Original Article

Quantitative Analysis of Histopathological Changes in a Mouse Model of Dinitrochlorobenzene-induced Atopic Dermatitis: Correlation With Total Serum IgE Levels

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ABSTRACT

Background: The mouse model of atopic dermatitis (AD) plays a vital role in the development of new therapies, facilitating the assessment of drug impacts and the investigation of disease mechanisms. Its genetic and physiological traits closely resemble those of mammals, making it an effective system for replicating symptoms and studying the molecular processes associated with inflammation and immune responses.

Objectives: The aim of this study was to evaluate the histopathological characteristics and identify quantitative metrics of the epidermis and dermis, as well as serum IgE levels, in a mouse model of AD induced by dinitrochlorobenzene (DNCB) in order to guide future studies trying to develop novel therapies for AD.

Methods: A total of 12 mice were divided into two groups: one group was induced with AD using DNCB, while the control group received a vehicle. Microscopic analysis of skin samples assessed dermal and epidermal thickness and eosinophilic infiltration using hematoxylin and eosin (H&E) staining. Mast cell counts were determined with toluidine blue staining, and serum levels of total IgE were measured using a murine-specific enzyme-linked immunosorbent assay (ELISA) kit.

Results: The evaluation of skin and serum samples indicated that dermal and epidermal thickness, as well as eosinophil and mast cell infiltration, and serum IgE levels were significantly elevated in the DNCB-induced group.

Conclusion: This study demonstrates that the DNCB-induced mouse model of AD effectively replicates key histopathological features, including increased dermal and epidermal thickness, eosinophil and mast cell infiltration, and elevated serum IgE levels. These findings enhance our understanding of the disease and support future therapeutic development. Our results can help researchers determine quantitative parameters measuring the efficacy of novel drugs proposed for alleviating atopic signs.

Keywords: BALB/c mouse, Dermal and epidermal thickness, Eosinophils, Experimental model, Mast cells, Skin inflammation

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Introduction

Atopic dermatitis (AD) is a prevalent inflammatory and pruritic skin condition affecting dogs, with prevalence estimates approximately 3-15% of the canine population. This multifactorial disorder arises from a complex interplay of genetic predisposition, immune system dysfunction, environmental allergens, and skin barrier impairment (Drechsler et al., 2024). Common allergens include pollen, dust mites, and certain food components, which trigger hypersensitivity reactions of both type I and type IV. The disease often manifests in young dogs, typically between six months and three years of age, leading to chronic discomfort characterized by severe itching and skin lesions (Drechsler et al., 2024). Traditional treatments, such as corticosteroids, provide only temporary relief and are associated with significant side effects, including corticosteroid-induced hepatopathy. This has driven researchers and veterinarians to seek alternative therapies that not only alleviate itching and inflammation but also offer longer-lasting effects with minimal adverse reactions (Fernandes et al., 2023).

Understanding the pathogenesis of AD is crucial for effective management. The compromised skin barrier allows allergens to penetrate more easily, resulting in heightened inflammation and susceptibility to secondary infections. Consequently, addressing both the symptoms and underlying causes of this condition is essential for improving the quality of life for affected dogs (Fernandes et al., 2023). As the search for innovative treatments continues, the focus remains on developing strategies that enhance patient well-being while minimizing potential side effects. The initial step in evaluating the therapeutic efficacy, as well as the potential toxicity and side effects of a treatment, involves investigating its effects in an appropriate animal model of the disease (Fernandes et al., 2023). For AD, commonly used animal models include BALB/c and NC/Nga mice, where various methods are employed to induce dermatitis. Two chemicals frequently utilized for this purpose are dinitrochlorobenzene (DNCB) and 903MC, both of which are applied topically to the skin of the mice to effectively induce dermatitis (Peng et al., 2018). In numerous studies, genetic engineering has been employed to create mouse models that secrete a range of inflammatory mediators, including interleukin-4 (IL-4), which have proving effective for investigating AD (Marsella & De Benedetto, 2017). Among these models, NC/Nga mice are unique as they are the only strain known to spontaneously develop symptoms of dermatitis. These mice, resulting from inbreeding, typically begin to exhibit dermatitis symptoms between 6 to 8 weeks of age and respond to various environmental allergens. This characteristic makes them a valuable model for studying the mechanisms and treatments of AD (Jayasinghe et al., 2024).

