# Alleviative Effect of Sinapoyl Choline versus Tacrolimus on DNCB-Induced Atopic Dermatitis Murine Model

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## ABSTRACT

**Background:** Atopic dermatitis (AD) is a long-lasting inflammatory skin disease characterized by erythematous, dry patches. Sinapoyl choline is a naturally occurring phenolic compound with anti-inflammatory and antioxidant properties.

**Aim:** This study aimed to evaluate the ameliorative effect of topical sinapoylcholine in a 2,4-dinitrochlorobenzene (DNCB)-induced mouse model of AD-like dermatitis.

**Methods:** Fifty male BALB/c albino mice were randomly assigned to five groups of 10 each. Except for the healthy control group, atopic dermatitis was induced by sensitization with 1% DNCB, followed by repeated challenges with 0.5% DNCB. Mice were treated topically once daily for 3 weeks with vehicle cream, 0.1% tacrolimus ointment, and 1% sinapoyl choline cream.

**Results:** Topical sinapoyl choline improved histopathological changes and significantly decreased clinical dermatitis scores compared to the induced group. Immunohistochemical analysis revealed that the treated mice showed increased filaggrin expression. Additionally, sinapoyl choline significantly reduced serum IgE and skin tissue IL-4, IL-13, and malondialdehyde (MDA) levels and restored reduced glutathione (GSH) levels, as demonstrated by enzyme-linked immunosorbent assay.

**Conclusion:** Sinapoyl choline mitigates DNCB-induced AD-like symptoms by modulating inflammatory and oxidative stress responses and enhancing the epidermal barrier.

**Keywords:** Atopic dermatitis; Sinapoyl choline, 2,4-Dinitrochlorobenzene, Filaggrin; Mice.

## INTRODUCTION

Atopic dermatitis (AD) is a chronic allergic skin condition characterized by relentless itching and eczematous plaques that recur (Courtney et al. 2023; Habbas et al. 2025). Sleep, education, employment, and family relationships can all be negatively impacted; visible deformity from redness, flaking, and bleeding excoriations can lead to depression, social stigmatization, and a lower quality of life (Simpson et al. 2016; Talamonti et al. 2021). AD affects about 4% of adults and 10% of children, and half of adults develop persistent skin disease (Leung and Guttman-Yassky 2017). The skin barrier protects the body from excessive trans-epidermal water loss and from external hazards such as chemicals, allergens, and infectious agents (Proksch et al. 2008; Baroni et al. 2012). The pathophysiology of the condition is multifaceted, involving psychological factors, external triggers, impaired epidermal barrier function, oxidative stress, and immune dysregulation (Agrawal and Woodfolk 2014; Ahn et al. 2020). Therapeutic management remains challenging. Topical or oral corticosteroids, systemic immunosuppressants, and biological agents are the current standard treatments (Wei et al. 2025). Although biological agents are the most commonly recommended treatment for AD, their long-term use is limited by high cost and dose-limiting side effects, such as cutaneous atrophy, telangiectasia, and hyperpigmentation (Riedl et al. 2023). Despite the use of corticosteroid-sparing agents such as cyclosporine, azathioprine, and methotrexate, therapeutic outcomes remain suboptimal (Nie et al. 2025). This highlights the need for safer and more sustainable treatment options (Lee and Im 2022).

The selected biomarkers were chosen for their well-established roles in the pathophysiology of atopic dermatitis. Serum IgE, along with IL-4 and IL-13, was measured as a key indicator of Th2-mediated immune

responses and allergic inflammation, both central features of AD (Mitroi et al. 2024; Čelakovská et al. 2025). Moreover, oxidative stress is important in the pathophysiology of chronic inflammatory skin conditions, as increased formation of reactive oxygen species (ROS), gives rise to skin barrier disruption, inflammation, and disease progression (Abbas et al. 2023; Ali et al. 2025c; Luo et al. 2025). As a result, assessing oxidative stress indices is critical for identifying the pathomechanisms and estimating treatment efficacy (Abbas et al. 2025b; Abdulbari et al. 2025; Kvedariene et al. 2025a). In the current investigation, oxidative stress was assessed via determining malondialdehyde (MDA), a commonly used indicator of lipid peroxidation that reflects oxidative injury to membrane lipids (Cordiano et al. 2023; Ali et al. 2025b; Shihab et al. 2025). Furthermore, reduced glutathione (GSH), a vital intrinsic antioxidant, was tested to figure out cellular redox balance and antioxidant defense mechanisms to combat reactive oxygen species (Bertino et al. 2020; Khorsheed and Abu Raghif 2024; Ali et al. 2025a). In addition, filaggrin expression was assessed by immunohistochemistry as a marker of epidermal barrier integrity, given that filaggrin deficiency is strongly associated with impaired skin barrier function in atopic dermatitis (Batista et al. 2015; Ilves et al. 2015).

Recent pharmaceutical advances, including biologic therapies targeting IL-4/IL-13 cytokines and small-molecule inhibitors, have underscored the importance of modulating inflammatory mechanisms while restoring barrier function. In the current context, plant-based constituents are gaining recognition as potential complements or adjunctive treatments (Hassan et al. 2022; Hussein et al. 2025). Sinapic acid is a naturally occurring hydroxycinnamic acid found in many plants, particularly in Brassicaceae seeds (Nguyen et al. 2021). It is a phenolic compound with potent antioxidant and anti-inflammatory properties. A lot of research has suggested that it is a strong antioxidant (Natella et al. 1999; Pekkarinen et al. 1999; Zou et al. 2002). Sinapoyl choline, a choline ester of sinapic acid, is particularly abundant in the seeds of rapeseed (Brassica napus), mustard (Brassica juncea), and other related cruciferous plants (Nićiforović and Abramovič 2014). In vitro assays showed that sinapoyl choline at concentrations typical of rapeseed pomace extracts, exhibited ≥50% antioxidant activity relative to the total extract, indicating its capacity to scavenge free radicals and protect biological molecules, such as DNA (Yates et al. 2019). Further in vitro studies demonstrated that sinapoyl choline's antioxidant capacity, as assessed by radical-scavenging assays, remained robust when embedded in protein-based hydrogels, thereby confirming its radical-neutralizing capabilities in complex media (Li et al. 2024).

Sinapoyl choline and its derivatives, including sinapic acid, have also shown anti-inflammatory effects in various biological models. In a high-fat diet-induced model of non-alcoholic fatty liver disease (NAFLD), supplementing mice with sinapoyl choline reduced expression of TNF-α and NF-κB, two markers of systemic inflammation, in intestinal and adipose tissues. This suggests that sinapoyl choline can suppress inflammatory signaling pathways linked to metabolic stress (Li et al. 2019). Proteomic studies examining how sinapoyl choline affects macrophages have shown that it can shift macrophages from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype, which is linked to improved lipid handling in immune cells and reduced inflammatory responses (Liu et al. 2023). Sinapoyl choline differs from other phytochemicals studied because it has both hydrophilic and robust antioxidant properties. This could make it more effective when applied to the skin (Martinović et al. 2019; Menezes and Campos 2024).

The combined antioxidant and anti-inflammatory properties of sinapoyl choline make it a promising therapeutic candidate for the treatment of atopic dermatitis. Accorfing to our knowlege, the current study is the first to investigate the topical application of sinapoyl choline in a 2,4-dinitrochlorobenzene (DNCB)-induced AD model.

## MATERIALS AND METHODS

### *Drugs and chemicals*

All chemicals used in the experiment were purchased from various sources. A DNCB was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., catalog no.: C465514-25g, lot #D2527134, while tacrolimus 0.1% ointment was supplied by Ajanta, India. All mouse ELISA kits were acquired from ELK Biotechnology. Sinapoyl choline was obtained from Chengdu Biopurify Phytochemicals Ltd (Chengdu, China) (Catalogue No.: BP1308; CAS No.: 18696-26-9; Lot No.: PRF23022444). The phytocompound had a

confirmed purity of 98% (manufacturer specification: 95-99%), a molecular formula of $C_{16}H_{24}NO_5^+$, and a molecular weight of 310.369 g/mol. Based on the manufacturer's certificate of analysis, the compound's identification was validated by mass spectrometry (MS) and nuclear magnetic resonance (NMR), while purity was established using HPLC-DAD and/or HPLC-ELSD.

Mouse IgE ELISA Kit, cat.: ELK2593, lot: 32475732. Mouse IL4 ELISA Kit, cat.: ELK1153, lot: 52775764. Mouse IL-13 ELISA Kit, cat.: ELK1145, LOT: 32675764. Mouse MDA ELISA Kit, cat.: ELK10925, lot: 52775764. Mouse GSH ELISA Kit, cat.: ELK10913, lot: 32675764. An immunohistochemistry antibody to filaggrin was purchased from Cloud-clone crop, cat. no.: PAJ103Mu01, lot: A20251125153. HRP-conjugated IgG with DAB chromogen was purchased from Elabscience, cat. no.: E-IR-R213, lot: DW0686N021246.

