PHARMACIA

Pharmacia 71: 1–14
DOI 10.3897/pharmacia.71.e114753

Research Article

Effects of topical Ivermectin on imiquimod-induced Psoriasis in mouse model – Novel findings

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Received 26 October 2023 ♦ **Accepted** 6 November 2023 ♦ **Published** 20 February 2024

Citation: Almudaris SA, Gatea FK (2024) Effects of topical Ivermectin on imiquimod-induced Psoriasis in mouse model – Novel findings. Pharmacia 71: 1–14. https://doi.org/10.3897/pharmacia.71.e114753

Abstract

Aim: investigate the possible anti-psoriatic effect of ivermectin in mice based on observational and histopathological outcomes and biomarkers.

Methods: Sixty male Swiss Albino Mice were divided into six groups (Groups I–VI); each group contained ten mice with shaved dorsal skin. The clinical, pathological, and laboratory effects were measured.

Results: Topical Ivermectin significantly decreased vascular endothelial growth factor levels. At the same time, the combination of ivermectin plus Clobetasol showed a more significant reduction in tumor necrosis factor-alpha (TNF-α) and Interleukin-17 (IL-17) levels. Regarding the Interleukin-10 (IL-10) level, the Ivermectin and Ivermectin/Clobetasol combination groups showed a significant increase in IL-10.

Conclusion: Topical Ivermectin's anti-psoriasis activity increases IL-10 levels and could be used efficiently to alleviate psoriatic symptoms. Its combination treatment with Clobetasol holds promise for the management of psoriasis.

Keywords

ivermectin, IL-10, TNF-a, VEGF, iL-17, psoriasis, imiquimod, histopathological study

Introduction

Psoriasis is an immune-mediated inflammatory disease with a chronic prolapsing nature. Many factors can provoke psoriasis; some are genetic or external, like mild trauma, infection and sunburns, stress, and systemic drugs (Burge et al. 2016). Certain medications also can exacerbate psoriasis, such as lithium, β-blockers, anti-malarial drugs, and nonsteroidal anti-inflammatory drugs (Kamiya et al. 2019). Skin lesions are localized or generalized, mostly symmetrical. Lesions can cause itching, stinging, and pain (Burge et al. 2016). The skin lesions in psoriasis patients can have different symptoms according to the type of psoriasis; they are mostly described as having red, scaly, irritated skin patches which sometimes can affect the whole body (Dinulos 2021).

Psoriasis is generally classified into pustular and non-pustular psoriasis (such as psoriasis vulgaris, guttate psoriasis, inverse psoriasis, and erythrodermic psoriasis), and all the types share some common characteristics like

erythema and thickening of the skin (Sarac et al. 2016). Histologically, the psoriatic skin in the dermis is usually thickened, and the dermis is thickened and characterized by an increased vascularity, and the cellular infiltrate is rich with macrophages and mast cells (Gudjonsson et al. 2007). Also, the thickened epidermis (acanthosis), reduction in the granular layer, and neutrophil accumulation in parakeratosis stratum corneum are characteristics of psoriasis lesions (Nakajima and Sano 2018).

Dermatological application of Imiquimod (IMQ) – which is a Toll-like receptor (TLR) 7/8 ligand, induces and possibly exacerbates psoriasis; in a treated mouse model, it exhibits similarities to human plaque-type psoriasis in terms of skin erythema, thickening, scaling, epidermal alterations (acanthosis, parakeratosis), neo-angiogenesis, and inflammatory infiltrate comprising T cells, neutrophils, and dendritic cells (van der Fits et al. 2009a; El Malki et al. 2013). Prior research has established the significance of various factors in this model, including the psoriasis area severity index (PASI) score, histological staining, and the involvement of inflammatory cytokines (van der Fits et al. 2009a; El Malki et al. 2013; Nadeem et al. 2015; Khamees et al. 2018; Khamees et al. 2020; Ahmed et al. 2022; Mohammed et al. 2022b).

Treatment of psoriasis usually focuses on treating the symptoms; there are different treatment options available for psoriasis, but none are curative, depending on the type, location, and severity of the lesions. Treatment may be topical, phototherapy, systemic, or a combination, depending on the disease severity (Globe et al. 2009). Topical treatment is usually given to mild-moderate cases of psoriasis. Such treatments include corticosteroids (CS) (Sawyer et al. 2013), vitamin D analogues (such as calcipotriene) (Mason et al. 2013), topical retinoids (such as tazarotene) (Abudlqader and Kadhim 2021), and many others.