The aim of this study was to investigate histopathological changes in the epidermis and dermis of a mouse model of AD induced by DNCB. Additionally, the study aimed to explore the correlation between alterations in serum IgE levels and the observed histopathological changes.

Materials and Methods

Sample size determination for mice in each group:

This pilot investigation determined the sample size using the resource equation method, as outlined by Charan & Kantharia (2013) The minimum number of animals required for each group was calculated with the following parameters: degrees of freedom (DF) were set at 10, and K represented the number of groups (two groups). As a result, a total of 6 mice were allocated to each group, calculated using the Equation 1:

1. N=(K/DF)+1 (Charan & Kantharia, 2013).

Twelve male BALB/c mice, 5 weeks old, were sourced from the Experimental and Comparative Studies Center of Iran University of Medical Sciences. After their arrival at the laboratory, the mice were acclimatized for one week. Throughout the study, they were housed individually in cages maintained at a temperature of 22 to 25 ˚C and a humidity level of 45%. The mice had unrestricted access to food (laboratory animal pellets) and water 24 hours a day. The light exposure was regulated intermittently, with a cycle of 12 hours on and 12 hours off (Ogasawara et al., 2024).

To induce AD, a solution of DNCB in a mixture of acetone and olive oil (1:3) was applied at specific concentrations (Jayasinghe et al., 2024). The mice were divided into two groups: the control group, comprising 6 mice, received applications of the vehicle (acetone and olive oil), while the second group, known as the DNCB group, also consisted of 6 mice and had their skin treated with DNCB solutions.

Dermatitis induction

One day prior to the study, four square centimeters of skin were shaved from the backs of all mice. On day 1, 150 µL of a 1% DNCB solution was applied to the backs of the mice in the second group, with an additional 20 µL of the same solution smeared on their faces and ears

On the fifth day, a 0.2% DNCB solution was similarly applied to the back, face, and ears of the mice, continuing this treatment three times a week for four weeks (on days 1, 5, 7, 12, 14, 16, 21, 23, 26, 28, 30, and 33) (Lee et al., 2010). In contrast, the first group received applications of the vehicle (acetone and olive oil in a 1:3 ratio) on their backs, faces, and ears.

Histopathological and serological evaluation:

At the end of the study (33rd day), the mice were anesthetized with 2% isoflurane, and relevant skin and blood samples were collected. The skin samples were fixed in 10% formalin for 24 hours before being sent to the pathology laboratory for paraffin embedding and preparation of tissue sections, followed by specific staining procedures. From the prepared tissue sections, 20 microscopic slides were selected at a magnification of 100× to measure the thickness of the epidermis and dermis using standard hematoxylin and eosin (H&E) staining, following a standardized light microscopic method to quantify the thicknesses of the dermal and epidermal layers (Figures 1 and 2). The number of mast cells was quantified using toluidine blue staining, while eosinophils were counted in each group across 20 microscopic sections at 40× magnification with H&E staining. The number of mast cells and eosinophils was evaluated using a wide field microscope at ×400 total magnification (the average counts across three high-power fields (HPFs) were about 0.264 mm^2).

After serum separation, blood samples were stored at -70 ˚C until analysis. Serum IgE levels were measured using a specific enzyme-linked immunosorbent assay (ELISA) kit that utilized horseradish peroxidase (HRP)-conjugated goat anti-mouse IgE as the secondary antibody (Biotime, China) (Lee et al., 2020).

Statistical analysis

The Mann-Whitney U test was employed to compare data collected from two groups of mice. A difference was considered significant at a 95% confidence interval (CI), indicated by a $P<0.05$. Additionally, to assess the correlation between IgE levels and other variables, we calculated Spearman correlation coefficients and performed linear regression analysis using SPSS software, version 22.