***Experimental animal maintenance and housing:***

The “Iraqi Center for Cancer Research and Medical Genetics” in Al-Yarmouk’s animal house served as the study site. 50 male Albino BALB/c mice, weighing 20-30 g and aged 6-9 weeks, were selected as the appropriate animal strain for the experiment and purchased from the Al-Rhazi Center of the Ministry of Industry and Minerals. The animals were housed in SPF settings with 12-hour light/dark cycles, hygienic, sterilized housing, unlimited access to water, and ad libitum feeding and were given one week to acclimate to their new environment. Throughout the experiment, a new, sterile substrate was added to the cage every two days.

***Study design and animal allocation***

A prospective, randomized, controlled animal study was conducted. This study included 50 male albino mice, which were randomly assigned to five groups:

**Group 1 (healthy control):** 10 healthy mice that received no medical care and had normal skin.

**Group 2 (induction):** 10 mice with induced atopic dermatitis that received no treatment.

**Group 3 (tacrolimus):** 10 mice with induced atopic dermatitis treated with a topical 0.1% tacrolimus ointment once daily.

**Group 4 (vehicle):** 10 mice with induced atopic dermatitis treated with a vehicle-based cream once daily.

**Group 5 (sinapoyl choline):** 10 mice with induced atopic dermatitis treated with a 1% sinapoyl choline cream once daily.

A schematic representation of the experimental design is illustrated in **Figure 1**.

***Induction procedure and treatment of Atopic dermatitis***

After a 7-day acclimation period, a precise electric shaver was used to carefully remove hair from the dorsal skin. Any remaining hair was then removed with hair removal cream, and the animals were given a day to rest. On day 1, 150 µL of 1% DNCB (dissolved in a 3:1 mixture of acetone and olive oil) was applied once to the dorsal skin (about 4 $cm^2$) of all mouse groups except the control group. Four days after the sensitization phase, the dose was boosted with 150 µL of 0.5% DNCB three times a week for 3 weeks, based on slightly modified versions of previously described protocols (Hassan et al. 2022; Kasim et al. 2024). Mice were given tested agents daily for three weeks following visual confirmation of skin sensitization and AD.

All animals in the experiment were sacrificed on day 31. Each mouse was anesthetized with 80 mg/kg ketamine and 10 mg/kg xylazine administered intraperitoneally (IP). All mice were euthanized by exsanguination (cardiac puncture) after full anesthesia; this method is suitable for tissue collection and preservation (Pierozan et al. 2017; Shihab et al. 2018; Sharif et al. 2026a). Skin and blood samples were collected for further analysis.

Animals were randomly assigned to experimental groups using a computer-generated randomization sequence to minimize selection bias. Allocation concealment was maintained by an independent investigator not involved in outcome assessment. Topical treatments were applied at consistent time intervals throughout the experimental period. Starting on day 10, topically applied agents were given once daily for 21 days. To guarantee therapeutic comparability, 100 mg of the designated preparation (1% sinapoyl choline cream, vehicle cream, or 0.1% tacrolimus ointment) was administered evenly to each mouse's around 4 $cm^2$ lesioned dorsal skin (about 25 $mg/cm^2$). All treatment groups followed identical administration protocols during the trial. To prevent observer bias, investigators who performed clinical grading, biochemical testing, and histological examination were blinded to group assignment.

***Tacrolimus Dose Justification***

Tacrolimus was administered topically at a concentration of 0.1%, which is a clinically authorized and frequently employed preparation for moderate-to-severe atopic dermatitis (Ashcroft et al. 2005; Wollenberg et al. 2008). This dose was also extensively utilized in mouse models to provide persistent immune-modulatory and anti-inflammatory benefits, serving as a credible reference standard for comparisons (Shallal et al. 2025; Elaf Mahmood et al. 2026).

***Sinapoyl choline 1% cream formulation***

Sinapoyl choline is a hydrophilic alkaloid with anti-inflammatory and antioxidant properties. A standard emulsification technique was used to prepare an oil-in-water cream containing 1% sinapoyl choline (Kurebayashi et al. 2024; Mahdi et al. 2026). After dispersing carbomer (0.5%) in purified water, glycerin (3%) and propylene glycol (5%) were added. The aqueous phase was then heated to 70°C, and sinapoyl choline was dissolved. Cetostearyl alcohol (2%) was melted separately at about 70°C and then gradually added to the aqueous phase while stirring. Triethanolamine was used to adjust the pH to 5.5, and phenoxyethanol (0.8%) was added as a preservative (Mohiuddin 2019; Abbas et al. 2025a). The cream was applied topically to the lesioned skin once daily and stored in sterile containers.

The concentration of sinapoyl choline (1%) was chosen in light of earlier research into sinapic acid and its derivatives, which found strong anti-inflammatory and antioxidative activities at equivalent amounts (Kaltalioglu 2023; Jin et al. 2025). Furthermore, this dosage was found to be sufficient to assure optimal skin penetration and pharmacological action in topical preparations (Malviya et al. 2023).

***Vehicle cream formulation***

After dispersing carbomer (0.5%) in purified water, glycerin (3%) and propylene glycol (5%) were added. To create an emulsion, cetostearyl alcohol (2%), separately melted at about 70°C, was gradually added to the aqueous phase while stirring. Triethanolamine was used to adjust the pH to 5.5, and phenoxyethanol (0.8%) was added as a preservative. For topical application, the vehicle cream was stored in sterile containers.

***Sample collection***

The experiment took 31 days to complete. Afterward, all animals were sacrificed to collect samples. Each animal received an anesthetic dose of 80 mg/kg ketamine and 10 mg/kg xylazine before sacrifice, and the heart was punctured to draw blood. To separate serum, blood was collected in a gel tube and centrifuged for seven minutes at 5000 rpm. The serum was collected in 2 mL Eppendorf tubes and kept at -20°C until the day of analysis (Idan et al. 2024; Mekkey et al. 2024). Following cervical dislocation, the animals were dissected to obtain two skin samples—one for ELISA tissue homogenate and the other for histopathological and IHC analysis.

***Preparation of skin tissue homogenates***

After euthanasia, dorsal skin specimens were extracted, washed with ice-cold phosphate-buffered saline solution (pH 7.2-7.4) to eliminate blood, and then kept at -20°C for testing (Jaafar et al. 2025; Shallal et al.

2025). The cutaneous tissues were weighed and homogenized at a fixed ratio of 1 g tissue in 9 mL of ice-cold phosphate-buffered saline with a glass homogenizer stored on ice. The homogenates were then centrifuged at 5,000 rpm for 15 minutes. The residues were gathered and maintained at −20°C for further biochemical analysis. To achieve consistency between samples, tissue biomarker levels were adjusted to tissue weight. IL-4 and IL-13 levels are represented as pg/g tissue, while MDA and GSH levels are indicated as ng/g tissue.

***Observational severity assessment***

According to the severity scoring of AD on human skin (SCORAD), four clinical signs were used to assess the severity of DNCB-induced AD-like skin lesions: erythema/redness, erosion/deep excoriation, edema/swelling, and scaly patches. Depending on intensity, the parameters were rated on a scale of 0 (no symptoms), 1 (mild symptoms), 2 (moderate symptoms), and 3 (severe symptoms). The total dermatitis scores were determined by adding the individual values (Riedl et al. 2023; Kim et al. 2024).

***Enzyme-linked immunosorbent assay (ELISA) technique***

After being cleaned with cold normal saline, skin tissues were rinsed with PBS (1X), blotted dry, and weighed. A Polytron homogenizer on ice was used to homogenize 100 mg of tissue in 1 mL of cold PBS (pH 7.2) containing protease inhibitors, 0.05% sodium azide, and 0.5% Triton X-100. After centrifuging the homogenates at 5,000 rpm for 10 minutes at 4°C, the supernatants were collected and stored at -20°C until further analysis. Sandwich ELISA kits were used to measure serum IgE levels according to the manufacturer's instructions. Tissue homogenates were used to measure skin tissue biomarkers, including IL-4, IL-13, MDA, and GSH. Sandwich ELISA kits were used to measure IL-4 and IL-13, and competitive/colorimetric assay kits were used to measure MDA and GSH according to the manufacturer's instructions. A microplate reader (Huma Reader®, HUMAN Diagnostics, USA) was used to measure absorbance at 450 nm for each assay, and concentrations were determined using the corresponding standard curves.

To ensure the tests were accurate, all ELISA measurements were performed twice, as the manufacturer specified. The kit manufacturer's specifications were met for assay sensitivity and detection limits. To reduce inter-assay variability, samples were processed consistently.

