ROS) production in the skin is closely related to the progression of psoriasis. Interestingly, Fumaderm[®], a drug displaying potent antioxidant activity, has been proven to be an effective agent for the treatment of psoriasis [13],

revealing the potential of oxidative stress as the target for psoriasis therapy.

In recent decades, traditional Chinese herb has aroused immense clinical interest as an alternative medicine for the treatment of psoriasis [14]. Tryptanthrin (TRYP) (Fig. 1A) is a natural alkaloid with anti-inflammatory and anti-breast cancer effects [15, 16], which could be derived from *Polygonum tinctorium*, *Isatis tinctoria*, and indigo plants [17]. These three herbs have been demonstrated to effectively alleviate psoriasis [18–20]. Some research suggested a potent effect of TRYP on IL-17A inhibition [21]. The potential of TRYP against oxidative stress has also been revealed in recent years [22]. In light of the regulatory role of TRYP in IL-17A and the oxidative stress effect, we hypothesized that TRYP may be a drug candidate for psoriasis therapy. However, there is still no literature supporting this point yet.

To verify our hypothesis, the present study evaluated the pharmacologic effects of TRYP on imiquimod (IMQ)-induced psoriatic mouse models and TNF- α -induced human keratinocytes. The underlying molecular mechanisms were also preliminarily explored in vivo and in vitro.

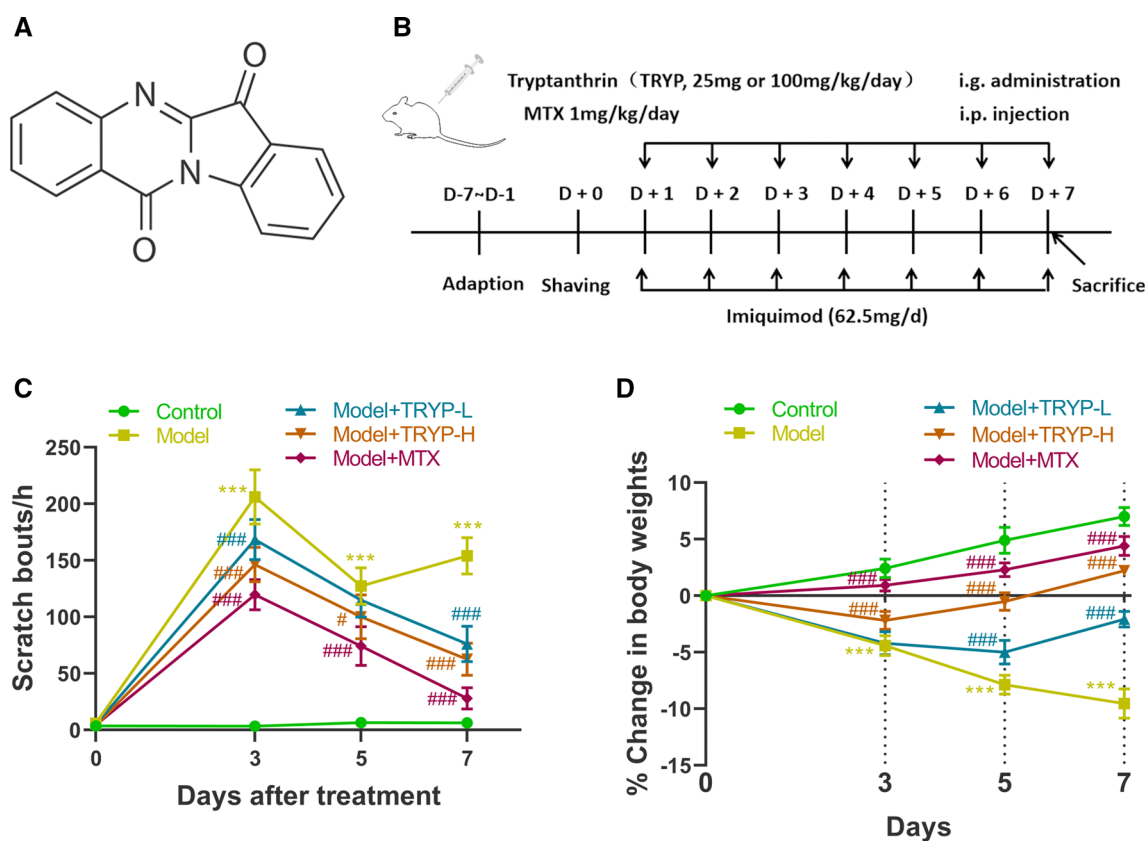


Fig. 1 The effect of TRYP on scratching behavior and body weight changes in psoriatic mouse models. **A** The chemical structure of TRYP. **B** Schematic experimental design of IMQ-induced psoriatic mouse models that received different treatments. **C** The scratch bouts

and **D** body weight changes of mice from each group on days 0, 3, 5, and 7 (** $p < 0.005$ significant difference from the control group, ### $p < 0.005$ significant difference from the model group)

Materials and methods

Psoriatic model establishment and treatments

The psoriatic mouse model was established by a topical application with 5% IMQ cream (62.5 mg/day) on a shaved back region for a week, as previously reported [23]. Given that methotrexate (MTX) is a widely used agent for the treatment of psoriasis [24], it was thus selected as the positive drug in animal experiments. TRYP (SML0310; purity 98%) used in this study was purchased from Sigma-Aldrich (MO, USA).

BALB/c male mice (18–25 g, 6–8 weeks) purchased from the Experimental Animal Center of Guangdong Province (Guangzhou, China) were arbitrarily divided into five groups ($n = 5/\text{group}$) after a 7-day acclimation as follows: control, model, model + TRYP-L (models received intragastric administration of a low dose (25 mg/kg) of TRYP), model + TRYP-H (models received intragastric administration of a high dose (100 mg/kg) of TRYP), and model + MTX (models received intragastric administration of 1 mg/kg MTX) groups. The animal experiment design is depicted in Fig. 1B. In brief, the back skin of the mice was shaved 1 day before IMQ treatment (day 0). All treatments were carried out at 12 h intervals after applying IMQ for seven consecutive days. The scratching behavior, body weight, and Psoriasis Area and Severity Index (PASI) of each mouse were recorded on days 0, 3, 5, and 7. The PASI was evaluated according to the extent of erythema, scales, and infiltration on the shaved back skin as follows: 0 means none; 1 means slight; 2 means moderate; 3 means marked; and 4 means severe. On day 7, mice were killed by cervical dislocation after 1 h of treatment to collect spleens, lymph nodes, and skin for further analysis.

To observe histological changes in the skin, the dorsal skin of each mouse was cut and fixed in formalin solution, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin (H&E).

The experimental protocol regarding animals was approved by Chongqing Traditional Chinese Medicine Hospital Animal Care and Use Committee.

Th17 cells quantification

Spleen samples and inguinal lymph node samples harvested from each group were ground to obtain single-cell suspensions. Cells were subjected to staining with FITC-conjugated anti-mouse CD4 antibody and Alexa Fluor 647-conjugated IL-17A antibody before the flow cytometric analysis. Finally, the percentage of CD4⁺/IL-17A⁺ was determined on flow cytometry (BD Biosciences, CA,

USA) to evaluate the infiltration of Th17 cells in the spleen and lymph nodes.

