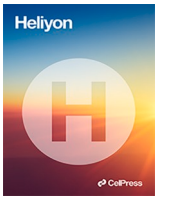
Heliyon 10 (2024) e35391

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Anti-atopic dermatitis effect of fraxinellone via inhibiting IL-31 in vivo and in vitro

Niuniu Yang [a,b,c,*], Jialin Deng [a,b,c], Huiwen Xu [d], Huijuan Dai [a,b,c], Han Jin [a,b,c], Haifeng Shao [a,b,c], Yanqing Liu [a,b,c]

[a] *Medical College, Yangzhou University, Yangzhou, 225009, China*
[b] *The Key Laboratory of Syndrome Differentiation and Treatment of Gastric Cancer of the State Administration of Traditional Chinese Medicine, Yangzhou University, Yangzhou, 225009, China*
[c] *Jiangsu Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Treatment of Senile Diseases, Yangzhou University, Yangzhou, 225009, China*
[d] *School of Nursing & Public Health, Yangzhou University, Yangzhou, 225009, China*

ARTICLE INFO

Keywords:
Fraxinellone
Atopic dermatitis
IL-31
IL-31RA
TRPV1
TRPA1

ABSTRACT

Chronic recurrent itch and skin inflammation are prominent features of atopic dermatitis (AD), which is closely related to the immune response driven by T-helper type 2 (Th2) cells. The expression of interleukin 31 (IL-31) is positively correlated with the severity of dermatitis. Anti-IL-31 receptor α (IL-31RA) targeted drugs have been used to treat AD, however, they are expensive and have side effects. Fraxinellone (FRA) is one of the main limonoid components in the dried root bark of *Dictamnus dasycarpus* Turcz.; however, its anti-inflammatory and antipruritic effects on atopic dermatitis (AD) have not been previously reported. In this study, we investigated the anti-dermatitis effect of FRA and its potential mechanism of action using a 2,4-dinitrofluorobenzene (DNFB)-induced AD-like mouse model and lipopolysaccharide (LPS)-stimulated HaCaT cells. FRA significantly inhibited chronic pruritus, epidermal thickening, and inflammatory infiltration in AD mice. FRA not only inhibited the levels of IL-31 in the serum and lesioned skin of AD mice but also significantly downregulated the mRNA expression and protein levels of IL-31, IL-31RA, transient receptor potential (TRP) V1, and TRPA1 in the lesioned skin and dorsal root ganglion (DRG) of AD mice. In LPS-stimulated HaCaT cells, FRA inhibited the production of iNOS and COX2, as well as the protein levels of IL-31, IL-31RA, TRPV1 and TRPA1, showing significant anti-inflammatory effects. In summary, our findings suggest that FRA exerts antipruritic and anti-inflammatory effects in AD by regulating the IL-31 pathway, and may hold promise for the clinical treatment of AD.

1. Introduction

Atopic dermatitis (AD) is an inflammatory skin disease that is prone to recurrent episodes and accompanied by intense pruritus [1]. AD has a high incidence rate in both developed and developing countries, affecting approximately 10 % of adults and 20 % of children [2,3]. Severe AD increases the risk of autoimmune diseases [4]. Pruritus, a hallmark symptom of AD, not only causes social

* Corresponding author. Department of Traditional Chinese and Western Medicine, Medical College, Yangzhou University, 136 Middle Jiangyang Road, Yangzhou, 225009, China.
E-mail address: nnyang@yzu.edu.cn (N. Yang).

https://doi.org/10.1016/j.heliyon.2024.e35391
Received 21 November 2023; Received in revised form 25 July 2024; Accepted 28 July 2024
Available online 29 July 2024

embarrassment and sleep difficulties, but also compromises the overall quality of life and increases the financial burden [5].

Current research indicates that AD is a systemic disease primarily orchestrated by T-helper type 2 (Th2) responses, and the cytokines released by activated Th2 cells play pivotal roles in driving skin inflammation and pruritus. Interleukin 31 (IL-31), a short-chain four-helix bundle cytokine, is mainly produced by activated Th2 cells, keratinocytes, mast cells, dendritic cells, and macrophages. It has been positively correlated with the severity and activity of AD and participates in itch and skin inflammation [6–8]. Increased IL-31 mRNA and protein levels are found in the serum and skin of patients with dermatitis, chronic spontaneous urticaria, cutaneous T-cell lymphomas, and prurigo nodularis [9–12]. IL-31, which co-opts with transient receptor potential (TRP) V1 and TRPA1, can also act as a neuro-immune link to drive sensory pathways through the activation of IL-31 receptor α (IL-31RA) on sensory neurons, resulting in itch [13]. The administration or overexpression of IL-31 can markedly induce scratching behavior in mice [7,14], which is attenuated in TRPV1 and TRPA1 knockout mice [15]. In addition, local application of 1.0 % capsaicin can inhibit the intense and prolonged scratching induced by IL-31 by desensitizing C-fibers and reducing the mRNA levels of IL-31RA in the dorsal root ganglion (DRG) [16]. Therefore, targeting the IL31-TRPV1/TRPA1 axis may be a promising strategy for the treatment of dermatitis.

Traditional Chinese medicine has long been used in the clinical treatment of dermatitis, and because of its low cost, good efficacy and safety, traditional Chinese medicine as a complementary and alternative medicine is of great significance in the development of AD treatment drugs. Cortex Dictamni, the dried root bark of *Dictamnus dasycarpus* Turcz., a perennial herb of the Rutaceae family, has been widely used to treat asthma, jaundice, coronary atherosclerosis, rheumatism, and multiple skin diseases including eczema, urticaria, and scabies for thousands of years [17–21]. Fraxinellone (FRA), one of the main active limonoid components of Cortex Dictamni, exhibits significant pharmacological properties, including anti-inflammatory, neuroprotective, anti-fibrotic, and anti-cancer effects [22–26]. However, research on the anti-dermatitis effects of FRA targeting IL-31 is rare. In this study, we aimed to evaluate the antipruritic and anti-inflammatory effects of FRA in AD through *in vitro* and *in vivo* experiments and to reveal its underlying mechanism.

2. Materials and methods

2.1. Reagents and chemicals

FRA (cat: A0488) was purchased from Mansite Biotechnology Co. Ltd. (Chengdu, China). 2,4-dinitrofluorobenzene (DNFB; cat: F830061) and carboxyl methyl cellulose (CMC-Na; cat: 9004-32-4) were purchased from Macklin (Shanghai, China). IL-31 (cat: SRP3209), lipopolysaccharide (LPS; cat: L8274) and toluidine blue (TB; cat: 89640) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A hematoxylin and eosin (H&E) staining kit (cat: G1120, Solarbio), mouse IL-31 enzyme-linked immunosorbent assay (ELISA) kit (cat: EK1100, Boster), mouse IL-4 ELISA kit (cat: TW10973, Tongwei), mouse Thymic stromal lymphopoietin (TSLP) ELISA kit (cat: E-EL-M0646, Elabscience) were used. For western blotting, primary antibodies against IL-31 (cat: PA5-115415, Thermo Scientific), IL-31RA (cat: YT5091, ImmunoWay), TRPV1 (cat: ab6166, Abcam), TRPA1 (cat: NB110-40763, Novus), iNOS (cat: 18985-1-AP, Proteintech), COX2 (cat: 12282T, Cell Signaling Technology) and β-actin (cat: 4970T, Cell Signaling Technology) were used. For the quantitative real-time polymerase chain reaction (RT-qPCR) experiment, the RNA Purification Kit (cat: 12183018A, Thermo Scientific), HiScript-Q-RT-SuperMix for qPCR Kit (cat: R123-01, Vazyme), and AceQ Universal SYBR Green qPCR Master Mix kit (cat: Q511-02/03, Vazyme) were used. The HaCaT cells (cat: ZQ0044) were purchased from Xinzhou Zhongqiao (Shanghai, China).

