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Calcifediol ameliorates DNCB-induced atopic dermatitis-like skin inflammation in mice with restoration of tight junction-associated Claudin expression

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Abstract: **Background**: Atopic dermatitis (AD) is a chronic, relapsing skin disease driven by epidermal barrier dysfunction and a dominant type 2 immune profile. Calcifediol (25-hydroxyvitamin D3), the major circulating vitamin D metabolite, has shown immunomodulatory potential in AD-like models; however, whether its benefits extend to regulating tight junction-associated claudin proteins, key determinants of epidermal barrier competence in AD, remains unclear. **Objectives**: To evaluate the therapeutic and preventive effects of calcifediol in a DNCB-induced chronic AD-like mouse model and determine whether these effects are associated with the restoration of tight junction proteins (Claudin-1, 3 and 8) expression in lesional skin. **Methods**: Female BALB/c mice were assigned to seven groups: Normal control, DNCB model, dexamethasone, low-dose calcifediol, medium-dose calcifediol, high-dose calcifediol and prophylactic calcifediol groups. AD-like lesions were established by repeated topical application of DNCB and calcifediol was administered intraperitoneally at 2, 4 and 8 µg/kg. Disease severity was assessed by dorsal lesion scoring, ear swelling, ear weight, spleen index, histopathology, serum IgE, IL-4 and IL-13 levels and cutaneous Claudin protein expression. **Results**: DNCB application produced AD-like skin inflammation, as shown by erythema, edema, scaling, epidermal thickening, inflammatory infiltration, increased ear swelling, a higher spleen index and elevated serum IgE, IL-4 and IL-13 levels. Calcifediol administration reduced lesion severity, lowered EASI scores, relieved ear inflammation and improved histopathological changes in the skin. It also decreased the spleen index and suppressed serum IgE, IL-4 and IL-13 levels. Moreover, calcifediol reversed the DNCB-induced reduction in Claudin-1, Claudin-3 and Claudin-8 levels in dorsal skin tissues. **Conclusion**: Calcifediol mitigated DNCB-induced AD-like inflammation in mice, possibly by attenuating type 2 immune activation and restoring the expression of tight junction-associated Claudin proteins. These results suggest that calcifediol deserves further investigation as a potential supportive strategy for atopic dermatitis.

Keywords: Atopic dermatitis; Calcifediol; Claudin; DNCB; Skin barrier

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INTRODUCTION

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease that affects nearly 15–25% of children and 3–7% of adults globally, with its prevalence steadily increasing in recent decades (Weidinger and Novak, 2016; Guttman-Yassky *et al.,* 2025). Beyond its cutaneous manifestations, AD imposes a substantial psychological burden on affected individuals (Fan *et al.,* 2025). Despite decades of research, AD pathogenesis remains incompletely understood and existing treatments rarely address the underlying causes of the disease, focusing instead on symptom management (Drucker *et al.,* 2018). This treatment limitation, combined with the direct medical expenses of patients and their families, underscores the urgent need for safer and more efficacious therapeutic approaches for AD management (Drucker *et al.,* 2017; Prados-Carmona *et al.,* 2025).

Vitamin D, a crucial fat-soluble nutrient, plays an important role in the development and progression of numerous diseases, including malignancies, atopic dermatitis, autoimmune diseases and cardiovascular disease (Mendes *et al.,* 2020). Once absorbed, vitamin D undergoes sequential metabolism to calcifediol (25-hydroxyvitamin D3) and subsequently to calcitriol (1,25-dihydroxyvitamin D3), an active form that targets the vitamin D receptor (Cantorna *et al.,* 2019). Notably, calcifediol deficiency has been linked to increased susceptibility to AD, suggesting a critical role in maintaining epidermal barrier integrity (Zeng *et al.,* 2023). Preclinical and clinical studies have demonstrated that vitamin D supplementation improves skin lesions and reduces AD severity in animal models and patients (Alosaimi *et al.,* 2022; Cabalin *et al.,* 2023; Lu *et al.,* 2023; Park *et al.,* 2023). However, the specific mechanisms underlying vitamin D action, particularly its influence on

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epidermal barrier structures such as tight junction proteins, remain insufficiently characterized.

To address this gap, the present study employed a well-established DNCB-induced murine model of AD-like dermatitis to evaluate the therapeutic and prophylactic effects of calcifediol—the primary circulating form of vitamin D. Calcifediol was chosen for its superior pharmacokinetic profile over cholecalciferol (vitamin D3), as it bypasses hepatic 25-hydroxylation and enables more direct and predictable regulation of circulating vitamin D levels (Landheer *et al.,* 2015). In contrast, calcitriol, although active, carries a higher risk of hypercalcemia and local adverse reactions, limiting its safety margin for clinical applications (Lu *et al.,* 2023). Although previous studies have highlighted the general anti-inflammatory and barrier-enhancing properties of vitamin D compounds in AD-like models, the specific role of calcifediol in modulating claudin family proteins, a key component of epidermal tight junctions, remains largely unexplored.

Tight junctions are specialized intercellular structures that prevent water loss and maintain epidermal permeability. Claudin proteins are central to their function and exhibit subtype-specific roles in skin barrier regulation. Specifically, Claudin-1, -3 and -8 have been identified as critical mediators of epidermal homeostasis, with their downregulation strongly implicated in the compromised barrier function and exaggerated immune responses characteristic of AD (Katsarou *et al.,* 2023; Wang *et al.,* 2025). Accordingly, this study assessed whether the anti-inflammatory and barrier-restorative effects of calcifediol are associated with the restored expression of Claudin-1, Claudin-3 and Claudin-8. By investigating the link between calcifediol and tight junction components, the present findings may advance current vitamin D research in AD and provide insights into the molecular basis for its therapeutic application.