***Histopathological assessment***

The dorsal skin was fixed in 10% neutral buffered formalin at room temperature, and the tissues were cleaned with tap water for a day. The tissues were embedded in paraffin and then cut into 5 µm sections using a rotary microtome (Zeiss AG). The tissues were stained with hematoxylin and eosin (H&E) after 3 minutes of deparaffinization and 3 minutes of rehydration. All staining reactions were conducted at room temperature. Histopathological alterations, including acanthosis, hyperkeratosis, parakeratosis, erosion, dermal inflammation, and extracellular edema, were observed. The evaluation was conducted using a semi-quantitative scoring system (0 = no anomaly, where 1+ indicates mild, 2+ moderate, and 3+ severe (Watanabe et al. 2009).

For each animal, three non-consecutive cutaneous sections were cut from the lesioned dorsal skin, and five randomly chosen, non-overlapping microscopic fields were analyzed from each section. All specimens were taken from the same DNCB-treated dorsal skin area to guarantee anatomical uniformity among animals. Histopathological analysis of hematoxylin and eosin-stained segments at ×100 magnification employing a semi-quantitative scoring method examining acanthosis, hyperkeratosis, parakeratosis, epidermal erosion, dermal inflammatory cell infiltration, and extracellular edema. The values recorded from all studied fields were averaged to produce a single histopathological score for each animal, which was subsequently applied in statistical analyses. All histopathological examinations were completed by an expert pathologist who was blind to the treatment groups during the research process.

***Immunohistochemistry analysis***

After being embedded in paraffin, the skin tissue blocks were sectioned onto positively charged slides and deparaffinized for 30 minutes at 60°C. The sections were rehydrated with ethanol solutions of decreasing

concentration and then treated with the antigen retrieval solution included in the Filaggrin IHC kit. Slides were incubated with 3% $H_2O_2$ for ten minutes at room temperature in the dark to block endogenous peroxidase activity. The kit's primary anti-filaggrin antibody was incubated with the samples overnight at 4°C. After that, the samples were washed with PBS and incubated for 20 minutes with the secondary antibody. Hematoxylin was used as a counterstain for nuclei, and DAB was used to visualize positive staining. While staining intensity was rated as 0 (no staining), 1 (weak positivity), 2 (moderate positivity), and 3 (strong positivity), the percentage of positive cells was rated as 0= no staining, 1 = <25%, 2 = 25–50%, 3 = 51-75%, and 4 = >75%. The total staining score (IRS) was calculated by summing the staining intensity score (0–3) and the percentage/proportion score (0–4), yielding a total score ranging from 0 to 7. With some minor adjustments to fit the current experimental design, the scoring method was taken from a previously published study (Nagano et al. 2021). Immunohistochemical scores were determined by a pathologist blinded to the experimental groups. For each animal, five random microscopic fields per section were examined at ×400 magnification. The animal was used as the unit of analysis, and the average score across all fields analyzed for each animal was used for statistical analysis.

### *Statistical analysis*

The Shapiro-Wilk test was employed to analyze the data distribution. Normally distributed data was evaluated using one-way ANOVA and Tukey's post hoc test. Non-normally distributed and ordinal data (clinical scores, histopathological scores, and immunohistochemistry scores) were assessed with the Kruskal-Wallis test followed by Dunn's multiple comparisons test. Adjustments for multiple comparisons were performed. The related figure legend specifies the statistical tests employed for each outcome. Statistical analysis of all data was performed using the Statistical Package for the Social Sciences (SPSS) 26.0. A p-value $\leq 0.05$ was considered statistically significant (Hasan et al. 2024; Drais et al. 2025).

### *Ethical approval*

The National Institutes of Health's animal care guidelines and the American Veterinary Medical Association's (AVMA) 2020 guidelines were followed for euthanasia, experimental design, and animal handling (Leary 2020). The Animal Ethical Committee of the Institutional Review Board (IRB) of the College of Medicine/Al-Nahrain University issued an approval statement (approval number 1364, date: October 7, 2025). The authors adhered to ARRIVE 2.0's recommendations (du Sert et al. 2020).

## RESULTS

### *The impact of tested agents on observational severity scores*

The observational severity scores for atopic dermatitis exhibited a significant increase in the induction and vehicle groups relative to the normal control group ($P < 0.05$); however, no substantial difference was noted between the induction and vehicle groups ($p > 0.05$). Both the standard treatment (tacrolimus) and sinapoyl choline significantly lowered the severity score in comparison to the induction group ($P < 0.05$), with no substantial differences noticed between the two treatment groups, as illustrated in **Table 1** and **Figures 2** and **3**.

### *Impact of tested agents on serum IgE, skin Th2 cytokines (IL-4, IL-13) in experimental atopic dermatitis*

The induction and vehicle groups exhibited significantly greater serum IgE, skin tissue IL-4, and IL-13 levels than the controls ($p < 0.05$), as indicated in **Table 2** and **Figure 4**. In contrast to the induction group, the tacrolimus-treated and sinapoyl choline-treated groups showed dramatically reduced levels of IL-4, IL-13, and IgE. However, no significant differences were identified between sinapoyl choline and tacrolimus in terms of IgE, IL-4, or IL-13 ($p > 0.05$).

### *Impact of tested agents on oxidative stress markers in experimental atopic dermatitis*

Compared with the healthy control group, the induction (DNCB) group showed a significant decrease in GSH

levels and a significant increase in skin tissue MDA levels ($p < 0.05$), as illustrated in **Table 3** and **Figure 5**. There were no significant differences between the vehicle and induction groups regarding MDA or GSH.

In contrast to the induction group, the treatment groups significantly reduced MDA levels and restored GSH levels. Importantly, there were no statistically meaningful variations between the tacrolimus and sinapoyl choline groups with respect to the level of MDA or GSH ($p > 0.05$).

***Impact of tested agents on alterations in skin histopathology***

The histological study demonstrated that the healthy control group's skin structure was typical, with no pathological changes. Conversely, the induction (DNCB) group had a substantial rise in all examined histopathological metrics, comprising acanthosis, hyperkeratosis, parakeratosis, erosion, dermal inflammation, and extracellular edema, in comparison to the healthy controls. There were no substantial distinctions between the vehicle-treated and induction groups, suggesting that the vehicle had no effect on the development of the disease.

The tacrolimus-treated and sinapoylcholine-treated groups dramatically diminished all histopathological characteristics relative to the induction group ($p < 0.05$) (**Table 4, Figures 6** and **7**). Interestingly, no statistically substantial variations were seen between the tacrolimus and sinapoyl choline groups in any of the variables tested ($p > 0.05$), implying partially similar histological improvement under the current experimental settings.

***Impact of tested agents on Immunohistochemistry expression of Filaggrin***

Using a semi-quantitative scoring system, the immunohistochemical expression of filaggrin in skin tissues was assessed across the various experimental groups. The immunohistochemistry score (IRS) was computed as the sum of the intensity

and percentage (proportion) scores for both staining intensity and the percentage of positively stained cells. The induction and vehicle groups showed sihnifiv=cant reductions in immunohistochemical expressions of filaggrin compared to controls.

Compared to healthy controls, the DNCB-induction and vehicle groups revealed substantially reduced filaggrin expression, with weak and discontinuous staining, indicating epidermal barrier degradation ($p < 0.05$). The tacrolimus-treated group exhibited substantial enhancements in filaggrin expression compared to the induction group. Likewise, sinapoyl choline (1%) treatment effectively corrected filaggrin expressions relative to the induction group ($p < 0.05$), resulting in greater staining intensity and a higher proportion of positively stained keratinocytes. A comparison of the sinapoyl choline and tacrolimus groups displayed no statistically noteworthy distinction in filaggrin immunoreactivity ($p > 0.05$), indicating that both therapies had equal effects on restoring epidermal barrier protein expression. **Table 5** and **Figure 8** summarize the findings by providing mean ± SD values for each parameter throughout the tested groups, while **Figure 9** displays representative images

## DISCUSSION

Atopic dermatitis is a widespread autoinflammatory and allergic skin disorder characterized by recurrent eczematous patches with redness, dryness, and itching triggered by a complicated interaction of cutaneous barrier breakdown, immunological disturbance, and oxidative damag (Hameed et al. 2020; Hassan et al. 2023). Considering the risks and negative side effects resulting from long-term administration of traditional medicines, investigating naturally occurring, plant-based, bioactive compounds became a key literature priority (Tahir ; Kim et al. 2023; He et al. 2025).

The current investigation employed a well-known mouse model in which repeated administration of the hapten DNCB effectively mimicked a human AD-like phenotype, as demonstrated by substantial increases in clinical dermatitis scores, increased concentrations of inflammatory Th2 cytokines (IL-4 and IL-13), elevated serum

IgE amounts, raised lipid peroxidation markers (MDA), and decreased skin antioxidant (GSH) and barrier protein (filaggrin) levels. Moreover, typical atopic dermatitis-like changes, such as hyperkeratosis, epidermal thickening, and inflammatory cell infiltration, were confirmed by histopathological examination. Taken together, these results confirm that the AD model was successfully established.