2.2. Mice

Male 6-8-week-old C57BL/6 mice were purchased from Yangzhou University Laboratory Animal Center (Jiangsu, China). The mice were housed in a specific pathogen-free (SPF) environment at 23 ± 2 °C, with a 12-h light-dark cycle and freedom to eat and drink (Irradiation-sterilized maintenance feed). All experiments were performed in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the Yangzhou University under a Project License (No. YXYLL-2022-161).

2.3. Acute itch behavior experiment

The mice were placed in a box for more than 30 min before the start of the experiment. After 2 h of intragastric administration of FRA (2.5, 5, and 10 mg/kg, 200 μl) or CMC-Na (0.5 %, 200 μl), the mice were administered IL-31 (19 ng, 100 μl) via subcutaneous injection into the nape and were immediately recorded for 30 min [27]. No treatment was given to the normal group. A researcher who was unaware of the experiment counted the number of scratches.

2.4. DNFB-induced AD-like mouse model and FRA treatment

A DNFB-induced AD-like mouse model was established, as previously described [28]. The abdominal hair of the mice was shaved on day −5. On day −3, the hair on the nape was shaved, and 100 μl of 0.5 % DNFB (dissolved in a 4:1 mixture of acetone and olive oil) was applied to the exposed abdomen. From days 0–14, the mice were stimulated with 50 μl of 0.2 % DNFB every 2 days, to induce an AD-like mouse model (Fig. 2A). In this study, the mice were randomly divided into: normal group, vehicle group, DNFB group, FRA (2.5, 5, and 10 mg/kg FRA) group, and DEX (1 mg/kg DEX) group. FRA and DEX were dissolved in 0.5 % CMC-Na for oral gavage. The DNFB group was treated with an equal volume of CMC-Na solution. No treatment was given to the normal group, and the vehicle group was stimulated with a mixture of acetone and olive oil without DNFB. Behavioral videos were recorded every two days during the

experimental period. After the mice were euthanized under isoflurane anesthesia, the blood, nape-lesion skin, and DRG were collected for further examination.

2.5. Dermatitis score, weight assessment, and splenic index

The nape-lesioned skin of the mice was assessed once every two days during the experiment. Skin lesions were scored based on the severity of skin lesion symptoms (erythema/hemorrhage, edema, excoriation/erosion, and scaling/dryness), and can be classified into four levels as follows: 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) [29]. Photographs of the skin lesions were captured using a high-resolution camera on days 0, 7, and 14. The mice were weighed every two days. The splenic index was calculated as = spleen weight (mg)/[body weight (g) × 10] [30].

2.6. Histological staining

Skin samples were fixed with 4 % paraformaldehyde (PFA) overnight, followed by dehydration in 20 % and 30 % sucrose solutions until the skin samples sank to the bottom. Subsequently, the skin samples were embedded in an optimal cutting temperature (OCT) medium and sectioned (10 μm) by a cryostat (Leica, CM1900) [31]. The skin sections were stained with H&E and TB to detect the epidermal thickness and number of mast cells, respectively.

2.7. ELISA

Blood was centrifuged at 3500 rpm for 15 min to obtain serum. The lesioned skin samples were treated with PBS and centrifuged at 3000 rpm for 15 min to obtain a protein homogenate. IL-31, IL-4, and TSLP levels were measured using ELISA kits, following the manufacturer's protocol [30]. The reaction products were measured using a microplate reader at a wavelength of 450 nm.

2.8. qRT-PCR

Total RNA was extracted from the lesioned skin and DRG tissues using RNA purification kits, and RNA was reverse-transcribed using HiScript-Q-RT-SuperMix for qPCR. Next, the AceQ qPCR SYBR Green Master Mix kit was used for qRT-PCR with 3 replicate wells for each sample [28]. The relative gene expression levels of the mRNAs were calculated using the $2^{-\Delta\Delta CT}$ method. PCR primers used in this study are as follows: IL-31 forward (F): CCACACAGGAACAACGAAGCCT and reverse (R): CCCGGTCCAGGCTGAAACACG; IL-31RA forward (F): CCAGAAGCTGCCATGTCGAA and reverse (R): TCTCCAACTCGGTGTCCCAAC; TRPV1 forward (F): CGAGGATGGGAAGAATAACTCACTG and reverse (R): GGATGATGAAGACAGCCTTGAAGTC; TRPA1 forward (F): GTCCAGGGCGTTGTCTATCG and reverse (R): AGCACTTCACACGAAGAACCA; and GAPDH forward (F): AGGTCGGTGTGAACGGATTTG and reverse (R): GGGGTCGTTGATGGCAACA.

2.9. Western blotting

HaCaT cells were preincubated with or without the indicated concentrations of FRA for 2 h and then stimulated with LPS (1 μg/ml) for 24 h. Total proteins were extracted from mouse skin tissues, the DRG, and HaCaT cells and were quantified using a BCA protein assay kit. Protein lysates were separated using 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After SDS-PAGE, a 0.45 μm polyvinylidene fluoride (PVDF) membrane was used for protein transfer and blocked with 5 % bovine serum albumin (BSA), then incubated with the primary antibodies against β-actin (1:2000), IL-31 (1:2000), IL-31RA (1:1000), TRPA1 (1:1000), TRPV1 (1:1000), iNOS (1:600), and COX2 (1:1000) at 4 °C overnight. Membranes were then incubated with a secondary antibody (1:5000) for 2 h. An ECL detection kit and a gel imaging instrument (Bio-Rad, Hercules, CA, USA) were used to visualize the protein bands. Gray values were quantified using the ImageJ software. All data were normalized by the value of β-actin [32].

2.10. Cell culture and cell viability assay

HaCaT cells were cultured in DMEM (Solarbio, China) supplemented with 10 % FBS and 1 % penicillin-streptomycin. Cell viability was measured by MTT assay. HaCaT cells were seeded into 96-well cell culture plates at a density of 5×10^3 cells/well. After 24 h, cells were treated with 0, 6.25, 12.5, 25, 50, and 100 μM FRA for 24, 48, and 72h. Then, MTT (20 μl, 0.5 mg/ml) was added to each well and cultured for 4 h. The culture medium was removed, and DMSO (150 μl/well) was used to dissolve the formazan crystals, which were measured at 490 nm using a microplate reader (PerkinElmer, USA) [33].

2.11. Statistical analysis

GraphPad Prism 8 was used to analyze all data. Data are presented as mean ± standard error of the mean (SEM). Different treatment analyses among groups were analyzed using an unpaired Student's *t*-test or one-way analysis of variance (ANOVA) with Dunnett's test. *P-values* < 0.05 were considered statistically significant.

3. Results

3.1. FRA administration inhibited IL-31-induced acute scratching behavior

IL-31 induces severe scratching in mice and plays a pivotal role in AD-related itch [7]. To investigate the role of FRA in IL-31-induced itch, FRA (Fig. 1A) or CMC-Na was administered to mice, followed by subcutaneous injection of IL-31 in the nape of the neck. The scratching behavior caused by IL-31 was noticeable (171.3 ± 8.8, n = 6). However, the strong scratching behavior induced by IL-31 was dose-dependently inhibited by FRA (138.3 ± 7.9, 114 ± 5.5, and 66.0 ± 5.9 scratching counts for 2.5, 5, and 10 mg/kg of FRA, respectively. n = 6) (Fig. 1B and C). These findings suggest a potential inhibitory role of FRA in IL-31-mediated itching.