Ivermectin (IVM) originates from a group of natural macrocyclic lactones known as avermectins, and it was originally isolated from the soil actinomycete *Streptomyces avermectinius* (Ventre et al. 2017). Ivermectin is used as an anti-parasitic in both human and veterinary medicine, and it also has anti-inflammatory properties that are useful in medicine (Ōmura and Crump; 2014). It is a crystalline powder that is white to yellowish-white in color; it is insoluble in water and soluble in alcohol (Zargari et al. 2016). It was approved in 1987 for use in humans, and it is used systemically for many parasitic infections like Ascariasis, Strongyloidiasis, and Onchocerciasis, and also for scabies and pediculosis (Crump and Ōmura 2011; Barańska-Rybak and Kowalska-Olędzka 2019). It is also available as a 1% topical cream for the treatment of severe papulopustular rosacea, and it has shown a strong ability to reduce the skin inflammatory lesions number; therefore, it is a promising agent for inflammatory diseases of the skin (Stein et al. 2014). Ivermectin works by inhibiting the reactivation of allergen-specific T cells in the skin that induce inflammatory symptoms (Ventre et al. 2017).

Ivermectin is a powerful angiogenesis inhibitor that can also inhibit proliferation and induce apoptosis in cells (Liu et al. 2016) and exerts anti-inflammatory effects in several ways, such as inhibiting lipopolysaccharide (LPS)-induced inflammatory cytokines, such as tumor necrosis factor-alpha, IL-6, and IL-1 (Castillejos-López et al. 2022), and upregulates the anti-inflammatory cytokine IL-10 (Sia et al. 2020), and can also suppress the LPS-induced NF-kB translocation (Zaidi and Dehgani-Mobaraki 2022). It also has a strong anti-bacterial action against certain *S. aureus* isolates (Ashraf et al. 2018), which may have a role in the pathogenesis of psoriasis (Teng et al. 2021). Additionally, there are reports that when given either topically or systemically, ivermectin employs anti-inflammatory effects in allergic inflammation murine models (i.e., asthma and atopic dermatitis). It can block the activation of the NF-kB pathway and inhibit toll-like receptor 4 (TLR4) signaling (Smith et al. 2016).

In this study, the main objective is to investigate the effect of topical ivermectin as a novel approach to treating psoriasis using the IMQ-induced mouse model. This model can assess ivermectin's therapeutic potential in addressing psoriasis's key pathological features, such as epidermal hyperplasia, inflammatory cell infiltration, and altered cytokine profiles. In addition to investigating the therapeutic effect of ivermectin/clobetasol combination on IMQ-induced mice model of psoriasis.

Methods

Study design

Male (Swiss Albino mice) weighing 28–32 g aged 2–3 months were randomly divided into six groups of 10 animals (60 mice). The animals were identified by marking different parts of the body. The mice were obtained from the (Animal Facility of the Al-Nahrain University – Biotechnology Research Center, Baghdad, Iraq), housed in polypropylene cages under a temperature-controlled environment (25 °C), with an inverted light-dark cycle (12/12 hours) and acclimated for seven days before starting the Experiment at the same center where the mice were obtained (Animal Facility of the Al-Nahrain University – Biotechnology research center, Baghdad, Iraq). The animals were maintained on a standard pellet diet and free access to water *ad libitum* supplied by Al-Nahrain University – Biotechnology Research Center. Before starting the Experiment, the animals were examined for the presence of any skin lesions, and only mice with apparently healthy skin and coat were included in the study; all animals that were included in the study underwent shaving from the dorsal region to expose an area of the back skin that measures about 1 × 2 cm for experimental purposes using an electric razor, followed by the application of a hair removal cream (Veet®, Reckitt Benckiser Pvt. Ltd., India), the remaining hair was then wiped away using gauze.