Results

In the microscopic examination, skin tissue samples were stained with H&E. The thickness of the epidermis and dermis in each mouse from the two study groups was measured at ×100 magnification, with the results presented in Table 1. The thickness of both the epidermis and dermis, as well as the number of eosinophils and mast cells, along with IgE levels, were significantly higher in the DNCB group (Table 1 and Figures 1, 2, and 3).

These results indicate a strong positive correlation between increased IgE levels and the thickness of both the dermis and epidermis, as well as with the number of eosinophils and mast cells. The Spearman's correlation coefficients (rs) ranging from approximately 0.7 to 0.8 suggest a very strong direct relationship between eosinophil counts and IgE levels. The results of the multiple linear regression analysis indicated a very strong collective significant effect of dermis and epidermis thickness on IgE levels ($F=24.42$, $P=0.001$, adjusted $R^2=0.72$) (Table 1). Additionally, eosinophil and mast cell counts also showed a significant relationship with IgE levels ($F=26.88$, $P<0.001$, adjusted $R^2=0.74$) (Table 1).

Discussion

The mouse model of AD serves as an essential resource for scientific research aimed at developing new treatments. This model not only facilitates the examination of drug effects but also enhances our understanding of the underlying mechanisms of the disease. Such insights could prepare the way for significant advancements in the management and treatment of AD (Kwon et al., 2024). Mouse models are extensively utilized to replicate AD. Due to their genetic and physiological similarities to mammals, mice effectively mimic the symptoms of the disease. This model provides a platform for testing new drugs and treatments, enabling researchers to explore the molecular and biochemical mechanisms linked to inflammation and the immune response (Li et al., 2023).

The results of the present study revealed a significant increase in the thickness of both the dermis and epidermis in the DNCB-induced group compared to the control group, challenged with vehicle. This thickening of the dermal and epidermal layers suggests chronic inflammation and structural alterations resulting from immune reactions (Figure 4). Such changes may be driven by the activity of immune cells, including mast cells and eosinophils, which, in turn, stimulate the secretion of cytokines and inflammatory factors. Typically, an increase in skin thickness is associated with elevated levels of extracellular matrix proteins, indicating a pronounced inflammatory response (Kim et al., 2024).

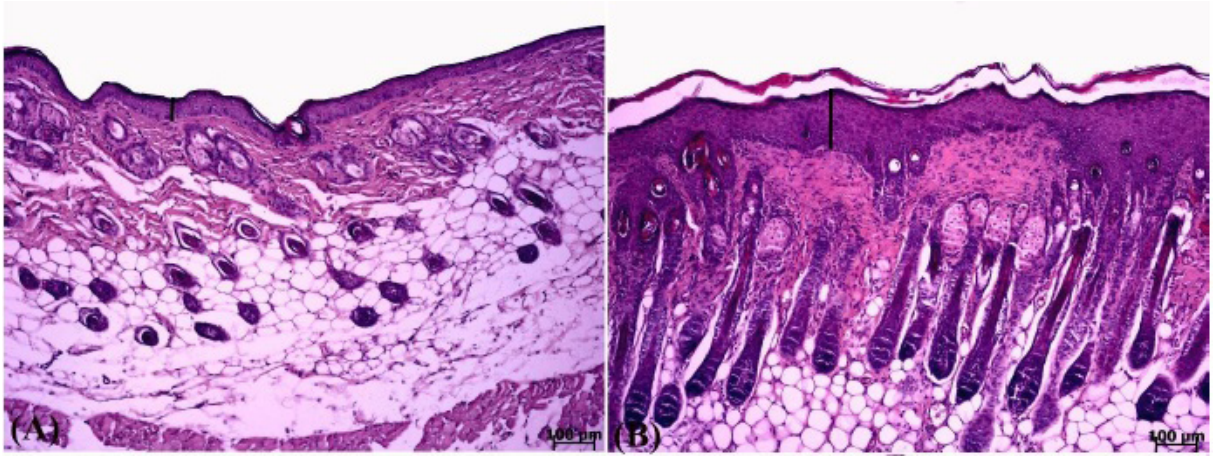

Figure 1. Epidermal thickness (black line) in the vehicle-challenged group (A) and the DNCB-challenged group (B) under a light microscope (×100 magnification)

Note: Epidermal thickness (black line) was significantly greater in the DNCB-challenged group (B) than in the vehicle-challenged group (A) (P<0.0001, U-value=-148.25, df=1).

