

Research article

Comparing the effects of Sodium lauryl sulphate and *Acacia concinna* in *Dermatophagoides* extract ointment induced atopic dermatitis

Jyothi Basini^{*1}, Darshini Amudhala¹, Sumalatha G², Mallikarjuna G¹, Niranjan Babu M¹ and Sorta Ganesh³

¹Department of Pharmacology, Seven Hills College of Pharmacy (Autonomous), Venkatramapuram, Tirupati, A.P, India- 517561

²Vikas Institute of Pharmaceutical Sciences, Nidigatla Road, Near Airport, Rajahmundry, East Godavari Dist., A.P-533102.

³Toxgene AR Biolabs Private Limited, Plot no 31,32,41&42, IP Ramireddy Palli, Narasinga Puram, Andhra Pradesh, 517102.

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***Corresponding Author:** Dr Jyothi Basini, Professor, Department of Pharmacology, Seven Hills College of Pharmacy (Autonomous), Venkatramapuram, Tirupati, AP, India,
Email id: jyothipharmacologyvmk@gmail.com

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Abstract

Background: The obvious dermatologic symptoms, psychological stress, sleep problems, and poor social and occupational functioning that accompany an inflammatory skin condition known as atopic dermatitis (AD), substantially diminish the quality of life for those who suffer from it. Animal models, particularly those induced by relevant allergens like *Dermatophagoides farinae* (DNFB), are crucial for studying the pathophysiology and potential treatments for AD. **Objective:** This study aims to compare the effects of Sodium Lauryl Sulfate (SLS), a common ingredient in shampoos, with *Acacia concinna*, a natural alternative, on AD-like symptoms. In this study, we evaluate the effectiveness of these chemicals by applying DNFB extract ointment to a mouse model of atopic dermatitis, concentrating on clinical manifestations, skin histopathology, and immune responses. **Methods:** Along with disturbance of their skin's protective barrier, regular application of DNFB extract ointment to the backs of mice produced skin lesions similar to those seen in atopic dermatitis. Using clinical scoring, histological analysis, and inflammatory marker measurement, the degree of dermatitis was evaluated. The effects of SLS and *Acacia concinna* were compared to those of a corticosteroid treatment. **Results:** Dermatitis scores, which show symptoms including redness, swelling, erosion, and dry skin, were significantly elevated after multiple applications of DNFB extract ointment. Both the dermis and the epidermis thickened, according to the histology report, heightened transepidermal water loss, and higher levels of inflammatory cytokines, closely mirroring human atopic dermatitis. Treatment with *Acacia concinna* and corticosteroids effectively reduced these symptoms, suggesting potential therapeutic benefits. **Conclusion:** The DNFB-induced murine model of AD is a valuable tool for studying the disease and evaluating treatments. *Acacia concinna* shows promise as a safer alternative to SLS in managing AD, warranting further investigation.

Introduction

Intense itching and redness are symptoms of atopic dermatitis (AD), an inflammatory skin disorder that can occur repeatedly throughout time [1-3]. Among adults, it affects 1-3 percent, and among children, it affects 10-20 percent. This makes it a huge public health concern

worldwide. Despite the increasing prevalence of atopic dermatitis, our understanding of its pathophysiology is still limited. Atopic dermatitis is typically treated with calcineurin inhibitors and corticosteroids, although there is an increasing demand for less toxic topical anti-inflammatory therapies [4].

Various mouse models have been developed to study AD, including a spontaneously developed dermatitis model in specific mouse strains and hapten-induced dermatitis models. These models, while useful, do not fully replicate the pathogenesis of human AD. NC mice, hence it was expected to be brought on by rodent-specific parasites that do not impact humans. Similarly, the triggers in other mouse models are not directly relevant to human AD [5]. Individuals with atopic dermatitis (AD) frequently have heightened sensitivity to house dust mite (HDM) allergens, as demonstrated by affirmative outcomes in radioallergosorbent, scratch, and patch tests. House dust mites, specifically *Dermatophagoides farinae* (DNFB), are a major source of environmental allergies in Japan. Given this relevance, DNFB extract was explored for its potential to induce dermatitis in mice, with previous studies indicating its ability to create AD-like skin lesions when applied as a suspension. On the other hand, these models had a delayed dermatitis start, likely due to the rapid disappearance of allergens from the skin. As a result, an ointment containing DNFB extract was developed with the assumption that it would increase the duration of allergen presence and, thus, hasten the development of skin sores. Inducing AD-like skin lesions in rats was achieved by repeatedly applying DNFB extract ointment and disrupting the skin's protective barrier; substantial dermatitis was noticed as early as two weeks following the initial exposure. Due to its striking immunological, histological, and clinical resemblance to human AD, this model served as a valuable tool for investigating the aetiology of the disease and testing potential treatments. Another piece of evidence supporting the model's usefulness is its robust reaction to corticosteroid treatment (Betamethasone dipropionate ointment) [6].

Soap, cosmetics, toothpaste, mouthwash, and shampoos are just a few of the many products that contain sodium laurate (SLS), an anionic surfactant. Although SLS works well in these uses, studies have shown that it can have negative side effects owing to its surfactant characteristics, which can disrupt cell membranes and change protein structure. For this reason, most personal care product manufacturers advise using SLS no more than twice weekly to reduce the frequency with which its negative effects are seen [7].

In contrast, *Acacia concinna* Linn., a medicinal herb utilised in Ayurveda, provides a natural alternative. The fruits of the plant, referred to as Shikakai, are traditionally utilised for hair cleansing, stimulating hair growth, and functioning as an expectorant, emetic, and purgative [8].