Increased serum IgE is characteristic of atopic dermatitis, with about 80% of patients displaying elevated IgE levels (Gether et al. 2025). Moreover, IL-4 and IL-13 are pivotal in the pathogenesis of AD by facilitating B-cell class switching to IgE, recruiting eosinophils, and sustaining allergic inflammation (Kim et al. 2014; Rossi et al. 2023). Upregulated IgE levels confirm systemic hypersensitivity and are strongly associated with disease severity (Brunner et al. 2017). The current results also showed a marked increase in MDA levels, suggesting increased oxidative damage and lipid peroxidation (Najim et al. 2007; Ji and Li 2016; Kvedariene et al. 2025b). Simultaneously, a significant reduction in GSH was observed, indicating weakened antioxidant defenses (Ji and Li 2016; Sharif et al. 2026b).

This study also found no statistically significant differences in major structural endpoints between the vehicle-treated group and the DNCB-induced untreated group in mice. Park et al. (2020) reported analogous findings, indicating no significant disparity in histopathological scores between the vehicle-treated and untreated groups (Park et al. 2020). Another study found that the total serum IgE and skin tissue IL-4, IL-13, and MDA levels were not significantly different between the groups treated with the vehicle and those that were not (Kim et al. 2014; Shallal et al. 2025).

Elevated Th2 cytokines, especially IL-4 and IL-13, which are known to suppress filaggrin expression and worsen barrier dysfunction, may be the cause of this persistent decrease in filaggrin, which is consistent with atopic dermatitis pathology (Weidinger and Novak 2016; Langan et al. 2020). These results imply that the pathological and immunological alterations in atopic dermatitis are not considerably impacted by vehicle treatment (Cho et al. 2015).

Topically applied corticosteroids are still a primary choice of therapy for atopic dermatitis and several other dermatoses (Ingurgio 2024; Khafaji et al. 2024), but the present investigation did not include them as a comparator group, which would provide a broader therapeutic context. The main goal was to compare sinapoyl choline to tacrolimus, a non-steroidal immune-modulating drug that is frequently prescribed for those whose prolonged steroid administration is restricted because of undesirable side effects including skin shrinkage and inhibition of the hypothalamic-pituitary-adrenal system (Pena et al. 2023; Al-Rajhi et al. 2025; Ghazy et al. 2026). Prospective research that incorporates corticosteroid-comparing agents would offer an increasingly detailed assessment of therapy.

Tacrolimus ointment 0.1% is a safe and efficient nonsteroidal macrolide option for long-term AD treatment. Tacrolimus is an effective topical immunomodulator that reduces T-lymphocyte activation and inflammatory cytokine production by inhibiting calcineurin phosphatase activity (Pascual and Fleisher 2004; Kraaijeveld et al. 2019; Umar et al. 2022). Topical tacrolimus has been shown in prior research to alleviate atopic dermatitis by inhibiting Th2 cytokines, specifically IL-4 and IL-13, which are key mediators of allergic inflammation and IgE production (He et al. 2019). Furthermore, a decrease in total serum IgE levels has been linked to tacrolimus treatment (Mo Ku et al.). The histopathological scores also revealed a significant decrease in epidermal thickness, as well as reductions in hyperkeratosis, parakeratosis, erosion, inflammation, and edema versus the DNCB induced group. This outcome is consistent with previous studies demonstrating a similar reduction in dermatitis severity scores following tacrolimus treatment (Walters and Lane 2020; Shallal et al. 2025).

The administration of 1% sinapoyl choline cream once daily for 21 days resulted in significant improvements in both macroscopic and microscopic disease characteristics. Comparative analysis indicated that the therapeutic effectiveness of 1% sinapoyl choline was partially similar to that of the clinical reference standard, 0.1% tacrolimus ointment, with no statistically significant differences identified between the two active treatments with respect to all studied parameters.

The observed decrease in serum levels of IgE, IL-4, and IL-13 suggests that sinapoyl choline may regulate Th2-mediated immune responses, which are critical components of atopic dermatitis pathogenesis (Kang et al. 2025). IL-4 is recognized for its ability to induce IgE class switching in B lymphocytes and, in conjunction with IL-13, to enhance allergic inflammation, impair skin barrier function, and facilitate the recruitment of inflammatory cells (Bernstein et al. 2023). Sinapoyl choline is a choline ester of sinapic acid, a polyphenolic constituent recognized for its anti-allergic actions, which are ascribed to its capacity to inhibit critical mediators such as IL-4, IL-13, and IgE (Saeedavi et al. 2022a). The inhibition of the allergic immunological responses seen with sinapoyl choline therapy in this research could thus be attributable, at least partially, to the same fundamental mechanisms. This immune-modulatory impact is expected to have contributed to the amelioration in clinical symptoms, as well as the notable decrease in histological abnormalities and skin inflammation reported in treated mice. Besides, the decline in IgE levels provides evidence for the concept that sinapoyl choline efficiently controls Th2-driven immune responses, thus mitigating AD severity. These results are in line with earlier research showing sinapoyl choline reduced Th2 cytokines and IgE (Saeedavi et al. 2022b).

Regulation of important oxidative and inflammatory signaling mechanisms might provide a molecular explanation for the medical properties of sinapoyl choline. Sinapoyl choline probably suppresses the production of pro-inflammatory cytokines like IL-4 and IL-13 through inhibiting NF-κB activation (Gong et al. 2023). Furthermore, filaggrin expressions could potentially be restored, and Th2 polarization may be reduced as a result of STAT6 signaling blockade (Dębińska 2021a).

The reported reduction in MDA production, together with the enhancements of GSH activity, suggests that sinapoyl choline has a robust antioxidative impact. This outcome implies that sinapoyl choline efficiently attenuates lipid peroxidation while also boosting endogenous antioxidant defenses, hence lowering oxidative stress-related skin damage (Styrczewska et al. 2019; Yang et al. 2020). The decline in MDA levels could be related to sinapoyl choline's ability to suppress reactive ROS-induced lipid peroxidation, most likely via its phenolic architecture obtained from sinapic acid, which allows for the elimination of free radicals ("Sinapine-enriched rapeseed oils reduced fatty liver formation in high-fat diet-fed C57BL/6J mice - RSC Advances (RSC Publishing) DOI:10.1039/D0RA00215A" ; Li et al. 2022). In parallel, an increase in GSH concentrations may occur from a restoration of intracellular redox balance, either by diminishing oxidative burden or by stimulating antioxidant defense mechanisms, such as GSH formation and regeneration (Sharquie et al. 2005; Liu et al. 2024; Zhang et al. 2024). Furthermore, suppressing cutaneous inflammation could indirectly assist in alleviating oxidative injury since inflammatory cells are an important supplier of ROS in allergic and inflammatory dermatoses (AlBairmani et al. 2025; Kvedariene et al. 2025a). Together, these immune-modulatory and antioxidant effects can boost the integrity of the epidermal barrier and lessen the clinical manifestations associated with atopic dermatitis.

More importantly, sinapoyl choline therapy yielded substantially decreased observational severity scores and histopathological features, such as acanthosis, hyperkeratosis, parakeratosis, epidermal erosion, and extracellular edema, relative to both the induction and vehicle-treated groups. Alongside this, there was a significant decrease in parakeratosis, which may indicate that aberrant keratinocyte proliferation and epidermal thickening were suppressed (Langan et al. 2020).

Filaggrin is a structural protein necessary for hydration, defense against environmental allergens, and maintenance of the epidermal barrier’s integrity (Dębińska 2021b). It's notable that filaggrin expression is negatively regulated by IL-4 and IL-13, which also contribute to epidermal barrier dysfunction in atopic dermatitis. Thus, elevated IL-4 and IL-13 cytokine activity may contribute to epidermal dysfunction in atopic dermatitis by downregulating filaggrin and other barrier-related genes in human skin (Furue 2020; D'Avino et al. 2026).

There were no visible signs of skin irritation, worsening of erythema, or adverse reactions in the animals administered sinapoyl choline in this study. However, it deserves to be pointed out that sinapoyl choline was designed in a custom cream base, while tacrolimus was given as a commercial ointment. Variations in vehicle composition could have an impact on penetration of the skin and local pharmacokinetics, thus influencing therapeutic effectiveness. This is a research weakness. Furthermore, the lack of a healthy control group

receiving sinapoyl choline restricts conclusions on its local tolerability in non-irritated skin. However, despite no obvious irritations being noted, a full safety assessment warrants additional examination. Moreover, a full toxicological evaluation, including studies on skin toxicity and systemic absorption, is needed before it can be used in humans.

## STUDY LIMITATIONS

This study has certain limitations that should be acknowledged when interpreting the findings. The use of a single concentration (1%) of sinapoyl choline without evaluation of a dose–response relationship limits the ability to determine the optimal therapeutic dose. The absence of comprehensive toxicity evaluation, including dermal irritation testing and systemic exposure assessment, restricts conclusions regarding the safety profile of topical sinapoyl choline. The formulation was not subjected to detailed physicochemical characterization (e.g., stability, permeability, drug release kinetics), which may influence translational applicability. Mechanistic insights remain limited, as key signaling pathways (e.g., NF-κB, STAT6, and MAPK) were not directly evaluated at the molecular level, making the proposed mechanisms largely inferential.

