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Research Article

The mitigating effect of a topical preparation of amlodipine on imiquimod-induced psoriasis-like lesion in mice

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Abstract

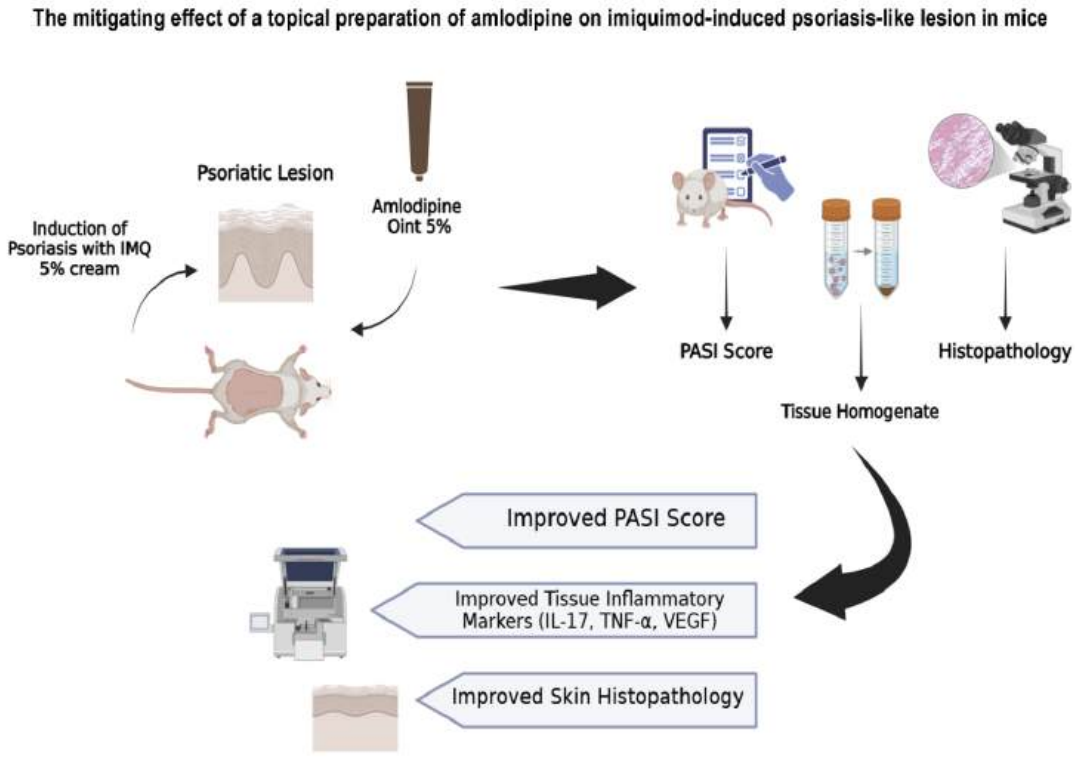
Introduction: Psoriasis is a chronic inflammatory skin disease depicted by deforming, inconsistent penetrations, and repeated proliferative and inflammatory skin ailment. Local treatment with topical agents is one of the substantial modalities in the remediation of this ailment. Efforts are directed toward the development of novel efficacious topical medicaments. The study examined the potential anti-psoriatic effect of topical amlodipine as a 5% ointment in mice based on observational, histopathological, and biomarker outcomes.

Materials and Methods: Forty-five male Swiss Albino mice were randomly allocated into five distinct groups (I-V), with 9 mice in each group (n=9) that underwent shaving of the dorsal hair. In Group I; animals served as control, while animals of other groups received imiquimod (IMQ) on their shaved backs for six consecutive days to induce psoriasis. Groups from III to V received continuous application of IMQ following day 6 along with a vehicle, clobetasol, and amlodipine 5% ointment for 8 consecutive days. During the timeline of the experiment, observation changes were assessed daily, followed by animal euthanasia and sample collection for biochemical and histopathological evaluation.

Results: Topical amlodipine ointment markedly reduced the inflammatory signs of psoriatic lesions, and histopathological inspection confirmed these findings. The levels of interleukin-17 (IL-17), tumor necrosis factor-alpha (TNF-a), and vascular endothelial growth factor (VEGF) were significantly ameliorated by topical amlodipine Ointment in comparison with the untreated imiquimod-induced psoriatic mice group.

Conclusion: The study concludes that topical amlodipine showed an anti-psoriatic effect comparable to that of topical clobetasol owing to antioxidant and anti-inflammatory effects making amlodipine a promising agent in the future of psoriasis management as an adjuvant treatment to the standard.

Graphical abstract



Keywords

amlodipine, calcium channel blockers, vascular endothelial growth factor, interleukin-17, psoriasis, imiquimod

Introduction

Psoriasis is considered a chronic skin condition of complicated etiologies mainly relating to a combination of genetic, immunological, and environmental factors; it is described by inconsistent penetrations, deforming, and repeated inflammatory and proliferative ailment of skin, which overall affects 1% ~ 3% of people worldwide at any age, but occurs most frequently in two age groups: the first age group is between 20 and 30 years and the second is between 50 and 60 years (Alwan and Nestle 2015; Saleh et al. 2022). Clinical features of psoriatic lesions involve fully demarcated, symmetrical erythematous plaques with adherent silvery or white scale from hyperproliferation of epidermal keratinocytes. It commonly affects the scalp and extensor surfaces (Sarac et al. 2016).

Pathogenesis of psoriasis is complex. In the last three decades, hundreds of clinical studies and research have been done, but the pathogenesis of the disease is still not completely understood. Pathophysiology of psoriasis comprises abnormal proliferation of keratinocytes and dermal and epidermal infiltration of immune cells which involve both innate (nonspecific) as well as adaptive (specific) immune system components, with dendritic cells and T cells playing the most crucial role (Balak and Hajdarbegovic 2017; Ni and Lai 2020).