Given the rising concerns over chemical formulations like SLS, there is increasing interest in exploring the potential of *Acacia concinna* as a safer alternative for managing conditions like AD. On this interest we explored the comparative analysis of sodium lauryl sulfate and *Acacia concinna* shampoos on atopic dermatitis.

Material and methods

Animals

Feminine mice, six weeks old, bought from Kedar Biolabs in Mahbubnagar, Telangana, India. The animals were housed in stable polycarbonate tanks that had carefully managed environmental conditions. These included a temperature range of $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$, humidity levels that varied from 30-70%, and a 12-hrs light or dark cycle. Mice were given time to adjust to their new environment one week prior to the trial's start. The study was given the go light by the test facility's Institutional Animal Ethics Committee (IAEC) under the project named "Comparing the Effects of SLS in Shampoos and *Acacia concinna* on Atopic Dermatitis," with the IAEC number TAB/IAEC/IDT/01/24.

Plant collection

We scoured the Tirupati market for *Acacia concinna* fruit. By comparing its features with descriptions in different floras and herbariums, as well as with the help of the Department of Botany at SV University, Tirupati, AP, India, and others, the plant's authenticity and identification were verified. Over the course of many days, the fruits were left to dry in the shade. We pounded them into a fine powder using a grinding machine when they had dried completely, and then we sealed the container to keep the powder fresh.

Extraction of *Acacia concinna*

For the extraction, 100 g of *Acacia concinna* fruit powder was used. The powder was extracted using two different solvents: deionised water and 99% ethanol. Twelve hours of immersion in ethanol followed by one hour of boiling in a water bath set at 60 degrees Celsius to extract the distilled water was used to prepare the samples. After two filtrations through a nylon membrane filter with a pore size of 0.45 μm , the samples were subjected to further filtration using Whatman No. 1 filter paper. The water-based extract was concentrated by means of a spinning evaporator in a water bath maintained at 60 degrees Celsius, whereas the alcohol-based extract was concentrated by means of a similar apparatus maintained at 80 to 90 degrees Celsius. Finally, both extracts were dried using a freeze dryer at -62°C [9].

Preparation of *dermatophagoides farinae* extract ointment

Dermatophagoides farinae bodies (DNFB) were cultured and isolated from their faeces and culture medium using flotation in a saturated sodium chloride solution. Using an agate mortar and pestle, the floating mites were collected and mixed with phosphate-buffered saline (PBS) until they were evenly suspended. Before centrifugation, the suspension was stirred all night. To create the DNFBb ointment, the liquid above the solid was subjected to dialysis and then lyophilised. Then, 5 milligrammes of DNFBb extract per gramme of hydrophilic petrolatum was added to the mixture [10].

Experimental procedure

The back fur of the mice was delicately peeled off with the use of clippers and a razor. The depilated dorsal epidermis and both auricular surfaces were treated with a topical application of DNFBb ointment or ointment base (hydrophilic petrolatum) in the amount of 100 mg in order to elicit a response from the ears. In order to break down the barrier, one hundred and fifty microlitres of a sodium dodecyl sulphate (SDS) solution with a concentration of four percent was applied to the dorsal skin and both sides of each ear 3 hours before applying the DNFBb ointment. The depilated dorsal skin was treated with the SDS solution once on days one and four. At first, there were four groups of five mice each: A 50 mg/kg dose of DNFB and SLS was given to a control group. One group received DNFB and *Acacia concinna* at a dosage of 100 mg/kg. A 100-milligram dose of betamethasone dipropionate ointment was administered to the shaved dorsal skin. The treatment duration extended from day 7 to day 13. On days 7, 10, and 13, a 0.2% DNFB solution was administered to the dorsal skin surface of sensitised mice. On the other hand, the equivalent amounts of vehicle were administered to the control groups (table 1).

Table 1. Treatment schedule.

S. No.	Group	Treatment Schedule
1	Group I	Control group treated with Distilled Water
2	Group II	DNFB+ Sodium Lauryl Sulphate (50mg/kg)
3	Group III	DNFB+ <i>Acacia concinna</i> (100mg/kg)
4	Group IV	Betamethasone dipropionate Ointment (100mg)

Assessment of AD

Severity of dermatitis

Using a grading system, the extent of the dermatitis was assessed under a microscope. The severity of the redness, swelling, erosion, and scaling was assessed using a scale ranging from 0 (none) to 3 (extreme). The total score for every mouse was arrived at by adding together the scores from these five symptoms; the range for this score was 0 to 12. An investigator who was not aware of the groups' assignments administered the test [11].

Trans-epidermal water loss (TEWL)

On the thirteenth day, under controlled settings ($21 \pm 2^\circ\text{C}$ and 50-55% humidity), TEWL was recorded at the centre of the shaved dorsal area of each mouse to assess skin dryness. After about 30 seconds of pressing the probe to the skin, measurements were obtained once the TEWL readings had stabilized [12].

Inflammatory markers

Centrifugation was performed for 10 minutes at a temperature of 4°C in a velocity of 10,000 rpm to isolate plasma from mouse blood samples. Prior to the analysis, the plasma was preserved at -80°C . Following the instructions

provided by the manufacturer, to measure the amount of mast cells and IgE, enzyme-linked immunosorbent assays (ELISA) were employed. Through the utilisation of a BioMag separation column, we were able to separate CD4+ T cells and CD8+ monocytes from the draining lymph nodes of the animals. In RPMI-1640 medium that was supplemented with 10% foetal bovine serum, β -mercaptoethanol, and glutamine, T cells were stimulated using anti-CD3 and anti-CD28 antibodies. The concentrations of IL-4 and IFN- γ produced during stimulation were determined using an ELISA assay.