The DNCB-induced murine model, while widely used, does not fully replicate the complexity of human atopic dermatitis, particularly regarding genetic predisposition and microbiome interactions, thereby limiting direct clinical extrapolation. Despite sinapoyl choline having already been researched in several experimental modalities, clinical trials are required to demonstrate its therapeutic efficacy in humans. The current study provides crucial preclinical evidence for its possible function in AD; however, additional research, including clinical trials, is needed to validate these results. Furthermore, future studies should address current limitations by incorporating dose optimization, extended treatment duration, mechanistic validation, safety profiling, and potentially translational clinical investigations.

## CONCLUSION

Topically applied sinapoyl choline exhibited considerable anti-inflammatory, antioxidant, and barrier-restorative properties in a DNCB-induced murine model of atopic dermatitis. These results indicate that sinapoyl choline may be a promising candidate for further exploration as a topical therapeutic agent. Nonetheless, due to the preclinical nature of this study, further investigation is necessary to clarify its molecular mechanisms, refine dosing strategies, determine safety profiles, and validate efficacy in clinical environments.

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### Conflict of Interest

There is no competing interest to declare.

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### Author Contributions

Noor A. Abdulredha was responsible for conceptualization, methodology, experimental work, data collection, data analysis, and drafting of the manuscript. Ahmed R. Abu-Raghif contributed to study design, supervision, critical revision of the manuscript, and final approval of the version to be published. All authors have read and approved the final manuscript.

**Data Availability Statement**

All data generated or analyzed supporting the findings of this study are available from the corresponding author upon reasonable request.

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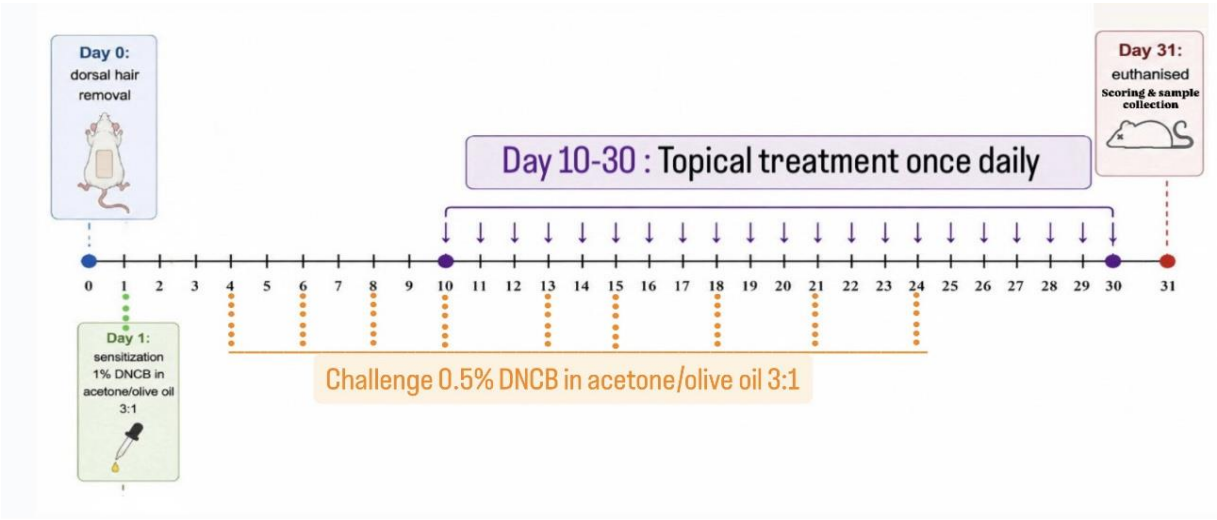



**Figure 1**. **Schematic representation of the study design**. Mice were first sensitized with 1% DNCB on day 1. Starting from day 4, they received 0.5% DNCB three times weekly for three weeks. Beginning on day 10, they were treated topically once daily for three weeks with 1% sinapoyl choline, a vehicle cream, and 0.1% tacrolimus. On day 31, the mice were assessed using SCORAD and then sacrificed for blood and skin sample collection.

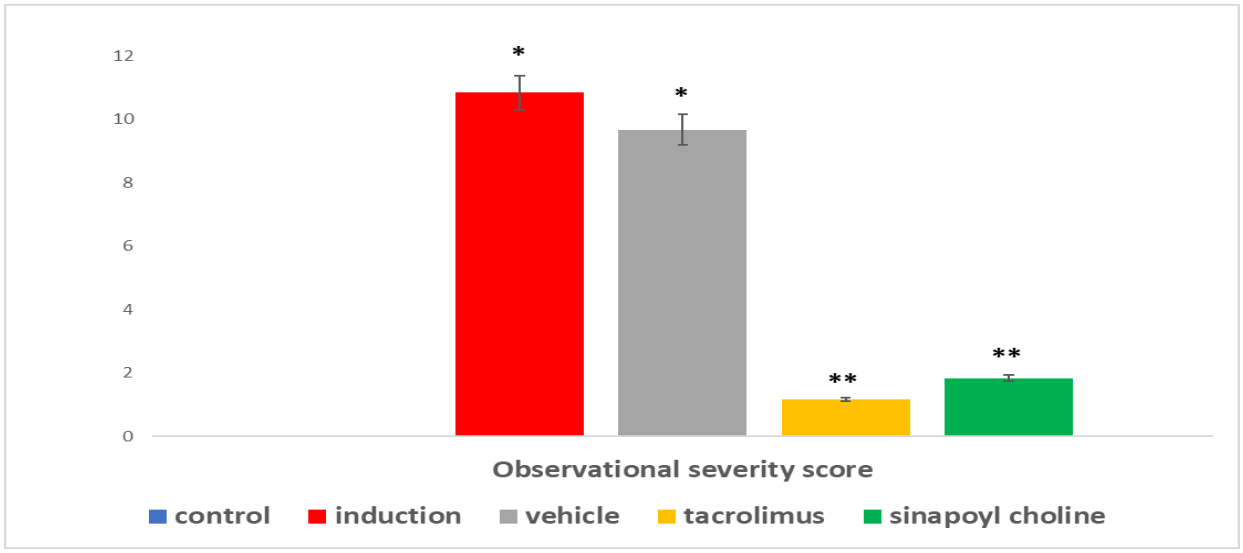



**Figure 2: Impact of tested agents on observational severity scores.** Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.

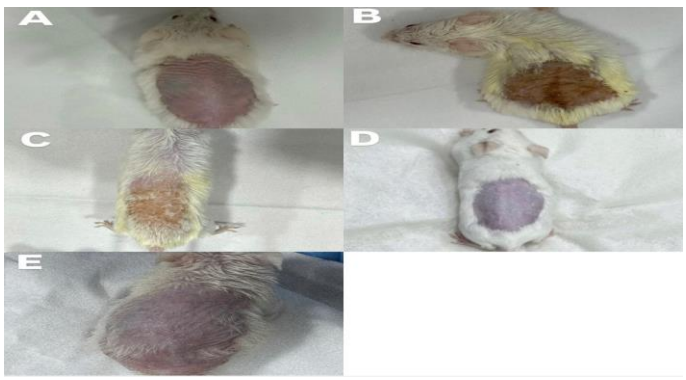
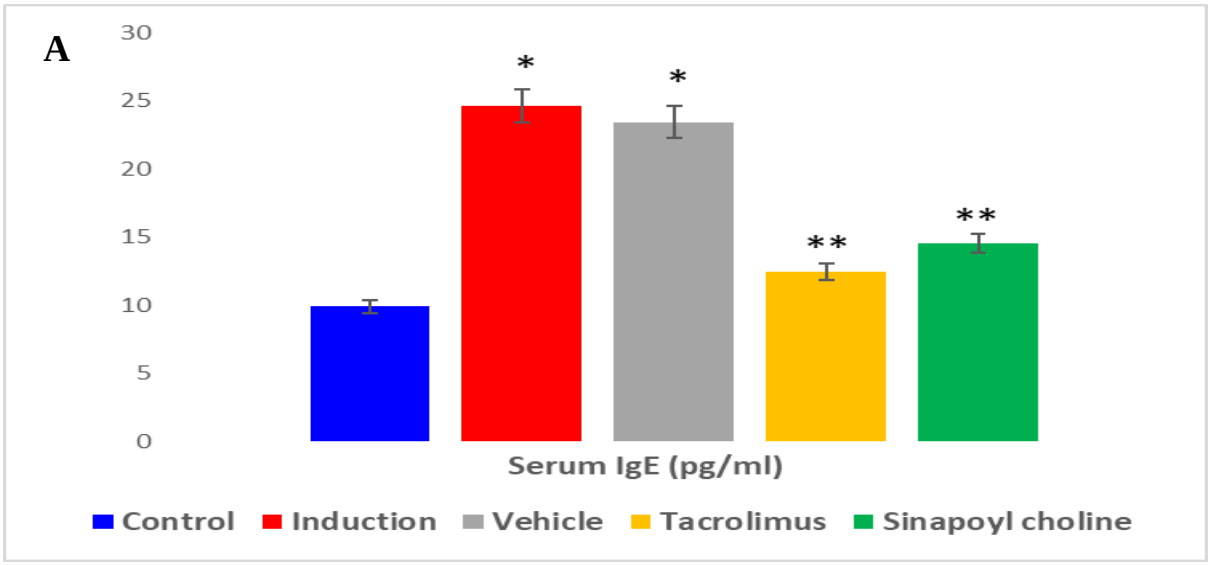