MATERIALS AND METHODS

Animals

Six-to-eight-week-old female BALB/c mice (18–22 g) were purchased from the Hangzhou Medical College Animal Center (SCXK (Zhe) 2024-0002) and maintained under specific pathogen-free (SPF) conditions with food and water provided ad libitum. Female mice were selected because of their heightened susceptibility to Th2-skewed immune responses, a pathological feature commonly associated with atopic dermatitis (AD). Humane endpoints were applied throughout the study to minimize animal suffering, including weight loss exceeding 20%, severe skin ulceration and persistent lethargy. Animals meeting these criteria were euthanized promptly. All experimental procedures adhered to the principles of the 3Rs and were approved by the Animal Ethics Committee of Quanzhou Medical College (Approval No. 2025089). All personnel involved in lesion scoring or sample analysis were blinded to group assignments.

Animal grouping, model induction and drug administration

Forty-two female BALB/c mice were allocated into seven experimental groups (six animals per group) using a random number table for group randomization (Lu *et al.,* 2023): a normal control group (NC, healthy mice receiving vehicle), a model group (AD + vehicle), a positive control group (AD + dexamethasone, 1 mg/kg), three treatment groups (AD + calcifediol at 2, 4, or 8 μg/kg) and a prophylactic group (AD + calcifediol, 8 μg/kg).

Chronic AD-like dermatitis was induced using epicutaneous DNCB application (Sigma), following established protocols. On day 0, the dorsal hair (3×2 cm area) was shaved to expose the skin. Sensitization was achieved with 2% DNCB (150 μL to the dorsal skin and 20 μL to the right ear) on days 1, 3 and 7, followed by dermatitis maintenance with 0.5% DNCB on days 9, 12, 15, 18 and 21 (100 μL to the dorsal skin and 10 μL to the right ear), as described previously (Tamakuwala *et al.,* 2016). Normal control mice received only the vehicle (acetone: olive oil = 3:1, Aladdin Biochemical Technology Co., Ltd., Shanghai). Successful model induction was validated by visible dorsal skin lesions (edema, scaling and excoriation) and by significantly increased dermatitis scores compared with controls. To evaluate therapeutic outcomes, calcifediol and dexamethasone were administered intraperitoneally beginning on day 9, with injections given every 2 days (7 doses in total). Intraperitoneal injection was selected because it has been shown to deliver stable calcifediol concentrations in vivo (Zhou *et al.,* 2018; Wang *et al.,* 2020). For prophylactic evaluation, calcifediol (8 μg/kg) was administered intraperitoneally three days prior to model initiation and continued every other day. On day 22, the mice were euthanized after lesion scoring and tissue samples were collected for biochemical analysis.

Eczema area and severity index assessment

Skin lesion severity was scored on day 22 using an adapted Eczema Area and Severity Index (EASI), a clinical tool for assessing eczema extent and severity (Tamakuwala *et al.,* 2016). Trained investigators blinded to group allocation independently evaluated four parameters—erythema, edema/papulation, excoriation/erosion and lichenification. Lesions were graded from 0 to 3 for each criterion, with the assigned numeric value corresponding directly to lesion severity.

Ear swelling and spleen index

Right ear thickness was measured with a digital vernier caliper (Mitutoyo 500-196-30, Mitutoyo Corp., Kawasaki,

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Japan) before DNCB sensitization (day 0) and after treatment (day 22) and ear swelling was calculated as the difference between the three measurements. After euthanasia, spleens were isolated and examined for gross morphology, color and size, then weighed to calculate the spleen index using the following formula: spleen index (mg/g) = spleen weight (mg) / body weight (g).

ELISA

On day 22, whole blood (~1 mL per mouse) was collected from the retro-orbital venous plexus under light anesthesia. After clotting at room temperature for 20 min, the samples were centrifuged at 2000 rpm (4°C, 20 min). The collected serum was stored at −20°C prior to ELISA.

Hematoxylin-Eosin (H&E) staining

After deep anesthesia, mice underwent transcardiac perfusion with phosphate-buffered saline (PBS). Harvested right ear and dorsal skin samples were fixed in 10% neutral-buffered formalin for 48 h. Following routine graded ethanol dehydration, tissues were paraffin-embedded and cut into 4-μm sections. Histopathological changes in the epidermis were evaluated using hematoxylin and eosin (H&E) staining. The sections were examined using a light microscope (Olympus, Tokyo, Japan). H&E staining was performed by investigators blinded to the group allocation.

Western blotting

After sufficient anesthesia, dorsal skin specimens were harvested, homogenized and lysed on ice for 30 min. After centrifugation, supernatants were collected and total protein content was quantified using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China). Fifty micrograms of protein were loaded into each electrophoresis well and all Western blot assays were performed in three independent biological replicates to ensure reproducible quantification. Protein homogenates (50 μg/lane) were fractionated via 12% SDS-PAGE prior to transfer onto PVDF membranes (Millipore).