Scratching behaviour

A set of custom-made plastic chambers with dimensions of $20 \times 30 \times 20$ cm were used to house the mice. Each chamber had a mirror for thorough observation and ventilation holes. The chamber's ceiling was fastened so it couldn't be opened. For one hour, we monitored the subjects' scratching habit and counted how many times they scratched.

Statistical analysis

The data was represented in Mean and standard deviation. Wilcoxon and Steel's test was used to discover if the dermatitis score was significant. To round out the statistical analysis, the Kruskal-Wallis test and the Student's t-test were applied for analysis. Statistical significance was defined as a p -value of less than 0.05.

Results

Severity of dermatitis, epidermal thickness and TEWL

The intensity of symptoms in mice treated with DNFB + SLS (50 mg/kg) escalated progressively with the frequency of DNFB exposures. The application of *Acacia concinna* and Betamethasone dipropionate ointment significantly diminished the progression of skin lesions resembling atopic dermatitis. Skin epidermal hyperplasia in mice can be induced by DNFB treatment. Regular DNFB treatments increased epidermal thickness, as expected.

As a protective barrier, the stratum corneum lessens perspiration loss and blocks harmful environmental rays. The epidermis's stratum corneum relies on filaggrin, a filament-aggregating protein, for structural integrity. The expression of filaggrin, an essential component of the epidermal barrier, is suppressed and altered by dust mite antigen and oxazolone treatment, according to recent studies. Repetition of DNFB treatment, as expected, reduced epidermal filaggrin expression; however, SLS, *Acacia concinna*, and Betamethasone dipropionate treatment restored epidermal filaggrin expression.

On days 1 and 4, mice were given 100 mg of DNFB ointment topically to induce lesions that resembled atopic dermatitis. The sensitised mice were applied a 0.2% DNFB solution topically to their backs on days 7, 10, and 13. As a control group, the mice were given normal saline. The experimental groups, on the other hand, were given DNFB +

SLS (50 mg/kg) or DNFB + *Acacia concinna* (100 mg/kg). Daily topical administration of betamethasone dipropionate ointment began on day 7, the first day of the DNFB challenge. The SCORAD index was utilised to assessed the

severity of atopic. From 0 (not present) to 3 (very severe), a severity level was given to each symptom. After tallying up each mouse's six symptoms, their ultimate score was calculated (Figure 1 and 2).

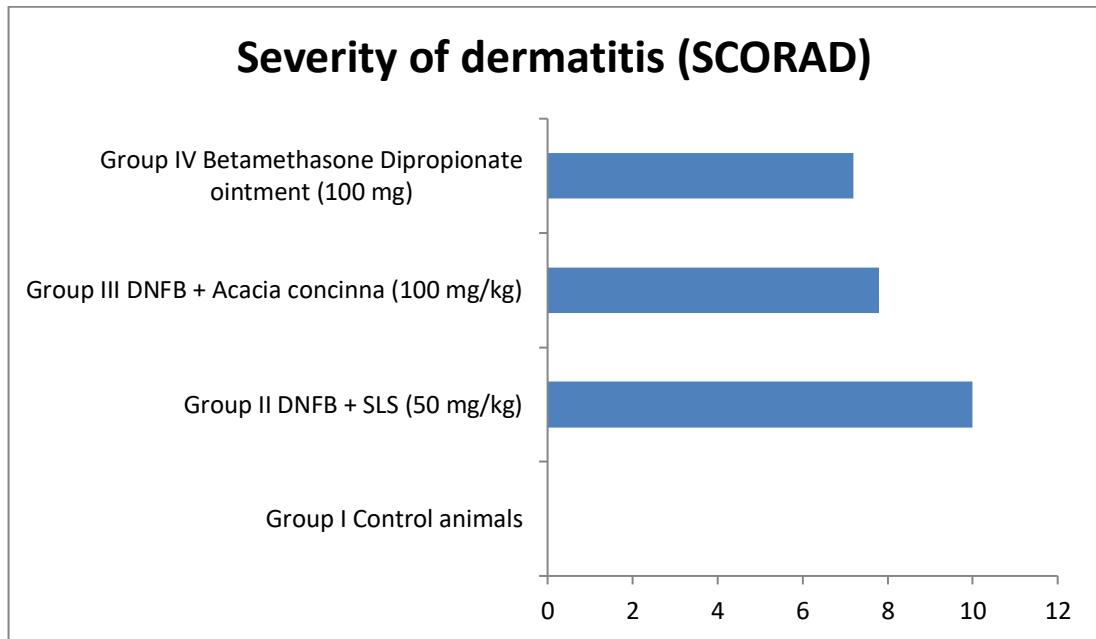


Figure 1. Effect of SLS, *Acacia concinna* and Betamethasone dipropionate ointment on AD-like severity of DNFB induced mice.