**Figure 3:** Images showing the severity of eczematous lesions in animals from various study groups. A: Control group. B. Induction group C. Vehicle group D. Tacrolimus E. Sinapoyl choline



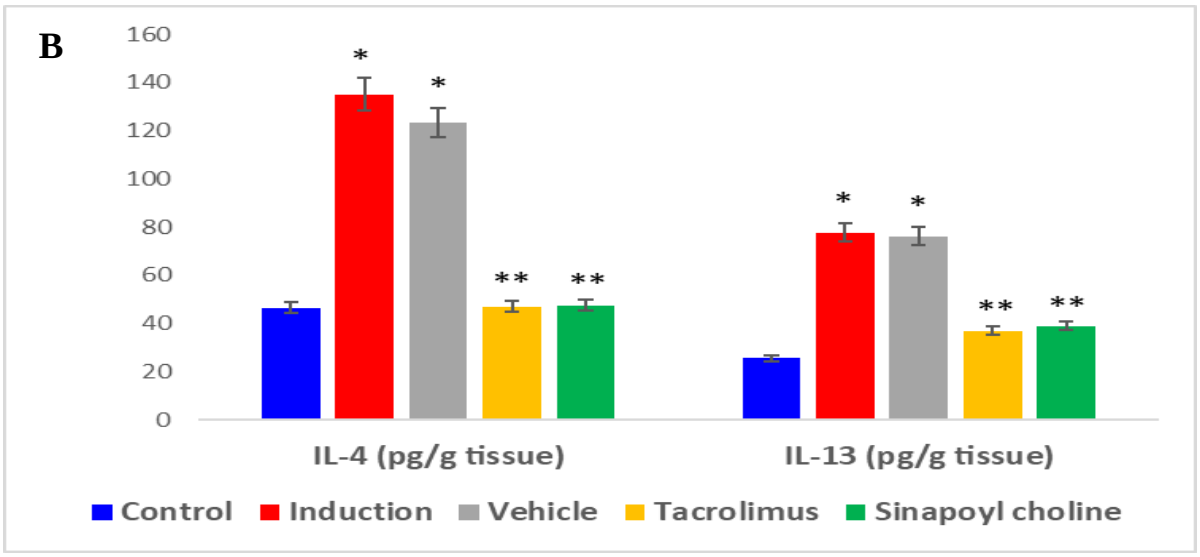
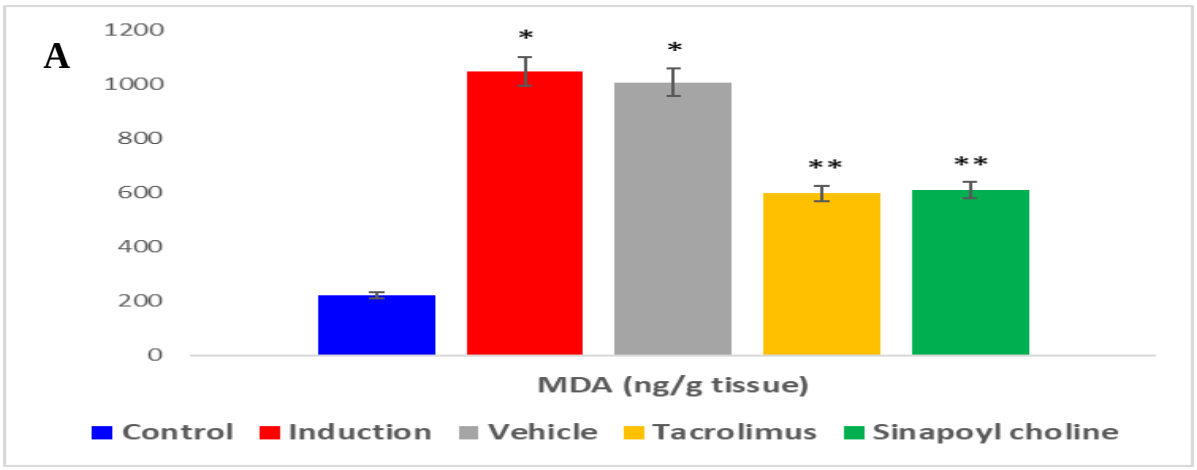




**Figure 4**: **Effects of tested agents on serum IgE and tissue IL-4 and IL-13 (A)** Effect of tested agents on serum IgE levels. **(B)** Effect of tested agents on skin tissue IL-4 and IL-13 levels in skin tissues. Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.



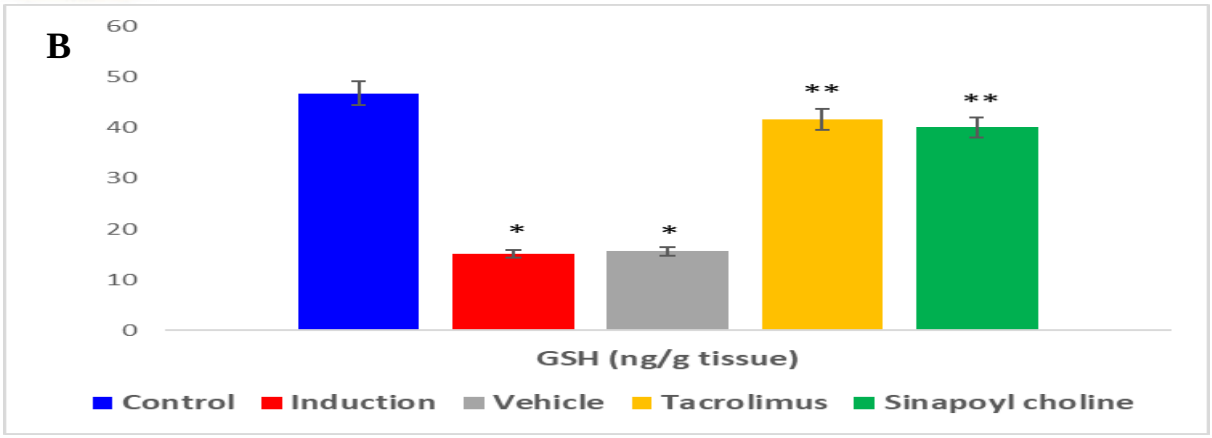



**Figure 5**: **Effects of tested agents on oxidative stress indicators (A)** Effect of tested agents on MDA levels. **(B)** Effect of tested agents on GSH levels. Data are displayed as mean ± SD. *= indicates significant differences ($p < 0.05$) compared to the control group; ** indicates significant differences ($p < 0.05$) versus the induction group.

