

Myricetin treatment has ameliorative effects in DNFB-induced atopic dermatitis mice under high-fat conditions

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Abstract

Atopic dermatitis (AD) is a chronic, inflammatory cutaneous disorder. Obesity is associated with increased prevalence and severity of AD for reasons that remain poorly understood. Myricetin, a dietary flavonoid found in fruits and vegetables, is known to have anti-inflammatory effects, but its role in AD is unclear. Thus, we investigated the effects of obesity on exacerbation AD lesions and evaluated the effects of myricetin on obese AD. Mice were fed normal diet (ND) or high-fat diet, and then 2,4-dinitrofluorobenzene was used to induce AD-like lesions. We found that obesity exacerbated AD lesions, and myricetin topical administration ameliorated symptoms and skin lesions of obese AD mice, such as dermatitis scores, scratching behavior, epidermal thickness, and mast cell infiltration. In addition, myricetin reduced the levels of immunoglobulin E and histamine, inhibited the infiltration of CD4⁺T cells, and modulated the expression of Th1, Th2, Th17, and Th22 cytokines and pro-inflammatory factors (CCL17, CCL22, IL-1 β , and TGF- β). Moreover, myricetin restored impaired barrier function by reducing transepidermal water loss, increasing lamellar body secretion, as well as upregulating the mRNA and protein expression of filaggrin. Western blot results showed that significantly increased levels of phosphorylated I κ B and NF- κ B p65 was observed in the obese AD mice compared with the AD mice fed ND, whereas the myricetin could downregulate the phosphorylations of I κ B and NF- κ B, and inhibited mRNA expression of iNOS and COX2. Taken together, our results suggest that myricetin treatment exhibits potentially protective effects against the obesity-associated AD by inhibiting inflammatory response and restoring skin barrier function.

Keywords: atopic dermatitis; obesity; myricetin; inflammatory cytokines; skin barrier; NF- κ B signaling pathway.

Atopic dermatitis (AD) is the most common chronic and inflammatory skin disease that affects approximately 20% children and significantly impairs the quality of patient life, which is marked by intense pruritus, skin redness, and thickened skin (Weidinger *et al.*, 2018; Williams *et al.*, 1999). The pathogenesis of AD is complex, multifactorial and involves many different mechanisms, including genetic susceptibility and environmental factors, immune dysregulation of both T-helper cell type 2 (Th2) and T-helper cell type 1 (Th1), production of immunoglobulin E (IgE), activation of mast cells and dendritic cells, leading the production of inflammatory mediators, and skin barrier dysfunction (Eyerich and Novak, 2013; Furue *et al.*, 2017). Dietary, inhalant allergens, and stress have been identified as risk factors for AD (Hanifin, 1997; Katsarou and Armenaka, 2011; Lever, 2001). Identification and control the risk factors are fundamental for the prevention and treatment of AD.

Obesity has become increasingly prevalent around world and is a major risk factor of chronic inflammation and various diseases, such as cardiovascular diseases, metabolic diseases, and dementia (Kopelman, 2000). It has also been increasingly linked to various skin disorders, including acanthosis nigricans,

psoriasis, and AD (Chiricozzi *et al.*, 2016; Ibuki *et al.*, 2012; García Hidalgo, 2002). Increasing evidence indicates that obesity has been linked with systemic chronic low-grade inflammation and skin barrier dysfunction, which is associated with the increasing incidence of AD (Aoki and Murase, 2019; Hajer *et al.*, 2008; Zhang and Silverberg, 2015). A cross-sectional study by Patel *et al.* (2014) found that adopting a Western diet (mainly high-fat content) increases the risk of childhood asthma and AD, and the risk of AD increases greatly with advancing weight. In addition, it has been reported that overweight subjects are more susceptible to be sensitized to food allergens, accompanied by a significant increase in serum IgE and C-reactive protein levels, which may induce sensitization and allergic reactions (Baumann and Lorentz, 2013). However, the reason why obesity aggravates AD remains poorly understood.

Corticosteroids or calcineurin inhibitors are the most widely prescribed medication used for the treatment of AD (Misery, 2011). They primarily depress T-cell immune responses and regulate Th2-mediated chemokines like CCL17 (thymus activation-regulated chemokine) and CCL22 (macrophage-derived chemokine), which are the major contributing factors in the

pathogenesis of AD (Beck *et al.*, 2014). Several studies reported that oral or topical corticosteroids can provide only symptomatic relief, and these treatments are often accompanied by adverse side effects, such as skin thinning and atrophy, dryness, rebound dermatitis (Coondoo *et al.*, 2014), and other serious complications including osteoporosis, cataract, venous thromboembolism, muscle atrophy, hypertension, and disturbance of mood and sleep (Schäcke *et al.*, 2002). Calcineurin inhibitors, such as tacrolimus and cyclosporine, have been reported as potential risk factors for lymphomas, leukemias, and cancer (Castellsague *et al.*, 2018; Schäcke *et al.*, 2002). Therefore, there is an urgent need to develop and research more effective and safer pharmacological intervention for AD.

Myricetin (3,5,7,3',4',5'-hexahydroxyflavone), is one of the most abundant dietary flavonoids mainly found in a number of foods, especially in vegetables, fruits, berries, tea, and red wine (Häkkinen *et al.*, 1999; Sellappan and Akoh, 2002). Various studies on the biological activity of myricetin and found that it has strong anti-cancer, anti-oxidant, anti-inflammatory, antibacterial, and analgesic effects (Semwal *et al.*, 2016). In recent years, more and more studies have shown that myricetin has a number of therapeutic effects on skin disorders, including anti-aging, anti-ultraviolet damage, anti-allergic, and skin cancer. Kim *et al.* (2015) found that myricetin significantly alleviated UVB-induced skin damage in the mouse models by inhibiting the activity of myeloperoxidase, and reducing the secretion of pro-inflammatory cytokines, such as IL-1 β and IL-10. Furthermore, myricetin has also been found to possess anti-aging and antioxidant capacity by reducing intracellular ROS generation, effectively scavenging of free radicals and improving the antioxidant effect of cells (Büchter *et al.*, 2015). Besides these effects, myricetin has been claimed to display anti-allergy effects, which is related to blockade the migration of lymphocytes to bronchial lymphocytes and reduce serum IgE (Medeiros *et al.*, 2008). Based on the various biological and pharmacological activities of myricetin, it would have ameliorative effects for AD.

Therefore, the present study aims to investigate the adverse impacts of obesity on inflammation and skin barrier during AD progression, as well as the preventive therapeutic effects of myricetin on AD skin lesions, in a high-fat diet (HFD)-induced obese mouse model.

Materials and methods

Animals

Six-week-old female KM mice were purchased from the Animal Experiment Center of Wuhan University (Wuhan, China). All mice were housed in the animal facility at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of $55 \pm 5\%$ with a 12 h light/dark cycle, given rodent chow and water *ad libitum* before our study. All animal care and experimental protocols were conducted in accordance with the guidelines established by the Institutional Animal Care and Use Committee (IACUC) of the Animal Experiment Center of Wuhan University (Permit Number: 42010200006385).

Drugs and chemicals

Myricetin was purchased from Aladdin (Shanghai, China). 2,4-dinitrofluorobenzene (DNFB) (purity = 99%) was purchased from Alfa Aesar (Heysham, UK). Dexamethasone (Dex) (purity = 98%) was obtained from Saen Chemical Technology Co Ltd (Shanghai, China). Olive oil was supplied by Aladdin. All enzyme-linked

immunosorbent assay (ELISA) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). CD4, CD8, NF- κ B p65, p-NF- κ B p65, I κ B- α , p-I κ B- α , and filaggrin (FLG) antibodies were obtained from Bioss Biotechnology Co Ltd (Beijing, China), as well as β -actin antibody was purchased from Cell Signaling Technology (CST). Secondary antibody, horse radish peroxidase-conjugated goat anti-rabbit IgG was obtained from Jackson (Lancaster, China).

Experimental model of diet-induced obesity and DNFB-induced AD-like lesions

After a week habituation period, the mice were randomly selected and assigned to 6 equal groups ($n=6$): (1) NOR group: a normal control group fed normal diet (ND) (10% fat); (2) HFD group: an obesity group fed HFD (60% fat); (3) DNFB group: an AD group fed ND for 8 weeks and then treatment with DNFB; (4) Co group: an obesity-AD group fed HFD for 8 weeks and then treatment with DNFB; (5) Co +0.4% myricetin group: same as the Co group, except topical administration with 0.4% myricetin, whereas DNFB was applied to the dorsal skin; (6) Co +0.05% Dex group: same as the Co group, except topical administration with 0.05% dexamethasone (Dex), whereas DNFB was applied to the dorsal skin. Mice were fed with ND or HFD, and body weight of each mouse was recorded every other day. A schematic of the experimental procedure is shown in Figure 1A. After 8 weeks of dietary intervention, the body weight of HFD-fed mice was significantly increased compared with ND-fed mice, indicating the diet-induced obesity mouse models were established. Then, hapten DNFB was applied to the dorsal skin of mice to induce AD-like symptoms as described previously, with some modifications (Motoyama *et al.*, 2018). Before sensitization, dorsal skin (approximately 3×2.5 cm) was shaved by using an electric clipper and 6% Na₂S to remove any remaining hair on day 0. For the sensitization process, 100 μ l of 1% DNFB (dissolved in acetone:olive oil = 3:1) was applied to the dorsal skin on days 1 and 3. After 4 days, 0.5% DNFB was applied to challenge the dorsal skin (50 μ l) at twice a week; 0.4% myricetin dissolved in propylene glycol/ethanol (3:1 [v/v], 100 μ l/mouse) and topically applied to the dorsal skin of obese mice every day when the DNFB-induced AD-like mice model was developed from days 1 to 13. As a positive control, 100 μ l of 0.05% Dex (dissolved in propylene glycol/ethanol [3:1 (v/v)]) was topically treated from days 1 to 13.