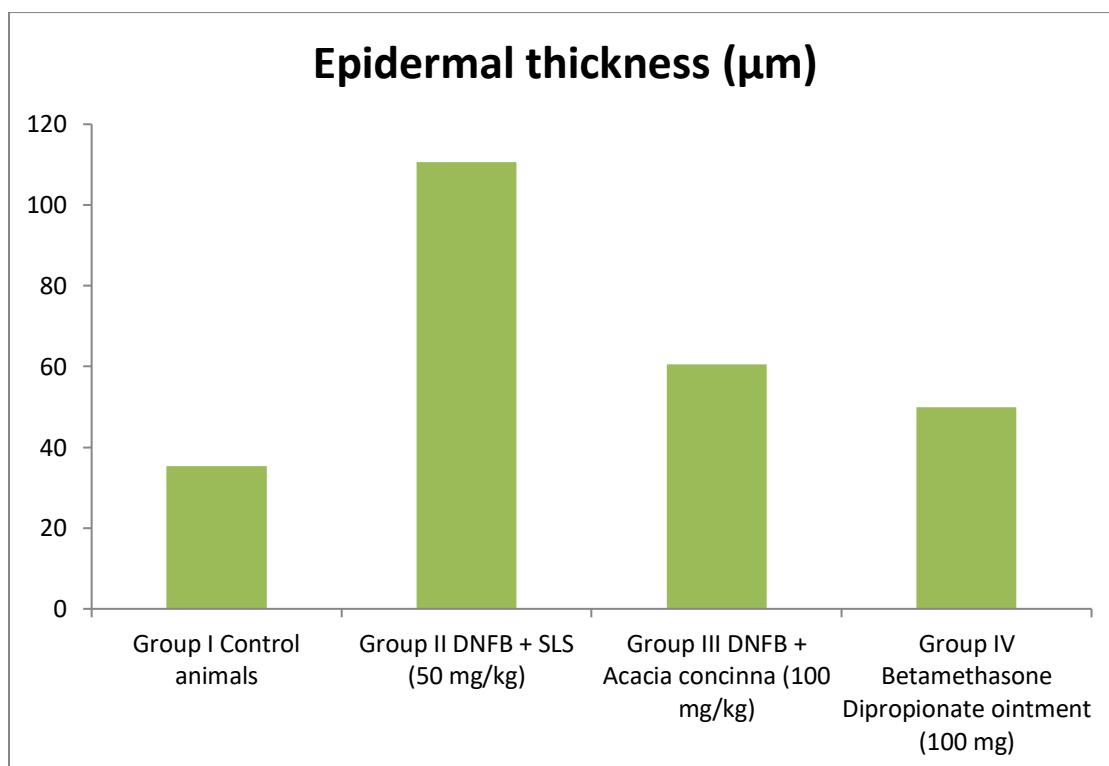


Figure 2. Effect of SLS, *Acacia concinna* and Betamethasone dipropionate ointment on AD-like epidermal thickness (μm) of DNFB induced mice.

Following each treatment, skin lesions were evaluated and rated. Following the sixth treatment, skin lesions from cryosections were generated using haematoxylin and eosin (H&E) staining for histological examination. Under the microscope, the thickness of the epidermis (in micrometres) was measured in 24 sections stained with H&E. This cell count is taken as an average of the five different parts. On the last day, the TEWL value was calculated on the dorsum to gauge skin moisture and barrier condition. In contrast to the upregulation of SLS, all DNFB treatments reduced TEWL value, *Acacia concinna* and Betamethasone dipropionate ointment treated group (Figure 3).

Inflammatory markers

The differentiation of CD4+ T cells into Th1 and Th2 subsets is regulated by cytokines that are produced within the draining lymph node's microenvironment. During the course of the progression of AD, it would appear that CD4+ T helper 1 or 2 cells play a critically important role. Th1 or Th2 predominant inflammation may manifest at different stages of atopic dermatitis, contingent upon the severity of the condition. Production of cytokine is a markers for differentiating Th1 and Th2 cells. IFN- γ and IL-2 are predominantly produced by Th1 cells, while Th2 cells are primarily associated with the secretion of IL-4, IL-5, and IL-13. To determine whether there was a change in Th1 and Th2 responses, we stimulated CD4+ T cells that had been isolated from different groups' lymph nodes.

The following treatments were used: Control, DNFB + SLS (50 mg/kg), DNFB + *Acacia concinna* (100 mg/kg), and Betamethasone dipropionate ointment. The production of IFN- γ was decreased after CD4+ T cell activation when Betamethasone dipropionate ointment and *Acacia concinna* were applied, while IL-4 production was unaffected (Figure 4).

Activated CD4+ T cells of DNFB-treated mice are being examined to assess the impact of *Acacia concinna* and betamethasone dipropionate ointment on the synthesis of IL-4 and IFN- γ . The day subsequently the last DNFB treatment, lymph node removal and CD4+ T cell recovery occurred. CD4+ T cells were activated by incubating with plate-bound anti-CD3 antibody (2C11; 5 μ g/mL) and soluble anti-mouse CD28 antibody (2 μ g/mL) for fifty hours. Prior to stimulation, cells were purified to a concentration of 1×10^6 cells/mL. Following T cell activation using an ELISA, we quantified IL-4 and IFN- γ . The presence of inflammatory cells within skin lesions is one of the characteristics that sets atopic dermatitis apart from other skin conditions. The main

cellular mediators of inflammatory dermatoses are T cells within the cutaneous lesions (18). As a result, we compared SLS therapy to *Acacia concinna* and Betamethasone dipropionate ointment to see whether the former reduced T cell infiltration in skin lesions. Ointments containing *Acacia concinna* and Betamethasone Dipropionate significantly reduced the number of CD4+ and CD8+ T lymphocytes that infiltrated affected skin areas (Figure 5).

Serum from mice treated with DNFB was tested for IgE levels using enzyme-linked immunosorbent assays. From the first day of DNFB (day 7), SLS, *Acacia concinna*, and Betamethasone dipropionate ointment were consistently administered topically. One day after the last injection, researchers measured total IgE levels in the designated groups.

When allergen-sensitive IgE activates mast cells, granules release a range of mediators, including prostaglandins, leukotrienes, and cytokines. So, we used Toluidine Blue staining to observe how SLS, *Acacia concinna*, and Betamethasone dipropionate ointment affected the infiltration and degranulation of mast cells in skin lesions. In mice, number of invading mast cells in skin lesions and degranulation were significantly reduced when given betamethasone dipropionate ointment and DNFB + *Acacia concinna* (100 mg/kg) (Figure 6).