Following a 1-h block in 5% (w/v) non-fat milk at room temperature, blots were incubated overnight (4°C) with primary antibodies targeting Claudin-1 (UpingBio, catalog number YP-mAb-16951, 1:800 dilution), Claudin-3 (UpingBio, catalog number YP-mAb-16957, 1:800 dilution), Claudin-8 (UpingBio, YP-mAb-16965, 1:800 dilution) and β-actin (Solarbio, catalog number K200058M, 1:1000 dilution). After secondary antibody incubation (HRP-conjugated, 1 h, RT), immunoreactive signals were captured using an enhanced chemiluminescence (ECL) substrate and imaged with a chemiluminescence imaging system (General Electric Company, Beijing, China). Band intensities were then quantified using ImageJ software. Claudin-1, Claudin-3 and Claudin-8 expression was normalized to β-actin and the results are presented as ratios. To ensure reproducibility, the experiments were performed in three biological replicates for each cohort.

Statistical analysis

In all graphical representations, values denote the mean, with error bars reflecting the standard error of the mean (SEM). Data normality and homogeneity of variance were evaluated before statistical analysis. Differences across experimental cohorts were evaluated using a one-way analysis of variance (ANOVA). Subsequent post hoc analyses were tailored to specific comparisons. Dunnett's procedure was used to compare treatments against the model group (assuming equal variances), whereas Tukey's method enabled comprehensive pairwise comparisons. Statistical significance was defined as $P < 0.05$.

RESULTS

Calcifediol ameliorated dorsal skin lesions in DNCB-induced AD-like mice

As shown in fig. 1A, repeated DNCB exposure successfully induced AD-like dermatitis in mice by day 22. Mice in the model group exhibited typical AD-related cutaneous lesions, including erythema, edema, scaling and ulceration, which were absent in the normal control group (Fig. 1B). Treatment with dexamethasone or calcifediol (2, 4, or 8 μg/kg) significantly reduced the dorsal skin lesions. EASI scores were recorded as follows: normal control group (0.00 ± 0.00); model group (8.50 ± 0.20); dexamethasone group (3.50 ± 0.34); calcifediol therapeutic groups (4.83 ± 0.33 for 2 μg/kg, 4.08 ± 0.35 for 4 μg/kg and 2.75 ± 0.31 for 8 μg/kg); and prophylactic group (1.83 ± 0.11; Fig. 1C). Notably, prophylactic calcifediol administration resulted in a lower total EASI score than dexamethasone treatment ($P < 0.01$). Throughout the experiment, no obvious signs of severe distress, aberrant locomotor activity, or treatment-related mortality were observed in any calcifediol-treated mouse group. Together, these findings indicate that calcifediol markedly alleviates skin lesion severity and facilitates barrier recovery in mice with AD.

Calcifediol attenuates epidermal hyperplasia and inflammatory infiltration in AD-like mice

Moreover, histopathological analysis revealed pronounced epidermal hyperplasia and prominent inflammatory cell infiltration in both the epidermal and dermal layers of the model mice. Notably, these pathological changes were attenuated following dexamethasone or calcifediol treatment, with prophylactic calcifediol administration yielding the most prominent histological improvement (Fig. 2).

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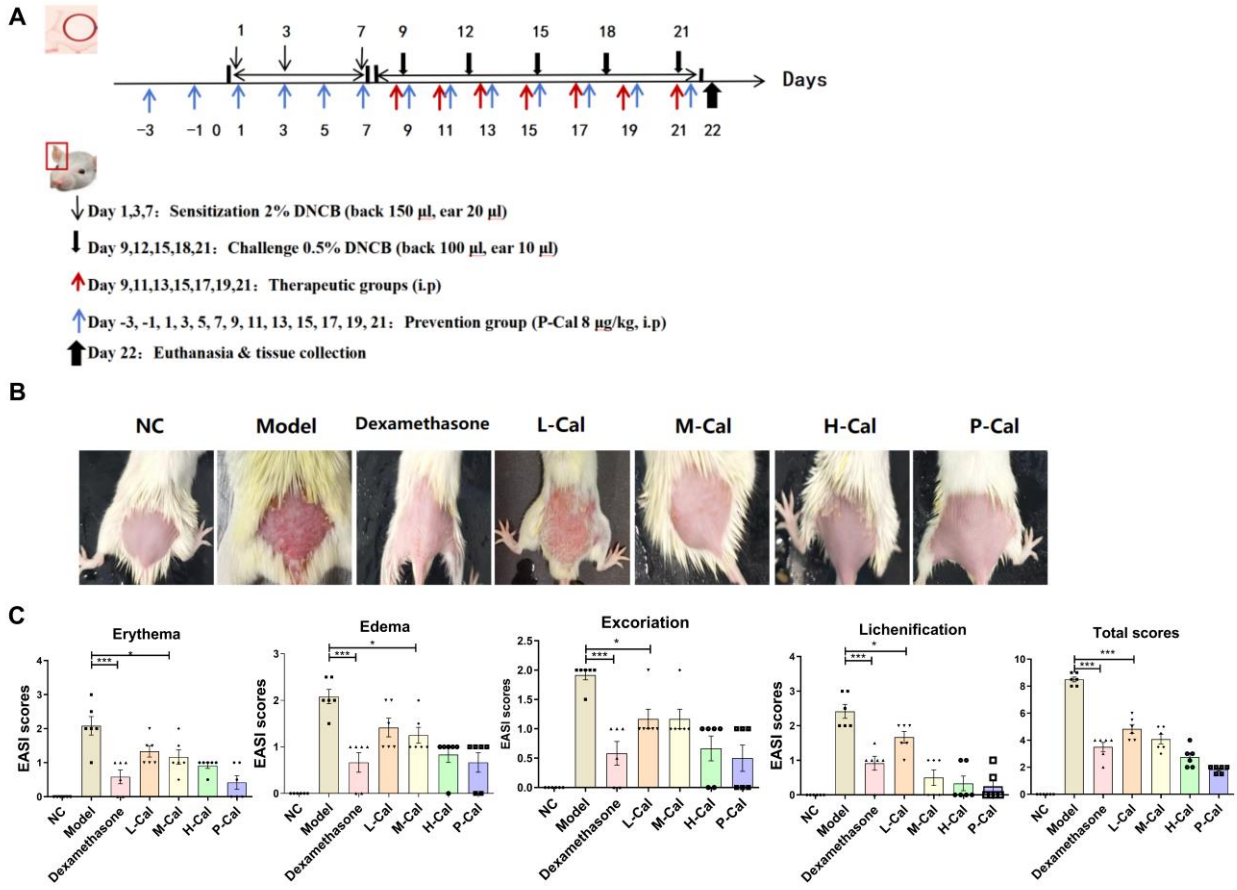