The total duration of the Experiment was 14 days from the starting day. Regarding the allocation of mice: Group I (Positive Control) consisted of 10 mice (n = 10) that remained as a control group with no intervention during

the experimental duration. Group II (Psoriatic Control) consisted of 10 mice (n = 10) that received Induction of psoriasis by a dose of 62.5 mg of Imiquimod cream 5% as a once-daily topical application on the shaved back skin for six days duration until the appearance of a psoriatic lesion as mentioned by van der Fits et al. study (van der Fits et al. 2009b) and this method was considered the standard of Induction for the current study, and mice in this group received no further intervention for the rest of the experimental days. Group III (Vehicle control) consisted of 10 mice (n = 10) that received Induction for six days based on the previously mentioned method (van der Fits et al. 2009b) and then received Petrolatum gel 15% as a twice-daily topical application on the shaved dorsal skin for eight days duration. Group IV consisted of 10 mice (n = 10) that received Induction for six days based on the previously mentioned method (van der Fits et al. 2009b) and then received Clobetasol propionate 0.05% ointment once daily topical application (at a dosage of 0.25 g/kg) for eight days duration. Group V consisted of 10 mice (n = 10) that received Induction for six days based on the previously mentioned method (van der Fits et al. 2009b) and then received a preparation of ivermectin 1% ointment w/w twice daily topical application on the shaved dorsal skin for eight days duration. Group VI consisted of 10 mice (n = 10) that received Induction for six days based on the previously mentioned method (van der Fits et al. 2009b) and then received a topical preparation of 0.025% Clobetasol propionate ointment combined with ivermectin ointment 0.5% w/w twice daily topical application on the shaved dorsal skin for eight days duration. Fig. 1 illustrates the flow chart of the study.

Imiquimod cream was supplied as (Aldara® 5% Cream, Meda Pharmaceuticals, Solna, Sweden), Petrolatum Gel 15% was supplied from the local market (Iraqi Federation of Industries, Baghdad, Iraq), Clobetasol propionate was supplied as ointment preparation (Dermovate®,