Mice were given 100 mg of DNFB ointment topically three times daily to treat their dorsal skin. This treatment was administered to the mice from day one through day four. The mice that had been sensitised were given a 0.2% DNFB solution that was applied topically to their backs on days 7, 10, and 13. The dosage that was administered to each group of animals was different: the control group was administered an equivalent volume of normal saline, the group that received DNFB and SLS received 50 mg/kg, and the group that received DNFB and *Acacia concinna* received 100 mg/kg. Beginning with day 7, the first DNFB challenge, betamethasone dipropionate ointment was given topically daily. Immunofluorescent staining was carried out on cryosections of back skin samples using fluorescein isothiocyanate after the last DNFB treatment (Figure 7).

Scratching behaviour

The scratching effects of SLS, *Acacia concinna* and Betamethasone dipropionate ointment were investigated in DNFB induced mice. The effect of SLS treated group animal animals shown more scratching when compared to *Acacia concinna* and Betamethasone dipropionate (Figure 8).

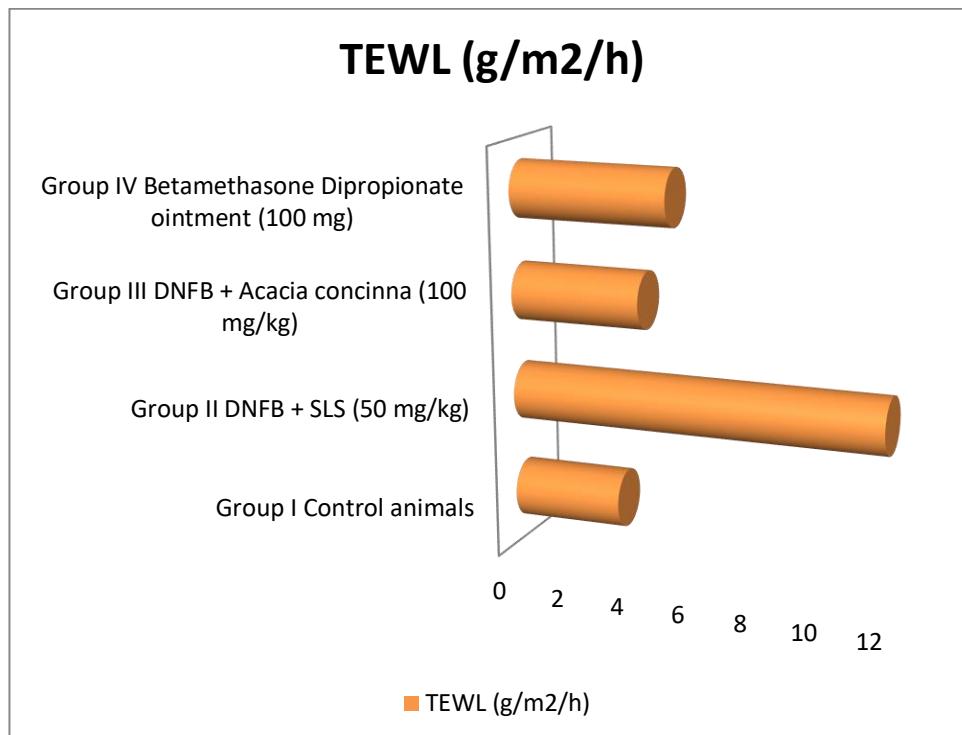


Figure 3. Effect of SLS, *Acacia concinna* and Betamethasone dipropionate ointment on AD-like Trans Epidermal Water Loss (g/m²/h) of DNFB induced mice.

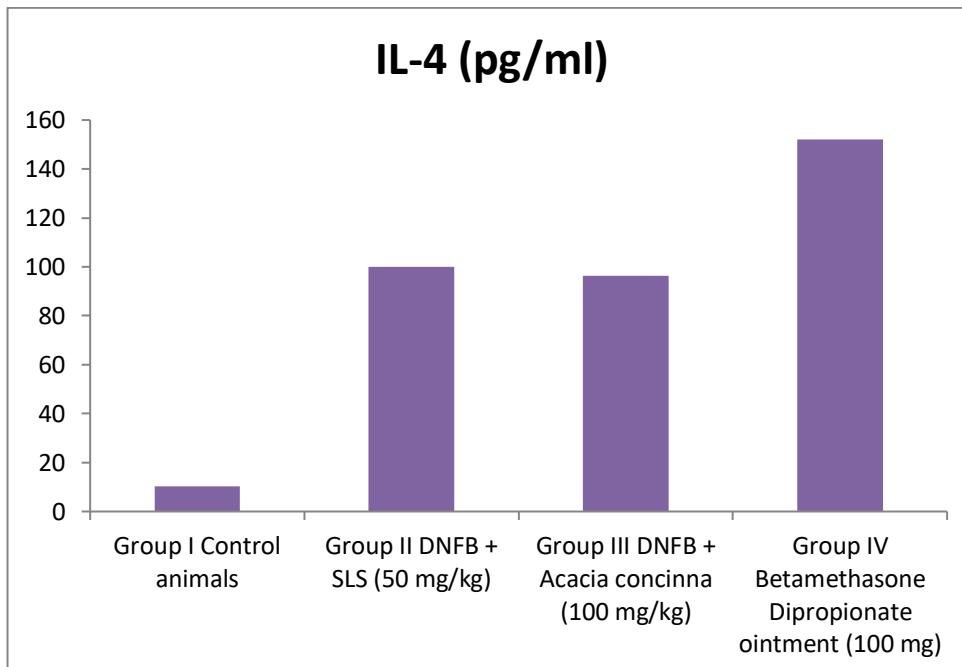


Figure 4. Effect of SLS, *Acacia concinna* and Betamethasone dipropionate ointment on AD-like IL-4 of DNFB induced mice.