Fig. 1: Calcifediol alleviated dorsal skin lesions in DNCB-induced AD-like mice.
(A) Schematic diagram of the experimental design for the establishment of an atopic dermatitis (AD) mouse model and drug administration; (B) Representative photographs of dorsal skin lesions in each group on day 22; (C) EASI scores of the dorsal skin lesions in each group. Data are expressed as mean± SEM. Data were analyzed via one-way ANOVA coupled with Dunnett's post hoc test. Asterisks denote significance relative to the model group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; n = 6 per group). NC, normal control. L-Cal, low-dose (2 μg/kg) calcifediol, M-Cal, medium-dose (4 μg/kg) calcifediol. H-Cal: high-dose (8 μg/kg) calcifediol. P-Cal, prophylactic (8 μg/kg) calcifediol.

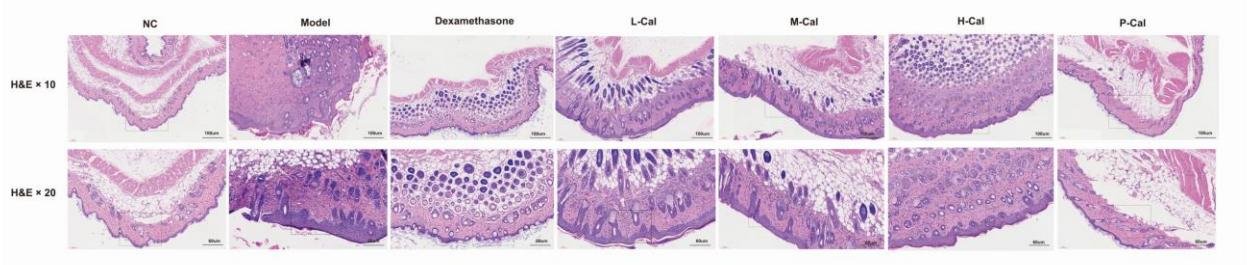


Fig. 2: Calcifediol attenuates epidermal hyperplasia and inflammatory infiltration in AD-like mice.
All dorsal skin samples from the groups underwent H and E staining. Micrographs at ×10 (top, scale bar = 100 μm) and ×20 (bottom, scale bar = 60 μm) magnifications are presented separately. Prominent rectangular annotations were used to highlight key pathological features, including epidermal hyperplasia and inflammatory cell infiltration.

Calcifediol reduced ear inflammation, swelling and weight in AD-like mice

Gross observation of the right ear on day 22 showed obvious erythema and scaling in the model mice, both of which were markedly reduced after treatment with dexamethasone or calcifediol (2, 4, or 8 μg/kg; Fig. 3A).