Following the exposure to some inciting event, either of infectious or of traumatic origin, epidermal activation of the innate immunity cells involving the natural killer (NK) T lymphocytes, the plasmacytoid dendritic cells (pDC), and neutrophils occurs. These cells work in the production of several pro-inflammatory cytokines, including tumor necrosis factor (TNF-α) alpha and interferon-α (IFN-α), which in turn, stimulate the activation of the myeloid dendritic cells in the skin tissue to produce cytokines that support T lymphocyte (CD4+) cells evolution and the proliferation into the T helper types 1 and 17 (Th1 and Th17) by the action of nonspecific immune system, this process followed by specific immune system activation; Th1 cells work in releasing TNF-α, IL-2 and IFN-γ which, in turn, cause amplification in the inflammatory cascade, acting on keratinocytes and dermal dendritic cells. T helper 17

(Th17) cells generate Interleukins including IL-17, IL-21, as well as IL-22, which, in turn, leads to the activation of the JAK/STAT pathway and promotes the development of epidermal keratinocytes (Alwan and Nestle 2015; Calautti et al. 2018; Alsamarai et al. 2019).

The angiogenesis induced by vascular endothelial growth factor (VEGF) also represents a pathogenetic mechanism in psoriasis (Abu-Raghif et al. 2015c); microvascular alterations are promoted by VEGF in the dermal papillae; these, in turn, facilitate the development and insistence of psoriatic lesions. It has been observed that psoriatic patients exhibit an elevation in the serum levels of VEGF and that the disease severity of psoriasis is correlated with serum VEGF levels (Marina et al. 2015). The overexpression of VEGF in psoriatic patient's skin biopsies represents evidence for VEGF's important role in taking part in the epidermis barrier normal function and for the presence of a linkage between epidermal VEGF and hyperplasia of keratinocytes. Hence, it may be sustained that VEGF is a key factor in the proliferation of keratinocytes (Sankar et al. 2017). Detmar et al. (1994) revealed that VEGF *in vitro* persuades mitotic activity of the keratinocytes and upregulates the vascular endothelial growth factor receptors (VEGFR-1) and (VEGFR-2) expression in both the endothelial cells (ECs) and keratinocytes. VEGFR-1 and 2 are both perceivable in the dermal lesions of psoriasis patients (Li et al. 2023).

Amlodipine is a dihydropyridine member of third-generation long-acting calcium channel blockers (CCBs) that was approved by the FDA in 1987 to be prescribed as a monotherapy or in combination with other drugs to treat hypertension in adults and children (6 years and older), Prinzmetal angina and angiographically documented coronary artery disease (Kaya et al. 2018). Several research studies concluded that amlodipine has the potential to inhibit inflammatory cytokines by lowering the concentration of intracellular calcium and the enhancement of antioxidant defenses (El-Morsy et al. 2015). Furthermore, by inhibiting the calcium flow into fibroblasts, amlodipine can suppress the growth and proliferation of fibroblasts, and the synthesis of extracellular matrix proteins (collagen, fibronectin, and proteoglycans) (Huang et al. 2014). All these properties appear useful for the treatment of keloids and hypertrophic scars and may also be relevant for the prevention of necrosis in skin flaps (Cohen et al. 2001; Ogretmen et al. 2014).

The current study is directed towards examining the potential anti-psoriatic effect of a topical preparation of amlodipine and compared to the standard clobetasol, through evaluation of different tissue biomarkers and histopathological changes.

Materials and Methods

Chemicals and reagents

Chemicals and reagents used in this study were purchased from different sources: Imiquimod 5% cream as sachets (Aldara™, MEDA pharmaceuticals, Sweden); Clobetasol 0.05% ointment (Dermovate™, GSK Pharma, United Kingdom); Xylazine HCl (XYL-M2, VMD ® Livestock Pharma, Belgium); Ketamine HCl (Ketamine 10%, Alfasan Nederland BV, Netherlands); Formaldehyde 37% (Sigma-Aldrich, Germany), and tissue lysis buffer Thermo Fisher Scientific, USA). All mouse ELISA kits were purchased from MyBioSource (USA).

Preparation of topical 5% ointment of amlodipine

Amlodipine ointment 5% was prepared by accurately measuring 6.9 grams of amlodipine besylate powder (Pioneer Co. for Pharmaceutical Industries, Sulaymaniyah, Iraq), which is equivalent to 5 grams of amlodipine, and dissolving it in propylene glycol (Sigma-Aldrich, Germany) in a 1:1 ratio in a water bath with continuous stirring to ensure dissolution. The preparation was subsequently combined with an ointment base consisting of Vaseline® (Sigma-Aldrich, Germany) and Lanoline (Skyrun Industrial Co. Ltd., China) in a 1:1 ratio by adding 47.5 grams of each. The mixture was then continuously stirred at room temperature until a homogeneous cream was obtained (Sheraz et al. 2016).

Experimental animal care and housing

A total of 45 male albino Swiss mice were purchased from the animal house of the College of Medicine / Al-Nahrain University, Baghdad, Iraq. Animals were selected based on the following criteria (healthy fur with no visible cuts or skin lesions, active and energetic, age ranging from 8 – 10 weeks, and weight ranging from 28 – 32 gm). Animals were allocated in the animal house of the College of Medicine / Al-Nahrain University and kept in three separate large cages of 15 animals each to help them acclimatize to the new environment for one week. Mice were fed a standard diet pellet of one portion daily with free access to clean water (water bottles were cleansed and refilled daily). Sterile wood shavings were used for cage padding and were changed every other day. The environment of the animal house was controlled at a temperature of 25°C ± 2 and a humidity level between 45 – 60% with 12-hour interval light/dark cycles.