Evaluation of dermatitis severity, scratching behavior, and transepidermal water loss

The severity of dermatitis on the dorsal skin was observed and the score was recorded on days 6 and 12 according to the criteria described previously (Kang *et al.*, 2019). The sum of the 4 individual scores was used as the total dermatitis severity score (0–12) for each mouse: 0 (none), 1 (mild), 2 (moderate), and 3 (severe) for each of the 4 symptoms: erythema (redness, hemorrhage), edema (swelling), erosion (excoriation), and dryness (scaling, crusting). On days 5 and 11, the scratching behavior was recorded with a digital camera, and the number of scratching bouts with the hind limbs on the dorsal skin was counted for 10 min after DNFB treatment for 30 min. Transepidermal water loss (TEWL) from dorsal skin was measured on days 6 and 12 by using Tewameter TM300 (CK electronic).

Histological observation

The dorsal skin from each mouse was fixed with 4% formaldehyde and embedded in paraffin to obtain longitudinal sections. Then 5- μ m paraffin sections were stained with hematoxylin

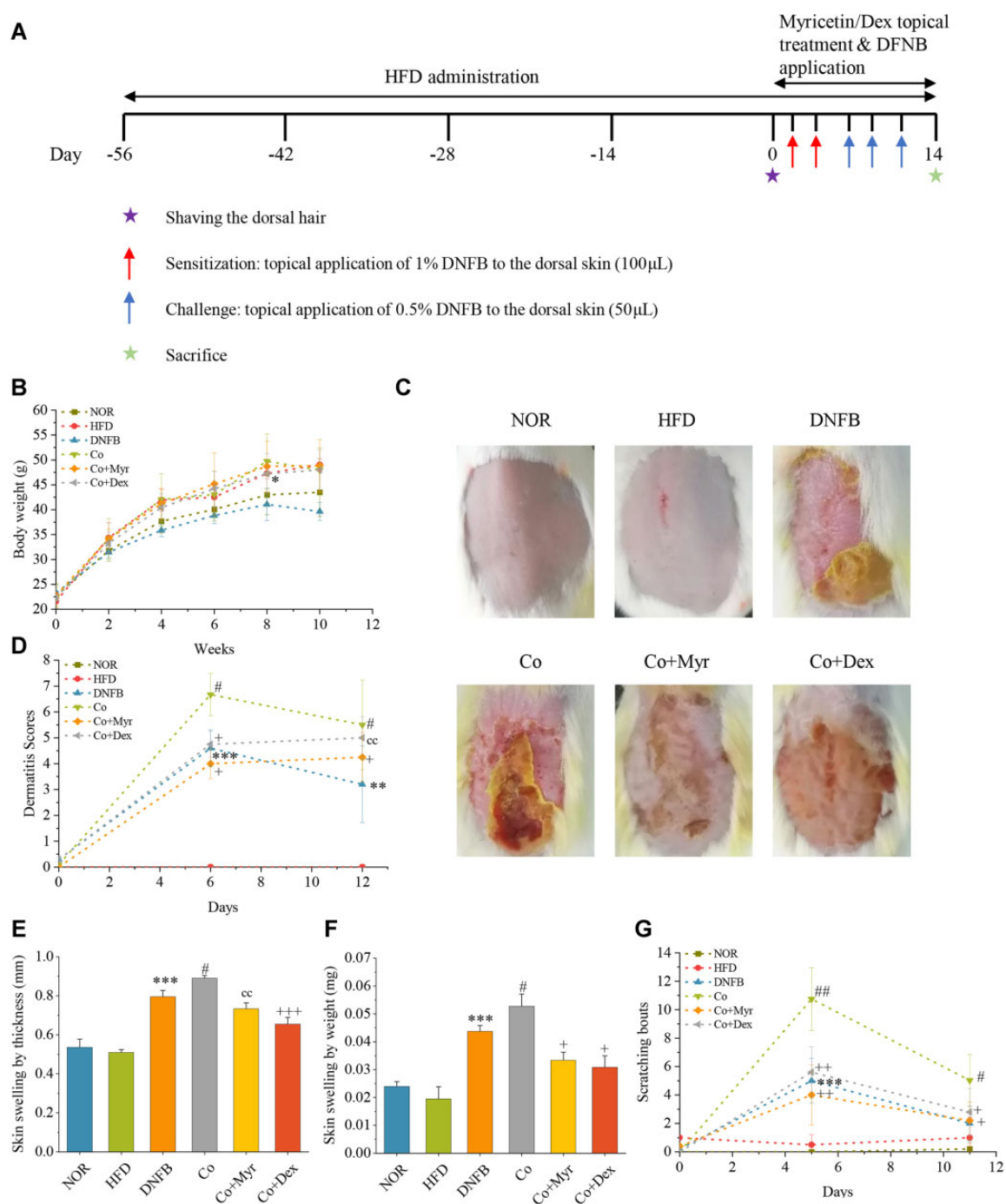


Figure 1. Myricetin efficiently alleviated DNFB-induced AD-like symptoms in obese mouse model. **A**, Scheme of the experimental procedure. The mice ($n = 5/\text{group}$) were fed ND (10% fat) or HFD (60% fat) for 8 weeks and then treatment with DNFB to induce AD symptoms. One percent DNFB was applied to the dorsal skin on days 1 and 3. After 4 days, 0.5% DNFB was applied to challenge the dorsal skin at twice a week; 0.4% myricetin or 0.05% Dex topically applied to the dorsal skin of obese mice every day when the DNFB-induced AD-like mice model was developed from days 1 to 13. **B**, The body weight changes of the mice were measured every 2 weeks during the experimental period. **C**, Typical photographs of dorsal skin in each group and comparison of AD-like skin lesions on day 6. **D**, The dermatitis severity was assessed on days 0, 6, and 12 from the sum of all individual scores. **E** Skin thickness (mm) and **F** skin weight (mg) (indicative skin swelling) were determined on day 14. **G**, The number of scratching bouts was counted within 10 min on days 0, 5, and 11. All data are expressed as mean $\pm$ SD ($n = 5$). * $p < .05$, ** $p < .01$, and *** $p < .001$ versus NOR group; # $p < .05$, ## $p < .01$, and ### $p < .001$ versus DNFB group; + $p < .05$, ++ $p < .01$, and +++ $p < .001$ versus Co group. cc: not statistically different at $p > .05$ versus Co group. Abbreviations: NOR, a normal control group; HFD, an obesity group; DNFB, DNFB-induced AD group; Co, DNFB-induced obese AD group; Co+Myr, myricetin treatment group; Co+Dex, dexamethasone treatment group; ND, normal diet; HFD, high-fat diet.

and eosin (H&E) and thickening of the epidermis was observed by microscope. For the measurement of mast cells, sections of dorsal skin were stained with toluidine blue (TB), after which the number of mast cells was counted. Epidermal thickness

and the number of mast cells were measured in 5 randomly selected fields from 3 randomly samples at 100 $\times$ magnification and 200 $\times$ magnification, respectively, by Image-Pro 6.0 Plus software.

Estimation of IgE and inflammatory chemokines

The mice were sacrificed at the end of the experiment. The whole blood was immediately withdrawn from eyeball, and the serum samples were collected from blood by centrifugation at $1500 \times g$ for 15 min at 4°C and stored at -80°C until use. At the same time, the samples of the dorsal skin and spleen tissues were obtained and homogenized in a 9-fold volume of precooling phosphate-buffered saline (PBS), and the homogenate was centrifuged at $2000 \times g$ for 15 min at 4°C to collect the supernatant for ELISA assay. The levels of IgE in serum and spleen were measured using a Mouse IgE ELISA kit in accordance with the manufacturer's instructions. The IL-4, IFN- γ , TNF- α , and IL-17 levels in the serum and skin homogenates were estimated using commercial ELISA kits.

Immunofluorescence staining

Immunofluorescence staining was performed as previously described (Singh et al., 2019). In brief, paraffin-embedded tissue slices (thickness $4.5 \mu\text{m}$) were deparaffinized, rehydrated, and immersed in ethylene diamine tetraacetic acid antigen repair buffer for antigen repair. Subsequently, the slices were rehydrated and blocked with 5% bovine serum albumin (BSA) for 30 min at 37°C . Immunostaining of histological sections was performed using anti-CD4 antibody (1:200), anti-CD8 antibody (1:200), and anti-FLG antibody (1:200) overnight at 4°C . They were then washed 3 times with PBS and incubated with a goat anti-rabbit IgG secondary labeled tetramethyl rhodamine isothiocyanate (TRITC) (1:200) for 1 h at room temperature. Finally, slices were counterstained with DAPI to label cell nuclei, and images were captured by using an inverted fluorescence microscope (Olympus, Japan).

Quantitative real-time PCR

Briefly, we extracted total RNA using a Biomarker Cell/Tissue Total RNA Isolation kit (Biomarker Technologies, Beijing, China) following manufacturer's protocol. After the *in vivo* experimental period, the dorsal skin was obtained and total RNA was isolated. The RNA concentration was assessed on a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific), and its purity was checked by determining A260/A280 and A260/A230 ratios. Then, BiomarkerScript II 1st Strand cDNA Synthesis Kit (Biomarker Technologies) was used for the reverse transcription as directed by the manufacturer's instructions. Quantitative real-time PCR analysis was conducted using a Bio-Rad Real-Time PCR detection system (Bio-Rad) with SYBR Green. The relative mRNA level of each targeted gene was normalized to the mRNA level of β -actin. All gene-specific primer sequences used for amplification were shown in [Supplementary Table 1](#).