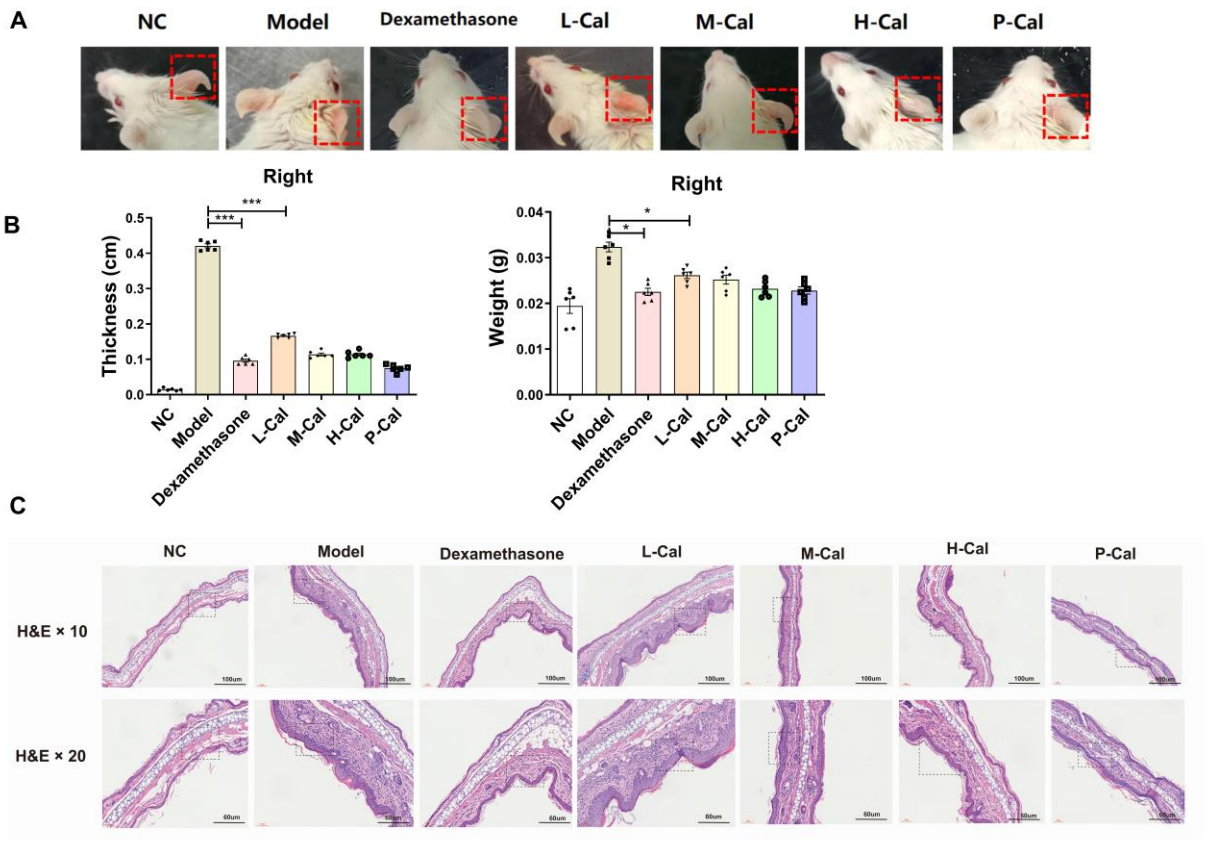


Fig. 3: Calcifediol reduced ear swelling and inflammatory infiltration in DNCB-induced AD-like mice.
(A) Photographs of right ear lesions in each group on day 22; (B) Quantitative analysis of right ear thickness and ear weight. Data are presented as mean ± SEM; n = 6 mice per group. Each mouse was considered an independent biological replicate. Statistical variance was analyzed via one-way ANOVA, utilizing Dunnett's procedure to contrast individual treatments with the model group (*$P < 0.05$, ***$P < 0.001$ vs. model; n = 6 independent replicates); (C) Hematoxylin and eosin-stained histological slices from mouse right ears were photographed at ×10 (scale bar = 100 μm, upper panel) and ×20 (scale bar = 60 μm, lower panel).

Quantitative analysis further demonstrated that calcifediol significantly decreased ear swelling and ear tissue weight compared to the model group ($P < 0.001$ and $P < 0.05$, respectively, Fig. 3B). Histological analysis showed that DNCB-induced epidermal thickening and inflammatory cell infiltration in ear tissues were also attenuated after treatment with calcifediol or dexamethasone (Fig. 3C). These results indicate that calcifediol effectively suppressed local inflammatory responses in ear lesions of AD-like mice.

Calcifediol decreased spleen index and serum total IgE, IL-4 and IL-13 levels

Compared with the normal control group, the spleens of the model mice were enlarged and darker in appearance (Fig. 4A). The spleen index was quantified as follows: NC (2.991 ± 0.140); DNCB-induced model group (7.883 ± 0.184); dexamethasone group (3.497 ± 0.093); calcifediol therapeutic groups (6.610 ± 0.272 for 2 μg/kg, 5.166 ± 0.181 for 4 μg/kg and 4.904 ± 0.159 for 8 μg/kg), prophylactic group (4.742 ± 0.171; Fig. 4B). ELISA quantification demonstrated significant elevations in serum total IL-4, IL-13 and IgE concentrations in the model group. For IL-4, NC (801.428 ± 68.935), Model (1304.148 ± 31.081), dexamethasone (1036.011 ± 41.001), L-Cal (1207.696 ± 21.392), M-Cal (1124.994 ± 25.793), H-Cal (1086.359 ± 41.497), prophylactic group (1016.521 ± 20.700). For IL-13, normal control group (223.265 ± 12.929), Model (406.003 ± 3.373), dexamethasone (281.189 ± 3.171), L-Cal (337.051 ± 1.948), M-Cal (324.501 ± 3.726), H-Cal (299.801 ± 0.721), prophylactic group (260.920 ± 8.063). For serum IgE, NC (11468.964 ± 770.655), Model (29832.480 ± 3460.785), dexamethasone (14513.347 ± 512.602), L-Cal (22745.177 ± 739.785), M-Cal (19088.327 ± 792.777), H-Cal (17629.340 ± 231.809), prophylactic group (15569.515 ± 718.302). Serum IL-4 was significantly decreased in the medium-dose, high-dose and prophylactic groups with similar downward trends observed for IgE and IL-13($P < 0.05$, $P < 0.01$, $P < 0.001$, Fig. 4C). Collectively, these findings support the notion that calcifediol exerts potent anti-inflammatory and immunomodulatory effects in murine models of AD.