3.2. FRA reduced chronic itch, lesion skin scores, and splenic index values in DNFB-induced AD-like mice

To determine whether FRA could alleviate chronic itch and inflammation induced by AD, all mice, except for those in the control groups, were modeled and treated as shown in Fig. 2A. Compared with the control group, the number of scratches progressively increased in the DNFB group (i.e., those treated with CMC-Na) throughout the experiment, but was significantly suppressed in the FRA and DEX groups. On day 14, scratching counts in the DNFB group were 182.83 ± 15.0 (n = 12); however, the scratching counts in FRA groups were decreased in a dose-dependent manner (2.5, 5, and 10 mg/kg of FRA had scratching counts of 109.8 ± 12.3, 61.2 ± 7.3, and 43.0 ± 4.7, respectively. n = 11) (Fig. 2C and D). The skin lesions of AD mice in each group on days 0, 7 and 14 are shown in Fig. 2B. DNFB-induced AD-like mice exhibited increasingly severe dermatitis lesions, such as crusting, epidermal peeling, skin thickening, and skin dryness, as the modeling time progressed. The skin lesion scores in the DNFB group were significantly higher than those in the FRA (5 mg/kg, and 10 mg/kg) and DEX groups on day 14; however, there was no significant difference compared to the low-concentration FRA group (Fig. 2E and F). Additionally, the splenic index showed that the spleens of mice in the FRA (5 mg/kg, and 10 mg/kg) groups were significantly smaller than those in the DNFB group (Fig. 2G–H). Moreover, the FRA group showed sustained weight gain throughout the experimental period (Fig. 2I and J). This indicated that FRA could alleviate chronic itch in AD mice and mitigate the severity of skin lesions in AD mice.

3.3. FRA restricted the epidermal thickening and inflammatory response in DNFB-induced AD-like mice

Repeated scratching can lead to epidermal thickening and infiltration of inflammatory cells. To evaluate the effect of FRA on the epidermal thickness and number of mast cells in AD mice, the lesion skin was stained with H&E and TB, respectively. As shown in Fig. 3A and C, compared with the control group, the epidermal thickness was markedly increased in the DNFB group, whereas FRA treatment notably reduced it. The TB staining results were consistent with these findings. In the FRA group, the increase in the number of mast cells was significantly inhibited (Fig. 3B and D).

Cytokines play a crucial role in regulating immune responses. Studies have shown that serum levels of IL-31 are positively correlated with the severity of AD [8]. To verify whether FRA can inhibit the levels of IL-31 in AD mice, serum IL-31 levels were measured using an ELISA kit. As expected, the serum levels of IL-31 in AD mice were markedly increased, and FRA treatment had a significant inhibitory dose-dependent effect on IL-31 levels. Moreover, the increased levels of IL-31 in the lesioned skin were also inhibited in the FRA groups (Fig. 4A and D). In addition, IL-31 can promote the release of some pro-inflammatory factors, such as IL-4 [34] and is positively correlated with TSLP levels in AD patients [35]. Therefore, the IL-4 and TSLP levels were measured. The results showed that the increased levels of IL-4 and TSLP in the lesion skin and serum of AD mice were also significantly reduced by FRA treatment (Fig. 4B, C, 4E, and 4F). These findings indicate that FRA effectively mitigated inflammation in AD mice.

3.4. FRA inhibited the IL-31 pathway in the lesion skin and DRG of DNFB-induced AD-like mice

By binding to IL-31RA, IL-31 activates TRPV1$^+$/TRPA1$^+$ sensory neurons, activates or sensitizes TRP channels, and plays an

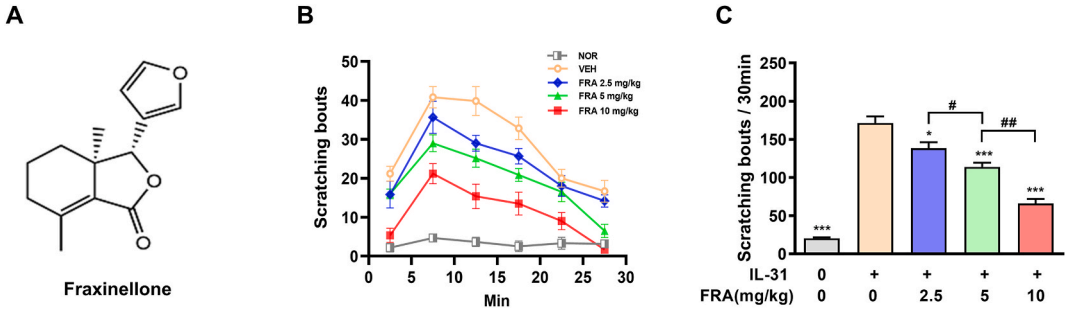


Fig. 1. Fraxinellone (FRA) inhibits the acute itch induced by IL-31. (A) Chemical structure of FRA. (B) Line chart of scratching behavior induced by IL-31 over 30 min. (C) Quantification of scratching behavior induced by IL-31 following administration of different concentrations of FRA ($^*P < 0.05$, $^{***}P < 0.001$, compared with IL-31 group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ comparison between groups; n = 6).

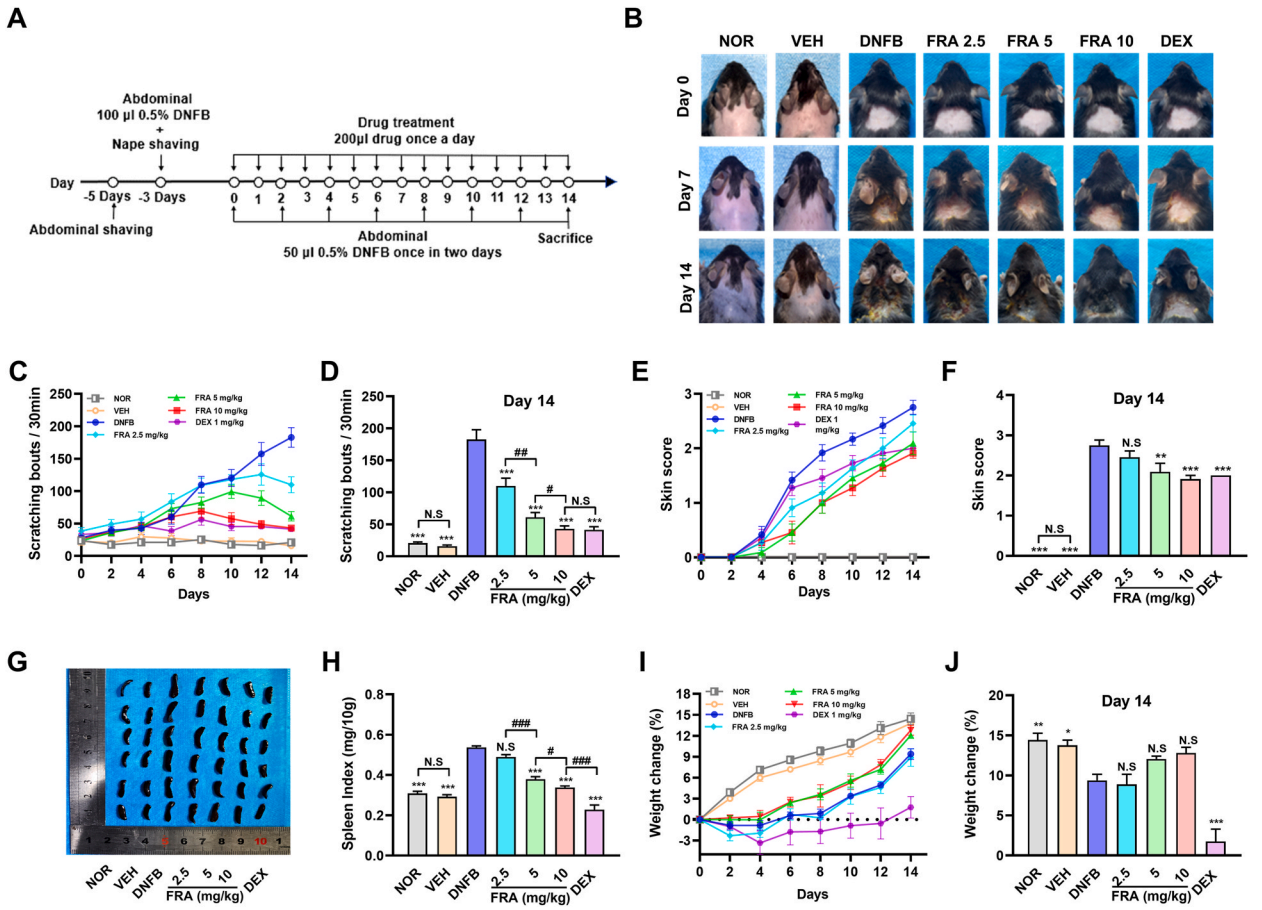


Fig. 2. Fraxinellone alleviates the symptoms of DNFB-induced AD-like mice. (A) Schematic representation of DNFB-induced AD-like mouse model. (B) Clinical manifestations of AD-like mice induced by DNFB in each group on days 0, 7, and 14. (C–D) Scratching behavior over time and quantification of scratching behavior of each group on day 14. (E–F) Skin severity scores during the experiment and the skin score on day 14. (G–H) Photographs of spleens of DNFB induced AD-like mice and the splenic index. (I–J) Changes of mouse body weights in different groups over time and comparison of body weights on day 14 ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus DNFB group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, comparison between groups; N.S, not significant; n = 6–12).