Western blot

For Western blot analysis, protein samples were prepared as previously described (Choi et al., 2014). Briefly, collected skin samples were homogenized in radio-immunoprecipitation assay lysis buffer with protease inhibitor and phosphatase inhibitor, and skin lysate was centrifuged at $10000 \times g$ for 15 min at 4°C to obtain supernatant. Equivalent amounts of proteins ($30 \mu\text{g}$) were electrophoresed by SDS-PAGE gel (8%–12%) and then transferred onto PVDF membranes. The membranes were blocked with 5% BSA for 2 h, then probed with specific primary antibodies and incubated with appropriate HRP-conjugated secondary antibodies. Following a repeat of the wash step with TBST, the membranes were visualized by chemiluminescence using enhanced

chemiluminescence reagents. Finally, the images were captured with chemi-Doc imaging system (Bio-Rad) and analyzed by Image Lab software (Bio-Rad). The primary antibodies used were as followed: rabbit anti-I κ B α (1:2000), anti-phospho-I κ B α (1:500), anti-NF- κ B (1:1000), anti-phospho-NF- κ B p65 (1:500), and anti- β -actin (1:1000).

Transmission electron microscopy

For transmission electron microscopy (TEM) analysis, the skin samples were fixed with 2% glutaraldehyde in 0.15 mM sodium buffer (pH=7.2), washed 3 times, and subsequently post-fixed for 2 h with 1% osmium tetroxide in 0.12 mM sodium buffer (pH=7.2). The samples were dehydrated in a graded series of ethanol, transferred to propylene oxide, and then embedded in a mixture of Epon according to standard procedures. Sixty- to 80-nm-thick ultrathin sections were cut with an ultramicrotome and collected on formvar-coated copper grids, following stained with uranyl acetate and lead citrate. Finally, the samples were examined under a Tecnai Spirit (200 kV) transmission electron microscope and digital images were recorded with the ITEM software package.

Statistical analysis

Statistical analyses were performed using IBM SPSS 26.0 statistical software. The data were expressed as the mean $\pm$ standard deviation (SD) of at least triplicate experiments. Statistical significance differences among multiple groups were compared using 1-way analysis of variance with Dunnett's multiple comparison test. * $p < .05$, ** $p < .01$, and *** $p < .001$ versus NOR group; # $p < .05$, ## $p < .01$, and ### $p < .001$ versus DNFB group; + $p < .05$, ++ $p < .01$, and +++ $p < .001$ versus Co group.

Results

Myricetin attenuated clinical severity of DNFB-induced AD symptoms in obese AD mice

To investigate the effect of myricetin on AD-like lesions in obese mice, we used the HFD-induced obesity mouse model followed by DNFB-induced AD model. Compared with ND-fed mice, the body weight of HFD-fed mice was significantly increased and became obese mice when they were fed with HFD for 8 weeks (Figure 1B). Subsequently, DNFB was topically applied on dorsal skin as well as 0.4% myricetin topically applied to the dorsal skin of mice every day from days 1 to 13. During the period of AD induction, HFD/DNFB-induced comorbidity mice developed more severe AD-like lesions, presenting as erythema, edema, hemorrhaging, and scarring with the dermatitis score (day 6: 6.7 ± 0.8 vs 4.6 ± 0.7 ; day 12: 5.5 ± 1.7 vs 3.2 ± 1.4) was significantly increased compared with AD mice fed with ND (Figs. 1C and 1D). Topical application with myricetin showed a significant decrease of dermatitis score (day 6: 4 ± 0.6 vs 6.7 ± 0.8 ; day 12: 4.25 ± 0.9 vs 5.5 ± 1.7) as compared with Co group (Figs. 1C and 1D). DNFB challenge-induced skin swelling reaction, presented by the thickness/weight of the dorsal skin created by 9-mm punch after mice was sacrificed at day 14, was also marked reduced by myricetin or Dex treatment (Figs. 1E and 1F).

Pruritus is one of the major symptoms of AD not only affect the quality of life of patients but also exacerbates AD progression (Kim et al., 2018), so scratching behavior of each mice was measured at days 0, 5, and 11 during the AD induction period. Consistent with the skin lesions development, mice exhibited significantly increased scratching episodes after repeated

application of DNFB onto the dorsal skin, with the HFD/DNFB-induced mice gaining more itching. Compared with the Co group, myricetin markedly reduced the scratching bouts on days 5 and 11 (day 5: 4 ± 2.1 vs 10.7 ± 2.2 ; day 11: 2.2 ± 1.3 vs 5.0 ± 1.8) (Figure 1G). Our finding strongly supports the notion that itch provokes a strong desire to scratch the affect skin and scratching results in significant damage to the skin and makes the dermatitis worse, thus leading to further itching.

Myricetin alleviated DNFB-induced hyperkeratosis and infiltration of mast cells in obese AD mice

According to previous study (Matsuoka et al., 2003), the skin lesions of AD are characterized by epidermal thickening and dermal infiltration of inflammatory cells. Therefore, we investigated the effects of myricetin on DNFB-induced histological changes. Results of H&E staining showed that the dorsal skin thickness of HFD/DNFB-induced mice was more severe compared with AD mice fed with ND, and myricetin treatment significantly reduced dorsal skin thickness compared with the obese AD group from 467.8 ± 30.9 to $234.3 \pm 35.2 \mu\text{m}$ (Figs. 2A and 2B). Mast cells have been shown to participate in the pathophysiology of AD by secreting histamine in response to itching and inflammation. Thus, we evaluated mast cell infiltration and the histamine levels in the serum and skin of AD-like murine model. Similarly, compared with DNFB-induced mice, HFD/DNFB-induced comorbidity mice exhibited a dramatic increase in mast cell infiltration (Figs. 2C and 2D) as well as elevated levels of histamine in serum and skin (Figs. 2E and 2F). Interestingly, these symptoms were alleviated to a certain extent by the myricetin topical administration (Figs. 2A–F).

Myricetin reduces the IgE and inflammatory cytokines in obese AD mice

The inflammatory of AD is characterized by 2 phases, which Th2 type cytokines are dominant in an acute phase with a transition to a chronic phase expressed Th1, Th17, and Th22 cytokines (Gittler et al., 2012). Therefore, this study investigated whether myricetin affected the Th2 or Th1 response in obese AD mice. The concentration of IgE in the serum and spleen tissue of the DNFB-induced obese mice was increased compared with the AD mice fed ND (serum: 23.3 ± 2.8 vs $17.2 \pm 1.0 \mu\text{g/ml}$; spleen: 1.0 ± 0.06 vs $0.6 \pm 0.02 \mu\text{g/mg}$), but myricetin decreased the concentration of IgE level in the serum and spleen compared with the obese AD group from 23.3 ± 2.8 to $13.1 \pm 1.8 \mu\text{g/ml}$ and 1.0 ± 0.06 to $0.6 \pm 0.09 \mu\text{g/mg}$ (Figs. 3A and 3B). The effect of myricetin on Th2/Th1 cytokines and pro-inflammatory factors was further measured. The levels of IL-4, IFN- γ , TNF- α , and IL-17 in the serum and the dorsal skin tissue obtained from HFD/DNFB-induced mice were significantly higher than those measured in serum and skin obtained from DNFB-induced mice. Topical treatment with myricetin markedly reduced the levels of them in DNFB-sensitized obese mice (Figs. 3C–J). Next, we examined whether myricetin could regulate the expression of pro-inflammatory genes by qRT-PCR. Compared with DNFB group, the mRNA expression levels of CCL17, CCL22, IL-1 β , and TGF- β in the Co group were significantly upregulated, but this upregulation was inhibited by myricetin (Figs. 3K and 3L).

The effect of myricetin on the infiltration of CD4⁺ and CD8⁺ T cells in dorsal skin of obese AD mice

CD4⁺ T cells and CD8⁺ T cells are major lymphocytes that are involved in the adaptive immune response, they differentiate

into diverse types of helper T cells that lead to the infiltration of inflammatory cytokines in AD lesions (Park et al., 2021). To analyze the role of myricetin in controlling infiltration of CD4⁺ and CD8⁺ T cells in obese AD mice, immunofluorescence staining was used to assess whether myricetin reduced the expressions of them in the dorsal skin. Compared with the DNFB group, strong positive reaction of CD4 (red) was observed in skin lesions obtained from the DNFB-induced obese mice (Figs. 4A and 4C). Topical treatment with myricetin exhibited a marked diminution in the infiltration of CD4⁺ T cells (Figs. 4A and 4C). However, the expression of CD8 was only elevated in the DNFB group, and no such difference was observed in the other groups (Figs. 4B and 4D).

Myricetin improve impaired skin barrier function in obese AD mice

It is well known that TEWL was positively associated with the disruption of skin barrier function in AD patients (Yosipovitch, 2004). Our results revealed that obese AD mice exhibited an increase in TEWL versus the DNFB-induced mice fed ND on days 6 and 12 (day 6: 63.1 ± 12.3 vs $42.3 \pm 11.2 \text{ g/m}^2/\text{h}$; day 12: 68.6 ± 4.1 vs $59.9 \pm 6.8 \text{ g/m}^2/\text{h}$). Topical application with myricetin markedly reduced the increase in TEWL (Figure 5A). Recent studies found that FLG, an important skin-barrier protein of the stratum corneum (SC), decreased markedly in skin lesions and can be a primary cause of skin barrier disruption (Mcaleer and Irvine, 2013). Therefore, we measured the FLG expression among all groups by immunofluorescence staining and qRT-PCR. The FLG expression (red fluorescence response) as well as mRNA level was observed in the dorsal skin obtained from normal mice, whereas the expression at the skin lesions of AD mice was significantly inhibited, especially in the HFD/DNFB-induced mice (Figs. 5B–D). Thankfully, the apparent decrease in the FLG expression was reversed with topical treatment with myricetin in the obese AD mice (Figs. 5B–D).