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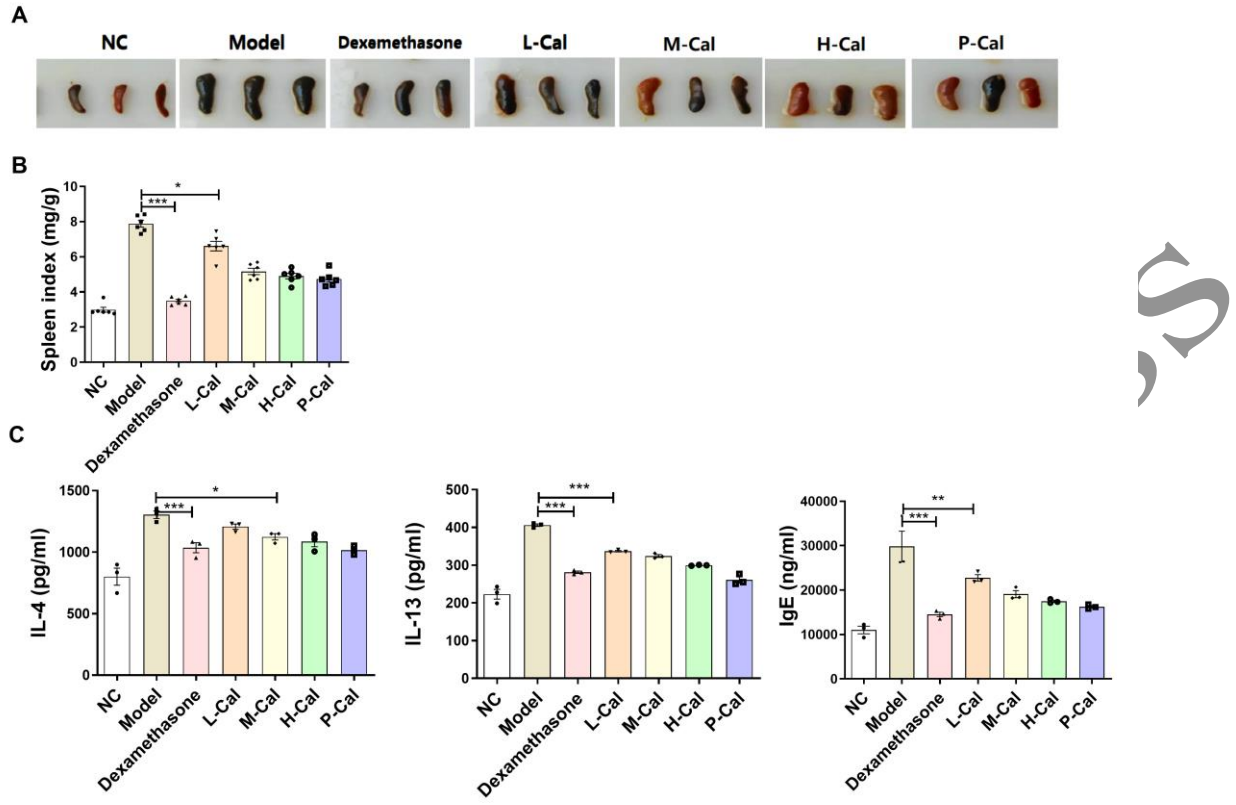


Fig. 4: Calcifediol decreased spleen index and serum inflammatory mediator levels in DNCB-induced AD-like mice. (A) Representative gross appearance of spleens from each group; (B) Quantitative analysis of the spleen index in each group. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. model group, n = 6; (C) IL-4, IL-13 and IgE levels were quantified via ELISA, with readouts expressed as mean ± SEM. Variance across groups was analyzed using one-way ANOVA, followed by Dunnett's procedure to assess significance relative to the model cohort (**P* < 0.05 vs. Model, three mice per group were analyzed, comprising three independent biological experiments).

Calcifediol upregulated Claudin family tight junction proteins in dorsal skin lesions

Calcifediol treatment restored the expression of these tight junction-associated proteins compared with the model group (Fig. 5A-D). Western blotting showed that DNCB exposure reduced Claudin-1 (Fig. 5B), Claudin-3 (Fig. 5C) and Claudin-8 (Fig. 5D) protein expression in dorsal skin tissues compared with the normal control group.

DISCUSSION

AD is a chronic, recurrent inflammatory skin disorder pathologically defined by impaired epidermal barrier integrity and aberrant immune system activation. Its global prevalence has increased in recent years, making it a major focus of dermatological research (de Bruin-Weller *et al.,* 2026). In the present study, a classical AD mouse model was successfully established that reproduced the major clinical and histopathological features of the disease, including marked erythema, edema, desquamation, epidermal hyperplasia and inflammatory cell infiltration, consistent with previous reports (Sitko *et al.,* 2025; Lee *et al.,* 2026). Clear relief of visible AD skin lesions was observed in DNCB-exposed mice receiving calcifediol treatment. As the primary circulating metabolite of vitamin D, calcifediol produced measurable therapeutic benefits even at the lowest tested dose (2 μg/kg), yielding markedly lower EASI scores than untreated model animals. Mice given calcifediol before DNCB sensitization also showed much milder dermatitis, suggesting a preventive benefit when this compound is supplied before allergen exposure. Direct comparison of efficacy between the prophylactic and therapeutic regimens is precluded by their distinct intervention windows. Prophylactic calcifediol was administered prior to lesion onset, whereas therapeutic treatment commenced post-inflammation. Consequently, the attenuated lesion severity in the prophylactic cohort does not necessarily indicate greater pharmacological potency. Rather, it likely reflects fortified baseline skin homeostasis that mitigates the subsequent DNCB-induced allergic response, consistent with the established role of vitamin D in maintaining barrier integrity.