Cell culture and viability assessment

Human keratinocyte HaCaT cells purchased from Beyotime (C6282) were cultured in DMEM containing 10% FBS, 100-U/mL penicillin, and 100- $\mu\text{g}/\text{mL}$ streptomycin. The cells were maintained in a cell incubator with 5% CO₂ at 37 °C.

To obtain a suitable concentration range of TRYP for treatment with HaCaT cells, HaCaT cells were incubated with diverse concentrations of TRYP (0, 10, 20, 50, 100, and 200 nM), followed by subjecting to cell viability analysis using CCK-8 assay. Briefly, after incubation with TRYP for 24 h, the CCK-8 reagent was added to the plates for 2 h additional incubation. Finally, the optical density (OD) at 450 nm was monitored with a microplate reader (Bio-Rad, California, USA).

To mimic psoriatic inflammation in vitro, 20 ng/mL of TNF- α was used to incubate with HaCaT cells for 4 h [25]. The treatment of TRYP on HaCaT cells was subsequently carried out for another 12 h.

Inflammatory cytokines detection

By using commercial ELISA kits, the concentrations of inflammatory cytokines in the protein isolated from skin tissues were determined. The concentrations of IL-1 β , IL-6, and IL-17A were also detected in the cell supernatants of diverse groups. The ELISA kits for mouse TNF- α (ab208348), mouse IL-22 (ab223857), IL-1 β (mouse: ab197742; human: ab46052), IL-6 (mouse: ab222503; human: ab178013), and IL-17A (mouse: ab199081; human: ab83688) were purchased from Abcam (Cambridge, UK).

RT-PCR

Total RNA was extracted from epidermis layers of skin tissues using TRIzol reagent (Thermo Fisher, Massachusetts, USA). cDNA synthesis was performed using PrimeScript RT reagent kit (Takara, Tokyo, Japan). Using a SYBR Premix EX TaqTM II kit (Takara), qRT-PCR was performed on a Bio-Rad system to detect the expression of studied genes, the primer sequences of which are listed in Supplementary Table 1. Finally, the relative gene expression was calculated with GAPDHs as internal control using the comparative Ct ($2^{-\Delta\Delta\text{CT}}$) method [26].

Oxidative stress parameter examination

The content of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), and catalase (CAT) was

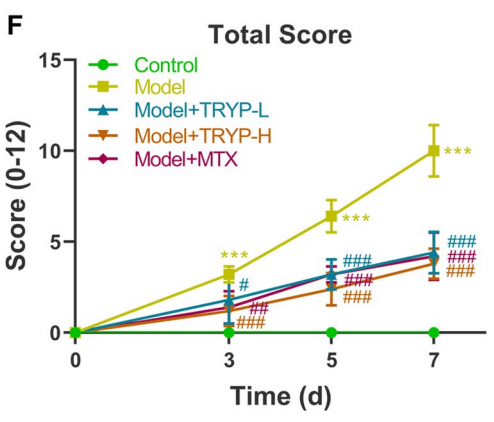
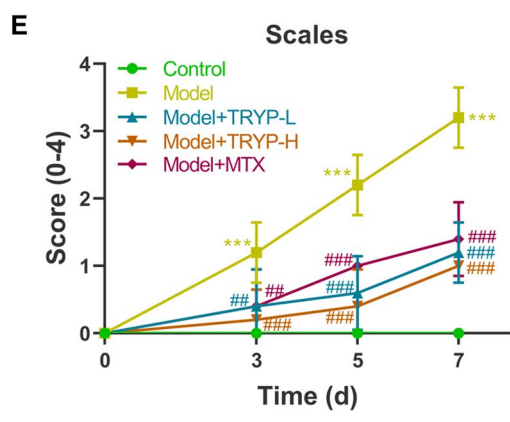
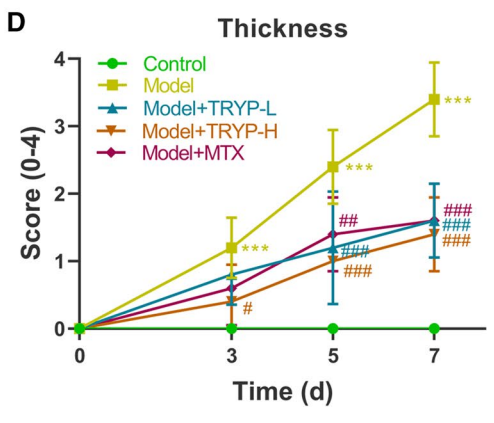
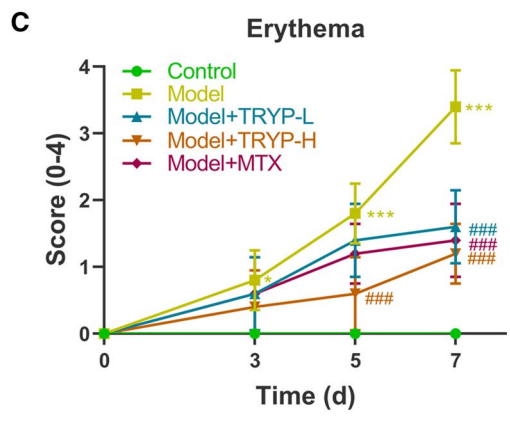
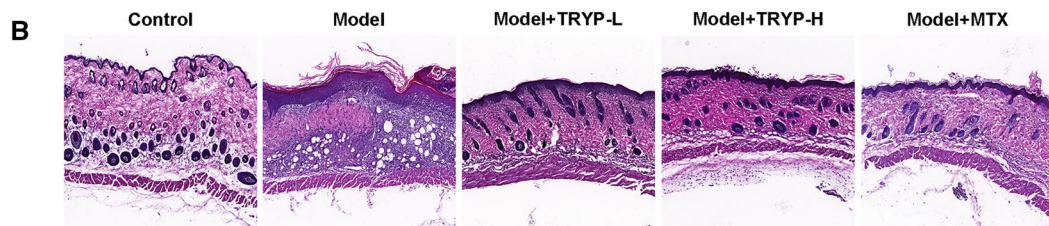
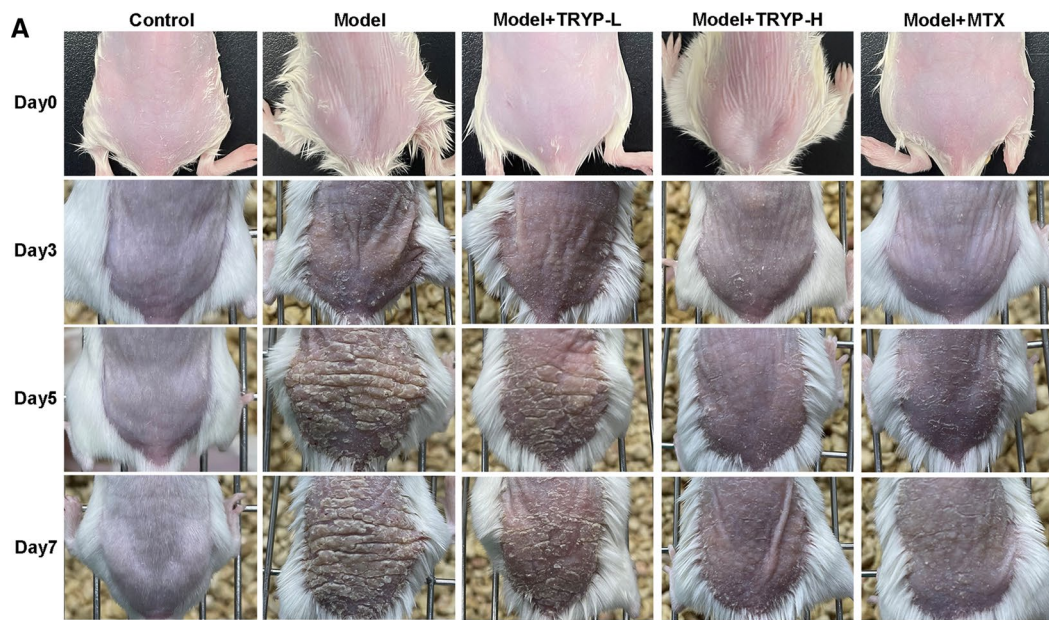