important role in AD pathogenesis [13]. In order to investigate whether FRA exerts anti-dermatitis effects through the IL-31-TRPV1/TRPA1 pathway, we detected the mRNA expression and protein levels of IL-31, IL-31RA, TRPV1, and TRPA1 in the lesion skin and DRG of AD mice. As shown in Fig. 5, FRA treatment significantly inhibited the mRNA and protein levels of IL-31, IL-31RA, TRPV1, and TRPA1 in the lesioned skin compared to those in DNFB group (Fig. 5A and C). Similarly, FRA treatment suppressed the upregulated mRNA and protein levels of IL-31RA, TRPV1, and TRPA1 in the DRG of AD mice (Fig. 5B and D).

3.5. Effect of FRA on LPS-induced iNOS and COX2 production and IL-31, IL-31RA, TRPV1, and TRPA1 protein levels in LPS-stimulated HaCaT cells

HaCaT cells have also been used to study AD-related inflammatory responses [33,36,37]. To further investigate the anti-dermatitis effects of FRA and the effects of FRA on the IL-31 pathway and the production of iNOS and COX2, we performed *in vitro* experiments using LPS-stimulated HaCaT cells. The MTT assay showed that FRA had no significant cytotoxic effects on HaCaT cells at concentrations of 0–50 μM (Fig. 6A). After stimulation by LPS (1 μg/ml), the iNOS and COX2 production increased in HaCaT cells, but treatement with 6.25, 12, and 25 μM FRA significantly inhibited the protein levels of iNOS and COX2 (Fig. 6B). To further explore the inhibitory effect of FRA on the IL-31 pathway, we measured the protein levels of IL-31, IL-31RA, TRPV1, and TRPA1. Consistent with the *in vivo* animal experiments, the protein levels of IL-31, IL-31RA, TRPV1 and TRPA1 were significantly reduced by FRA treatment in LPS-stimulated HaCaT cells (Fig. 6C), which further confirmed the inhibitory effect of FRA on the IL-31 pathway.

4. Discussion

In this study, we demonstrated that FRA regulates the IL-31-TRP pathway by exerting antipruritic and anti-inflammatory effects

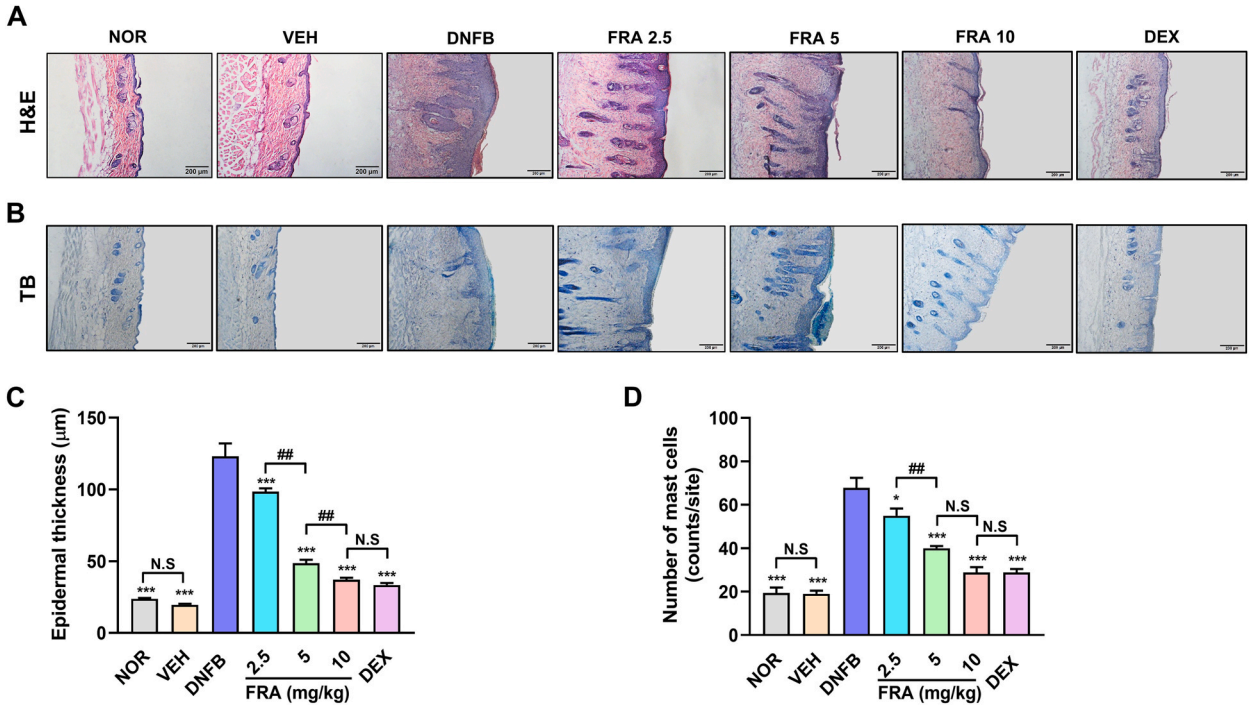


Fig. 3. Fraxinellone mitigates the epidermal thickening and mast cell infiltration in DNFB-induced AD-like mice. (A) H&E and (B) toluidine blue staining of the lesion skin of DNFB-induced AD-like mice in each group. (Scale bar: 200 μm) (C) Epidermal thickness of the lesion skin. (D) Number of mast cells ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus DNFB group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, comparison between groups; N.S, not significant; n = 5).