Myricetin recover SC abnormality by increasing lamellar body secretion in obese AD mice

Abnormalities in barrier function are associated with the defecation in lamellar body (LB) formation and secretion. Alteration of the lamellar lipid structure is often observed in human AD patients (Jung et al., 2011). To investigate the effect of myricetin on recovery of lamellar lipid during the barrier recovery process, TEM was performed to quantify the secretion areas of LB. A clear pool of LB was observed at SC-stratum granulosum interface in the normal mice. Repeated application with DNFB on the dorsal skin induced a significant reduction of the secretion areas, which was most prominent in HFD-DNFB-induced mice (NOR vs AD [ND] vs AD (HFD) = 0.37 ± 0.03 vs 0.17 ± 0.008 vs $0.13 \pm 0.01 \mu\text{m}^2/\mu\text{m}$). Thankfully, topical administration with myricetin significantly promoted LB ($0.27 \pm 0.01 \mu\text{m}^2/\mu\text{m}$) secretion compared with the obese AD group ($0.13 \pm 0.01 \mu\text{m}^2/\mu\text{m}$) (Figs. 5E and 5F).

Myricetin inhibited the activation of NF- κ B signaling pathway in obese AD mice

Existing evidences have reported that NF- κ B signaling pathway regulates various pro-inflammatory cytokines, and plays an important role in the pathogenesis of AD (Hseu et al., 2005). Accordingly, to investigate the mechanism by which myricetin relieved AD-like symptoms and inhibited the levels of pro-inflammatory cytokines, we detected the expression of I κ B and NF- κ B in the dorsal skin lesions by western blot analysis. As

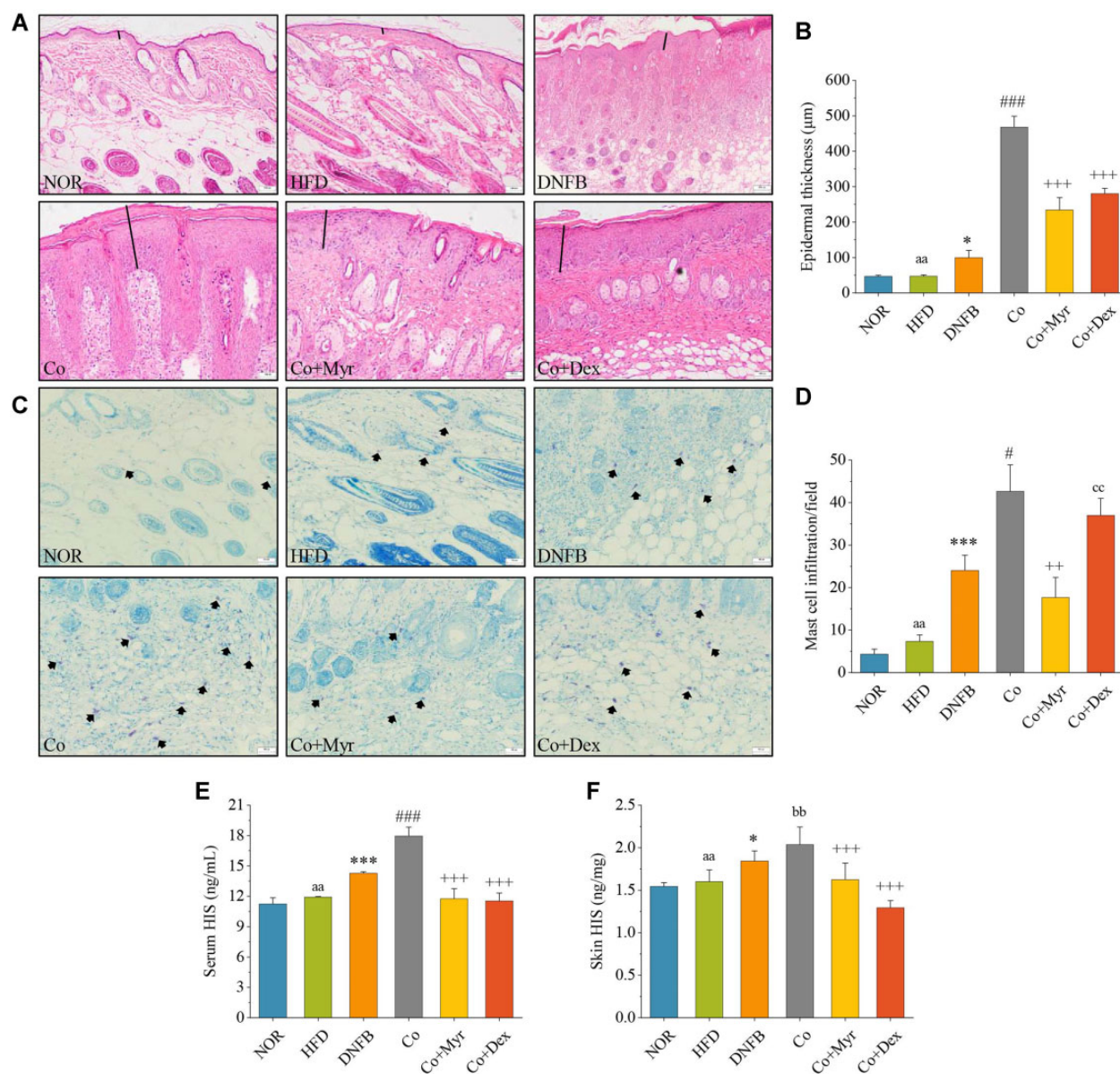


Figure 2. Myricetin alleviated histopathological symptoms of dorsal skin in obese AD mice. A, H&E staining (100× magnification, scale bars = 100 μm), straight line indicates the thickness of epidermis. B, Epidermal thickness was quantified by counting 5 randomly chosen fields per section (n = 3). C, Toluidine blue staining (200× magnification, scale bars = 50 μm), arrows indicate the mast cell. D, The number of mast cell was counted in 5 randomly selected fields per section (n = 3). E and F, Serum and skin level of histamine were measured by ELISA kit (n = 5). All data are expressed as mean ± SD. **p* < .05 and ****p* < .001 versus NOR group; #*p* < .05 and ###*p* < .001 versus DNFB group; +++*p* < .001 versus Co group. aa: not statistically different at *p* > .05 versus NOR group; bb: not statistically different at *p* > .05 versus DNFB group; cc: not statistically different at *p* > .05 versus Co group. Abbreviations: NOR, a normal control group; HFD, an obesity group; DNFB, DNFB-induced AD group; Co, DNFB-induced obese AD group; Co+Myr, myricetin treatment group; Co+Dex, dexamethasone treatment group.

shown in Figures 6A–D, HFD/DNFB-induced mice exhibited significant upregulations of phosphorylated IκB and NF-κB p65 when compared with the DNFB group (p-IκB: 1.62 ± 0.06 vs 0.92 ± 0.06 ; p-NF-κB p65: 1.46 ± 0.04 vs 1.34 ± 0.04), and myricetin significantly inhibited the protein expression of p-IκB and p-NF-κB p65 compared with obese AD group (p-IκB: 0.78 ± 0.03 vs 1.62 ± 0.06 ; p-NF-κB p65: 1.23 ± 0.02 vs 1.46 ± 0.04). However, no significant changes in total protein levels of IκB and NF-κB p65 were observed among the different groups.

Because iNOS and COX2 are the important target genes of NF-κB during inflammation. iNOS, a major pro-inflammatory gene, catalyzes the NO production and then exacerbates AD-like symptoms, such as scratching (Orita et al., 2011). COX2 can be

upregulated by various cytokines, bacterial endotoxins, and growth factors during the immune response of AD (Bajpai et al., 2018). As shown in Figures 6E and 6F, DNFB stimulation significantly increased the mRNA levels of iNOS and COX2, particularly in the conditions of co-existent HFD and DNFB. In contrast, topical treatment with myricetin exhibited inhibition of iNOS and COX2 expressions in the skin lesions.

Discussion

AD is a common allergic inflammatory skin disease, presenting chronically relapsing cutaneous inflammation (Leung and Soter, 2001). The initiation and progress of AD result from the combined