These findings align with previous studies indicating that chronic inflammation can result in structural changes within the skin. The increased presence of eosinophils and mast cells in the DNCB-induced area reflects the activation of the type 2 immune response, which is particularly associated with allergic diseases and AD (Kwon et al., 2018). Eosinophils are recognized as pivotal cells in allergic responses, playing a significant role in inflammation. Similarly, mast cells contribute critically to the development of clinical symptoms, such as itching and skin redness, through the secretion of histamine and other inflammatory mediators (Wong et al., 2020).

The increase in these cells signifies the severity of inflammation and immune system activity, which may exacerbate the clinical symptoms of the disease. Elevated

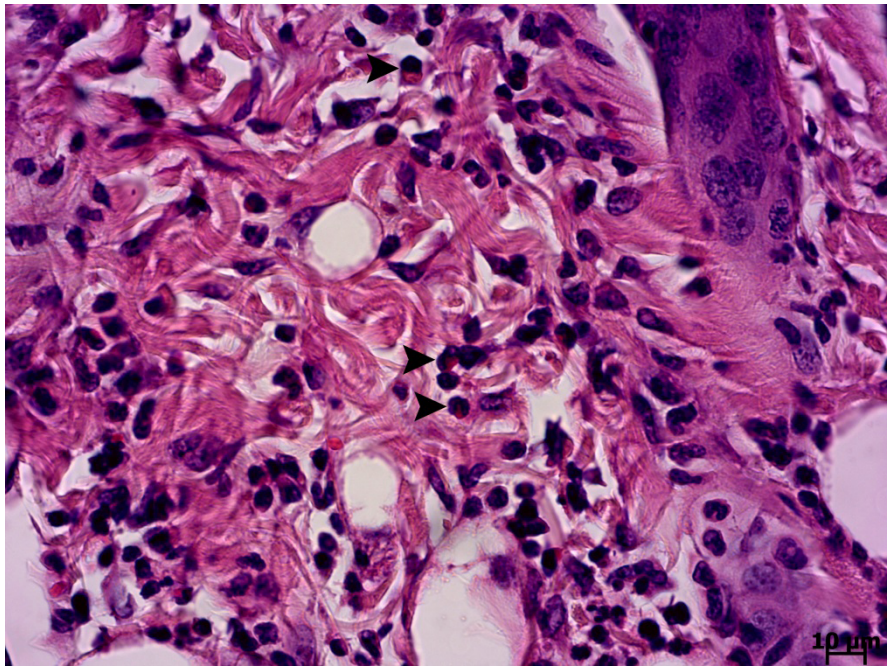

Figure 2. Infiltration of eosinophilic inflammatory cells (indicated by arrowheads) in the dermis of DNCB-challenged group (23.8±2.8 95% CI, 20.32%, 27.28%) (×100 magnification)

Note: A significant difference was reported between the two study groups (P<0.05, U-value=-56.13, df=1)