Study design and settings

The mice were randomly re-allocated after the period of acclimatization into five groups (I – V) of 9 animals each (n = 9) using block randomization. Ahead of beginning the experimental procedures, the animals were inspected for any skin abnormalities. Only mice with visibly healthy fur and skin were selected for the present study. All the animals included in the study underwent shaving of the dorsal region to expose a certain portion of the back skin measuring approximately 1x2 cm. An electric razor was used for this purpose. Subsequently, a hair removal cream was applied, and the shaving process was finalized by using gauze to wipe any remaining hair.

Each group underwent a different experimental treatment plan to compare the outcomes in an experiment that lasted for 14 days in total. Group I served as healthy control, in which the animals did not receive any intervention. Group II served as the induction, in which the animals received a daily application of imiquimod (IMQ) 5% cream on the animal's shaved backs for 6 days with no other medical intervention (van der Fits et al. 2009). Group III served as the vehicle (ointment base only). Group IV served as the standard treatment (clobetasol 0.05% ointment) (Carvalho et al. 2015) and Group V served as the test treatment (amlodipine 5% ointment). Groups III through V received a daily topical application of IMQ 5% cream for 6 days, followed by continuous daily application of IMQ together with

ointment base and amlodipine 5% ointment twice daily with an interval of 9 hours (7 AM and 4 PM), and clobetasol propionate 0.05% ointment once daily (at 7 AM) for 8 days to complete a timeline of 14 days as shown in Figure 1.

Induction of psoriasis-like lesion

Animal groups subjected to induction of psoriasis-like lesions with IMQ were inspected at the beginning for their general appearance and health. An area of 1x2 cm on the dorsal back of each animal was shaved and the remaining hair was removed using a hair remover cream that was applied for 15 seconds and then removed with the aid of medical gauze to minimize skin irritation. The area was then cleansed with warm water followed by a swab of 70% alcohol; then the animals were left for 24 hours before starting the application of IMQ. Induction of psoriasis-like lesions was done by applying a once-daily dose of 6.25 mg of IMQ 5% cream only to the shaved backs for 6 consecutive days (van der Fits et al. 2009). Following this time, animals that developed clear and visible signs of skin thickness, erythema, and evident scaling (Baek et al. 2012) were included for the remaining part of the experiment to receive IMQ and the topical intervention treatment, whereas animals that did not show clear psoriatic-like manifestations or with deteriorated health were excluded and kept in separate cages.

Assessment of psoriasis area severity index (PASI) score

The PASI clinical scoring system is a semiquantitative observational measure that was used to quantify the inflammation levels in the dorsal skin of mice. This was done to evaluate the success of the induction models and the effectiveness of the treatment plans. The process involved visually evaluating three distinct characteristics on the back skin of each of the sampled mice: erythema (redness), induration (thickness), and desquamation (scaling). Each attribute was assigned a numerical value on a scale of 0 to 4. A value of 0 represents an attribute's absence, while a value of 1 indicates a modest presence, 2 indicates a moderate presence, 3 indicates a marked presence, and 4 indicates a highly prominent presence. The outcome was a cumulative score that had a potential range of 0 to 12 (Fredriksson and Pettersson 2009). The whole assessment was done with the help of a dermatologist who was unaware of the animal grouping and the type of interventions.

Animal euthanasia and sample collection

Animals' euthanasia was done on day 15 of the experiment. Animals received a high dose of intraperitoneal (IP) anesthesia combination of ketamine (80mg/kg), and xylazine (10mg/kg), followed by cervical dislocation (Underwood and Anthony 2020). Skin tissues were excised and washed with phosphate buffer saline (PBS) then dried on filter paper and cut into two parts, one fixed in 10% formaldehyde for histopathological analysis, and the other part was kept in Eppendorf tubes and stored at -20°C to make tissue homogenates for further evaluation of tissue biomarkers (Hussein et al. 2024a).

Enzyme-linked immunosorbent assay (ELISA) technique

Skin tissue homogenate was prepared by adding 1 mL of cold homogenization buffer that contained 1 mM PMSF, one mcg/mL pepstatin A, one mcg/mL aprotinin, and one mcg/mL leupeptin in phosphate-buffered saline, pH 7.2, with 0.05% sodium azide and 0.5% Triton X 100 to 100 mg of skin tissue, followed by homogenization with an electrical tissue homogenizer (Staruar®, England) using an ice bucket to keep the sample cold. Homogenate samples were then centrifuged at 12,000 rpm for 10 min. Then, the supernatant was collected in Eppendorf tubes and stored at -20°C for later analysis (Calkins et al. 2001; Hussein et al. 2024b).

Biomarkers of skin tissue homogenate samples were assessed using the ELISA technique. Frozen samples were left to thaw at room temperature and processed based on the manufacturer's kit protocol. The biomarkers included in this study are tumor necrosis factor-alpha (TNF-α), interleukin-17 (IL-17), and vascular endothelial growth factor (VEGF), to which the sandwich ELISA protocol was applied. The absorbance was measured using a microplate reader (HumaReader®, USA) at a wavelength of 450 nm. The concentration of each sample was determined according to the standard curve supplied by each manufacturing kit. Analysis of the homogenate samples was done in a blinded