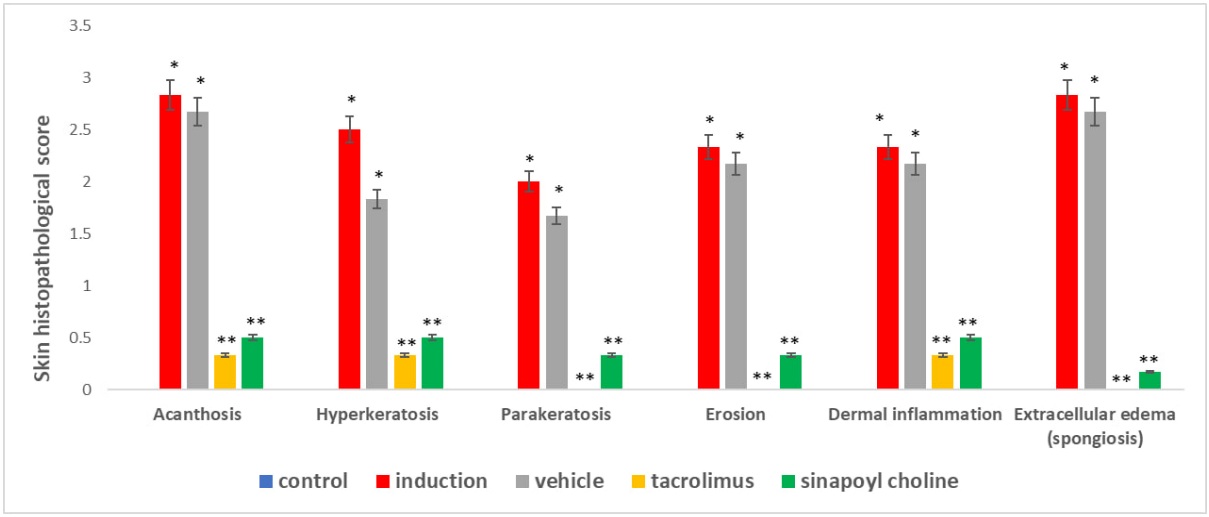
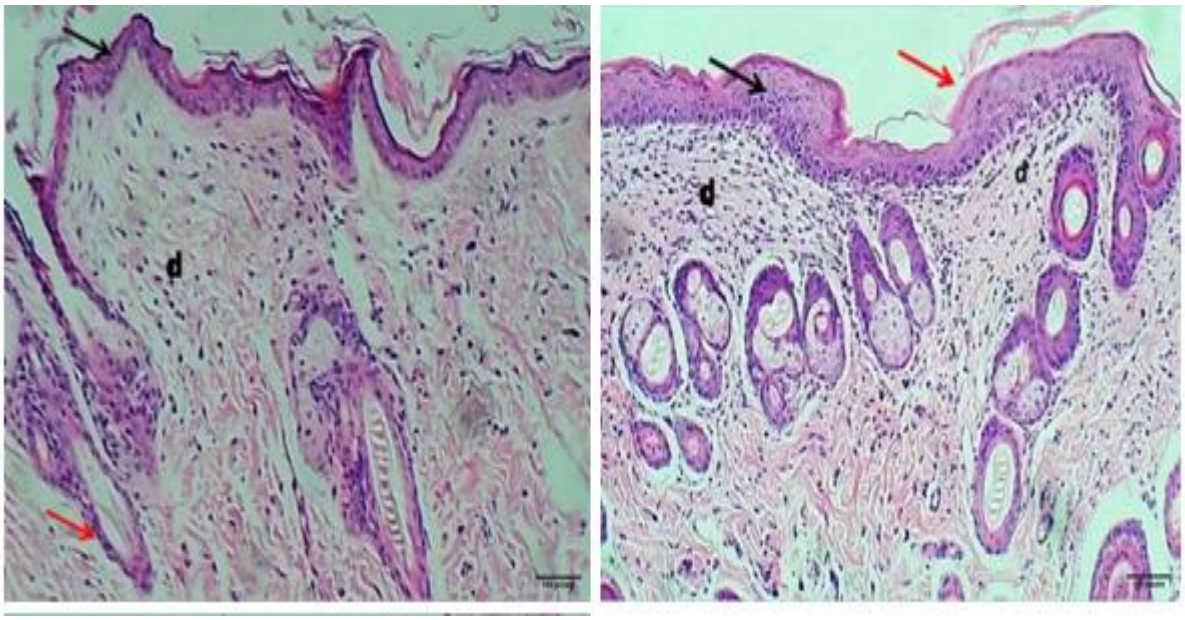



**Figure 6:** Impact of tested agents on skin histopathological score. Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.



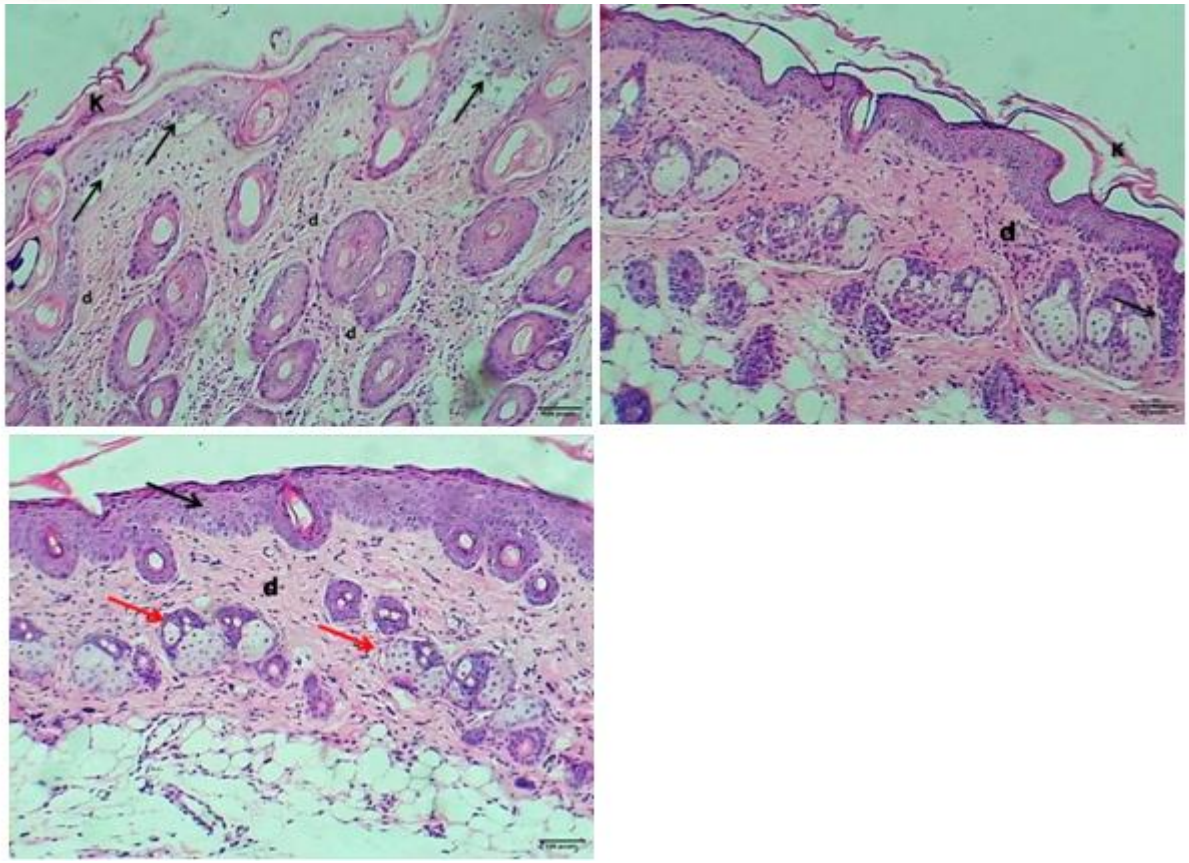



**Figure 7: Impact of studied agents on mouse skin histopathology alterations. (A) Cross-section of skin under light microscopy for a healthy control** presenting normal appearance of epidermis lining cells with normal keratin layer (black arrow), dermis fibrous tissue (yellow arrow), and hair follicles (red arrow). H&E stain, 100x. (**B). Cross-section of skin for induction (DNCB) group** showing acute atopic dermatitis with marked acanthosis with spongiosis featuring little hyperkeratosis (red arrow) and fibroplasia of dermis fibrous tissue with active angiogenesis (d), H&E stain, 100x (**C). Cross-section of skin for vehicle group** revealing severe degeneration with necrosis of lining cells and spongiosis from formation of epidermis (black arrow), hyperkeratosis and parakeratosis (k) & dermal papillae formation that revealed angiogenesis with perivascular and perifollicular infiltration of leukocytes (d). H&E stain. 100x. **(D). Cross-section of skin for the standard (tacrolimus) group** demonstrating little focal acanthosis of lining cells of the epidermis (black arrow), little hyperkeratosis (k), and dermis fibrous tissue infiltrated with leukocytes (d). H&E stain. 100x. **(E). Cross-section of skin for the sinapoyl choline group** exhibiting normal stratum basale cells of the epidermis with normal stratum corneum (black arrows) and normal dermis fibrous tissue (d). & related sebaceous glands (red arrows). H&E stain. 100x. Scale bar = 100 µm.

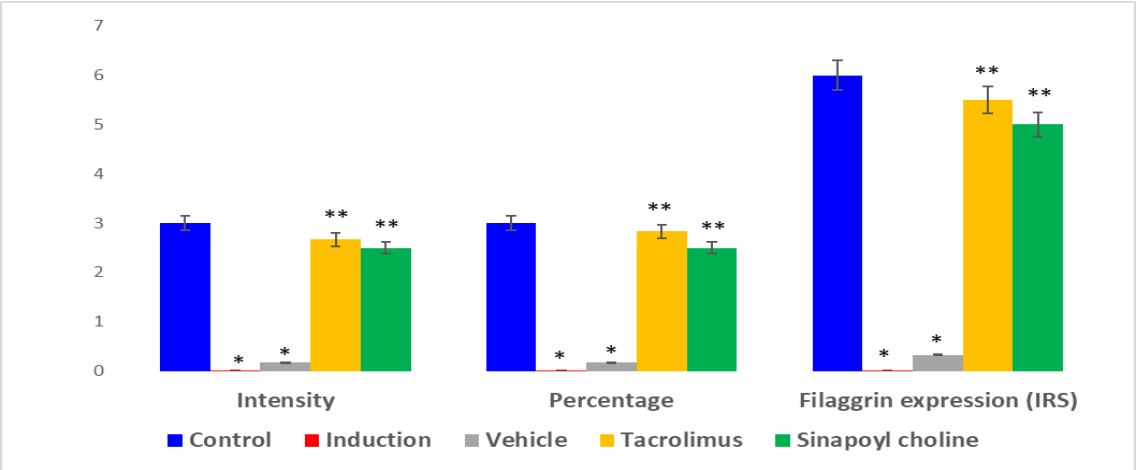



**Figure 8**: Effects of tested agents on immunohistochemistry scores of filaggrin expression in experimental atopic dermatitis model. Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$)

compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.