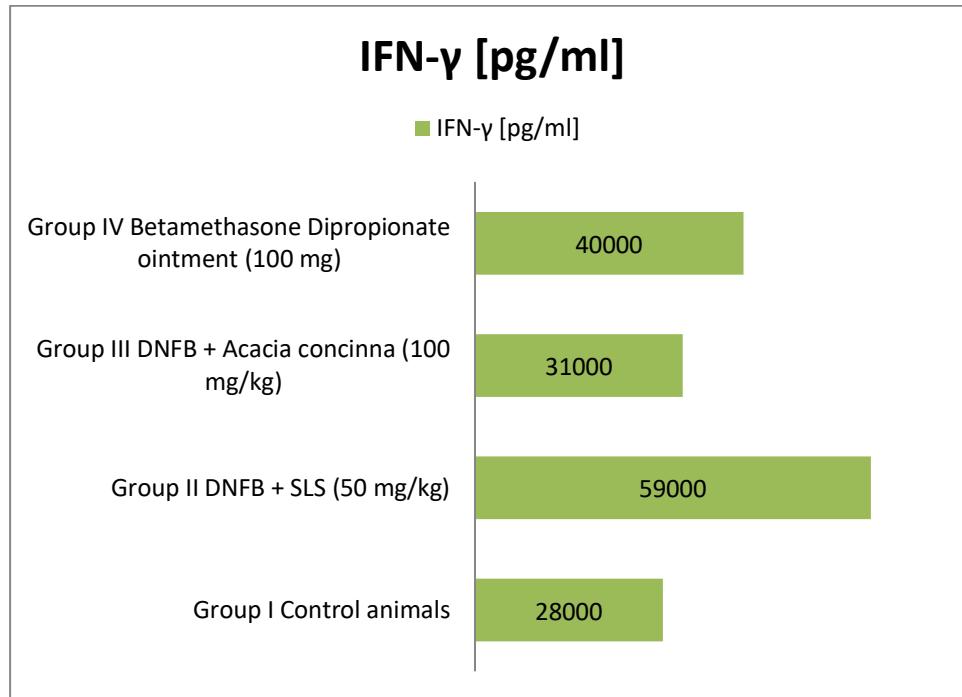


Figure 5. Effect of SLS, *Acacia concinna* and Betamethasone dipropionate ointment on AD-like IFN- γ of DNFB induced mice.

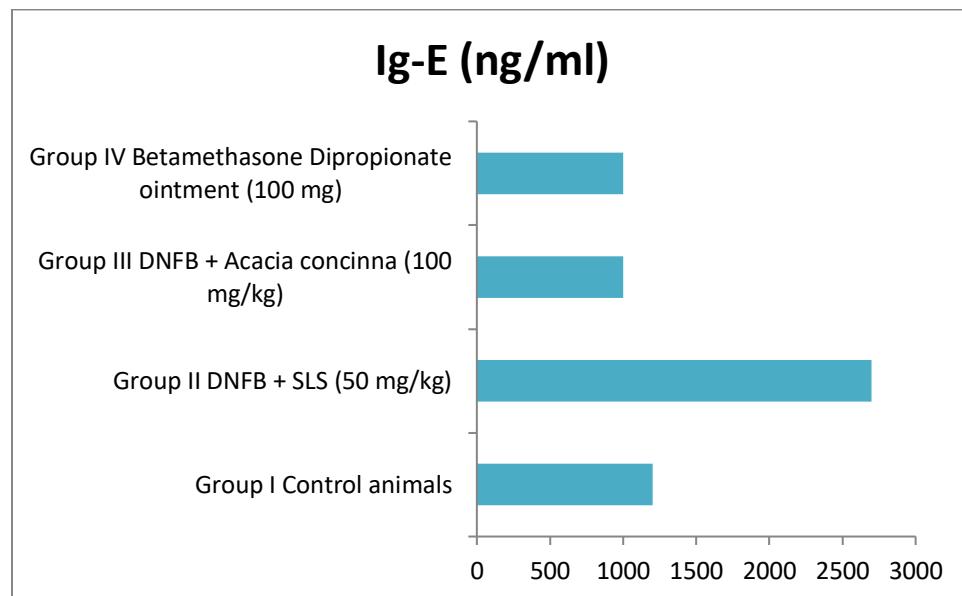


Figure 6. Effect of SLS, *Acacia concinna* and Betamethasone dipropionate ointment on AD-like Ig-E of DNFB induced mice.