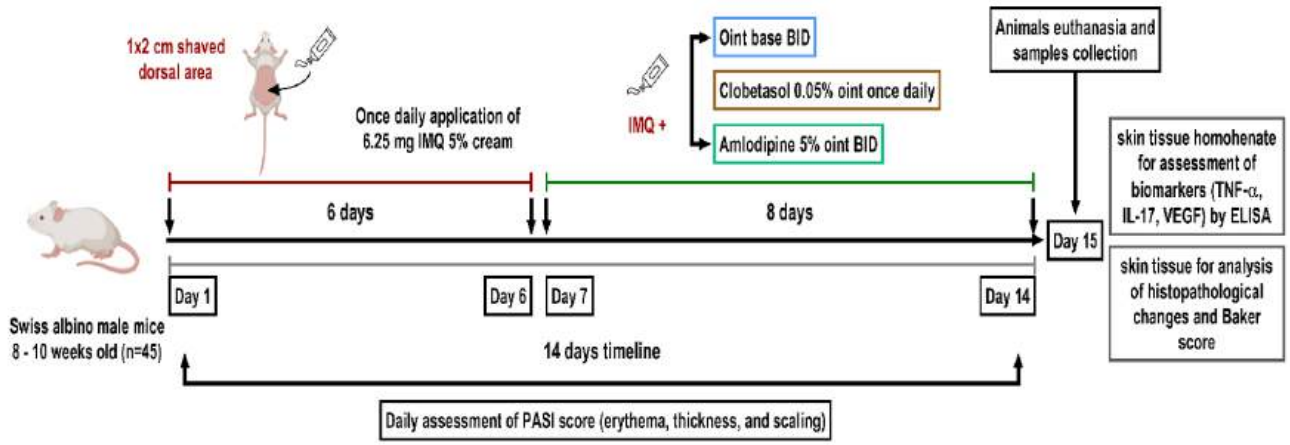


Figure 1. Flow chart of the study design timeline with interventions. ***Note:*** IMQ – imiquimod, oint – ointment, BID – twice daily, TNF-α – tumor necrosis factor-alpha, IL-17 – interleukin-17, VEGF – vascular endothelial growth factor, PASI – psoriasis area severity index.

manner to the bio-analyst.

Histopathological examination and scoring

One part of the excised skin tissue was fixed in 10% buffered formalin for 24 - 48 h at room temperature and then processed to obtain the final tissue-paraffin-embedded blocks. Sections (thickness of 5 µm) would be stained with hematoxylin and eosin (H&E). Two pathologists blinded to the animal groups of the experiment assessed histopathological changes, including the Baker's score of severity of skin inflammation, which is a semiquantitative scoring system ranging from 0 to 10 for the assessment of epidermal thickness, parakeratosis, hyperkeratosis, Munro abscess, acanthosis, lymphocytic infiltrate, and papillary congestion (Kang et al. 2016; Mohammed et al. 2022) as shown in table 1.

Table 1. Baker scoring system for severity of skin inflammation (Baker and Fry 1992)

Skin layer	Histological Feature	Score
Keratin	Munro abscess	**2**
	Hyperkeratosis	**0.5**
	Parakeratosis	**1**
Epidermis	Thinning over papillae	**0.5**
	Rete ridges appearance	**1.5**
	Acanthosis	**0.5**
	Lack of granular layer	**1**
Dermis	Mild lymphocytic infiltrate	**0.5**
	Moderate lymphocytic infiltrate	**1**
	Severe lymphocytic infiltrate	**2**
	Papillary congestion	**0.5**

Statistical analysis

Computing the sample size was performed using the G Power program (Faul et al. 2007), which is based on Cohen's principles (Charan and Kantharia 2013). The process of entering and analyzing data was carried out via Microsoft Excel 2010 and SPSS version 26. The representation of continuous variables was done using their mean and standard deviation (SD). The results of the Shapiro-Wilk test for normality indicated that the data did not follow a normal distribution. As a result, non-parametric testing, specifically the Mann-Whitney test and Kruskal-Wallis test, were employed. The level of statistical significance was determined via a P-value ≤ 0.05.

Results

Signs relating to the success of psoriasis induction including redness (erythema), increased skin thickness, and scaling on the area of skin where IMQ was applied started to appear four to five days after the beginning of the experiment. These gross observational signs progressively developed and worsened by day 6 of the induction, as depicted in Figure 2. An evident difference was observed between the normal control group and the induced group with IMQ in terms of psoriasiform signs. Throughout the whole duration of the experiment, animals were closely monitored for any alterations in the skin gross appearance. This evaluation was done using the PASI scoring criteria. In the healthy control group (Group I), in which no treatment was applied to the back skin, the general appearance of the skin remained constant with no signs of lesions, injuries, or color changes. Upon application of IMQ to the skin (Group II), there was a gradual increase in signs of inflammation (mostly redness) from day 3 of induction, lasting throughout the whole experimental period, in addition to increasing skin wrinkles and thickness, accompanied by the emergence of yellow spotting on the skin that progressively developed into flaky scales on the last day of the experiment.