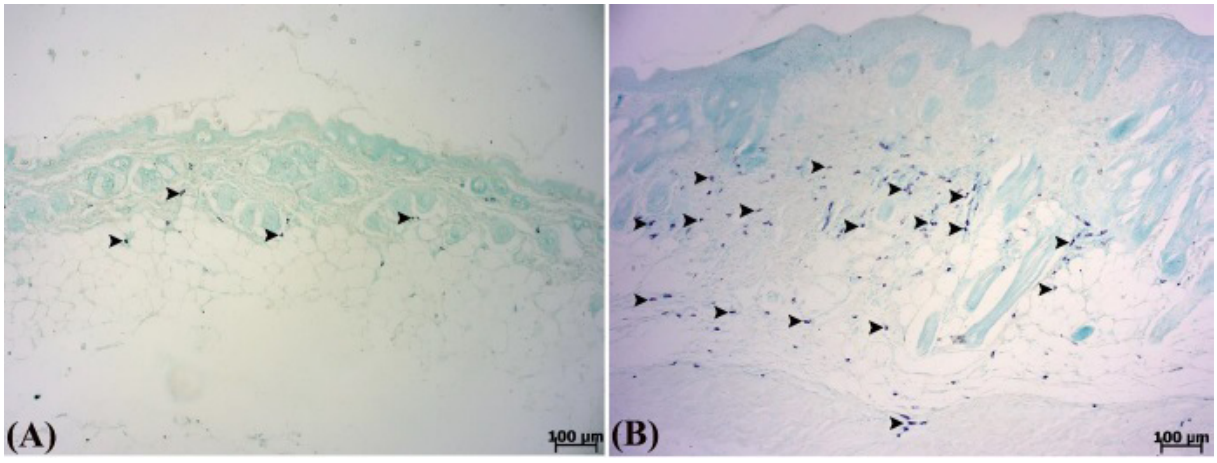

Figure 3. Mast cells infiltrating the skin tissue (indicated by arrowheads) in the vehicle- challenged group (A) and the DNCB- challenged group (B)

Note: A significant increase in the number of mast cells was observed in the DNCB-challenged group (P<0.0001, U-value=-174, df=1).

IgE levels are an indicator of allergic diseases, including AD (Figure 4). IgE serves as a crucial marker of sensitization, and its elevation is typically linked to the activation of mast cells and eosinophils (Kim et al., 2024). Serum levels of inflammatory cytokines, particularly IL-4 and IL-5, should be assessed, as they are responsible for the increased counts of eosinophils and mast cells in the dermis and epidermis of mice induced by DNCB. Park et al. (2022) indicated that IL-4 promotes the expression of IL-5, which is responsible for the maturation and release of eosinophils. While, IL levels are significant in the research area of allergic diseases, AD is primarily diagnosed based on clinical symptoms and historical information (Lugović-Mihić et al., 2023).

This finding indicates that DNCB-induced mice are sensitized, which can lead to more severe clinical symptoms. The DNCB-induced group exhibited significantly increased thickness in both the dermis and epidermis, along with elevated eosinophil and basophil cell counts, as well as IgE levels. In contrast, the control group of mice challenged with the vehicle demonstrated significant differences in epidermal and dermal thickness (Figure 4). These variations highlight the specific effects of DNCB on the induction of inflammation and immune response (Kim et al., 2023). The control group did not show a similar inflammatory response, underscoring the importance of using DNCB as an effective model for studying AD (Choi et al., 2024).

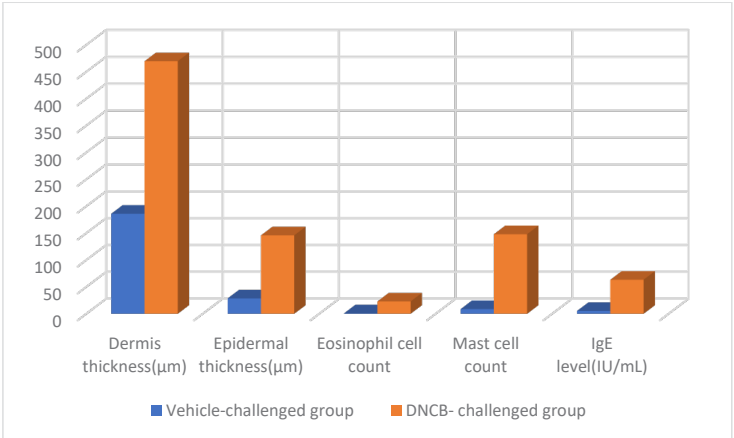

Figure 4. Mean values of the measured variables in mice from the two study groups

Note: The figure illustrates that the thickness of the dermis and epidermis, along with the numbers of eosinophils and mast cells, as well as IgE levels, were significantly higher in mice induced with DNCB.