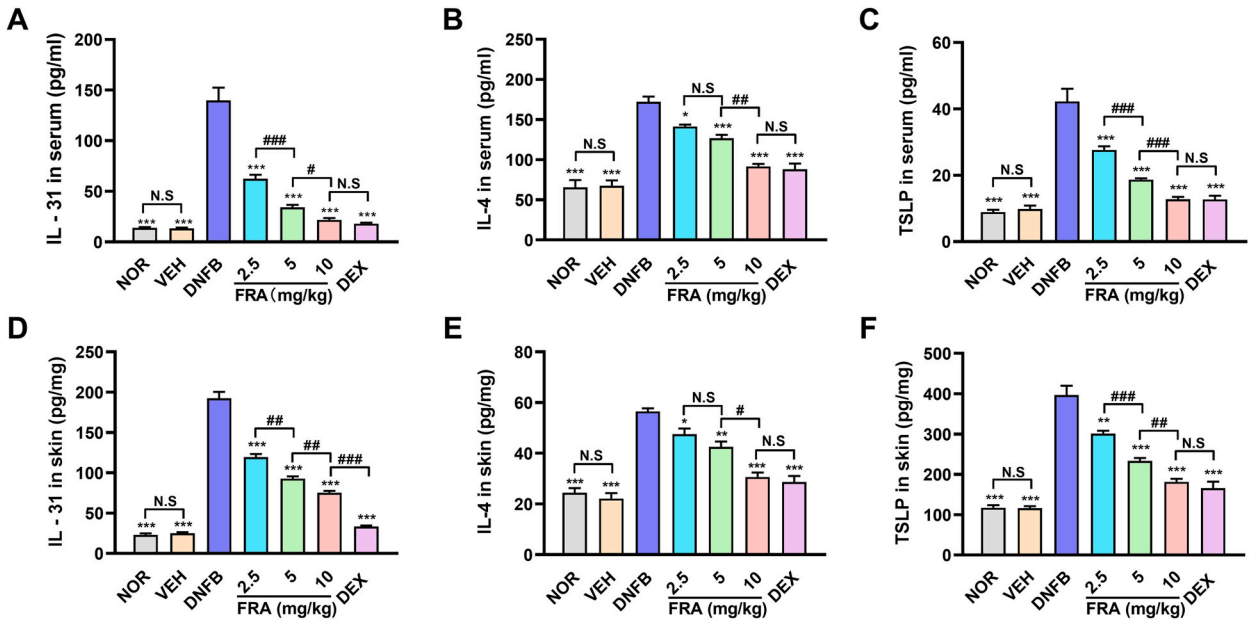


Fig. 4. Fraxinellone inhibits the expression of inflammatory cytokines in the lesion skin and serum of DNFB-induced AD-like mice. The levels of IL-31(A), IL-4 (B), and TSLP (C) in the serum of AD mice were measured by ELISA kit. The levels of IL-31(D), IL-4 (E), and TSLP (F) in the lesion skin in each group ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus DNFB group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, comparison between groups; N.S, not significant; n = 4).

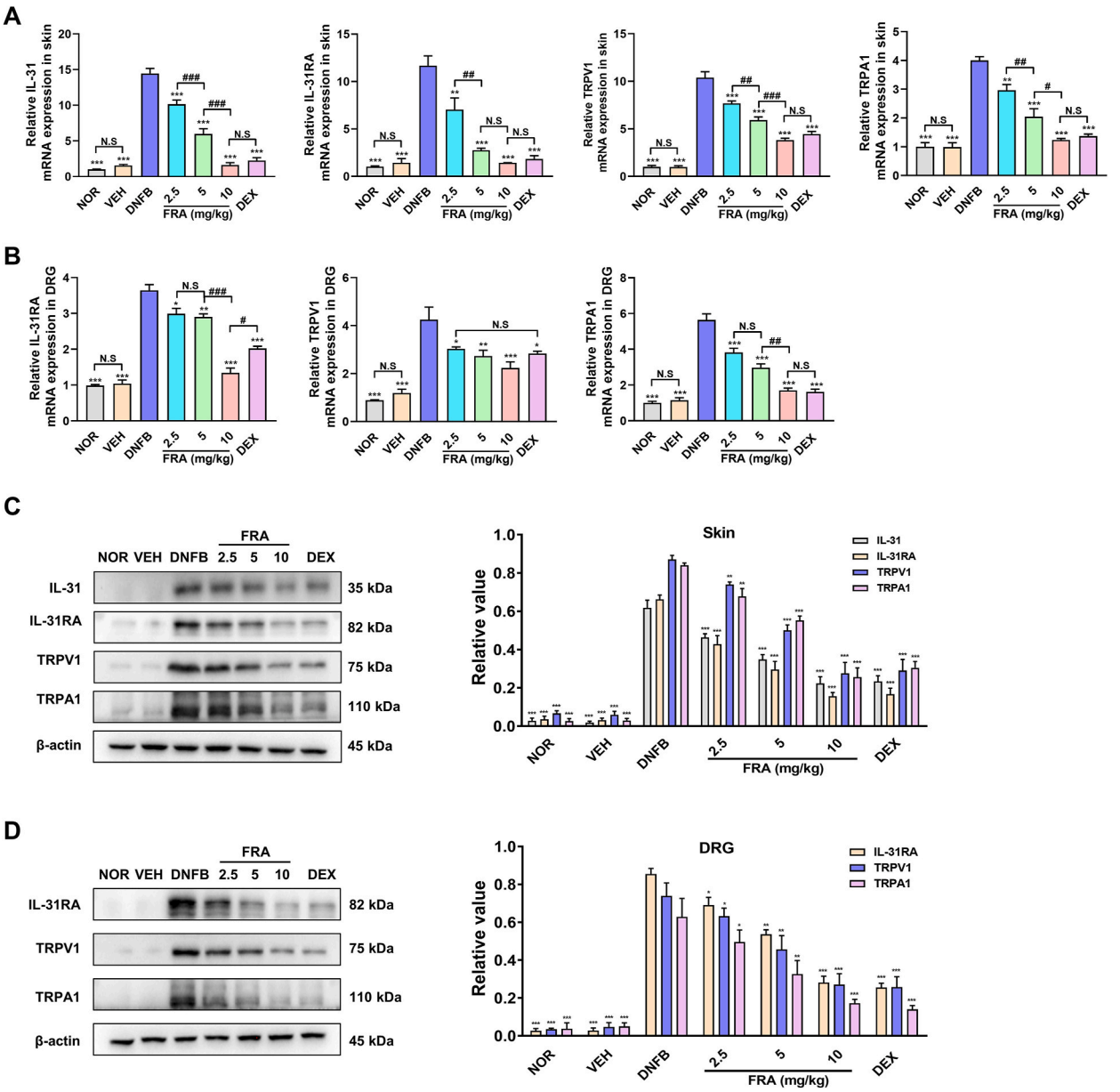


Fig. 5. Fraxinellone inhibits the mRNA expression and protein levels of IL-31, IL-31RA, TRPV1, and TRPA1 in the skin and DRG of DNFB-induced AD-like mice. (A) The relative mRNA expression of IL-31, IL-31RA, TRPV1, and TRPA1 in lesion skin. (B) The relative mRNA expression of IL-31RA, TRPV1, and TRPA1 in DRG. (C) The protein levels of IL-31, IL-31RA, TRPV1, and TRPA1 in lesion skin. (B) The protein levels of IL-31RA, TRPV1 and TRPA1 in DRG (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, versus DNFB group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, comparison between groups; N.S, not significant; n = 4).

using a DNFB-induced AD-like mouse model and LPS-stimulated HaCaT cells.

IL-31, as a cytokine, not only directly induces scratch behaviors, but also aggravates and amplifies the inflammation and itch in AD [38]. For chronic itch caused by moderate-to-severe AD, antihistamines are less effective [39]. Accumulating evidence indicates that Th2 cell-derived cytokines, including TSLP, IL-31, IL-13, IL-33, and IL-4 play an important role in AD-induced pruritus and inflammation, and the development of anti-cytokine drugs has become a research hot spot [40]. To investigate whether FRA could act on IL-31 to inhibit AD, we first observed the inhibitory effect of FRA on IL-31-induced itch. Interestingly, FRA significantly inhibited IL-31-induced scratching behaviors (Fig. 1).