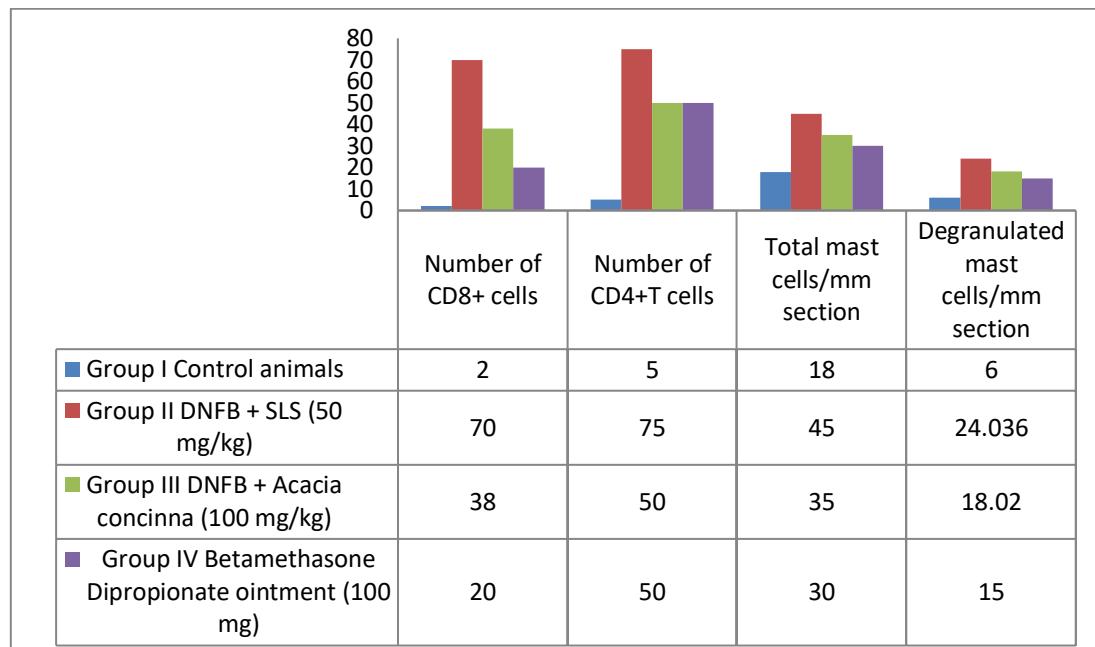


Figure 7. Dorsal skin lesions in DNFB-sensitive mice treated with ointments of SLS, *Acacia concinna*, and betamethasone dipropionate reveal the effects of infiltrating CD4+ and CD8+ T cells, mast cells, and degranulation.

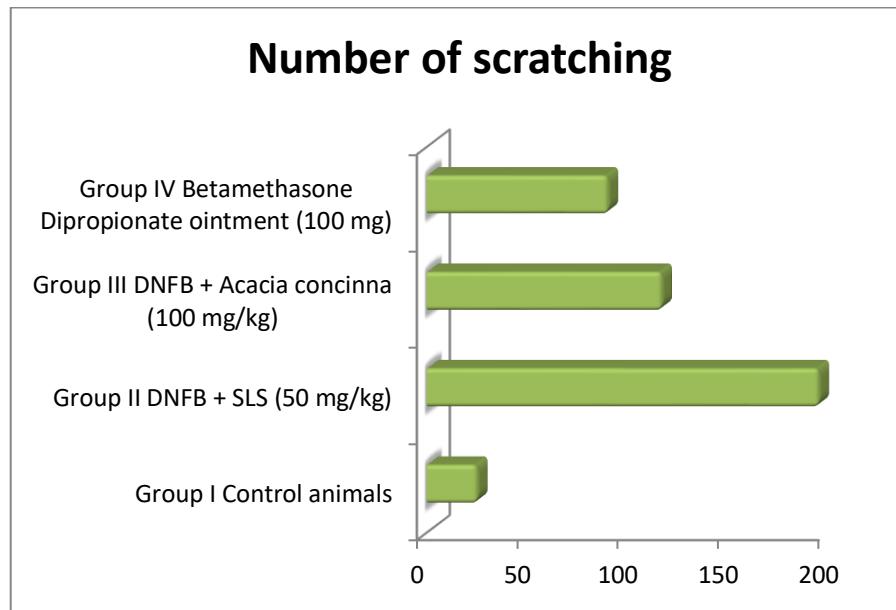


Figure 8. *Acacia concinna* and Betamethasone dipropionate ointment treatment reduces scratching behaviour counted for 1 hour.

Discussion

This paper compares DNFB-induced mice with SLS, *Acacia concinna*, and betamethasone dipropionate ointment. Atopic dermatitis is a frequently observed dermatological condition, characterised by erythema, oedema, excoriation, and scaling. In the absence of a beneficial impact of steroids on the treatment of AD, long-term management is unfeasible due to their adverse consequences. Consequently, researchers have been endeavouring to discover novel pharmaceuticals that are efficacious in AD [13]. For the treatment of AD, numerous effective medicines, including flavonoid

compounds, have been successfully developed. The following ingredients are included: (Betulin, Betulinic acid, Lupenone, Hexacosanol, Spinasterol, Calycotomine, Racimase-A, Oleanolic acid, Lupenone, Betulin, and Betulonic acid). A dandruff-fighting and hair-cleaning extract is made from its pods. Shikakai, or Vitamin C, which is plentiful in *Acacia concinna*, is good for the health of your hair. The natural acidophile properties of shikakai help to lower pH levels without stripping hair of its protective oils, leaving it looking healthy and shiny. Strengthening and nourishing hair is another area where it excels. Because of

their synergistic effects, amla, reetha, and shikakai are utilised to foster robust and shiny hair [14]. You may get all of these ingredients in powdered or dried fruit forms. For healthy, shiny hair free of frizz, dandruff, greying, and split ends, try a combination of Amla, Reetha, and Shikakai. These three ingredients work wonders on any hair type. AD is a skin condition that causes inflammation and flare-ups on a periodic basis. It is a significant public health concern on a global scale. House dust mite allergens cause severe skin reactions in people with atopic dermatitis. As a result, many people with atopic dermatitis believe that HDM allergens are environmental triggers for their condition. Since DNFB is the most frequent HDM species in Japan, it seems reasonable to employ it as a relevant antigen in an atopic dermatitis model.