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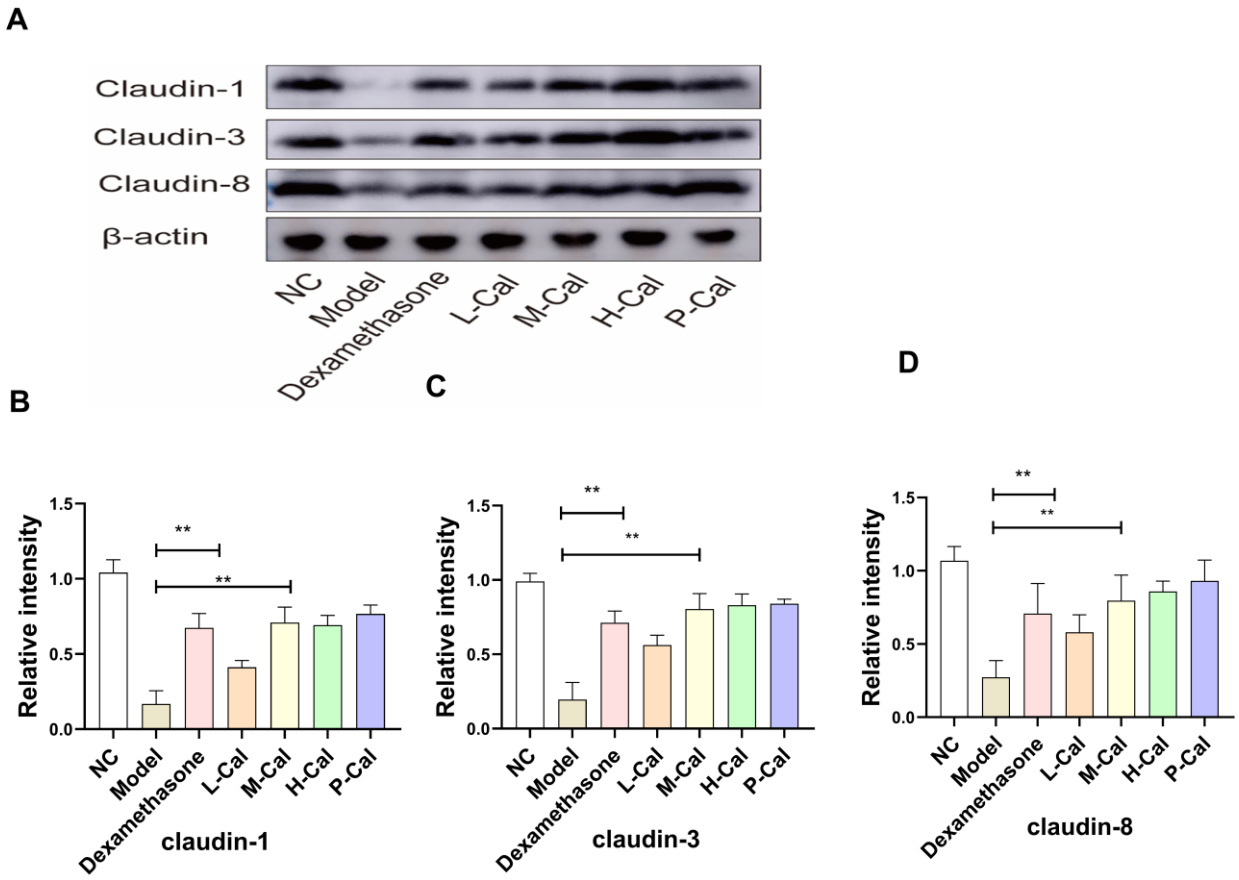


Fig. 5: Calcifediol upregulated the levels of Claudin family tight junction proteins in dorsal skin tissues of AD-like mice. (A) Representative Western blot bands showing Claudin-1, Claudin-3 and Claudin-8 protein expression in dorsal skin tissues from each group, with β-actin used as the loading control; (B) Densitometric quantification of Claudin-1 expression normalized to β-actin; (C) Densitometric quantification of Claudin-3 expression normalized to β-actin; (D) Densitometric quantification of Claudin-8 expression normalized to β-actin. Data are depicted as mean ± SEM (three mice per group were analyzed, comprising three independent biological experiments), with statistical variance assessed via a one-way ANOVA coupled with Dunnett's post hoc correction.

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Circulating calcifediol (25-hydroxyvitamin D) can be converted locally within skin tissue into bioactive 1,25-dihydroxyvitamin D, which activates cutaneous VDR signaling. VDR activity shapes keratinocyte maturation, reinforces epidermal barrier function, boosts antimicrobial peptide production and balances local immune activity. When given prophylactically, calcifediol may strengthen baseline skin barrier resilience and preserve tight junction structure, slowing allergen penetration and blunting the robust Th2 inflammatory cascade triggered by DNCB.

In short, the prophylactic group data illustrate a barrier-protective priming effect, whereas post-lesion dosing targets inflammation that has already become established—these two arms answer separate research questions and are not suitable for head-to-head efficacy comparison. In this study, calcifediol was administered by intraperitoneal injection. This precludes direct extrapolation to clinical settings because of inherent interspecies differences in pharmacokinetics, dosing regimens and delivery routes. Although the present preclinical data suggest a potential prophylactic benefit for AD-susceptible populations, well-controlled clinical trials are required to determine whether early calcifediol supplementation confers comparable skin barrier protection in humans.