Subsequently, we validated the antipruritic effects of FRA in a DNFB-induced AD-like mouse model. Consistent with our conjecture, FRA exhibited significant anti-dermatitis effects, effectively reducing chronic itch, skin scores, and inflammatory infiltration (Figs. 2 and 3). The chain reactions of recurrent scratching, skin barrier dysfunction, and allergic inflammation are closely associated with the

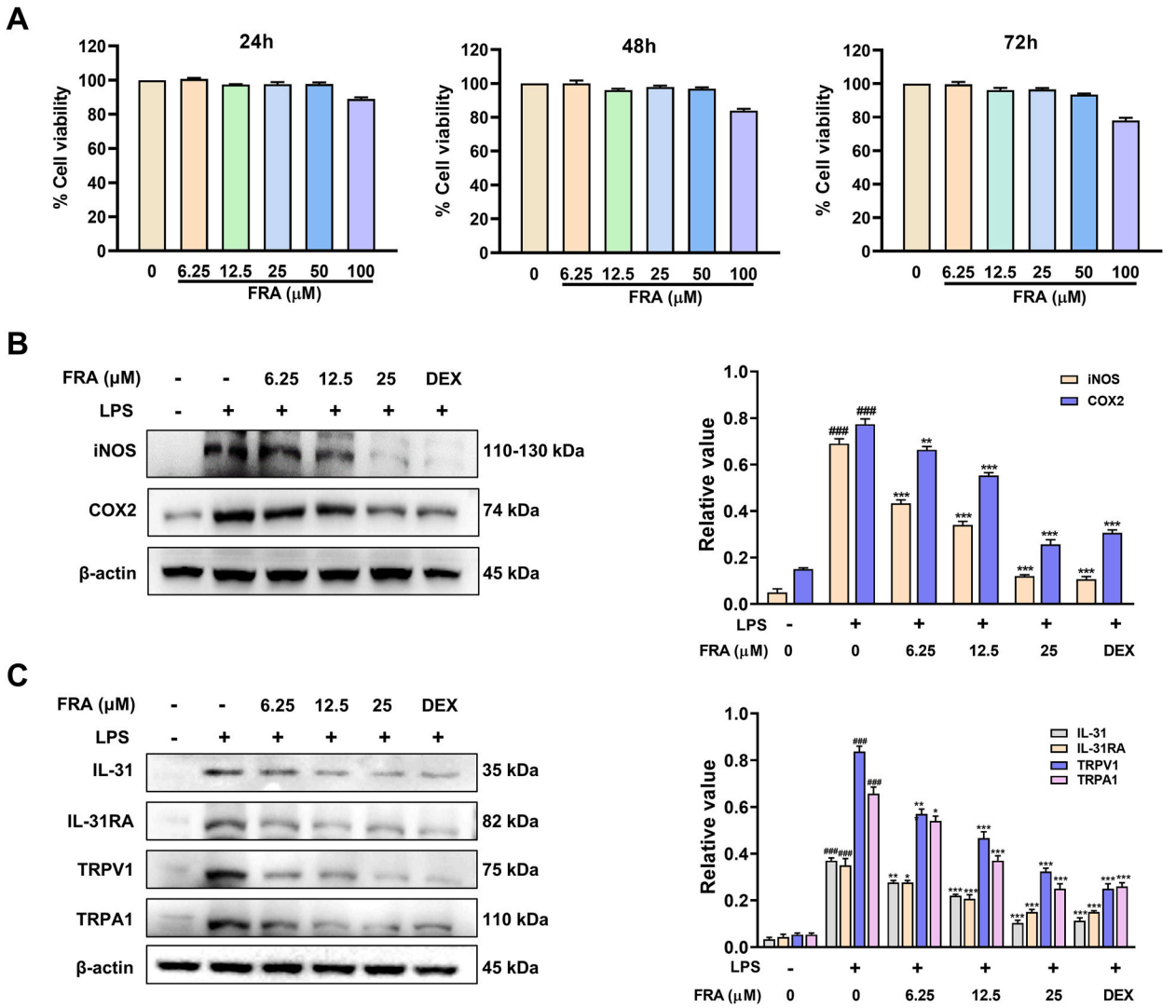


Fig. 6. Fraxinellone inhibits the inflammation response induced by LPS in HaCaT cells. (A) The effects of fraxinellone on HaCaT cells viability were measured using the MTT assay. HaCaT cell were preincubated with fraxinellone for 2 h, and then treated with LPS for 24 h. (B) LPS-induced iNOS and COX2 levels in HaCaT cells were determined using Western blotting. (C) The protein levels of IL-31, IL-31RA, TRPV1, and TRPA1 in LPS-stimulated HaCaT cells ($^{###}P < 0.001$, versus the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus the LPS-stimulated group; n = 4).

severity of AD. In particular, the vicious "scratch-itch-scratch" cycle involves not only the initiation and maintenance of AD skin inflammatory infiltration and damage, but is also the malignant result of AD [41]. In DNFB-induced AD-like mice, the skin showed obvious dryness, redness, swelling, exudation, and scabbing, with the increased number of scratching. FRA treatment, significantly improved skin lesions in DNFB-induced AD-like mice. Additionally, epidermal thickening and mast cell numbers were significantly inhibited. FRA has been suggested to exert protective effects on the skin barrier. Moreover, the treatment of FRA had no obvious effect on the body weight of AD mice, suggesting that FRA may have minor side effects.

Previous studies have shown that FRA inhibits the activation of various inflammatory molecules in the pancreas, thereby inhibiting acute pancreatitis [42]. FRA can also suppress the production of IL-17 and Th17 cell-related transcription factors, and significantly inhibit the activation of the STAT3 signaling pathway [43]. However, few studies have explored the anti-inflammatory effects of FRA on AD. Our findings revealed that FRA significantly reduced the levels of IL-31 in the serum and lesioned skin of DNFB-induced AD-like mice. Furthermore, FRA treatment decreased IL-4 and TSLP levels (Fig. 4), indicating its ability to attenuate inflammatory responses in DNFB-induced AD-like mice.

FRA treatment inhibited the decrease in IL-4 and TSLP levels, indicating its ability to attenuate the inflammatory response in AD mice. Th2 cytokines can activate or sensitize TRP channels to participate in the pathological development of AD via different mechanisms under different pain and itch conditions [40]. Previous studies have shown that IL-31 induces pruritus and regulates the pathogenesis of AD by activating $TRPV1^{+}/TRPA1^{+}$ sensory neurons and even sensitizing TRPV1 channels in a fast mode [13,40]. Our

results show that the mRNA and protein levels of IL-31, IL-31RA, TRPV1 and TRPA1 in the skin and DRG of DNFB-induced AD-like mice were inhibited by FRA treatment (Fig. 5). This further supports the notion that FRA exerts its anti-inflammatory and antipruritic effects in AD by modulating IL-31. TRPV1 and TRPA1 are expressed in sensory neurons and skin cells and play key roles in the transduction of inflammation, pain and itch signals [44–46]; we speculated that FRA may also have an inhibitory effect on some pain-related inflammatory diseases, such as enteritis and arthritis. However, this hypothesis requires further validation.

The anti-inflammatory effects of FRA and its inhibitory effect on the IL-31 pathway were further verified in LPS-stimulated HaCaT cells. Keratinocytes are one of the main cellular components and effector cells involved in inflammatory responses. Stimulating HaCaT cells with LPS can activate NO, and PGE2 production, and is an excellent model for anti-inflammatory drug screening [32]. The inflammatory response in the pathologic progression of AD plays a vital role. An imbalance in the production of the inflammatory mediators NO and PGE2, which are synthesized by iNOS and COX2 enzymes, is associated with the inflammatory response in AD. As shown in Fig. 6B, FRA significantly inhibited iNOS and COX2 protein levels in LPS-stimulated HaCaT cells. FRA has been reported to exert inhibitory effects on iNOS and COX2 production in LPS-stimulated RAW264.7 cells by inhibiting NFκB signal transduction [47]; however, in our study, we first confirmed the inhibitory effect of FRA on LPS-stimulated HaCaT cells. Importantly, the protein levels of IL-31, IL-31RA, TRPV1, and TRPA1 were inhibited by different FRA concentrations in LPS-stimulated HaCaT cells (Fig. 6C). This is consistent with the results of the animal experiments, thereby further elucidating the inhibitory effect of FRA on IL-31 and its downstream TRP channels.

Importantly, previous studies have demonstrated that oral administration of 100 mg/kg FRA dose not induce significant toxicological reactions in the vital organs of mice, including the liver, heart, kidney, lung and spleen [48]. No significant side effects of FRA were observed during the experiment. This indicates that FRA may have significant anti-inflammatory and antipruritic effects with minimal adverse effects in AD treatment, and has high research and development value in the clinical treatment of inflammation-related skin diseases.