Fig. 2 TRYP ameliorated psoriasis-like skin lesions in psoriatic mouse models. **A** Representative images of dorsal skin of mice from different groups on days 0, 3, 5 and 7. **B** Representative H&E staining of dorsal skin tissues of mice from different groups on day 7. **C** Erythema, **D** thickness, and **E** scales of the back skin was scored on days 0, 3, 5 and 7 on a scale from 0 to 4; **F** the corresponding cumulative score (0 to 12) was subsequently calculated (* $p < 0.05$ and *** $p < 0.005$, significant difference from the control group; # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.005$ significant difference from the model group)

measured in vitro and in vivo using the corresponding commercial kits (Beyotime, Shanghai, China) following protocols provided by the manufacturers.

Nucleoproteins extraction

Cell nuclei proteins from epidermis layers of skin tissues and HaCaT cells were extracted using a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Beijing, China). Samples were placed in 300 μL of extract buffer A containing 1 μL protease and phosphatase inhibitors. After incubation at 4 $^{\circ}\text{C}$ with agitation for 30 min and centrifugation at 2000 $\times g$ for 5 min, the supernatant was discarded and the precipitate was washed twice with PBS. Then, 100 μL of cold extract buffer B, containing 0.5 μL protease inhibitor and phosphatase inhibitor, was added. The samples were vigorously mixed at a high speed for 15 s, and the mixture was incubated at 4 $^{\circ}\text{C}$ with agitation for 30 min and subsequently centrifuged at 12,000 $\times g$ for 10 min. The supernatant contained the nucleoproteins.

Western blot

Total protein isolated from skin tissues or cells by using RIPA buffer (10X) (Cell signaling, Massachusetts, USA) was subjected to quantification with a BCA protein assay kit (Thermo scientific). The total protein or nucleoprotein was separated through SDS-PAGE gel before being transferred onto PVDF membranes. Afterward, membranes were blocked for 2 h and subsequently incubated overnight with primary antibodies. The information on primary antibodies used in this study is listed in Supplementary Table 2. Thereafter, membranes were rinsed and incubated with HRP-conjugated secondary antibody (ab7090; Abcam) for 2 h. Finally, protein bands were visualized with an ECL Western Blotting Substrate (Thermo Scientific, Massachusetts, USA).

NF- κB DNA-binding activity assay

After the nucleoprotein extraction from HaCaT cells, isolated nuclear protein fractions were then applied to assess NF- κB p65 binding activity by using NF- κB p65

Transcription Factor Assay Kit (ab133112; Abcam) according to the manufacturer's instructions.

Statistical analysis

GraphPad Prism (8.0.1 version; GraphPad Software, CA, USA) was applied in this study. Data were expressed as the mean \pm SD from at least three different experiments and analyzed using a one-way analysis of variance, followed by Tukey's post hoc test. P less than 0.05 represented that the difference was statistically significant.

Results

The effect of TRYP on scratching behavior, body weight changes, and psoriasis-like skin lesions in psoriatic mouse models

To investigate the therapeutic potential of TRYP on psoriasis, IMQ-induced psoriatic mouse models treated with TRYP or MTX (a reference drug that is effective in psoriasis) were utilized. After smearing IMQ cream to the shaved back skin of mice, the mice developed marked scratching behavior on the third day, which could be attenuated by both TRYP and MTX treatments (Fig. 1C). A significant weight loss was observed in psoriatic mouse models without treatment, suggesting systemic inflammation occurred in psoriatic mouse models since the body weight is considered an essential indicator of systemic inflammation (Fig. 1D). Interestingly, TRYP effectively rescued the weight loss of mice induced by IMQ (Fig. 1D).

The dorsal skin photographs of mice from each group showed that the model mice displayed typical psoriasis-like skin lesions on the shaved back, including the large areas of deep red plaques and the thickened and widely distributed scaling on the shaved back, compared to control mice (Fig. 2A). Intra-gastric administration of TRYP, especially of the high dose, considerably improved skin lesions across the treatment period (Fig. 2A). H&E staining was performed to observe the microenvironmental effects in skin lesions after diverse treatments. IMQ caused increased thickening of the epidermis layer and increased immune cell infiltration in the dermis layer. TRYP improved the pathological changes in skin lesions with reduced epidermal thickening, showing an effect similar to that in the MTX group (Fig. 2B). The result of daily PASI scores revealed that the degree of erythema, scale, and thickening in the model group became more and more serious, which was in line with the aforementioned finding (Fig. 2C–F). As expected, a significant decrease in the severity index of skin erythema, scaling, thickness, as well as cumulative scoring was observed in all treatment groups (model + TRYP-L, model + TRYP-H, and

model + MTX) as compared to the model group (Fig. 2C–F). These data revealed a comparable therapeutic effect of TRYP to MTX. Collectively, TRYP effectively ameliorated IMQ-induced scratching behavior, body weight loss, as well as psoriatic skin lesion in mice.

The effect of TRYP on IMQ-induced skin inflammation and oxidative stress in psoriatic mouse models

It has been reported that IMQ is capable of inducing psoriasis-like inflammation, accompanied by an increase of splenic Th17 cells in mice [27]. Hence, Th17 (IL-17A⁺CD4⁺) cell infiltration in the spleen and lymph nodes of psoriatic mouse models was investigated after the treatment of TRYP. A low percentage of IL-17A⁺CD4⁺ T cells in both the spleen and lymph nodes of the control mice was observed (Fig. 3A). Compared with the control group, the IL-17A⁺CD4⁺ cell frequency in both the spleen and lymph nodes was markedly increased in the model group (Fig. 3A). TRYP, at either low or high doses, clearly decreased the abundance of IL-17A⁺CD4⁺ T cells in lymph nodes, while TRYP at high doses, but not low doses, also significantly reduced the percentage of IL-17A⁺CD4⁺ T cells in the spleens (Fig. 3A). Besides, we demonstrated that treatment of TRYP considerably diminished the elevation of spleen index (ratio of spleen weight to body weight) induced by IMQ in mice (Fig. 3B), suggesting the anti-inflammatory activity of TRYP. Psoriasis-related inflammatory cytokines, such as TNF- α , IL-1 β , IL-6, IL-17A, and IL-22, were subsequently determined in the skin lesions. The outcomes from ELISA revealed that the release of the aforementioned inflammatory cytokines in the model group was more than those in the control group (Fig. 3C). On the other hand, treatments of TRYP, at either low or high doses, or MTX led to a significant reduction in inflammatory cytokines released from the skin of psoriatic mouse models (Fig. 3C). RT-PCR analysis showed that the mRNA expression levels of these inflammatory cytokines in the skin lesions from each group displayed a similar tendency to the result of ELISA (Fig. 3D), further revealing that TRYP effectively reduced the inflammation response in psoriatic skin tissue.