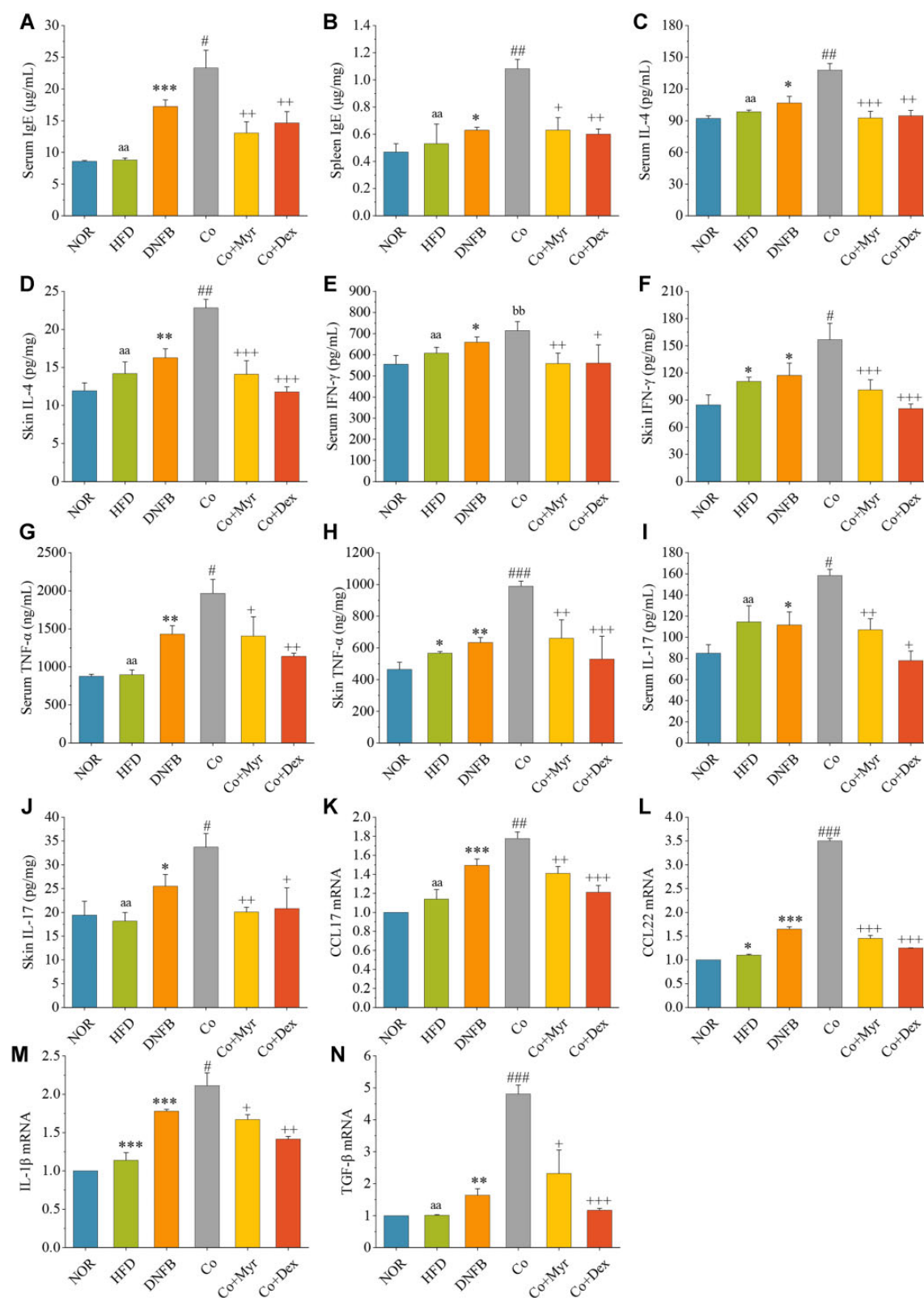


Figure 3. Myricetin regulated IgE, inflammatory cytokines levels, and pro-inflammatory genes expression in obese AD mice. Serum and spleen level (A, B) of IgE was measured by ELISA. C–J, Serum and skin levels of IL-4, IFN-γ, TNF-α, and IL-17 were determined by ELISA kits, respectively. K–N, qPCR analyses showed that myricetin inhibited the mRNA levels of CCL17, CCL22, IL-1β, and TGF-β. All data are expressed as mean ± SD (n = 5). **p* < .05, ***p* < .01, and ****p* < .001 versus NOR group; #*p* < .05, ##*p* < .01, and ###*p* < .001 versus DNFB group; +*p* < .05, ++*p* < .01, and +++*p* < .001 versus Co group. aa: not statistically different at *p* > .05 versus NOR group; bb: not statistically different at *p* > .05 versus DNFB group; cc: not statistically different at *p* > .05 versus Co group. Abbreviations: NOR, a normal control group; HFD, an obesity group; DNFB, DNFB-induced AD group; Co, DNFB-induced obese AD group; Co+Myr, myricetin treatment group; Co+Dex, dexamethasone treatment group.

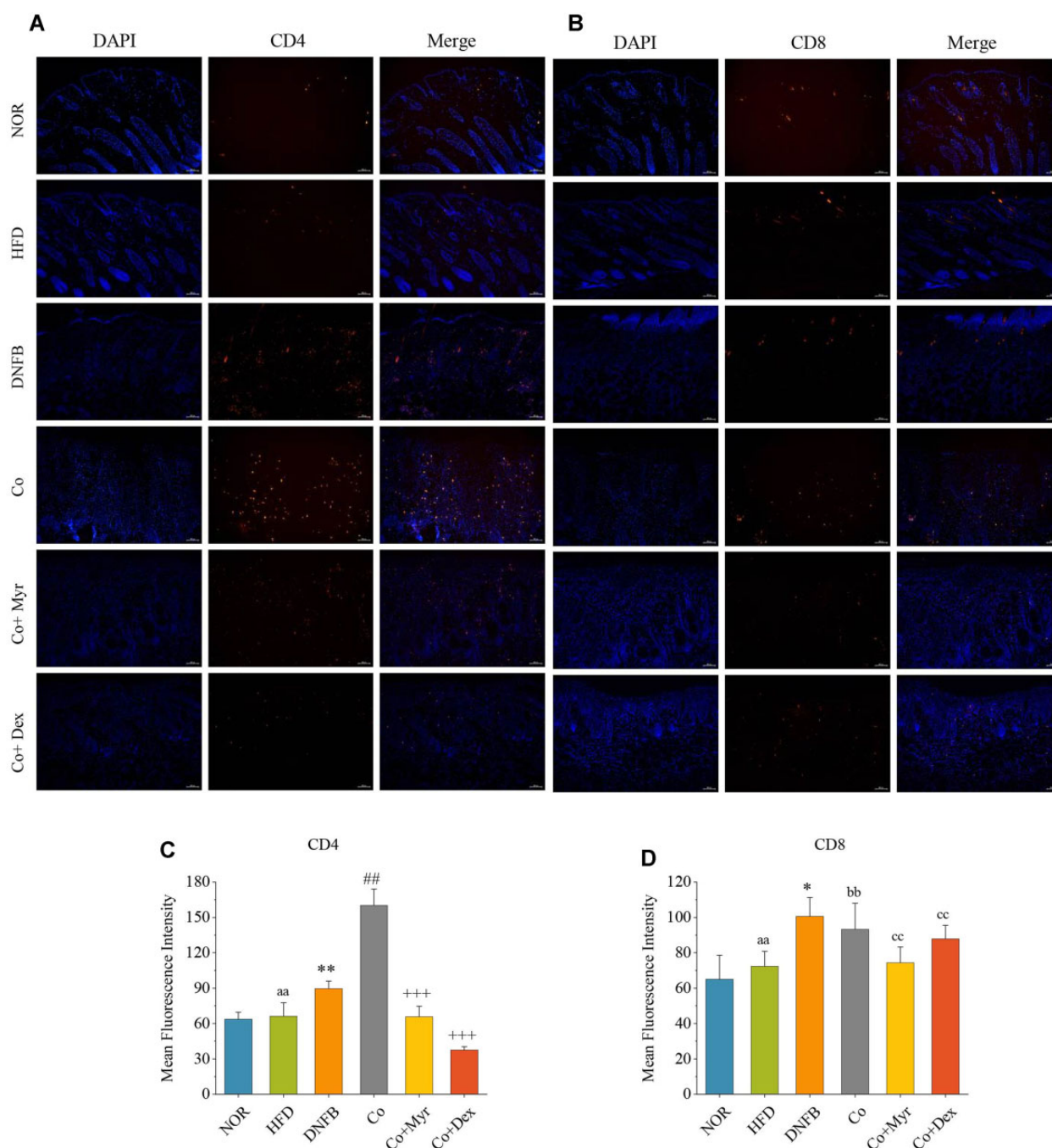


Figure 4. Effects of myricetin on the infiltration of CD4+ and CD8+ T cells in skin lesions in obese AD mice. Immunofluorescence staining of (A) CD4 and (B) CD8 (labeled with TRITC) in skin biopsies from AD mice. Cells were counterstained with nuclear DAPI (100× magnification, scale bar = 100 μm). The fluorescence intensity of (C) CD4 and (D) CD8 was measured using Image J from randomly selected 3 sections in each group. All data are expressed as mean ± SD (n = 3). * $p < .05$ and ** $p < .01$ versus NOR group; ## $p < .01$ versus DNFB group; + $p < .05$, +++ $p < .001$ versus Co group. aa: not statistically different at $p > .05$ versus NOR group; bb: not statistically different at $p > .05$ versus DNFB group; cc: not statistically different at $p > .05$ versus Co group. Abbreviations: NOR, a normal control group; HFD, an obesity group; DNFB, DNFB-induced AD group; Co, DNFB-induced obese AD group; Co+Myr, myricetin treatment group; Co+Dex, dexamethasone treatment group.

effects of genetic, environmental, and immunological factors (Novak and Bieber, 2005). Although various different mechanisms of AD have been proposed, the exact etiology remains unclear. Moreover, considerable evidence indicates a strong association between the metabolic and immune systems (Stoll, 2011). It is well known that obesity is associated with chronic low-grade inflammation, and AD is a comorbidity of obesity. AD and obesity often occur together, the prevalence and severity are affected by each other (Silverberg and Simpson, 2014). However, the exact

correlation mechanism is yet to be discovered. In this study, we hypothesized that HFD-diet may affect inflammatory responses, and then amplified the immune dysregulation and skin barrier disruption in the AD development. Based on this, we explored the preventive therapeutic effects of myricetin on inflammatory response and skin protection in DNFB-induced AD mice under HFD conditions. The results of the studies suggest that myricetin administration may be a safe and effective treatment for AD. Topical treatment with myricetin could attenuate inflammation