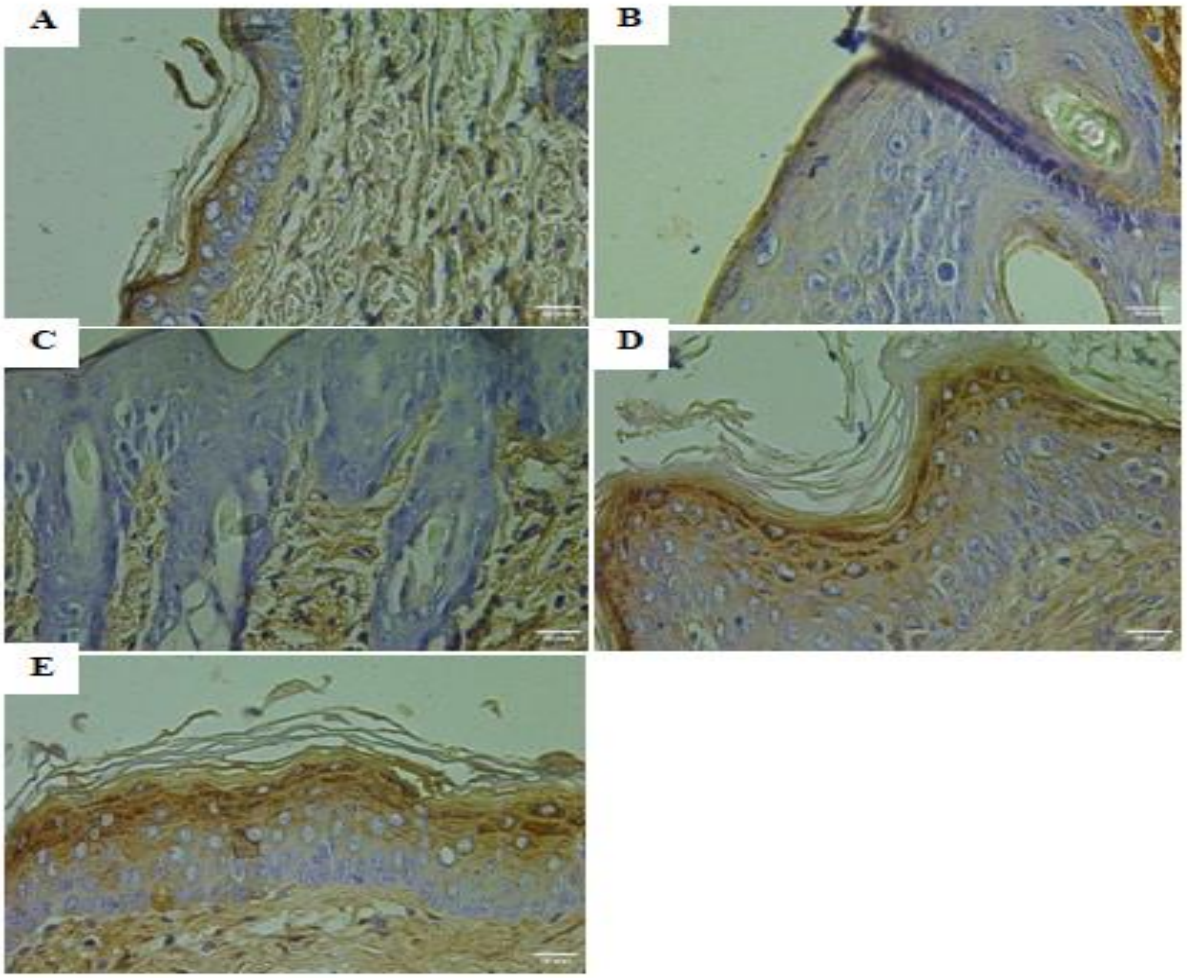



**Figure 9: Representative immunohistochemical staining of filaggrin expression in the skin of the experimental groups. (A)** The healthy controls had prominent cytoplasmic staining in the epidermis, notably in the stratum granulosum and stratum corneum (×400). **(B)** The Induction (DNCB-induced) group exhibited significantly reduced filaggrin expression in epidermal keratinocytes (×400). **(C)** The vehicle group demonstrated low filaggrin expression in epidermal keratinocytes (×400). **(D)** The standard (0.1% tacrolimus) group showed significant restoration of cytoplasmic filaggrin expression in the epidermis (×400). **(E)** The sinapoyl choline-treated group displayed high cytoplasmic filaggrin expression in the epidermis, affecting over 80% of keratinocytes (×400). Scale bar = 100 µm.

**Table 1:** Effects of tested agents on observational severity scores. Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.

| variables | Groups (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Healthy control | Induction (DNCB) | Vehicle | Tacrolimus | Sinapoyl choline |
| Observational severity scores | 0 ±0 | 10.83±0.75 | 9.66±0.12 | 1.16±0.07 | 1.83±0.07 |

**Table 2:** Effects of tested agents on anti-inflammatory markers (serum IgE, skin tissue IL-4, IL-13). Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.

| Variables | Groups (Mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Healthy | Induction (DNCB) | Vehicle | Tacrolimus | Sinapoyl choline |
| Serum IgE(pg/ml) | 9.90 ± 2.97 | 24.62± 1.52* | 23.43± 2.06* | 12.46± 3.42** | 14.54±4.31** |
| IL-4 (pg/g tissue) | 46.61±13.09 | 135.05±10.58* | 123.39±12.84* | 47.07±12.16** | 47.63±14.90** |
| IL-13 (pg/g tissue) | 25.61±6.65 | 77.78±13.22* | 76.10±12.90* | 37.04±8.82** | 39.07±8.76** |

**Table 3:** Effects of tested agents on oxidative stress markers (MDA and GSH). Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.

| Variables | Groups (Mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Healthy | Induction (DNCB) | Vehicle | Standard | Sinapoyl Choline |
| MDA (ng/g tissue) | 221.07±25.33 | 1045.61±165.59* | 1006.46±90.37* | 595.75±131.89** | 608.13±129.53** |
| GSH (ng/g tissue) | 46.80±9.37 | 15.10±4.35* | 15.59±4.64* | 41.62±11.80** | 40.08±9.44** |

**Table 4:** Effects of the investigated treatments on skin histopathological scores. Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group.

| variables | Groups (mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Healthy control | Induction (DNCB) | Vehicle | Standard (tacrolimus) | Sinapoyl choline |
| Acanthosis | 0±0 | 2.8±0.4* | 2.6±0.5* | 0.3±0.5** | 0.5±0.5** |
| Hyperkeratosis | 0±0 | 2.5±0.5* | 1.8±0.7* | 0.3±0.5** | 0.5±0.5** |
| Parakeratosis | 0±0 | 2.0±0.6* | 1.6±0.5* | 0±0** | 0.3±0.3** |
| Erosion | 0±0 | 2.3±0.8* | 2.1±0.9* | 0±0** | 0.3±0.3** |
| Dermal inflammation | 0±0 | 2.3±0.5* | 2.1±0.7* | 0.3±0.5** | 0.5±0.5** |
| Extracellular edema (spongiosis) | 0±0 | 2.8±0.4* | 2.6±0.5* | 0±0** | 0.1±0.4** |

**Table 5:** Effects of tested agents on immunohistochemical scores of filaggrin expression. Data were expressed as Mean ± SD. Data are displayed as mean ± SD. *=indicates significant differences ($p < 0.05$) compared to the control group; **=indicates significant differences ($p < 0.05$) compared to the induction group. IRS = immunohistochemical score.

| variables | Groups (Mean ± SD) | | | | |
|---|---|---|---|---|---|
| | **Healthy** | **Induction** | **Vehicle** | **Tacrolimus** | **Sinapoyl Choline** |
| **Intensity** | 3.00±0 | 0.33±0.05* | 0.67±0.08** | 2.67±0.51** | 2.50±0.54** |
| **Percentage** | 3.00±0 | 0.33±0.05* | 0.50±0.05* | 2.83±0.40** | 2.50±0.54** |
| **IRS=I+P** | 6.00±0 | 0.67±0.10* | 1.17±0.13* | 5.50±0.83** | 5.00±0.89** |