Except for inflammation, oxidative stress is considered another critical factor in the development of psoriasis; thus, the changes in MDA, GSH, SOD, and CAT were further examined in the skin lesions. The MDA level in the model group was higher than that in the control group, which could be reduced by TRYP at both high and low doses (Fig. 3E). Topical exposing IMQ induced a significant decrease in the levels of antioxidative related biomarkers (GSH, SOD and CAT) in the skin, and the treatment of TRYP restored the decreased levels (Fig. 3F–H). Moreover, the administration of MTX also displayed a suppression effect on IMQ-induced

oxidative stress, as demonstrated by the decrease of MDA content and the increase of GSH, SOD, and CAT levels in the skin of psoriatic mouse models (Fig. 3E–H). Notably, we found that the effect of TRYP at high doses on oxidative stress in psoriasis was slightly greater than that of MTX (Fig. 3E–H). These findings suggested that TRYP was potent in attenuating IMQ-induced oxidative stress *in vivo*.

TRYP regulated the NF- κ B/MAPK/Nrf2 pathways in psoriatic mouse models

Both the NF- κ B and MAPK pathways are considered important signals involved in the pathogenesis of psoriasis because of their critical role in inflammation [28]. To understand the mechanism behind the anti-inflammatory effect of TRYP in psoriatic mouse models, the expression and phosphorylation of I κ B, NF- κ B p65, and three MAPKs (JNK, ERK1/2, and p38 MAPK) were analyzed by western blot. The topical administration of IMQ caused a significant increase in the phosphorylation of I κ B, NF- κ B p65, JNK, ERK1/2, and p38 MAPK, as well as the NF- κ B nuclear translocation in the skin (Fig. 4A, B), suggesting that the NF- κ B and MAPK pathways were activated in psoriatic skin lesions. After treatment with TRYP, the phosphorylation level of I κ B, NF- κ B p65, JNK, and ERK1/2, and the NF- κ B nuclear translocation in psoriatic skin lesions was reduced, while that of p38 MAPK showed no remarkable change (Fig. 4A, B). Moreover, the regulatory role of TRYP in Nrf2 signaling, a prominent target for oxidative stress, was observed in psoriatic mouse models. In comparison with the control group, nuclear localization of Nrf2 in the model group was significantly increased, which was accompanied by the elevation of HO-1 expression (Fig. 4C). Upon treatment of TRYP, Nrf2 signaling was further activated (Fig. 4C), suggesting TRYP probably ameliorated oxidative stress in psoriatic skin via increasing Nrf2 activation.

TRYP suppressed the inflammation and oxidative stress induced by TNF- α via the NF- κ B/MAPK/Nrf2 pathways in HaCaT cells

To further confirm the therapeutic potential of TRYP on psoriasis-like traits, the anti-inflammatory and antioxidative effects were also explored *in vitro*. To select suitable concentrations of TRYP for *in vitro* study, HaCaT cells were pretreated with diverse concentrations of TRYP (0, 10, 20, 50, 100, and 200 nM), followed by a CCK-8 assay. As shown in Fig. 5A, at concentrations between 0 and 50 nM, TRYP did not cause cytotoxicity in HaCaT cells; however, up to 100 nM, TRYP began to impair the cell viability of HaCaT cells. Thus, the safe concentrations of TRYP (10, 20, and 50 nM) were chosen for subsequent experiments.