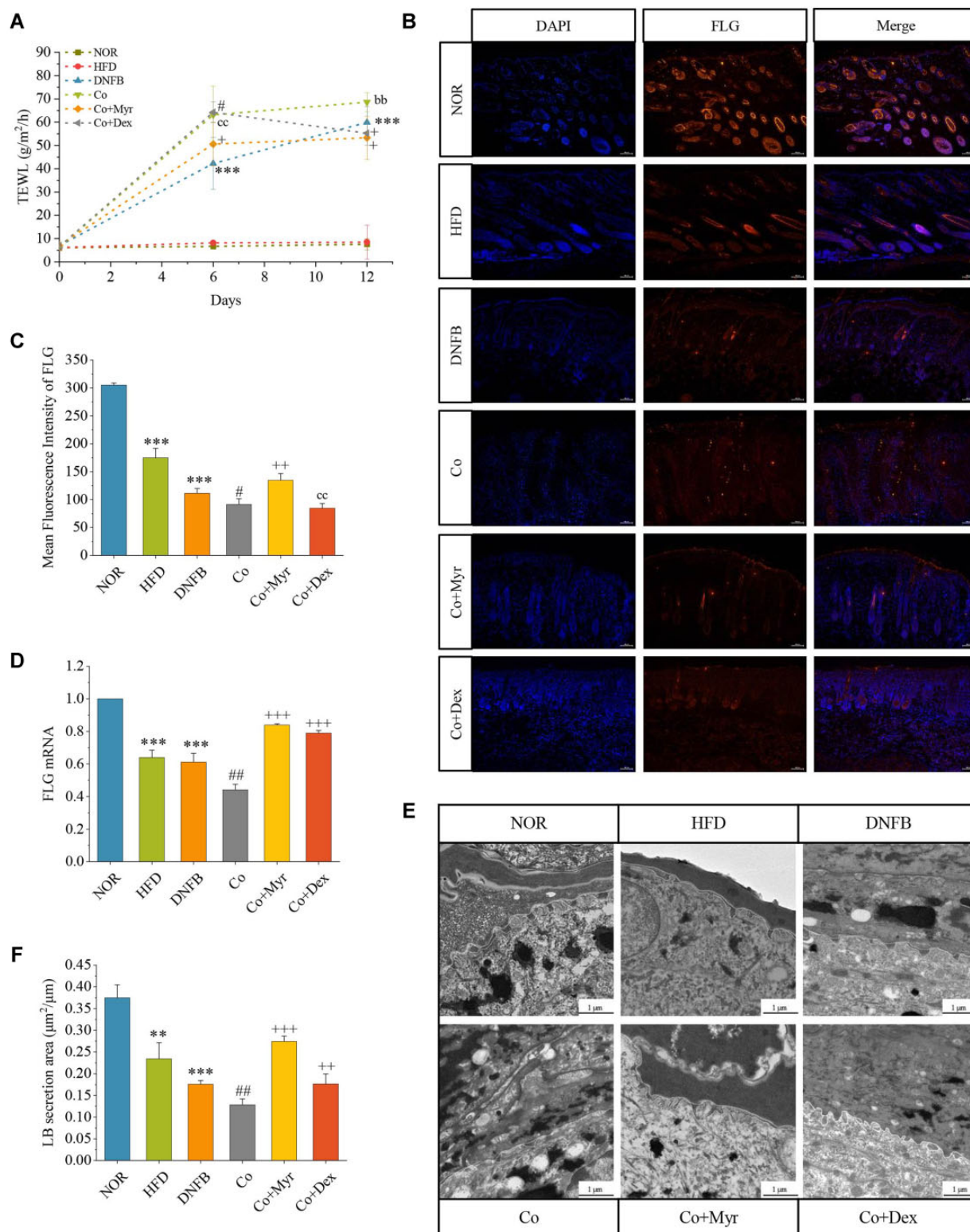


Figure 5. Topical administration myricetin recovered the impaired skin barrier. A, Measurement of TEWL (g/m²/h) at days 0, 6, and 12 (n=5). B, Immunofluorescence staining of FLG protein (labeled with TRITC) in skin lesions (n=3). Cells were counterstained with nuclear DAPI (100× magnification, scale bar = 100 μm). C, Quantitative fluorescence intensity of FLG was performed using from randomly selected 3 sections in each group (n=3). D, mRNA level of FLG was measured by qRT-PCR and normalized to β-actin (n=5). E, LB secretion in skin lesions were analyzed by transmission electron microscopy. Secretion areas, filled with the secreted LB content, at the stratum corneum and stratum granulosum interface, were delimited with dotted lines (scale bar = 1 μm) (n=3). F, Quantification of LB secretion area was performed as the ratio of the sum of the measured secretion areas (μm²) to the length (μm) of stratum corneum-stratum granulosum interface (n=3). All data are expressed as mean ± SD. **p* < .05, ***p* < .01, and ****p* < .001 versus NOR group; #*p* < .05 and ##*p* < .01 versus DNFB group; +*p* < .05, ++*p* < .01, and +++*p* < .001 versus Co group. bb: not statistically different at *p* > .05 versus DNFB group; cc: not statistically different at *p* > .05 versus Co group. Abbreviations: TEWL, transepidermal water loss; FLG, filaggrin; LB, lamellar body; NOR, a normal control group; HFD, an obesity group; DNFB, DNFB-induced AD group; Co, DNFB-induced obese AD group; Co+Myr, myricetin treatment group; Co+Dex, dexamethasone treatment group.

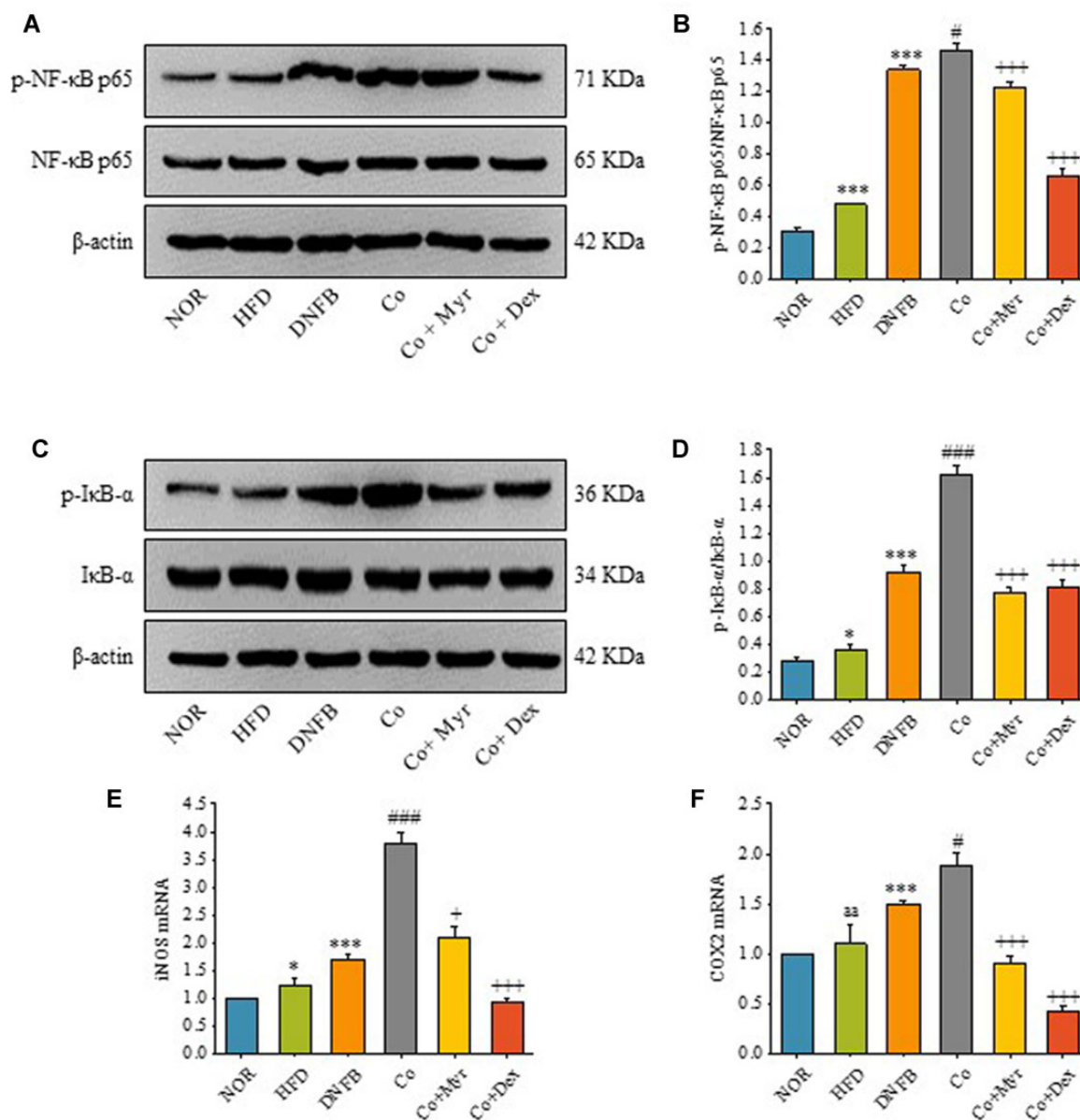


Figure 6. Myricetin inhibited NF- κ B activation and mRNA levels of iNOS and COX2 in skin lesions of obese AD mice. A, Western blot analysis for p-NF- κ B p65 and NF- κ B p65. B, Relative protein level of p-NF- κ B p65 was normalized to NF- κ B p65. C, Western blot analysis for p-I κ B- α and I κ B- α . D, Relative protein level of p-I κ B- α was normalized to I κ B- α . E and F, Expression of iNOS and COX2 was determined by qRT-PCR. All data are expressed as mean $\pm$ SD ($n = 3$). * $p < .05$ and *** $p < .001$ versus NOR group; $^{\#}p < .05$ and $^{###}p < .001$ versus DNFB group; $^{+}p < .05$ and $^{+++}p < .001$ versus Co group. aa: not statistically different at $p > .05$ versus NOR group. Abbreviations: NOR, a normal control group; HFD, an obesity group; DNFB, DNFB-induced AD group; Co, DNFB-induced obese AD group; Co+Myr, myricetin treatment group; Co+Dex, dexamethasone treatment group.

response and improve impaired barrier function in obese AD mice.

Skin lesions of AD are characterized by infiltration of inflammatory cells and epidermal thickening, which features increased with the HFD (Vahidnezhad et al., 2019). Histamine is the major chemical mediators of mast cells that can be released into the tissues and is associated with various symptoms, such as heat, redness, swelling, and itching (Oyoshi et al. 2009). Our results showed that HFD increased epidermal thickening, as well as dermal infiltration of mast cells in the AD lesions. These results aligned with P-D Moon et al.'s study (Vahidnezhad et al., 2019). Myricetin-treated mice exhibited decreased epidermal thickening and mast cells infiltration, with the inhibition of histamine in the serum and skin lesions.