Mice may develop skin lesions like to AD after multiple applications of an ointment containing DNFB extract. Dry skin was the first symptom, followed by mild redness, bleeding, and swelling in this form of infection. Skin thickening, severe redness, swelling, bleeding, scarring, erosion, and excoriation were the final symptoms. Histological examination revealed hyperkeratosis, parakeratosis, and significant dermal and epidermal thickening in the afflicted area. Enhanced levels of skin-inflammation-causing cells, including mast cells, eosinophils, and lymphocytes, were linked to these alterations. Comparing our model's histology and clinical findings to those of atopic dermatitis in humans, we discovered several remarkable parallels. Our model also exhibited scratching, a characteristic of AD. In a prior study, Researcher found that DNFB extract caused dermatitis in mice. They employed a DNFB extract suspension to bring on dermatitis, but the onset was painfully sluggish. Repeated administration of DNFB extract suspension did not significantly induce dermatitis in our initial experiment. The absence or non-onset of dermatitis in the mice was explained, we hypothesised, by the rapid elimination of the allergy from their bodies. The duration of action of DNFB extract ointment, as compared to DNFB extract suspension, was another of our predictions. We opted to use DNFB extract ointment to induce dermatitis in the hopes that the skin lesion would occur sooner. The DNFB extract ointment mimicked the symptoms of atopic dermatitis, which manifested rapidly and worsened continuously over time.

A connection has been established between skin lesions, topical eosinophilia, and high systemic IgE levels, also the expression of Th2 type cytokines, according to previous research on atopic dermatitis. Meanwhile, it was discovered that Th2 cytokines were the most commonly expressed in both acute and chronic atopic dermatitis lesions [15].

This is in contrast to healthy skin or skin that is not affected by atopic dermatitis.

The TEWL offers insights into the skin's barrier efficacy. The integrity of the internal-external barrier can be evaluated using an evaporimeter or a comparable instrument. A correlation exists between the inside-outside and outside-

inside barriers, though this is not always the case. Vasoconstriction, which occurs after corticosteroid treatment, may contribute to the reduction in TEWL following betamethasone administration. When blood vessels narrow, less blood flows into the dermis and epidermis, which should theoretically lower TEWL. A combination of the barrier and driving force components determines TEWL. Blood flow, quantified through laser Doppler flowmetry, is the paramount aspect of this component.

The dermatitis model developed in this work facilitates the assessment of topically administered medications, as the induced skin lesion persisted for a minimum of two weeks without additional topical allergen treatment. In individuals with AD, pruritus-induced scratching compromises the skin and exacerbates inflammation, thereby amplifying the itch sensation. Removing the mice's hind toenails improved the formed skin lesions, and we found that the mice scratched at the locations where the antigen was applied in our model (data not shown). Therefore, even in the absence of further antigen exposure, the dermatitis would remain due to the chronic scratching habit. We then tested whether our model could evaluate the effectiveness of drugs applied in human AD treatment. Corticosteroids are the principal pharmacological agents utilised in the treatment of AD. In addition, facial and cervical cutaneous lesions exhibit respons well to tacrolimus ointment, which was first released in Japan in 1999. Mice with spontaneously developed dermatitis show strong inhibitory effects of tacrolimus ointment, whereas those with corticosteroid ointment show relatively weak effects. Consequently, we investigated the impact of betamethasone dipropionate ointment, a steroid formulation, on dermatitis in our model. The application of these ointments biweekly significantly and distinctly diminished dermatitis ratings, with the inhibitory effects persisting throughout the research. The effectiveness of betamethasone dipropionate ointment on dermatitis scores was correlated with notable enhancement of histological alterations, including epidermal thickening and inflammatory cell infiltration. In conclusion, by consistently administering an ointment containing DNFB extract to mice, we were able to create a novel animal model of atopic dermatitis. Every immunological, histological, and clinical feature in this model closely matched those seen in individuals with atopic dermatitis. Furthermore, we confirmed that *Acacia concinna* and SLS worked well in this particular model. Consequently, *Acacia concinna* is proposed to be more efficacious than SLS.

Conclusion

In conclusion, our study successfully established a novel animal model of atopic dermatitis using DNFB extract ointment, which effectively replicated the clinical, histological, and immunological characteristics of the disease seen in humans. The model demonstrated significant skin lesions, including erythema, edema, and excoriation,

accompanied by a marked increase in inflammatory cells, aligning closely with the pathophysiology of AD. Our findings highlighted the efficacy of traditional and natural treatments, such as *Acacia concinna*, which showed superior effects in alleviating dermatitis symptoms compared to SLS. Notably, while corticosteroids like betamethasone are first-line treatments for AD, their long-term use poses risks of side effects, underscoring the need for alternative therapies. The positive response of the established model to both *Acacia concinna* and SLS suggests their potential as effective treatments in managing AD. Moreover, our observations on the scratching behavior of the mice indicated the chronic nature of the condition, reinforcing the importance of exploring both symptomatic relief and therapeutic interventions. This research not only advances our understanding of AD but also opens avenues for developing eco-friendly and safe alternatives for treating skin disorders, aligning with modern needs for sustainable healthcare solutions. Future studies should aim to further elucidate the mechanisms behind the beneficial effects of *Acacia concinna* and explore its potential integration into AD management protocols. Overall, this model serves as a valuable tool for evaluating the efficacy of various treatments in the quest for effective AD management.

Acknowledgments

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Ethics Approval and Consent to Participate

All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), TAB/IAEC/IDT/01/24, under the project Comparing the Effects of SLS in Shampoos and *Acacia concinna* on Atopic Dermatitis.

Consent for Publication

All authors have provided their consent for publication and affirm that they approve the final version of the manuscript.

Conflict of Interest

Nil

Declaration of generative AI and AI-assisted technologies in the writing process

Nil

Author Contribution

All authors are contributed equally in the research

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