Skin barrier dysfunction is a central pathogenic feature of AD and ear swelling together with local inflammatory infiltration are characteristic manifestations of cutaneous inflammation in AD mouse models (Kim *et al.,* 2019; Sitko *et al.,* 2025). In the present study, DNCB induction caused marked ear swelling, increased ear tissue weight, epidermal hyperplasia and inflammatory cell infiltration in ear tissue, whereas calcifediol treatment substantially attenuated these pathological changes. As a readily observable lesion site within the DNCB model, mouse ear

tissue further corroborates calcifediol's widespread anti-inflammatory capacity against AD. Consistent with the earlier phenotypic analysis, improvements recorded in the prophylactic cohort stem from early barrier defense rather than curative action on fully developed dermatitis. The protective effects of calcifediol were evident both macroscopically and histologically, with largely restored tissue structure. Collectively, these phenotypic shifts help rebuild intact skin homeostasis and interrupt the self-perpetuating vicious cycle linking defective epidermal barriers and persistent inflammation typical of AD.

A dysregulated Th2 immune response is a central pathological feature in the development of AD which is typified by increased serum IgE levels and overproduction of type 2 cytokines, including IL-4 and IL-13. These factors jointly drive inflammatory cell infiltration, pruritus and skin barrier damage (Park and Im, 2020). Existing molecular evidence confirms that IL-4 and IL-13 can directly suppress the transcription of claudin family proteins, forming a pathogenic link between systemic Th2 inflammation and local skin barrier damage. The present results showed that serum IgE, IL-4 and IL-13 levels were drastically elevated in DNCB-induced model mice, whereas calcifediol lowered these Th2 cytokines in a dose-related manner. The medium-dose, high-dose and prophylactic groups showed the strongest suppression of IL-4 secretion. While the spleen index serves as a broad indicator of systemic immune activation, the comprehensive evaluation of serum cytokines provides a more precise reflection of the suppression of allergic inflammation. These immunological observations align well with the previously reported immunomodulatory capacities of calcifediol (Lu *et al.,* 2023).

Within the skin barrier, claudins are essential components of tight junctions and play a pivotal role in maintaining barrier integrity, tight junction stability and epidermal permeability control (Mansour *et al.,* 2020). Members of the claudin protein family have been widely reported to participate in the pathological processes of numerous inflammatory skin diseases, such as psoriasis, atopic dermatitis and contact dermatitis (Jin YM *et al.,* 2021; Katsarou *et al.,* 2023). Previous studies have reported that key tight junction components, including claudins, are downregulated in skin lesions of both adult and pediatric patients with AD (Bergmann *et al.,* 2020). In particular, accumulating evidence shows that Claudin-1, Claudin-3 and Claudin-8 are functionally important tight junction molecules closely linked to the pathogenesis of AD. Aberrant downregulation of these three proteins contributes prominently to cutaneous barrier dysfunction in AD (Yamaga *et al.,* 2018; Bergmann *et al.,* 2020; Wang *et al.,* 2025; Ye *et al.,* 2026). Given their clear pathological correlation with AD barrier dysfunction, these three proteins were selected as core mechanistic indicators in the present study. Western blot analysis revealed a marked reduction in Claudin-1, -3 and -8 levels in DNCB-challenged skin, which was partially rescued by calcifediol administration. These findings suggest that calcifediol's barrier-protective efficacy in AD-like dermatitis is likely mediated by preserving tight junction integrity.

This study has several limitations. First, only female mice were used, so sex-dependent differences in AD pathophysiology and calcifediol responsiveness were not evaluated. Second, histopathological assessment was qualitative and lacked standardized quantitative scoring, limiting the statistical rigor of morphological conclusions. Third, direct barrier-function assays (e.g., transepidermal water loss (TEWL), dye penetration) were not performed; barrier recovery was inferred indirectly from tight-junction protein levels and EASI scores. Finally, although calcifediol administration was associated with restored Claudin expression, a direct causal, VDR-dependent link was not established and elevated Claudins may be a secondary consequence of reduced inflammation rather than a primary protective driver. The DNCB model also may not capture the chronic, heterogeneous nature of human AD. Future work should combine transcriptomic profiling, TEWL measurement and targeted gene manipulation to clarify the VDR–Claudin pathway and its clinical relevance.

CONCLUSION

In conclusion, calcifediol ameliorated DNCB-induced AD-like dermatitis in mice by reducing skin inflammation and improving epidermal barrier-related alterations. The restoration of Claudin-1, Claudin-3 and Claudin-8 expression suggests that maintaining tight junction integrity contributes to its barrier-protective effects. These data support the potential of calcifediol as a candidate intervention for AD-like inflammatory skin disease.

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Authors' contributions
Qundan Wu and Qian Ling: Conceived and designed the study; Shaomei Lin and Yuanzhi Hong: Performed the animal experiments; Qundan Wu and Huidan Zheng: Conducted ELISA, histological staining and Western blot

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analyses; Cuiping Huang and Mingzhu Chen: Collected and analyzed the data; Qundan Wu: Obtained funding and drafted the manuscript; Shaomei Lin and Mingzhu Chen: Revised the manuscript critically for important intellectual content; Wanting Liao: Supervised the study. All authors read and approved the final version of the manuscript for publication.

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Data availability statement
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval
All procedures were performed in accordance with the principles of the 3Rs and were approved by the Animal Ethics Committee of Quanzhou Medical College (Ethical Review No. 2025089). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflicts of interest
The authors declare no conflicts of interest.

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