The Th cells are the vital components of the adaptive immune response and play important roles in the immune response to pathogens. Based on the different surface glycoprotein, mature Th cells mainly divide into CD4+T cells and CD8+T cells, which play a key role in driving and maintaining AD inflammation (Ohkusu-Tsukada et al., 2018). CD4+T cells are subjected to differentiation into Th2 cells that secrete IL-4 and IL-13, Th1 cells that secrete IFN- γ , or Th17 cells that secrete IL-17 and IL-22 (Bhattacharyya et al., 2017). Among these, obesity conditions are associated with Th1 cells, and levels of Th1 cytokines (IFN- γ , TNF- α , and IL-6) are increased in the obese individuals (Pacifico et al., 2006). We also observed the same trends, obese mice that were not treated with DNFB showed increased levels of IFN- γ and TNF- α in skin tissue compared with normal mice. In addition, our

results suggested that HFD conditions could exacerbate AD-associated inflammation, shown as increased levels of IL-4, IFN- γ , and TNF- α , as well as pro-inflammatory cytokines expression of CCL17, CCL22, IL-1 β , TGF- β , iNOS, and COX2. This was potentially because of the low-grade inflammation caused by obesity makes mice more sensitive to DNFB stimulation. Topical application with myricetin significantly suppressed production of inflammatory cytokines in obese mice stimulated by DNFB. However, a previous article (Vahidnezhad et al., 2019) found that AD-like skin lesions were markedly exacerbated under HFD conditions, but the levels of IL-4, IL-13, IFN- γ , and TNF- α did not show significant differences. The discrepancies observed in these studies could be explained, in part, due to differences in the genetic background and modeling period of the mice.

One of the common features of AD is skin barrier dysfunction, which is considered to confer greater susceptibility to various allergens and irritants to induce the production of inflammatory cytokines. Impaired barrier function tends to be exacerbated by mechanical injury inflicted by scratching (Kabashima, 2013). In addition, diet-induced obesity is a harmful factor that impair skin function (Kabashima, 2013; Nino et al., 2012). Obesity-related skin showed dry, itching, and skin barrier dysfunction, with concomitant reduction of epidermal keratins expressions, such as KRT5 and KRT14 (Aoki and Murase, 2019). Some studies demonstrated that the impaired skin function associated with obesity is mostly due to obesity-induced inflammatory changes, oxidative stress, cutaneous insulin resistance, and consequent decreases in keratins expression (Aoki and Murase, 2019). Therefore, impaired barrier of the obesity-induced skin can exacerbate AD progression and reconstruction of barrier function is critical for the prevention and treatment of AD. During the progression of AD, impaired skin barrier function leads to an increased TEWL and defection in LB formation and secretion (Takahashi et al., 2014). FLG is a critical important skin barrier protein, and its loss-of-function mutations have been identified as the strongest genetic risk factor for AD (Elias et al., 2017). In our study, normal obese mice that were not treated with DNFB showed impaired skin function, as evidenced by the decreased FLG expression and LB secretion, but TEWL and skin thickness did not show significant difference. However, severe barrier damage, such as increased TEWL, decreased LB secretion, and FLG expression, was detected after DNFB stimulation, especially in the obese AD mice. Thankfully, a marked restoration of the impaired barrier function was observed in the myricetin-administered mice, compared with the AD (HFD) group.

NF- κ B is an important transcription factor involved in immune and inflammatory responses of various skin diseases including AD. In the resting state, NF- κ B is bound to the inhibitory protein I κ B to form inactive complex, and exists in the cellular cytoplasm. Upon stimulation, I κ B undergoes phosphorylation and degradation, and NF- κ B p65 is released and phosphorylated, leading to the activation of NF- κ B, which enhance the production of inflammatory cytokines and chemokines, including iNOS and COX2 (Baldwin, 1996). In this study, repeated challenge with DNFB, phosphorylation of I κ B- α and NF- κ B p65 was significantly increased in skin lesions of AD mice, suggesting activation of NF- κ B pathway. And, this activation was enhanced in AD skin lesions with HFD. However, topical administration of myricetin effectively inhibited these increases. In addition, NF- κ B activation was also observed in the normal obese mice that were not treated with DNFB, which might represent an underlying mechanism of the low-grade inflammation in obese mice.

Conclusions

In summary, we demonstrated that myricetin treatment was effective in DNFB-induced AD mice under HFD conditions. Myricetin could alleviate AD-like symptoms by regulating Th1/Th2 differentiation, inhibiting the mast cells infiltration and production of inflammatory cytokines. In addition, myricetin improved epidermal barrier function by decreasing TEWL, restoring LB secretion, and FLG expression. Myricetin prevented the production of inflammatory cytokines, mostly likely through the inhibition of NF- κ B activity. In conclusion, these results suggested that myricetin could potentially be applied as an effective and alternative therapeutic approach in the management of AD. Further studies are needed to clarify the detailed mechanism underlying these therapeutic effects.

Supplementary data

Supplementary data are available at *Toxicological Sciences* online.

Author contributions

J.-F.G.: Conceptualization, Data curation, Writing—original draft preparation. L.T.: Conceptualization, Investigation. F.L.: Methodology, Software. L.C.: Software, Validation. Y.-Y.Z.: Visualization, Investigation. H.D.: Supervision, Writing—review & editing.

Declaration of conflicting interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

- Aoki, M., and Murase, T. (2019). Obesity-associated insulin resistance adversely affects skin function. *PLoS One* **14**, e223528.
- Bajpai, V. K., Alam, M. B., Quan, K. T., Ju, M.-K., Majumder, R., Shukla, S., Huh, Y. S., Na, M., Lee, S. H., and Han, Y.-K. (2018). Attenuation of inflammatory responses by (+)-syngaresinol via MAP-Kinase-mediated suppression of NF- κ B signaling in vitro and in vivo. *Sci. Rep.* **8**, 9216.
- Baldwin, A. J. (1996). The NF-kappa B and I kappa B proteins: New discoveries and insights. *Annu. Rev. Immunol.* **14**, 649–683.
- Baumann, S., and Lorentz, A. (2013). Obesity—A promoter of allergy? *Int. Arch. Allergy Immunol.* **162**, 205–213.
- Beck, L. A., Thaçi, D., Hamilton, J. D., Graham, N. M., Bieber, T., Rocklin, R., Ming, J. E., Ren, H., Kao, R., Simpson, E., et al. (2014). Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl. J. Med.* **371**, 130–139.
- Bhattacharyya, M., Madden, P., Henning, N., Gregory, S., Aid, M., Martinot, A. J., Barouch, D. H., and Penaloza-MacMaster, P. (2017). Regulation of CD4 T cells and their effects on immunopathological inflammation following viral infection. *Immunology* **152**, 328–343.
- Büchter, C., Ackermann, D., Honnen, S., Arnold, N., Havermann, S., Koch, K., and Wätjen, W. (2015). Methylated derivatives of myricetin enhance life span in *Caenorhabditis elegans* dependent on the transcription factor DAF-16. *Food Funct.* **6**, 3383–3392.
- Castellsague, J., Kuiper, J. G., Pottgård, A., Anveden Berglind, I., Dedman, D., Gutierrez, L., Calingaert, B., van Herk-Sukel, M. P., Hallas, J., Sundström, A., et al. (2018). A cohort study on the risk of