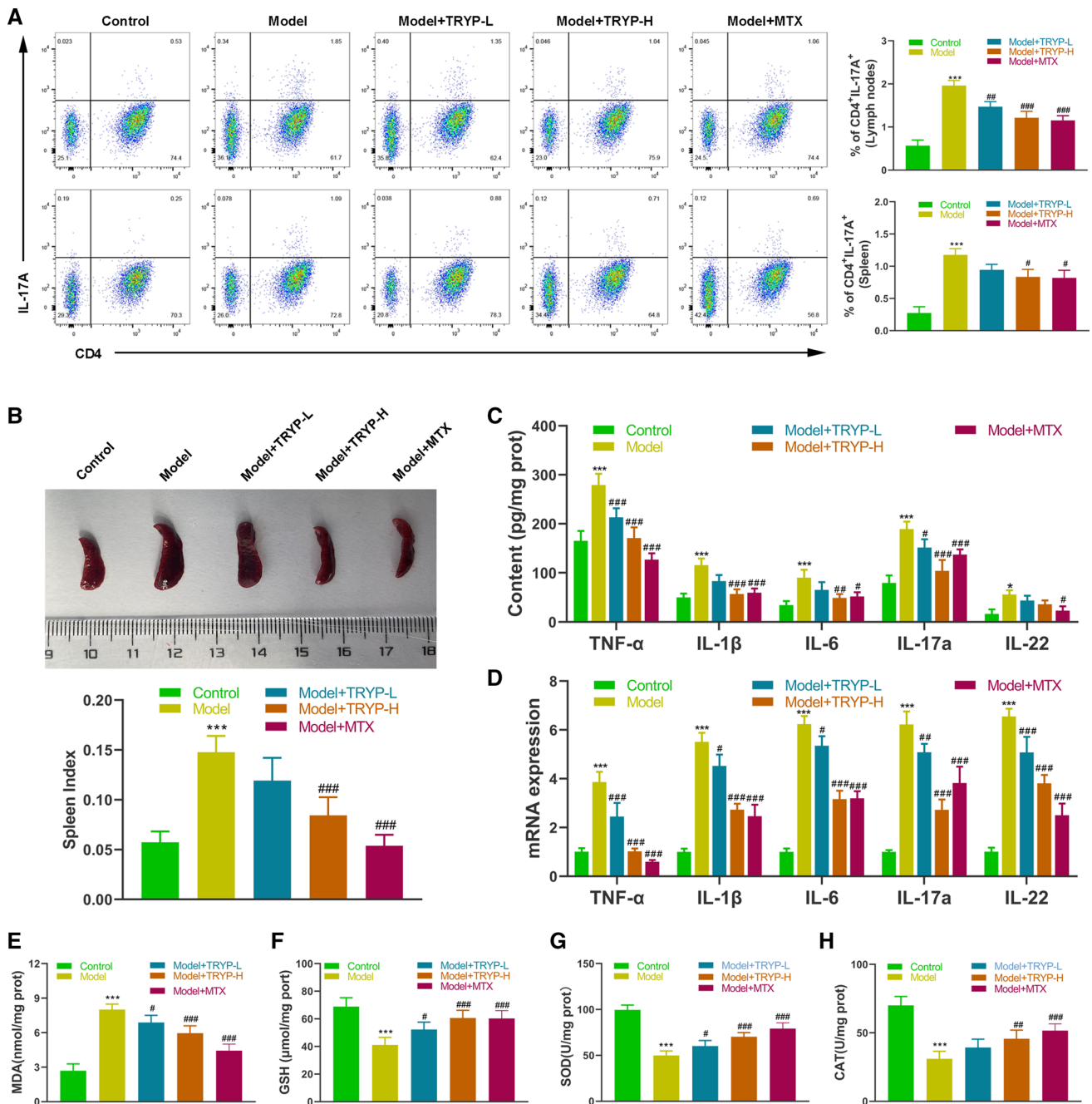


Fig. 3 The effect of TRYP on IMQ-induced skin inflammation and oxidative stress in psoriatic mouse models. **A** The percentage of IL-17A⁺CD4⁺ T cells in the total live cells in the spleen (top) and lymph nodes (bottom) isolated from mice of each group. **B** Representative photographs of the spleen (top) and spleen index (bottom) of mice from each group. **C** The secretion and **D** mRNA expression of TNF-

α , IL-1 β , IL-6, IL-17A, and IL-22 cytokines of skin from each group of mice. The contents of **E** MDA, **F** GSH, **G** SOD, and **H** CAT in the skin from each group of mice (* $p < 0.05$ and *** $p < 0.005$, significant difference from the control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.005$ significant difference from the model group)

Interestingly, TNF- α -treated HaCaT cells exhibited a significant elevation in the levels of secretion of IL-1 β , IL-6, and IL-17A, while this increase was blocked by TRYP treatment in a concentration-dependent way (Fig. 5B–D). Besides, the stimulation of TNF- α caused also increased

levels of MDA (Fig. 5E) and decreased GSH, SOD, and CAT levels (Fig. 5F–H), suggesting the occurrence of oxidative stress in HaCaT cells. Treatment with TRYP at various concentrations (10, 20 and 50 nM) significantly attenuated the oxidative stress of HaCaT cells induced by TNF- α , as

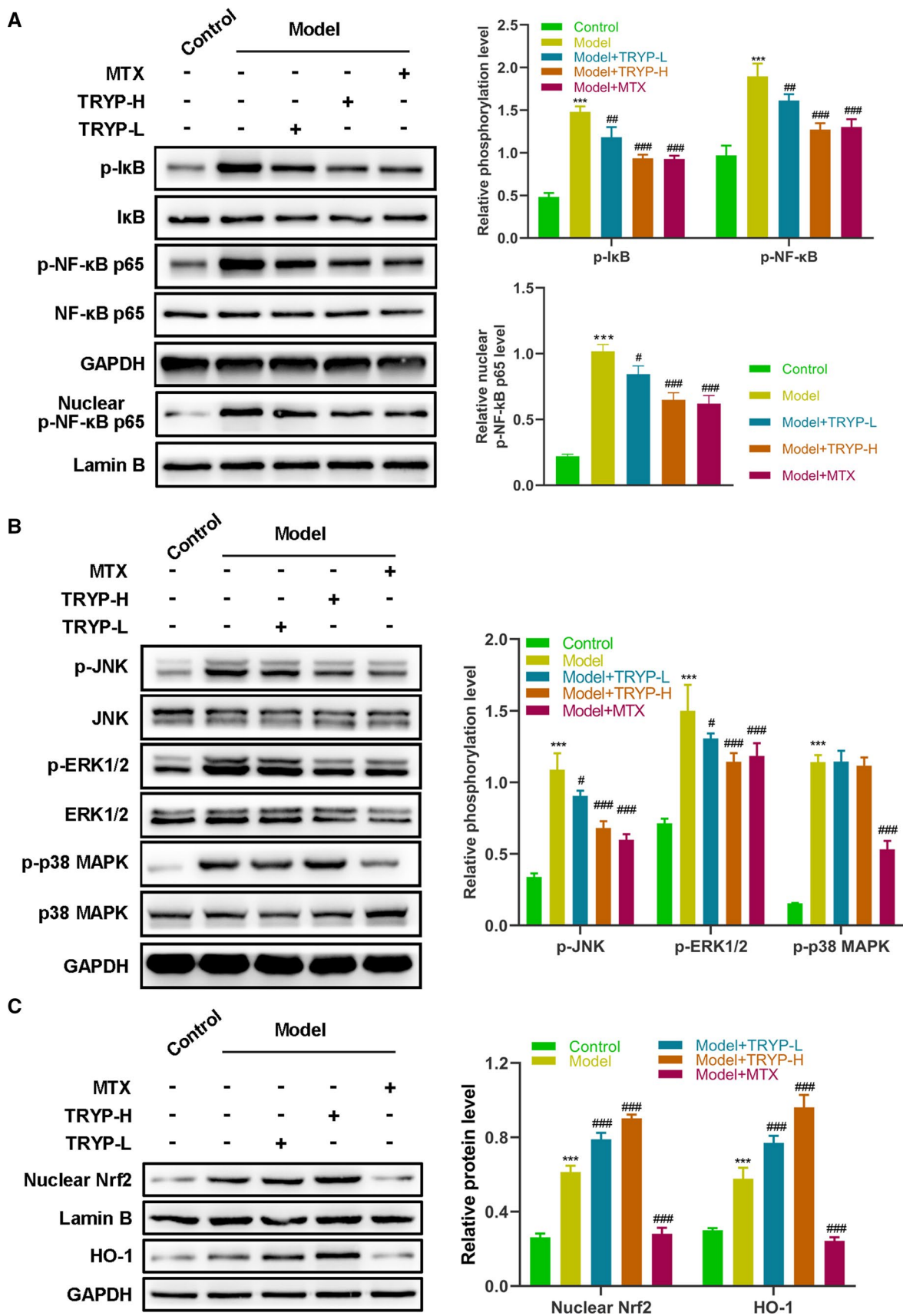


Fig. 4 TRYP regulated the NF- κ B/MAPK/Nrf2 pathways in psoriatic mouse models. **A** The expression and phosphorylation levels of I κ B and NF- κ B p65 proteins in the skin of each group of mice. **B** The expression and phosphorylation levels of JNK, ERK1/2, and p38 MAPK in the skin of each group of mice. **C** The expression levels of nuclear translocation of Nrf2 and HO-1 in the skin of each group of mice (* $p < 0.05$ and *** $p < 0.005$, significant difference from the control group; # $p < 0.05$ and ### $p < 0.005$ significant difference from the model group)

revealed by the reduction of MDA content and the elevation of GSH, SOD, and CAT levels (Fig. 5E–H).