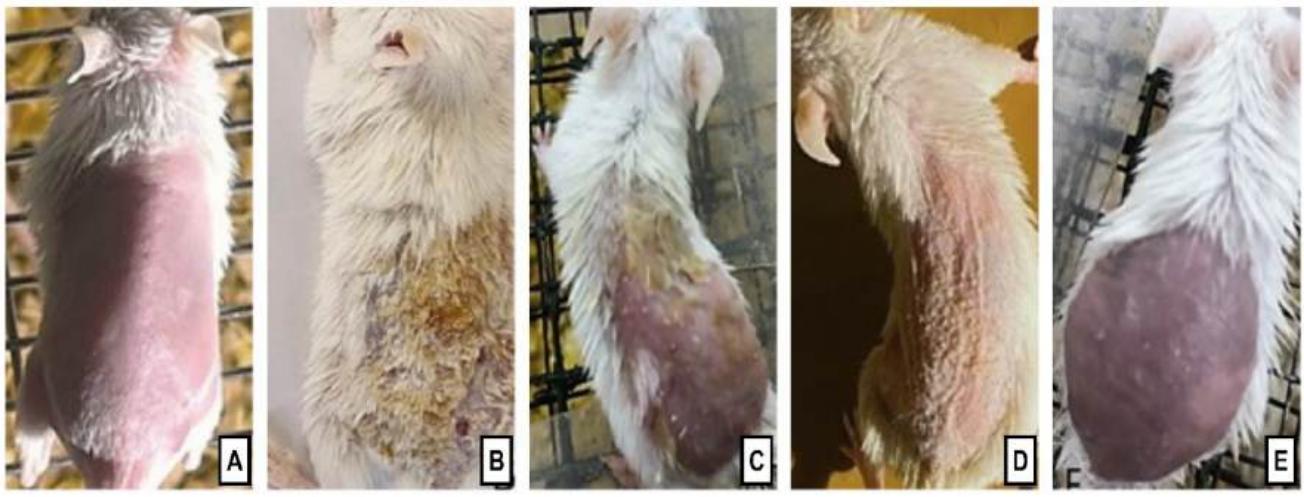


Figure 2. Gross images showing varying levels of skin inflammation severity on the dorsal skin where the test substances were applied on day 7 of the experiment and lasted for 8 days. **Note: (A)** shows the healthy control group (Group I), **(B)** Psoriatic IMQ-induced group (Group II), **(C)** IMQ + ointment base group (Group III), **(D)** group treated with IMQ + clobetasol (Group IV), and **(E)** group treated with IMQ + amlodipine ointment (Group V).

The skin of animals that received the ointment base only (Group III) did not exhibit any marked changes after the application of the ointment base, apart from a modest reduction in skin scaling. However, marked skin changes were observed when clobetasol and amlodipine were applied. Clobetasol-treated mice (Group IV) experienced a significant decrease in redness and scaling of the skin, as well as a reduced thickness and decreased skin wrinkling and puckering in the treated back area. These signs of improvement lasted until the finalization of the experiment. However, in the amlodipine-treated group (Group V), there was a minor reduction in skin redness and thickness, whereas a marked decrease in scaling was observed. These improvements started 3 days following the application of treatments as shown in Figure 3A.

The histopathologic section of the healthy control group (Group I) revealed normal spread of the keratin layer, normal thickness of the epidermis, and granular layer existence in the dermis. The histopathological section of the induction group (Group II) revealed a sloughing that covered a wide area, in addition to severe dense infiltration of neutrophils (Munro's abscesses) in the keratin layer. Additionally, there was an abnormal thickening of the epidermis (indicated by the black line), the presence of parakeratosis and hyperkeratosis, the absence of the granular layer, and an increase in the rete ridges (indicated by the red line) with the thinning of the papillary layer (Fig. 4B). On the other hand, the histopathological section of animals' skin that received the ointment base only revealed hyperkeratosis in certain areas of the epidermis, as well as focal Munro's abscesses, rete ridges, with the thinning of the papillary layer. The histopathological analysis of the clobetasol-treated group (Group IV) revealed a reduction in the thickness of both the epidermis and dermis. There was minimal thinning observed over the papilla, but Munro's abscess, Parakeratosis, and Rete's ridges were absent. However, hyperkeratosis remained present. The inflammation in this group can be described as mild to moderate, in contrast to the severe inflammation observed in the induction group (Fig. 4D). The histopathological examination of the amlodipine-treated group (Group V) revealed a reduction in thickness of both the epidermis and dermis. There was minimal thinning observed over the papilla, but Munro's abscess, Parakeratosis, and Rete's ridges were absent. Only a minimal inflammatory response was present, along with persistent hyperkeratosis.

The skin tissue levels of IL-17, TNF-a, and VEGF, histopathological scores (Baker score), and observational score (PASI score) were significantly elevated in the IMQ-induced psoriasiform group (Group II) compared to the healthy control group (Group I) ($P<0.001$). The results of Group III revealed no significant change ($P\geq0.05$) in the tissue levels of the inflammatory markers (IL-17 and TNF-α) except for the skin tissue level of VEGF, where there was a significant reduction ($P\leq0.001$) compared to the induced group (Group II) as demonstrated in Figure 5. In addition, the histopathological Bakers score and PASI score for the animals that received the ointment base (Group III) were comparable to those of the induced group (Group II) ($P\geq0.05$). The skin tissue levels of TNF-α and VEGF were significantly decreased ($P\leq0.01$) for the groups treated with clobetasol and amlodipine except for IL-17 in which animals treated with clobetasol showed no significant difference from the induction group (Group II) ($P\geq0.05$), whereas amlodipine-treated animals managed to exhibit a statistically significant reduction ($P\leq0.0001$) as shown in Figure 5. The histopathological and observational scores (Baker score and PASI score) significantly decreased in the