Table 1. Average measured variables in mice from the two study groups, including between-group comparisons and IgE correlations

Measured Variables	Vehicle-challenged Group (n=6)	DNCB-challenged Group (n=6)	P	Spearman's Correlation Between IgE Levels and the Variables	Multiple Regression Results With IgE as the Dependent Variable and the independent Variables as Predictors
Dermis thickness (micrometers)	186.1±1.08 95% CI [185.23, 186.96]	469.6±1.67 95% CI [468.26, 470.94]	P<0.0001 U-value=-317.96, df=1	rs=0.69728, P=0.02501	F=24.42, P=0.001, R^2=0.75, R^2adj=0.72
Epidermal thickness (micrometers)	28.7±0.67 95% CI [28.16, 29.23]	145.9±1.6 95% CI [144.62, 147.18]	P<0.0001 U-value=-148.25, df=1	rs=0.74621, P=0.01319	
Eosinophil cell count	1±0.35 95% CI [0.72, 1.28]	23.8±0.84 95% CI [23.12, 24.47]	P<0.05 U-score=-56.13 df=1	rs=0.82113, P=0.00359	F=26.88, P<0.001, R^2=0.77, R^2adj=0.74
Mast cell count	8.8±1.15 95% CI [7.88, 9.72]	148±1.37 95% CI [146.9, 149.1]	P<0.0001 U-score=-174, df=1	rs=0.81496, P=0.00408	
IgE level	5.43±2.96 95% CI [3.06, 7.79]	63.38±26.19 95% CI [42.42, 84.33]	P<0.05 U-score=-2.51, df=1	rs=1, P=0.0001	

The mouse model of AD is recognized as a crucial tool in skin disease research, with various methods available for inducing this condition in mice. One common approach is chemical induction using DNCB, which involves applying the compound to the skin, resulting in inflammation and itching. This method effectively simulates the symptoms of AD, providing a valuable platform for studying the disease and testing potential treatments. While it produces clinical signs quickly and allows for the examination of the immune response, it may also cause long-term damage to the skin (Sakamoto & Nagao, 2023).

Another method involves using genetic models, such as allergy-sensitive BALB/c mice, which are naturally predisposed to develop symptoms of AD. These models can more accurately simulate the genetic and immune mechanisms underlying the disease; however, they tend to be time-consuming, expensive, and require specialized maintenance (Riedl et al., 2023).

Induction using allergens is another technique, where mice are sensitized with allergic proteins, like egg ovalbumin or casein to elicit an immune response. This approach can trigger an immune and inflammatory response similar to that observed in humans, but it typically requires more time for symptoms to develop, and results may vary between individual mice (Riedl et al., 2023).

Finally, combination models utilize both chemicals and allergens simultaneously to enhance the severity of symptoms, providing a comprehensive approach to studying AD (Takahashi et al., 2024). The advantages of this approach include providing more accurate insights into the interactions between environmental and genetic factors. However, it also presents challenges, such as increased complexity in experimental design and data analysis (Takahashi et al., 2024). Each method for inducing AD in mice has its own set of advantages and disadvantages. The choice of the appropriate method depends on the research objectives, the type of data required, and the available resources. Utilizing these models enables researchers to gain a deeper understanding of the disease mechanisms and to develop new treatments (Hashemi et al., 2017).

Animal models, particularly mice and rats, can effectively simulate human diseases due to their genetic and physiological similarities to humans (Etebar et al., 2023). This capability allows researchers to assess the potential effects of drugs before conducting tests in humans. In vivo experiments using animal models are crucial for evaluating the safety and efficacy of drugs. These experiments provide valuable insights into how the body responds to treatments, information that is not obtainable through in vitro studies (Mahalmani et al., 2022). Researchers utilize pig models to study heart disease, which has facilitated investigations into the effects of drugs on heart health. Mouse models are employed to simulate the symptoms of Parkinson's disease and aid in the development of new treatments (Kumar & Smith, 2023).