Finally, the regulation of TRYP on the NF- κ B/MAPK/Nrf2 pathways was investigated in TNF- α -stimulated HaCaT cells to verify our previous *in vivo* studies. In HaCaT cells, we found that TNF- α stimulation increased the phosphorylated levels of I κ B and NF- κ B p65 proteins and the NF- κ B nuclear translocation, whereas TRYP treatment concentration-dependently rescued these protein alterations (Fig. 6A). These results suggested that TRYP treatment could suppress the activation of the NF- κ B pathway. Consistent with this finding, NF- κ B DNA-binding activity assay revealed that the increased DNA-binding activity of p65 induced by TNF- α was reversed by TRYP treatment in a dose-dependent way (Supplementary Figure S1). In addition, the increased level of JNK and ERK1/2 phosphorylation was blocked by TRYP treatment in TNF- α stimulated HaCaT cells (Fig. 6B). There was no obvious change in the phosphorylation of p38 MAPK after exposing HaCaT cells to TNF- α (Fig. 6B). Furthermore, it was obviously observed that the Nrf2 pathways (the expression of Nrf2 and HO-1) were activated by TRYP treatment concentration-dependently in TNF- α -stimulated HaCaT cells (Fig. 6C). Taken together, our results showed that TRYP suppressed oxidative stress and inflammation by regulating the NF- κ B/MAPK/Nrf2 pathways.

Discussion

Psoriasis is considered an autoimmune disorder; thus, it is mainly treated with immunosuppressive agents, such as cyclosporin and MTX [24, 28]. Since conventional immunosuppressive drugs have side effects, such as infections and nephrotoxicity, biological treatments with anti-inflammatory antibodies are attracting immense interest. Though several biological treatments show better efficacy, they are also much more expensive than conventional immunosuppressive agents. Therefore, it remains of critical importance to seek a safe and cost-effective drug for the treatment of psoriasis. TRYP is a natural product found in many traditional herbal plants with a potent anti-inflammatory activity [16, 21]. A recent study has shown that TRYP is an active compound for the treatment of psoriasis by its anti-Th17 effect [29]. Moreover, TRYP reportedly suppresses IL-17A production

during Th17 polarization [30]. The present study explored for the first time whether TRYP ameliorates psoriatic features in an IMQ-induced psoriatic mouse model and TNF- α -treated keratinocytes *in vitro*.

Topical administration of IMQ is currently one of the most widely accepted approaches for the establishment of psoriasis animal models in mice, since it is easy to operate and can reproduce histological characteristics and pathological inflammatory phenotype of psoriasis [31]. In this study, TRYP exhibited a similar efficacy to MTX for alleviating psoriatic skin lesion and increased the PASI scores. As noted in previous research, approximately 10% of body weight loss was observed in mice after exposing IMQ for 7 days, which could be restored by TRYP treatment. Th17 cells are considered to contribute to the pathogenesis of psoriasis [10]. TRYP also reduced IMQ-induced Th17 cell infiltration in both spleen and lymph nodes and spleen enlargement, indicating the suppression effect of TRYP on systemic inflammation in psoriatic mouse models.

As reported by previous laboratory and clinical research, various inflammatory cytokines, such as TNF- α , IL-22, and IL-17A, are secreted by immune and inflammatory cells during the progression of psoriasis, which probably and directly act on human keratinocytes, thereby resulting in a variety of pathological symptoms [32]. The level of cytokines, such as IL-17A, IL-22, and TNF- α , secreted by Th17 cells was elevated in the serum of psoriasis patients [33]. Our data showed that TRYP effectively reduced the secretion of TNF- α , IL-1 β , IL-6, IL-17A, and IL-22, and also their transcription level in psoriatic skin lesions. As oxidative stress plays a key role in psoriasis pathogenesis [7], our study verified that the effect of TRYP on the psoriatic mouse models was also closely related to its restoration role in the imbalance of reactive oxygen species (ROS) and antioxidants. Consistent with previous reports [34], the up-regulation of MDA and the down-regulation of GSH, SOD, and CAT were observed in the skin lesions of psoriatic mouse models, which confirmed the generation of oxidative stress in the psoriatic skin lesions. The changes in MDA, GSH, SOD, and CAT contents induced by IMQ were partly reversed by the intervention with TRYP. This could explain another mechanism behind TRYP on psoriasis *in vivo*. Intriguingly, our *in vitro* experiments based on TNF- α -treated HaCaT cells showed similar findings *in vivo*, which revealed that TRYP could ameliorate the psoriatic features by suppressing inflammation and oxidative stress *in vivo* and *in vitro*.

As a key pro-inflammatory signal, NF- κ B has been proven to be involved in inflammation dysregulation during psoriasis [35]. It has been reported that suppressing NF- κ B signaling is attributed to relieving symptoms of psoriasis in both psoriatic patients [36] and psoriatic mouse models [34]. Previous studies also have suggested the crucial role of the MAPK pathway in the pathogenesis of psoriasis [37], which