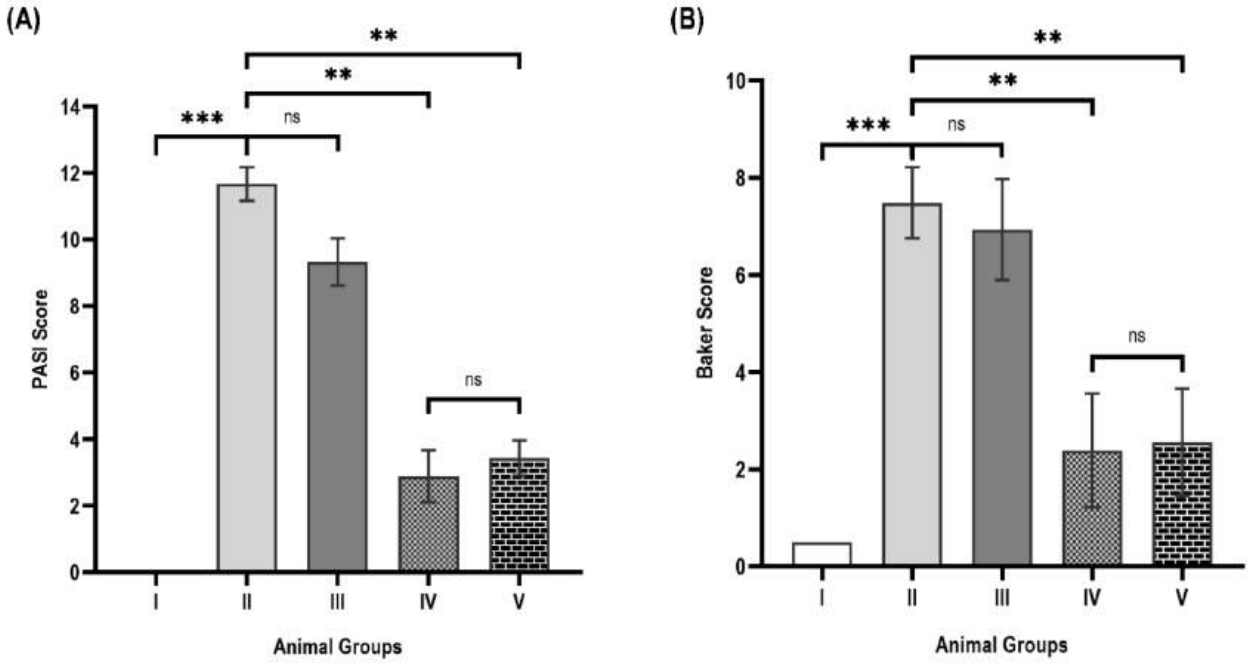


Figure 3. A gross observational and histopathological scoring system for the inflammation severity. ***Note:*** **(A)** represents the observational PASI score and **(B)** represents the histopathological Baker's score. Data are present as mean ± SD; animals in each group (n=9); p-value was set at ≤0.05; * indicates p≤0.05; ** indicates p≤0.001; *** indicates p≤0.0001, and ns = no significance (p≥0.05).