- lymphoma and skin cancer in users of topical tacrolimus, pimecrolimus, and corticosteroids (Joint European Longitudinal Lymphoma and Skin Cancer Evaluation—JOELLE study). *Clin. Epidemiol.* **10**, 299–310.
- Chiricozzi, A., Raimondo, A., Lembo, S., Fausti, F., Dini, V., Costanzo, A., Monfrecola, G., Balato, N., Ayala, F., Romanelli, M., et al. (2016). Crosstalk between skin inflammation and adipose tissue-derived products: Pathogenic evidence linking psoriasis to increased adiposity. *Expert Rev. Clin. Immunol.* **12**, 1299–1308.
- Choi, J. K., Oh, H., Lee, S., Kwon, T. K., Shin, T., Rho, M., and Kim, S. (2014). Salvia plebeia suppresses atopic dermatitis-like skin lesions. *Am. J. Chin. Med.* **42**, 967–985.
- Coondoo, A., Phiske, M., Verma, S., and Lahiri, K. (2014). Side-effects of topical steroids: A long overdue revisit. *Indian Dermatol. Online J.* **5**, 416–425.
- Elias, M. S., Long, H. A., Newman, C. F., Wilson, P. A., West, A., McGill, P. J., Wu, K. C., Donaldson, M. J., and Reynolds, N. J. (2017). Proteomic analysis of filaggrin deficiency identifies molecular signatures characteristic of atopic eczema. *J. Allergy Clin. Immunol.* **140**, 1299–1309.
- Eyerich, K., and Novak, N. (2013). Immunology of atopic eczema: Overcoming the Th1/Th2 paradigm. *Allergy* **68**, 974–982.
- Furue, M., Chiba, T., Tsuji, G., Ulzii, D., Kido-Nakahara, M., Nakahara, T., and Kadono, T. (2017). Atopic dermatitis: Immune deviation, barrier dysfunction, IgE autoreactivity and new therapies. *Allergol. Int.* **66**, 398–403.
- García Hidalgo, L. (2002). Dermatological complications of obesity. *Am. J. Clin. Dermatol.* **3**, 497–506.
- Gittler, J. K., Shemer, A., Suárez-Fariñas, M., Fuentes-Duculan, J., Gulewicz, K. J., Wang, C. Q. F., Mitsui, H., Cardinale, I., de Guzman Strong, C., Krueger, J. G., et al. (2012). Progressive activation of TH2/TH22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J. Allergy Clin. Immunol.* **130**, 1344–1354.
- Hajer, G. R., van Haeften, T. W., and Visseren, F. L. J. (2008). Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur. Heart J.* **29**, 2959–2971.
- Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., and Törrönen, A. R. (1999). Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *J. Agric. Food Chem.* **47**, 2274–2279.
- Hanifin, J. M. (1997). Critical evaluation of food and mite allergy in the management of atopic dermatitis. *J. Dermatol.* **24**, 495–503.
- Hseu, Y.-C., Wu, F.-Y., Wu, J.-J., Chen, J.-Y., Chang, W.-H., Lu, F.-J., Lai, Y.-C., and Yang, H.-L. (2005). Anti-inflammatory potential of *Andropogon camphorata* through inhibition of iNOS, COX-2 and cytokines via the NF- κ B pathway. *Int. Immunopharmacol.* **5**, 1914–1925.
- Ibuki, A., Akase, T., Nagase, T., Minematsu, T., Nakagami, G., Horii, M., Sagara, H., Komeda, T., Kobayashi, M., Shimada, T., et al. (2012). Skin fragility in obese diabetic mice: Possible involvement of elevated oxidative stress and upregulation of matrix metalloproteinases. *Exp. Dermatol.* **21**, 178–183.
- Jung, Y. J., Jung, M., Kim, M., Hong, S. P., and Choi, E. H. (2011). IL-1 α stimulation restores epidermal permeability and antimicrobial barriers compromised by topical tacrolimus. *J. Invest. Dermatol.* **131**, 698–705.
- Kabashima, K. (2013). New concept of the pathogenesis of atopic dermatitis: Interplay among the barrier, allergy, and pruritus as a trinity. *J. Dermatol. Sci.* **70**, 3–11.
- Kang, M., Cho, K., Lee, J., Subedi, L., Yumnam, S., and Kim, S. (2019). Effect of resveratrol-enriched rice on skin inflammation and pruritus in the NC/Nga mouse model of atopic dermatitis. *Int. J. Mol. Sci.* **20**, 1428.
- Katsarou, A., and Armenaka, M. C. (2011). Atopic dermatitis in older patients: Particular points. *J. Eur. Acad. Dermatol. Venereol.* **25**, 12–18.
- Kim, Y.-J., Choi, M. J., Bak, D.-H., Lee, B. C., Ko, E. J., Ahn, G. R., Ahn, S. W., Kim, M. J., Na, J., and Kim, B. J. (2018). Topical administration of EGF suppresses immune response and protects skin barrier in DNCB-induced atopic dermatitis in NC/Nga mice. *Sci. Rep.* **8**, 11895.
- Kim, K. H., Park, S. J., Lee, Y. J., Lee, J. E., Song, C. H., Choi, S. H., Ku, S. K., and Kang, S. J. (2015). Inhibition of UVB-induced skin damage by exopolymers from *Aureobasidium pullulans* SM-2001 in hairless mice. *Basic Clin. Pharmacol. Toxicol.* **116**, 73–86.
- Kopelman, P. G. (2000). Obesity as a medical problem. *Nature* **404**, 635–643.
- Leung, D. Y. M., and Soter, N. A. (2001). Cellular and immunologic mechanisms in atopic dermatitis. *J. Am. Acad. Dermatol.* **44**, S1–S12.
- Lever, R. (2001). The role of food in atopic eczema. *J. Am. Acad. Dermatol.* **45**, S57–S60.
- Matsuoka, H., Maki, N., Yoshida, S., Arai, M., Wang, J., Oikawa, Y., Ikeda, T., Hirota, N., Nakagawa, H., and Ishii, A., (2003). A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* **58**, 139–145.
- McAleer, M. A., and Irvine, A. D. (2013). The multifunctional role of filaggrin in allergic skin disease. *J. Allergy Clin. Immunol.* **131**, 280–291.
- Medeiros, K. C. P., Figueiredo, C. A. V., Figueiredo, T. B., Freire, K. R. L., Santos, F. A. R., Alcantara-Neves, N. M., Silva, T. M. S., and Piuvezam, M. R. (2008). Anti-allergic effect of bee pollen phenolic extract and myricetin in ovalbumin-sensitized mice. *J. Ethnopharmacol.* **119**, 41–46.
- Misery, L. (2011). Therapeutic perspectives in atopic dermatitis. *Clin. Rev. Allergy Immunol.* **41**, 267–271.
- Motoyama, K., Tanida, Y., Sakai, A., Higashi, T., Kaneko, S., and Arima, H. (2018). Anti-allergic effects of novel sulfated polysaccharide sacran on mouse model of 2,4-dinitro-1-fluorobenzene-induced atopic dermatitis. *Int. J. Biol. Macromol.* **108**, 112–118.
- Nino, M., Franzese, A., Ruggiero Perrino, N., and Balato, N. (2012). The effect of obesity on skin disease and epidermal permeability barrier status in children. *Pediatr. Dermatol.* **29**, 567–570.
- Novak, N., and Bieber, T. (2005). The role of dendritic cell subtypes in the pathophysiology of atopic dermatitis. *J. Am. Acad. Dermatol.* **53**, S171–S176.
- Ohkusu-Tsukada, K., Ito, D., Okuno, Y., Tsukada, T., and Takahashi, K. (2018). Signs of atopic dermatitis and contact dermatitis affected by distinct H2-haplotype in the NC/Nga genetic background. *Sci. Rep.* **8**, 2586.
- Orita, K., Hiramoto, K., Kobayashi, H., Ishii, M., Sekiyama, A., and Inoue, M. (2011). Inducible nitric oxide synthase (iNOS) and α -melanocyte-stimulating hormones of iNOS origin play important roles in the allergic reactions of atopic dermatitis in mice. *Exp. Dermatol.* **20**, 911–914.
- Oyoshi, M. K., He, R., Kumar, L., Yoon, J., and Geha, R. S. (2009). Cellular and molecular mechanisms in atopic dermatitis. *Adv. Immunol.* **102**, 135–226.
- Pacifico, L., Di Renzo, L., Anania, C., Osborn, J. F., Ippoliti, F., Schiavo, E., and Chiesa, C. (2006). Increased T-helper interferon- γ -secreting cells in obese children. *Eur. J. Endocrinol.* **154**, 691–697.
- Park, H. J., Lee, S. W., Park, S. H., Kaer, L. V., and Hong, S. (2021). Selective expansion of double-negative iNKT cells inhibits the development of atopic dermatitis in Va14 TCR transgenic NC/

- Nga mice by increasing memory-type CD8D T and regulatory CD4D T cells. *J. Invest. Dermatol.* **141**, 1512–1521.
- Patel, S., Custovic, A., Smith, J. A., Simpson, A., Kerry, G., and Murray, C. S. (2014). Cross-sectional association of dietary patterns with asthma and atopic sensitization in childhood—In a cohort study. *Pediatr. Allergy Immunol.* **25**, 565–571.
- Schäcke, H., Döcke, W., and Asadullah, K. (2002). Mechanisms involved in the side effects of glucocorticoids. *Pharmacol. Ther.* **96**, 23–43.
- Sellappan, S., and Akoh, C. C. (2002). Flavonoids and antioxidant capacity of georgia-grown vidalia onions. *J. Agric. Food Chem.* **50**, 5338–5342.
- Semwal, D., Semwal, R., Combrinck, S., and Viljoen, A. (2016). Myricetin: A dietary molecule with diverse biological activities. *Nutrients* **8**, 90.
- Silverberg, J. I., and Simpson, E. L. (2014). Association between obesity and eczema prevalence, severity and poorer health in US adolescents. *Dermatitis* **25**, 172–181.
- Singh, B., Covelo, A., Martell-Martínez, H., Nanclares, C., Sherman, M. A., Okematti, E., Meints, J., Teravskis, P. J., Gallardo, C., Savonenko, A. V., et al. (2019). Tau is required for progressive synaptic and memory deficits in a transgenic mouse model of α -synucleinopathy. *Acta Neuropathol.* **138**, 551–574.
- Stoll, M. L. (2011). Interactions of the innate and adaptive arms of the immune system in the pathogenesis of spondyloarthritis. *Clin. Exp. Rheumatol.* **29**, 322–330.
- Takahashi, H., Tsuji, H., Minami-Hori, M., Miyauchi, Y., and Iizuka, H. (2014). Defective barrier function accompanied by structural changes of psoriatic stratum corneum. *J. Dermatol.* **41**, 144–148.
- Vahidnezhad, H., Youssefian, L., Saeidian, A. H., Mansoori, B., Jazayeri, A., Azizpour, A., Hesari, K. K., Yousefi, M., Zeinali, S., Jouanguy, E., et al. (2019). A CIB1 Splice-site founder mutation in families with typical epidermodysplasia verruciformis. *J. Invest. Dermatol.* **139**, 1195–1198.
- Weidinger, S., Beck, L. A., Bieber, T., Kabashima, K., and Irvine, A. D. (2018). Atopic dermatitis. *Nat. Rev. Dis. Primers.* **4**, 1.
- Williams, H., Robertson, C., Stewart, A., Ait-Khaled, N., Anabwani, G., Anderson, R., Asher, I., Beasley, R., Björkstén, B., Burr, M., et al. (1999). Worldwide variations in the prevalence of symptoms of atopic eczema in the international study of asthma and allergies in childhood. *J. Allergy Clin. Immunol.* **103**, 125–138.
- Yosipovitch, G. (2004). Dry skin and impairment of barrier function associated with itch—New insights. *Int. J. Cosmet. Sci.* **26**, 1–7.
- Zhang, A., and Silverberg, J. I. (2015). Association of atopic dermatitis with being overweight and obese: A systematic review and meta-analysis. *J. Am. Acad. Dermatol.* **72**, 606–616.e4.