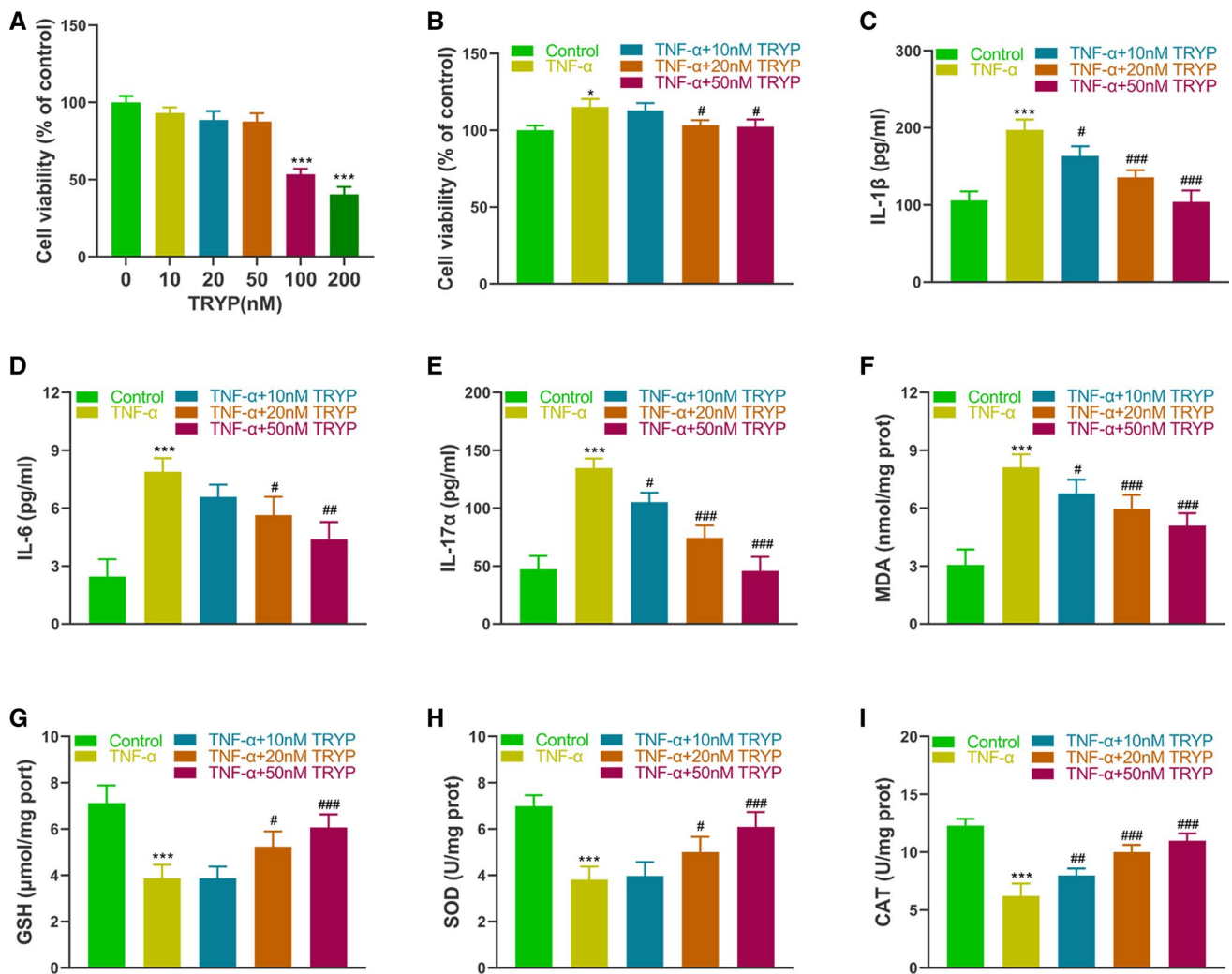


Fig. 5 TRYP suppressed the TNF- α -induced activation of inflammation and oxidative stress in HaCaT cells. **A** The cell viability of HaCaT cells upon different concentrations (0, 10, 20, 50, 100 and 200 nM) of TRYP. HaCaT cells were treated with TNF- α to mimic the psoriasis in vitro and then added 0, 10, 20, and 50 nM TRYP. HaCaT cells without any treatment is considered the control. **B** The cell viability of HaCaT cells from diverse groups. ELISA determined

the levels of **C** IL-1 β , **D** IL-6, and **E** IL-17A cytokines in the cell supernatant of HaCaT cells from diverse groups. The contents of **F** MDA, **G** GSH, **H** SOD, and **I** CAT in the cell supernatant of HaCaT cells from diverse groups (* $p < 0.05$ and *** $p < 0.005$, significant difference from the 0 nM or control group; # $p < 0.05$ and ### $p < 0.005$ significant difference from the TNF- α group)

was closely related to NF- κ B signaling activation in multiple inflammatory diseases [38]. Previous studies have reported increased ERK1/2 activation in cell extracts from lesional psoriatic skin and in the human psoriatic lesion as compared to that in the normal human epidermis [39]. Moreover, it has been demonstrated that JNK is not active in healthy human epidermis, but its activity is increased in psoriasis [40]. To uncover the molecular mechanism underlying the effect of TRYP, our study subsequently investigated whether TRYP attenuates psoriatic skin lesions by suppressing pro-inflammatory activity mediated by the NF- κ B/MAPK pathways. Consistent with previous studies, the phosphorylation of JNK and ERK1/2 in IMQ-induced psoriatic skin lesions

and TNF- α -treated HaCaT cells both significantly increased compared with those in the control. The data showed that TRYP effectively repressed the NF- κ B/MAPK pathways in IMQ-induced psoriatic skin lesions and TNF- α -treated HaCaT cells, including the activation of I κ B α and NF- κ B, as well as phosphorylation of JNK and ERK1/2.

As one of the key triggers of the antioxidant response, Nrf2 can regulate the transcription of antioxidant genes to protect cells from injury and inflammation induced by oxidative damage, which has recently been considered a promising target in psoriasis [41]. The MAPK signaling system has been implicated in Nrf2 induction by many previous reports [42, 43]. Given that TRYP is capable of enhancing

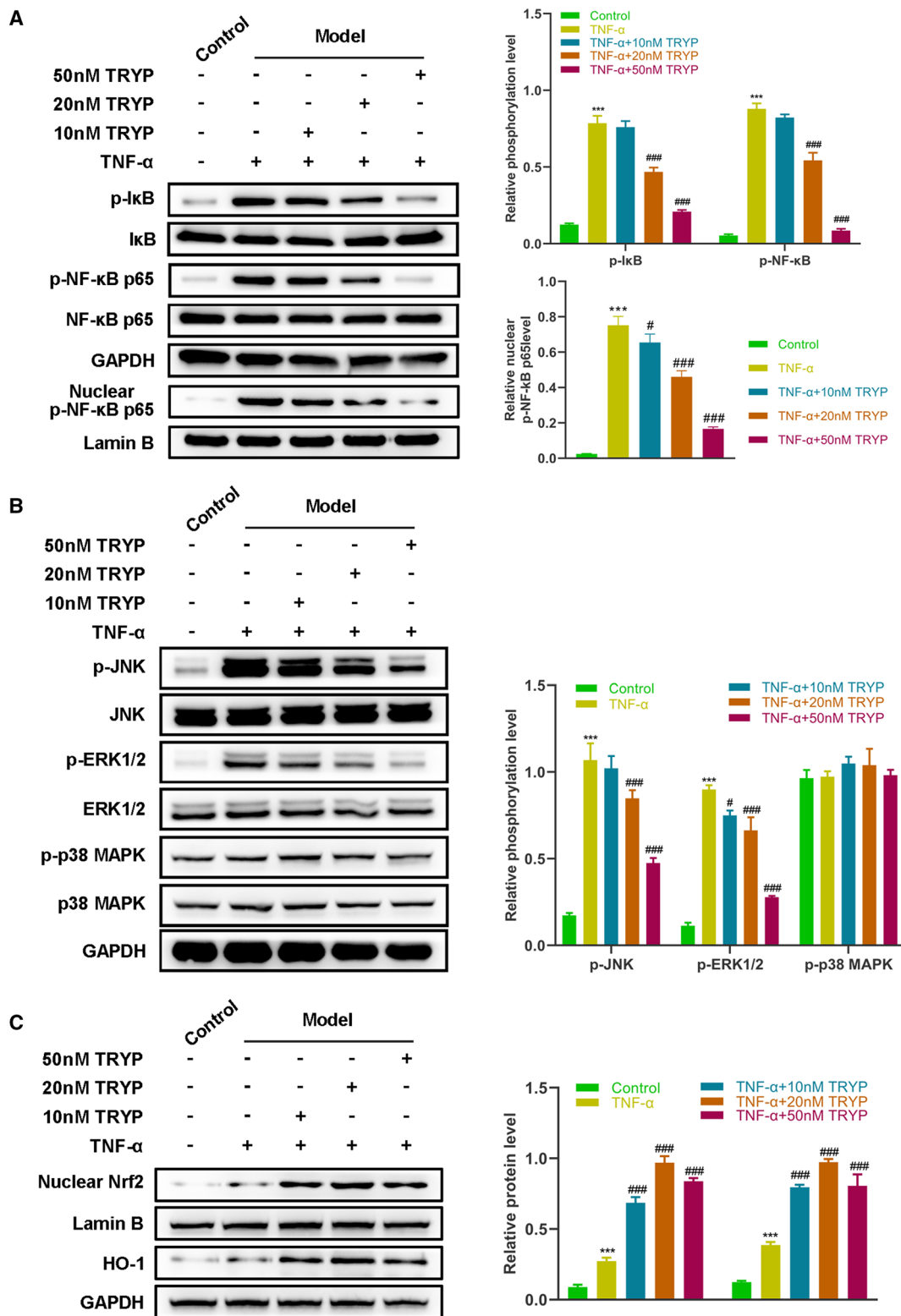


Fig. 6 TRYP regulated the NF- κ B/MAPK/Nrf2 pathways in TNF- α -induced HaCaT cells. **A** The expression and phosphorylation levels of I κ B and NF- κ B p65 proteins in HaCaT cells of each group. **B** The expression and phosphorylation levels of JNK, ERK1/2, and p38 MAPK in HaCaT cells of each group. **C** The expression levels

of nuclear translocation of Nrf2 and HO-1 in HaCaT cells of each group (** $p < 0.005$, significant difference from the control group; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.005$ significant difference from the TNF- α group)