However, some limitations of this study should be acknowledged. Although we clarified the inhibitory effect of FRA on IL-31, IL-31RA, TRPA1, and TRPV1, further investigations are warranted to delineate the precise mechanisms by which FRA acts on IL-31RA or TRP channels (e.g., whether FRA can inhibit or sensitize TRP channels).

5. Conclusion

We demonstrated the antipruritic and anti-inflammatory effects of FRA in DNFB-induced AD-like mice and LPS-stimulated HaCaT cells. FRA significantly inhibited chronic itch, epidermal thickening, and skin inflammation in DNFB-induced AD-like mice by regulating IL-31 and its downstream TRP pathway. These results suggest that FRA holds promise as a potential drug for the clinical treatment of AD.

Ethics declarations

Animal experiments were performed in strict accordance with the procedures and policies approved by the Ethic Committee of Medical College of Yangzhou University (No. YXYLL-2022-161, approval on September 17, 2022).

Data availability statement

The data underlying the results presented in this paper are not publicly available at this time but may be obtained from the corresponding authors upon reasonable request.

Funding

This work was supported by a Special Funding Project from the China Postdoctoral Science Foundation (2020T130562), the National Natural Science Foundation of China (NSFC) (81904212), and the Ministry of Education of the China Spring Sunshine Program (HZKY20220165).

CRediT authorship contribution statement

Niuniu Yang: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Jialin Deng:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Huiwen Xu:** Methodology, Investigation, Funding acquisition. **Huijuan Dai:** Methodology, Investigation. **Han Jin:** Methodology, Investigation. **Haifeng Shao:** Methodology, Investigation. **Yanqing Liu:** Writing – review & editing, Supervision, Resources.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35391.

References

[1] S. Mohammad, M.R. Karim, S. Iqbal, et al., Atopic dermatitis: pathophysiology, microbiota, and metabolome - a comprehensive review, Microbiol. Res. 281 (2024) 127595, https://doi.org/10.1016/j.micres.2023.127595.

[2] S. de Lusignan, H. Alexander, C. Broderick, et al., The epidemiology of eczema in children and adults in England: a population-based study using primary care data, Clin. Exp. Allergy 51 (3) (2021) 471–482, https://doi.org/10.1111/cea.13784.

[3] R. Chovatiya, Atopic dermatitis (eczema), JAMA 329 (3) (2023) 268, https://doi.org/10.1001/jama.2022.21457.

[4] D.K. Chu, L. Schneider, R.N. Asiniwasis, et al., Atopic dermatitis (eczema) guidelines: 2023 American academy of allergy, asthma and immunology/American College of allergy, asthma and immunology joint task force on practice parameters GRADE- and institute of medicine-based recommendations, Ann. Allergy Asthma Immunol. 132 (3) (2024) 274–312, https://doi.org/10.1016/j.anai.2023.11.009.

[5] K. Eyerich, M.J. Gooderham, J.F. Silvestre, et al., Real-world clinical, psychosocial and economic burden of atopic dermatitis: results from a multicountry study, J. Eur. Acad. Dermatol. Venereol. 38 (2) (2024) 340–353, https://doi.org/10.1111/jdv.19500.

[6] M.S. Fassett, J.M. Braz, C.A. Castellanos, et al., IL-31-dependent neurogenic inflammation restrains cutaneous type 2 immune response in allergic dermatitis, Sci Immunol 8 (88) (2023) eabi6887, https://doi.org/10.1126/sciimmunol.abi6887.

[7] I. Arai, S. Saito, Interleukin-31 receptor A expression in the dorsal root ganglion of mice with atopic dermatitis, Int. J. Mol. Sci. 24 (2) (2023) 1047, https://doi.org/10.3390/ijms24021047.

[8] U. Raap, K. Wichmann, M. Bruder, et al., Correlation of IL-31 serum levels with severity of atopic dermatitis, J. Allergy Clin. Immunol. 122 (2) (2008) 421–423, https://doi.org/10.1016/j.jaci.2008.05.047.

[9] M.M. Neis, B. Peters, A. Dreuw, et al., Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis, J. Allergy Clin. Immunol. 118 (4) (2006) 930–937, https://doi.org/10.1016/j.jaci.2006.07.015.

[10] C.T. Dobrican-Băruța, D.M. Deleanu, I.A. Muntean, I. Pintea, C.M. Florea, G.A. Filip, IL-31-Pruritus interleukin: serum values and clinical impact in chronic spontaneous urticaria-A Romanian retrospective study, J. Clin. Med. 12 (18) (2023) 5957, https://doi.org/10.3390/jcm12185957.

[11] X. Wen, H. Yu, L. Zhang, et al., The relationship and clinical significance of serum cytokine expression level and skin pruritus in patients with Hodgkin lymphoma and angioimmunoblastic T-cell lymphoma, Int. Immunopharm. 131 (2024) 111777, https://doi.org/10.1016/j.intimp.2024.111777.

[12] S. Chaowattanapanit, R. Wongjirattikarn, N. Chaisuriya, et al., Increased IL-31 expression in serum and tissue protein in prurigo nodularis, Ther Adv Chronic Dis 13 (2022) 20406223221112561, https://doi.org/10.1177/20406223221112561.

[13] F. Cevikbas, X. Wang, T. Akiyama, et al., A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: involvement of TRPV1 and TRPA1, J. Allergy Clin. Immunol. 133 (2) (2014) 448–460, https://doi.org/10.1016/j.jaci.2013.10.048.

[14] S.R. Dillon, C. Sprecher, A. Hammond, et al., Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice, Nat. Immunol. 5 (7) (2004) 752–760, https://doi.org/10.1038/ni1084.

[15] S. Pitake, P.C. Ralph, J. DeBrecht, S.K. Mishra, Atopic dermatitis linked cytokine interleukin-31 induced itch mediated via a neuropeptide natriuretic polypeptide B, Acta Derm. Venereol. 98 (8) (2018) 795–796, https://doi.org/10.2340/00015555-2977.

[16] I. Arai, M. Tsuji, H. Takeda, N. Akiyama, S. Saito, Capsaicin suppresses interleukin-31-induced itching partially involved in inhibiting the expression of dorsal root ganglion interleukin-31 receptor A in male mice, Neurobiol Pain 11 (2022) 100088, https://doi.org/10.1016/j.ynpai.2022.100088.

[17] M. Lv, P. Xu, Y. Tian, et al., Medicinal uses, phytochemistry and pharmacology of the genus Dictamnus (Rutaceae), J. Ethnopharmacol. 171 (2015) 247–263, https://doi.org/10.1016/j.jep.2015.05.053.

[18] T. Wei, L. Liu, X. Zhou, Cortex Dictamni extracts inhibit over-proliferation and migration of rat airway smooth muscle cells via FAK/p38/Bcl-2 signaling pathway, Biomed. Pharmacother. 102 (2018) 1–8, https://doi.org/10.1016/j.biopha.2018.03.039.

[19] S.P. Choi, C.Y. Choi, K. Park, et al., Glabretal-type triterpenoid from the root bark of Dictamnus dasycarpus ameliorates collagen-induced arthritis by inhibiting Erk-dependent lymphocyte proliferation, J. Ethnopharmacol. 178 (2016) 13–16, https://doi.org/10.1016/j.jep.2015.10.043.

[20] N. Yang, H. Shao, J. Deng, Y. Liu, Network pharmacology-based analysis to explore the therapeutic mechanism of Cortex Dictamni on atopic dermatitis, J. Ethnopharmacol. 304 (2023) 116023, https://doi.org/10.1016/j.jep.2022.116023.

[21] Y.H. Lin, Y.C. Chen, S. Hu, H.Y. Chen, J.L. Chen, S.H. Yang, Identifying core herbal treatments for urticaria using Taiwan's nationwide prescription database, J. Ethnopharmacol. 148 (2) (2013) 556–562, https://doi.org/10.1016/j.jep.2013.04.052.