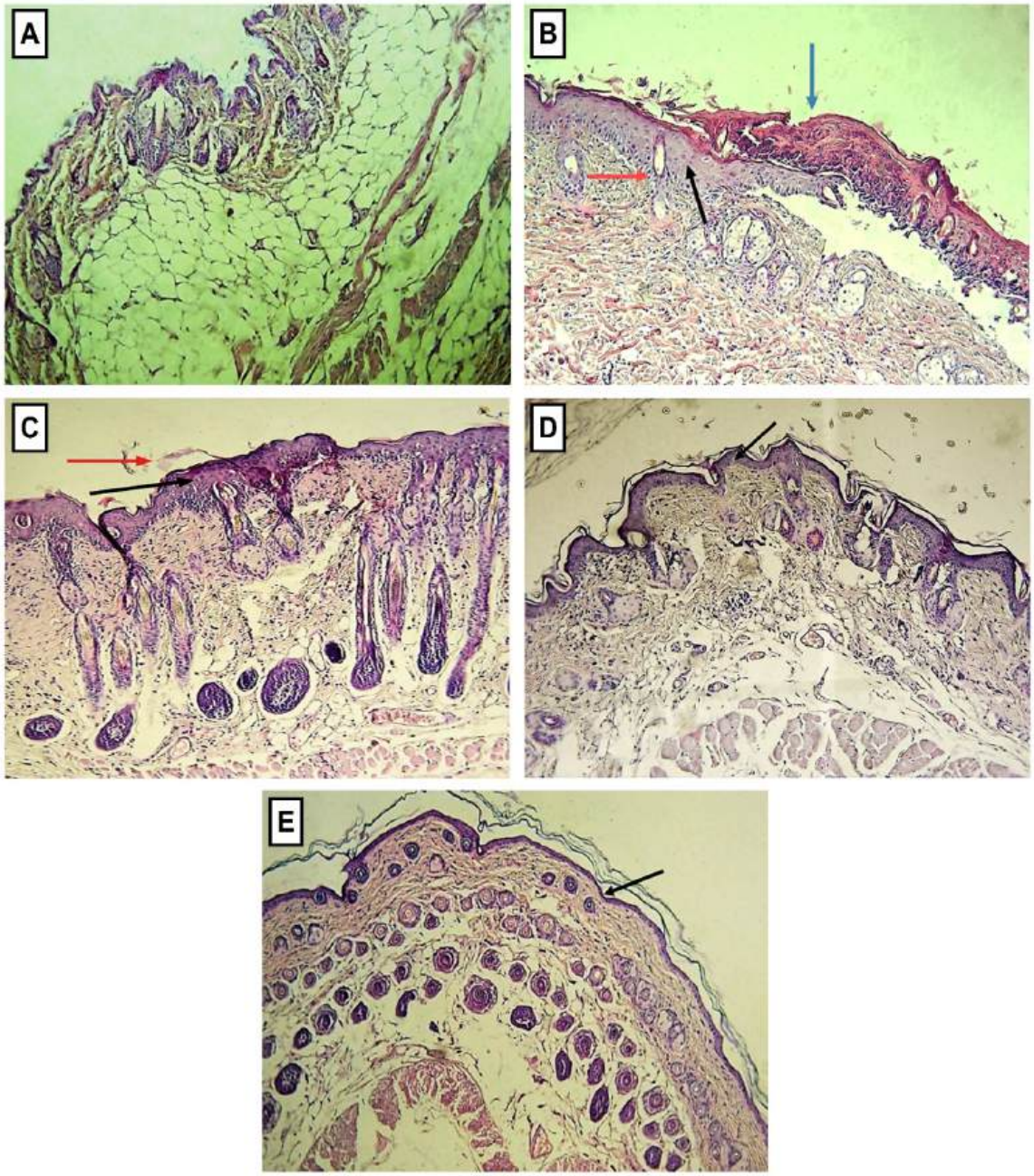


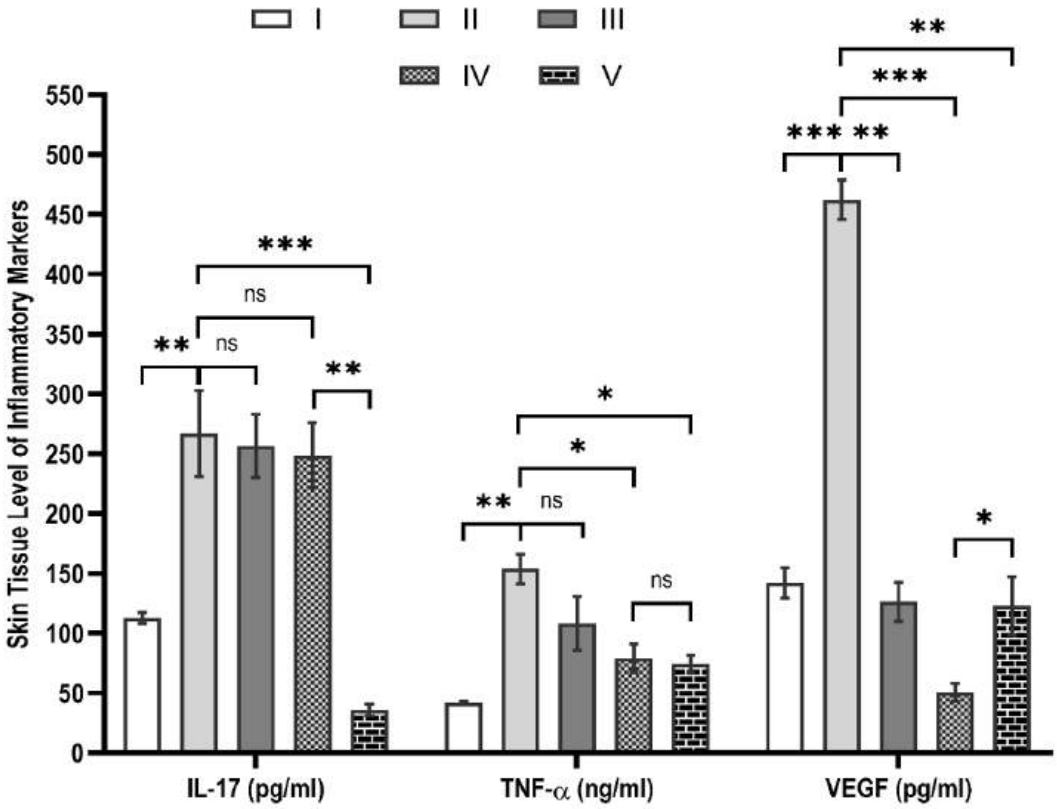
Figure 4. Histopathological examination of dorsal skin tissue among animal groups. ***Note:*** **(A)** shows the healthy control group (Group I); **(B)** psoriatic IMQ-induced group (Group II); **(C)** IMQ + ointment base group (Group III); **(D)** group treated with IMQ + clobetasol (Group IV), and **(E)** group treated with IMQ + amlodipine ointment (Group V). The examination was done at 100x magnification with (H & E). The blue arrow indicates the keratin layer, the black arrow indicates the epidermis, and the red arrow indicates the absence or presence of a rete ridge.

treated groups (groups of mice treated with clobetasol or amlodipine) compared to the induced group that was not treated ($P \leq 0.001$).

The findings of the study indicated a significant decrease in skin tissue IL-17 levels in the amlodipine-treated group (Group V) compared to the clobetasol-treated group (Group IV), with $p \leq 0.001$. However, the results indicate a significant decrease in skin tissue VEGF levels in the group treated with clobetasol compared to the group treated with amlodipine, with a high level of statistical significance ($p \leq 0.001$). Moreover, the levels of TNF-α in the skin tissue, as well as the histological and observational scores (Baker score and PASI score), were comparable in both treatment groups, with no statistically significant difference observed ($p \geq 0.05$).

Discussion

Psoriasis is considered a prevalent hyperproliferative skin disorder in which case the activated dendritic skin cells engage in interactions with the nearby keratinocytes on the surface of the skin, which consequently leads to redness, scaling, and thickness (Glitzner et al. 2014). In recent years, enormous progression has been made in understanding psoriasis pathogenesis, and these findings led to the development of different biological agents with excellent therapeutic efficacy. However, many of these agents are costly and their use is associated with unwanted effects which may limit their benefits. Therefore, further studies need to be conducted to find



Figures 5. Changes in skin tissue homogenate inflammatory biomarkers among animal groups. *Note:* Data are present as mean ± SD; animals in each group (n=9); p-value was set at ≤0.05; * indicates p≤0.05; ** indicates p≤0.001; *** indicates p≤0.0001; and ns = no significance (p≥0.05).

new agents that have the same therapeutic efficacy with minor adverse effects and at a reasonable price (Kerdel and Don 2018).

The presence of an exemplary animal model that reflects the molecular, immunological, and histopathological features of psoriasis continues to be an urgent issue. Mice are considered an optimal model for psoriatic induction because of their pathophysiological similarity with humans in terms of hyperkeratosis, skin inflammation, dendritic skin cell infiltration, the antioxidant system, and CD4 cell activity in vents of psoriasis (Kang et al. 2016).