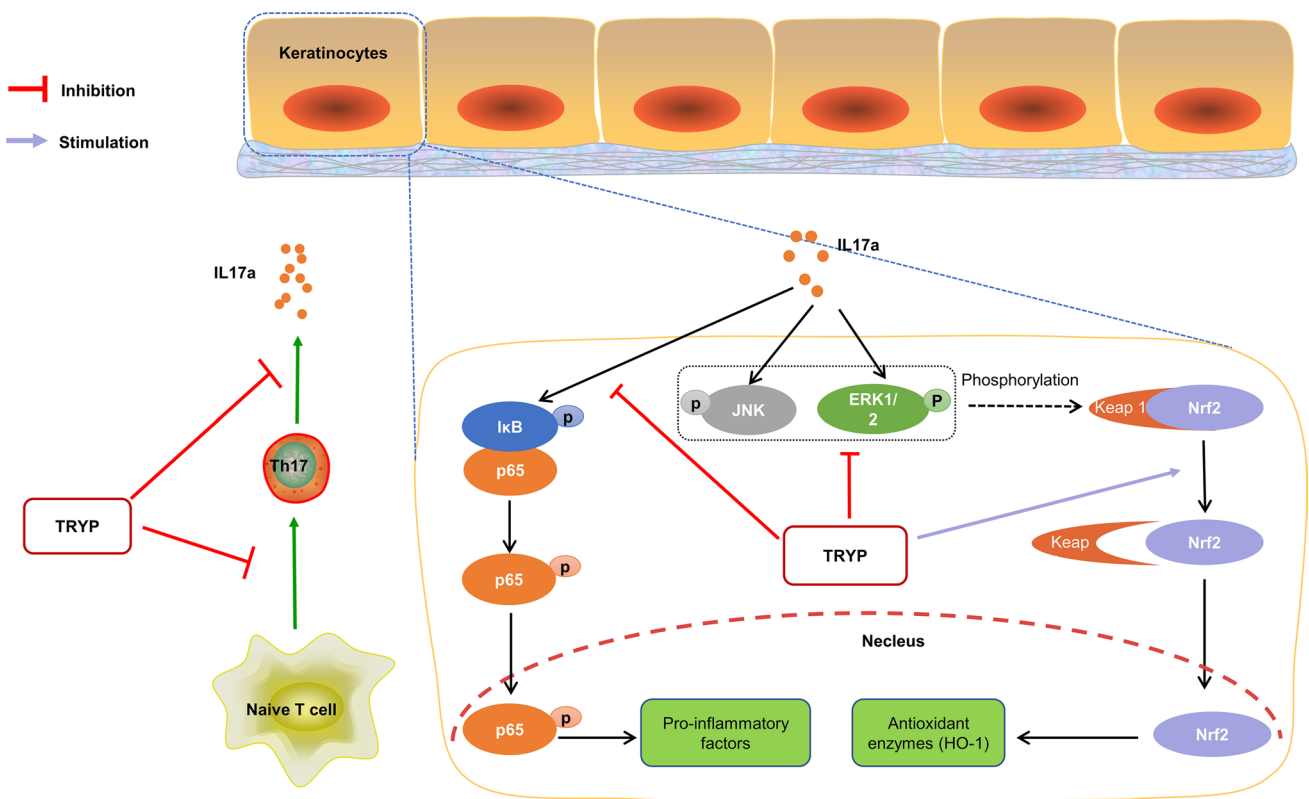


Fig. 7 The schematic depicts the probable mechanisms underlying the suppressive effects of TRYP on inflammation and oxidative stress in vivo

the antioxidant enzyme levels in psoriatic skin lesions, we wonder whether TRYP regulates Nrf2 signaling to attenuate oxidative stress in psoriasis. ERK1/2 and p38 phosphorylation can lead to an activation of Nrf2 in several pathological conditions [44]. This may explain that the activation of Nrf2 was observed in IMQ-induced psoriatic skin lesions. Nrf2 is mainly localized and binds to KEAP1 protein in the cytoplasm under basal conditions, which will quickly translocate into the nucleus to transactivate the antioxidant response once stimulated by oxidative stress or inflammation [45]. In this study, TRYP significantly increased the localization of Nrf2 in the nuclear region in psoriatic models both in vivo and in vitro. Furthermore, previous studies demonstrated that TRYP protected several cells against oxidative stress or inflammation via Nrf2 signaling, implying the effect of TRYP on psoriatic features might be attributed to the activation of the Nrf2 pathway. After the administration of TRYP, the activation of Nrf2 was strengthened but not weakened by the decreased phosphorylation of JNK and ERK1/2. That might be because the phosphorylation of Nrf2 at multiple sites by MAP kinases has a limited contribution to modulating the Nrf2-dependent antioxidant response [46]. These data suggested that the effect of TRYP on Nrf2 activation relied on other mechanisms, but not the MAPK signaling system. It has been widely accepted that Nrf2 regulates

antioxidant-responsive element-mediated induction of cytoprotective genes in response to oxidative stress after nuclear localization [47]. As a phase II antioxidant enzyme regulated by Nrf2, HO-1 is an important inducible stress response protein that converts hemoglobin to CO and Fe^{2+} and reduces the above products to bilirubin, thereby playing antioxidant and anti-inflammatory roles [48]. In line with previous studies [49], our data showed that the expression of HO-1 was significantly increased in psoriasis-like lesions compared to that in normal skin. After the strong activation of Nrf2 by TRYP, HO-1 was further up-regulated, thereby attenuating the inflammation and oxidative stress in both in vivo and in vitro models of psoriasis.

Collectively, TRYP is beneficial for psoriasis-like lesions due to its suppressive effect on immune responses exerted by related cytokines and oxidative stress via regulating NF- κ B/MAPK/Nrf2 pathways and IL-17 A-producing Th17 cells (Fig. 7). In the future, we will further elucidate the specific mechanism of TRYP and evaluate its safety in the IMQ-induced psoriatic mouse model.

In conclusion, we found that TRYP treatment could not only ameliorate local symptoms, but also control the systemic immune responses in IMQ-induced psoriatic mice. It is possible that TRYP diminished the infiltration of Th17 cells and the activation of the NF- κ B/MAPK pathways,

and activated the Nrf2 pathway to attenuate inflammation and oxidative stress in TNF- α -stimulated keratinocytes and psoriatic skin of mice, thereby exerting an anti-psoriatic effect. Our findings provide some scientific information for TRYP as a new drug for psoriasis in the future.

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Data availability All data generated or used during the study appear in the submitted article.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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