In veterinary medicine research, foot-and-mouth disease is a viral illness that can be studied in livestock using animal models, such as cows and pigs. Research involving these models has contributed to the development of effective vaccines (Habiela et al., 2014). Similarly, Newcastle disease is a prevalent condition in birds, and animal models have played a crucial role in helping researchers develop new vaccines and treatments to control the disease (Bello et al., 2018). Animal models, such as dogs, are utilized to study diabetes and the effects of various medications on blood sugar control. This research has facilitated the development of new treatments (Yagihashi, 2023). Additionally, mouse models are employed to simulate breast cancer and test new drugs. These models provide valuable insights into cancer mechanisms and the effects of treatments (Halim et al., 2022). Animal models, such as sheep with arthritis, are used to study the condition and evaluate the impact of new treatments. This research has contributed to the identification of more effective drugs (Hawkes & McGowan, 2023). The use of animal models in veterinary research not only enhances our understanding of disease mechanisms but also allows for the testing of drug safety and efficacy before they are administered to animals. This is crucial for providing new and effective treatments (Hawkes & McGowan, 2023).

Animal models enable researchers to develop new therapies and investigate their various effects. The use of animal models can significantly reduce the cost and time required for drug research. By simulating diseases in animals, researchers can obtain results more quickly and minimize unnecessary testing on humans. Despite the significance of animal models, it is crucial to adhere to ethical principles in their use. Researchers should aim to minimize the number of animals used and enhance their living conditions (Kiani et al., 2022).

Animal models are recognized as effective tools for treating animal diseases and are essential in veterinary, medical, and pharmaceutical research. These models enable researchers to study biological processes and diseases under conditions that closely resemble real-life scenarios (Mahalmani et al., 2022).

The findings from the Kumar and Smith' study on the development of a novel sample preparation method align with our study on AD by emphasizing the value of precise analytical techniques (Kumar & Smith, 2023). Both approaches highlight the significance of accurate measurements—whether for evaluating histopathological changes in dermatitis models or assessing selenium in pharmaceuticals—in advancing our understanding of complex biological and chemical systems (Pal & Rasha, 2024).

A study assessing the anti-inflammatory effects of allopurinol gel highlights the importance of studying inflammation in skin conditions, supporting our focus on histopathological changes and serum IgE levels in AD, as both investigations underscore the significance of evaluating histopathological changes and serum IgE levels in understanding the inflammatory mechanisms underlying skin conditions, thereby contributing to the development of targeted therapeutic strategies (Al-Saedi et al, 2022).

The findings of Fallahi et al. on alopecia in a rabbit model support our study by highlighting the role of inflammatory processes in skin conditions, reinforcing the importance of histopathological analysis and serum IgE levels in understanding the mechanisms of DNCB-induced AD (Fallahi et al., 2012).

The study by Maghami et al. on mycotic dermatitis in sheep can inform our discussion on AD by illustrating the role of infectious agents in skin inflammation, which parallels our findings in the mouse model of AD, highlighting the multifactorial nature of dermatological disorders (Maghami et al., 1978).

Conclusion

This study confirms the utility of the DNCB-induced mouse model for elucidating the disease mechanisms of AD. By providing insights into the underlying pathophysiology, this model serves as a critical platform for testing new therapeutic approaches. The findings underscore the importance of understanding the inflammatory pathways involved in AD, which could lead to the identification of novel molecular targets for intervention. Future research should prioritize the development and evaluation of targeted therapies aimed at reducing inflammation and alleviating clinical symptoms. This may include exploring biologic agents that specifically inhibit key inflammatory cytokines, small molecules that modulate immune responses, or innovative topical treatments that enhance skin barrier function. Additionally, studies could investigate the efficacy of combination therapies that address multiple aspects of the disease simultaneously. Moreover, it is essential to assess the long-term safety and effectiveness of these emerging treatments in both preclinical models and clinical trials. By advancing our understanding of AD and refining treatment strategies, we can ultimately improve patient outcomes and quality of life for those affected by this chronic condition. This comprehensive approach will not only benefit individual patients but also contribute to broader public health efforts aimed at managing allergic diseases effectively.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran (Code: IR.UT.VETMED.REC.1403.010).

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Authors' contributions

Methodolohy: Hamidreza Jahani; Experiments: Hamidreza Jahani and Maryam Nouri; Data collection and analysis: Fateme Jalousian; Data interpretation: Sara Shokrpour, Hamidreza Jahani and Fateme Jalousian; Writing the original draft: Hamidreza Jahani and Fateme Jalousian; Review, editing and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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