Imiquimod topical cream is commonly used in mouse models to provide psoriasis features. In the current study, imiquimod cream application induced psoriasiform skin lesions within 6 days of the experimental course in mice regarding erythema by either direct mast cell degranulation induction mediated by IgE-mediated mechanism or by IgE-independent mechanisms (Yu et al. 2016). Scaling can be attributed to the induction of psoriasiform skin inflammation in mice via inducing the IL23/IL17 axis that closely resembles the pathogenesis of psoriasis in humans leading to acanthosis, parakeratosis, inflammatory cell infiltration, epidermal thickening, the manifestation of Munro micro-abscess in the epidermis keratin, thinning over the papillae, enlargement in the rete ridges, as well as loss of granular layer in plurality of samples (Girolomoni et al. 2017).

Skin tissue biomarkers assay, in addition to Baker's scores, and the observational PASI scores were increased in a significant manner among the imiquimod-induced psoriasis group in comparison with the health control mice group. These findings of the study were consistent with several publications that have already reported these findings and proposed that imiquimod cream application can spur and exacerbate psoriasis in mice groups. Thus, the IMQ-induced psoriasis mice model can be considered a perfect model for assessing both psoriatic disease pathogenesis and anti-psoriatic drug efficacy (Chen et al. 2017; Khorsheed et al. 2024; Hussein et al. 2020; Ridha-Salman et al. 2024; Almudaris and Gatea 2024).

Topical steroids with high potency were thought once as a gold standard for psoriasis management, and their effectiveness was ascribed to several immune-suppressive and anti-inflammatory mechanisms of this class of drugs (Abed-Mansoor and Abu-Raghif 2022). Despite efficacy of clobetasol in psoriasis treatment, its use has been observed to be associated with well-recognized side effects such as striae, telangiectasias, and atrophy, besides systemic side effects which make it inappropriate in the long-term, thus there is an emerging need for a novel medication with fewer unwanted effects (Kwatra and Mukhopadhyay 2018; Ghazy and Abu-Raghif 2021; Hasan and Gatea 2024).

Amlodipine is a potent long-acting calcium channel antagonist that has been shown to exhibit not only antihypertensive effects but also anti-inflammatory and antioxidant effects (Sun et al. 2016). Several previous *in vitro* experiment results also showed that amlodipine acts on keratinocytes to affect the immune response and exert anti-inflammatory and anti-allergic effects that can be beneficial in psoriasis (Bao and Reinhardt 2015). In this

study, amlodipine topical 5% ointment significantly ameliorated IMQ-induced psoriasiform inflammation in the treated mice group. Skin tissue IL-17, TNF-α, and VEGF assays along with histopathological and observational scores in mice skin lesions of the imiquimod-induced group were all significantly ameliorated by amlodipine 5% ointment. Amlodipine could improve psoriasis through its anti-inflammatory effect and by inhibiting the degranulation of mast cells seen in an *in vitro* study, which makes it useful in cases of urticaria (Li et al. 2021). Several studies revealed that amlodipine plays an important role in immune regulation (Li et al. 2021). Furthermore, accumulating evidence indicates that calcium (Ca2+) serves as a common and universal second messenger in TLR signaling (Liu et al. 2008), thus amlodipine could improve psoriasis through its inhibitory effects on "Th17-associated cytokines through toll-like receptor 7 (TLR7) signaling" and central transcription factor nuclear factor-κB, the activation of which is mediated by Ca2 +/ calmodulin/Akt (Jeon et al. 2018).

Activation of these pathways by imiquimod resulted in increased expression of many cytokines, including TNF-α, and IL-17, leading to desquamation, epidermal hyperproliferation, and infiltration of lymphocytes; these findings are consistent with many previous studies. The level of these cytokines was found to be significantly increased in psoriatic skin lesions by imiquimod application, and it is accompanied by the increasing expression of TLR7 according to a study by Li et al. (2019); these changes propose that imiquimod may aggravate or initiate psoriasis through TLR7 induction that causes a rise in Th17-associated cytokines (Li et al. 2019; Mahdi et al. 2024). In the current study, skin tissue assay of TNF-a, Baker's score, and PASI score were significantly reduced by topical amlodipine and were comparable to such in clobetasol-treated group with no significant difference. Geng et al. (2018) studied the effect of Matrine (MT) as one of the medicinal herbs *Sophorae Flavescentis Radix* (SFR) main constituent and found that Matrine had the effect of antipruritic both in acute and chronic itch models and caused a significant reduction in scratching behavior of the chronic pruritus models in mice in a dose-depended manner when its mechanism of action was investigated; it has been revealed that its effects are likely due to the inhibition of presynaptic N-type calcium channel, which is in agreement with our model (Geng et al. 2018).

Limitations

Animal models of psoriasis offer useful insights into the processes of the disease and prospective treatments. However, it is important to note that there are intrinsic physiological and immunological response variations between mice and humans. Hence, although this study shows the effectiveness and safety of using amlodipine topically, additional research is required to confirm these results through human clinical trials. Moreover, the study primarily concentrated on a specific set of observable signs and histological factors to assess topical amlodipine's effectiveness in treating psoriasis. Including other outcome measures, such as analyzing cytokine levels, characterizing immune cell types, and evaluating pruritus or quality of life, could enhance our understanding of amlodipine's therapeutic effects and mechanisms of action.

Conclusion

The effect of topical amlodipine 5% ointment in psoriasis was comparable to that of clobetasol, making it promising in treating psoriasis as an alternative to or adjunct to steroids.

Ethical statement

Animal care, handling, and experimental procedures followed the National Center of the 3Rs (NC3Rs) and the ARRIVE 2.0 guidelines (Percie du Sert et al. 2020). The study was approved by the Institutional Review Board (IRB) of Kirkuk University/College of Pharmacy (Approval No. 7/26/2350 on the 11th of October 2023).

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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Data availability

All data are included in the research paper and further access to supplementary results will be granted upon request from the correspondent author.

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