[22] X.F. Wu, Z.J. Ouyang, L.L. Feng, et al., Suppression of NF-κB signaling and NLRP3 inflammasome activation in macrophages is responsible for the amelioration of experimental murine colitis by the natural compound fraxinellone, Toxicol. Appl. Pharmacol. 281 (1) (2014) 146–156, https://doi.org/10.1016/j.taap.2014.10.002.

[23] X. Han, H. Chen, J. Zhou, et al., The inhibitory effect in Fraxinellone on oxidative stress-induced senescence correlates with AMP-activated protein kinase-dependent autophagy restoration, J. Cell. Physiol. 233 (5) (2018) 3945–3954, https://doi.org/10.1002/jcp.26169.

[24] J. Shi, S. Sun, S. Xing, et al., Fraxinellone inhibits progression of glioblastoma via regulating the SIRT3 signaling pathway, Biomed. Pharmacother. 153 (2022) 113416, https://doi.org/10.1016/j.biopha.2022.113416.

[25] B. Zheng, M. Yuan, S. Wang, et al., Fraxinellone alleviates kidney fibrosis by inhibiting CUG-binding protein 1-mediated fibroblast activation, Toxicol. Appl. Pharmacol. 420 (2021) 115530, https://doi.org/10.1016/j.taap.2021.115530.

[26] Y. Xing, C. Mi, Z. Wang, et al., Fraxinellone has anticancer activity in vivo by inhibiting programmed cell death-ligand 1 expression by reducing hypoxia-inducible factor-1α and STAT3, Pharmacol. Res. 135 (2018) 166–180, https://doi.org/10.1016/j.phrs.2018.08.004.

[27] H. Ma, T. Gao, J.E.T. Jakobsson, et al., The neuropeptide Y Y2 receptor is coexpressed with nppb in primary afferent neurons and Y2 activation reduces histaminergic and IL-31-induced itch, J. Pharmacol. Exp. Therapeut. 372 (1) (2020) 73–82, https://doi.org/10.1124/jpet.119.262584.

[28] N. Yang, H. Shao, J. Deng, et al., Dictamnine ameliorates chronic itch in DNFB-induced atopic dermatitis mice via inhibiting MrgprA3, Biochem. Pharmacol. 208 (2023) 115368, https://doi.org/10.1016/j.bcp.2022.115368.

[29] A.P. Oranje, E.J. Glazenburg, A. Wolkerstorfer, F.B. de Waard-van der Spek, Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score, Br. J. Dermatol. 157 (4) (2007) 645–648, https://doi.org/10.1111/j.1365-2133.2007.08112.x.

[30] H. Dong, C. Feng, X. Cai, et al., 7-Methoxyisoflavone ameliorates atopic dermatitis symptoms by regulating multiple signaling pathways and reducing chemokine production, Sci. Rep. 12 (1) (2022) 8760, https://doi.org/10.1038/s41598-022-12695-3.

[31] F. Li, C. Wang, D. Hu, et al., mMrgprA3/mMrgprC11/hMrgprX1: potential therapeutic targets for allergic contact dermatitis-induced pruritus in mice and humans, Contact Dermatitis 86 (4) (2022) 286–294, https://doi.org/10.1111/cod.14051.

[32] S.Y. Kim, S.D. Han, M. Kim, et al., Mentha arvensis essential oil exerts anti-inflammatory in LPS-stimulated inflammatory responses via inhibition of ERK/NF-κB signaling pathway and anti-atopic dermatitis-like effects in 2,4-dinitrochlorobezene-induced BALB/c mice, Antioxidants 10 (12) (2021) 1941, https://doi.org/10.3390/antiox10121941.

[33] J.M. Lim, B. Lee, J.H. Min, et al., Effect of peiminine on DNCB-induced atopic dermatitis by inhibiting inflammatory cytokine expression in vivo and in vitro, Int. Immunopharm. 56 (2018) 135–142, https://doi.org/10.1016/j.intimp.2018.01.025.

[34] U. Raap, M. Gehring, S. Kleiner, et al., Human basophils are a source of - and are differentially activated by - IL-31, Clin. Exp. Allergy 47 (4) (2017) 499–508, https://doi.org/10.1111/cea.12875.
[35] U. Nygaard, M. Hvid, C. Johansen, et al., TSLP, IL-31, IL-33 and sST2 are new biomarkers in endophenotypic profiling of adult and childhood atopic dermatitis, J. Eur. Acad. Dermatol. Venereol. 30 (11) (2016) 1930–1938, https://doi.org/10.1111/jdv.13679.
[36] C.H. Park, S.Y. Min, H.W. Yu, et al., Effects of apigenin on RBL-2H3, RAW264.7, and HaCaT cells: anti-allergic, anti-inflammatory, and skin-protective activities, Int. J. Mol. Sci. 21 (13) (2020) 4620, https://doi.org/10.3390/ijms21134620.
[37] S.Y. Min, C.H. Park, H.W. Yu, Y.J. Park, Anti-inflammatory and anti-allergic effects of saponarin and its impact on signaling pathways of RAW 264.7, RBL-2H3, and HaCaT cells, Int. J. Mol. Sci. 22 (16) (2021) 8431, https://doi.org/10.3390/ijms22168431.
[38] A. Rabenhorst, K. Hartmann, Interleukin-31: a novel diagnostic marker of allergic diseases, Curr. Allergy Asthma Rep. 14 (4) (2014) 423, https://doi.org/10.1007/s11882-014-0423-y.
[39] J. Buddenkotte, M. Maurer, M. Steinhoff, Histamine and antihistamines in atopic dermatitis, Adv. Exp. Med. Biol. 709 (2010) 73–80, https://doi.org/10.1007/978-1-4419-8056-4_8.
[40] J. Meng, Y. Li, M.J.M. Fischer, M. Steinhoff, W. Chen, J. Wang, Th2 modulation of transient receptor potential channels: an unmet therapeutic intervention for atopic dermatitis, Front. Immunol. 12 (2021) 696784, https://doi.org/10.3389/fimmu.2021.696784.
[41] M. Tominaga, K. Takamori, Peripheral itch sensitization in atopic dermatitis, Allergol. Int. 71 (3) (2022) 265–277, https://doi.org/10.1016/j.alit.2022.04.003.
[42] M.J. Kim, G.S. Bae, I.J. Jo, et al., Fraxinellone inhibits inflammatory cell infiltration during acute pancreatitis by suppressing inflammasome activation, Int. Immunopharm. 69 (2019) 169–177, https://doi.org/10.1016/j.intimp.2019.01.043.
[43] S.M. Jung, J. Lee, S.Y. Baek, et al., Fraxinellone attenuates rheumatoid inflammation in mice, Int. J. Mol. Sci. 19 (3) (2018) 829, https://doi.org/10.3390/ijms19030829.
[44] C. Moore, R. Gupta, S.-E. Jordt, Y. Chen, W.B. Liedtke, Regulation of pain and itch by TRP channels, Neurosci. Bull. 34 (1) (2018) 120–142, https://doi.org/10.1007/s12264-017-0200-8.
[45] O. Gouin, K. L'Herondelle, N. Lebonvallet, et al., TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory response induced by their activation and their sensitization, Protein Cell 8 (9) (2017) 644–661, https://doi.org/10.1007/s13238-017-0395-5.
[46] S. Sun, X. Dong, Trp channels and itch, Semin. Immunopathol. 38 (3) (2016) 293–307, https://doi.org/10.1007/s00281-015-0530-4.
[47] J.H. Kim, Y.M. Park, J.S. Shin, et al., Fraxinellone inhibits lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase-2 expression by negatively regulating nuclear factor-kappa B in RAW 264.7 macrophages cells, Biol. Pharm. Bull. 32 (6) (2009) 1062–1068, https://doi.org/10.1248/bpb.32.1062.
[48] B. He, W. Zhang, J. He, Fraxinellone has anticancer activity by inducing osteosarcoma cell apoptosis via promoting excessive autophagy flux, Front. Pharmacol. 12 (2021) 653212, https://doi.org/10.3389/fphar.2021.653212.