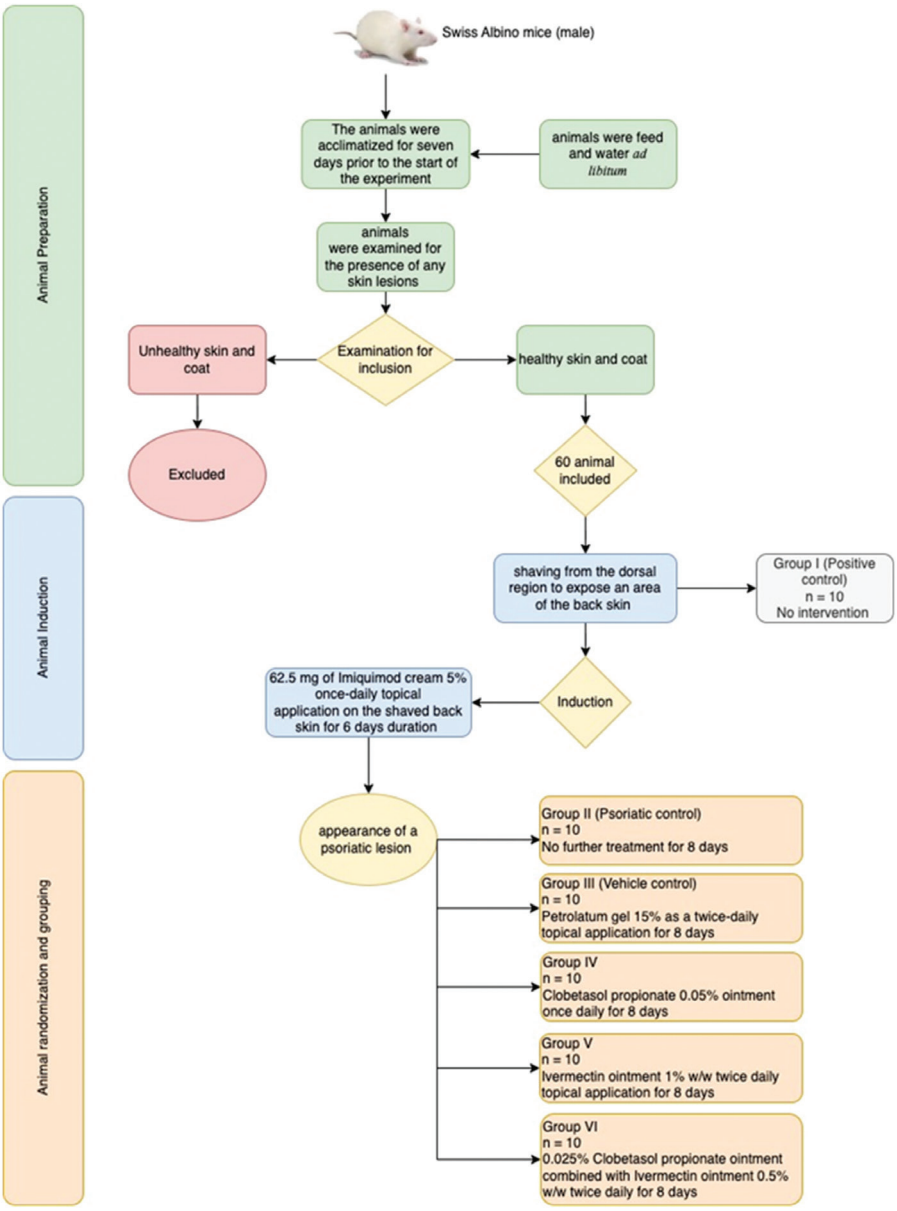

Figure 1. Flow chart of the study.

GlaxoSmithKline, Brentford, UK), and ivermectin was supplied as powder by (Hyperchem, Batch no. C1105113, Hangzhou, China) and later prepared as an ointment.

Induction of Psoriasis

Induction of Psoriasis was done by using Imiquimod cream 5% as a once-daily topical application on the shaved back skin for six days until the appearance of a psoriatic lesion (van der Fits et al. 2009b). The success of this model was determined by using the Psoriasis Area Severity Index (PASI) score (Fredriksson and Pettersson 2009) mice, in which we observed the following changes during the induction phase of the animals: skin erythema, increased skin thickness, and scaling; which were developed in animals before day 6 of Induction, this considered a successful induction model (Baek et al. 2012).

Psoriasis Area Severity Index (PASI) score

The PASI clinical scoring system was employed to examine the intensity of the inflammation of the mouse dorsal skin as a part of the assessment of induction models' success and treatment efficacy in this study. It involved a visual evaluation of three characteristics on the back skin of each mouse, which are erythema (redness), induration (thickness), and desquamation (scale). Each characteristic was assigned a number between 0 and 4 (0 = None, 1 = Slight, 2 = Moderate, 3 = Marked, 4 = Very marked), resulting in a total score ranging from 0 to 12 (Fredriksson and Pettersson; 2009). In this study, the evaluation was done individually by one researcher.

Preparation of Ivermectin ointment 1% w/w

Ivermectin, 1% ointment, was prepared according to the available concentration in the market that is approved by the FDA (2022); the formula is prepared according to Ansel's (Fuhrman 2006) where 250 mg of Ivermectin powder is used to prepare 1% (w/w) ointment after adding it to 18.75 g of petrolatum melted in a water bath and mixing with a stirrer, after that topping and mixing with more petrolatum until reaching the desired amount (25 g).

Ivermectin and clobetasol combination ointment

Commercial clobetasol ointment (Dermovate®) containing 0.05% clobetasole propionate (CP) was used with the prepared Ivermectin 1% ointment. An equal amount of both ivermectin and clobetasol (half concentration of both) was taken and well mixed by a spatula to obtain the final concentration of 0.5% Ivermectin and 0.025% clobetasol combination ointment (Convention 2014).

Outcome measures

The end of day 14 marked the end of the experimental period, and the outcome evaluation was done the next day. The evaluation of the treatment efficacy on the tested groups was based on the PASI Score (Fredriksson and Pettersson 2009) (Mentioned above), where the mean of all three characteristics was computed following observation.

After calculating the PASI score, all mice were anesthetized intraperitoneally (IP) with 80 mg/kg of ketamine and 10 mg/kg of xylazine. Following total anesthesia, all mice were terminated by exsanguination (cardiac puncture), a procedure suitable for tissue harvest and conservation (Pierozan et al. 2017; Underwood and Anthony 2020).

Tissue samples were harvested from the dorsal shaved skin (1 cm) and divided into two pieces. The first piece was prepared for histopathological analysis by dehydration and immersion in liquid paraffin at a 55–60 °C temperature range. The slide was then prepared based on this method discussed by previous studies (Cardiff et al. 2014). Histopathological analysis was then done based on Baker's grading system (Kang et al. 2016).

The second piece of skin tissue was prepared for biochemical analysis to measure the essay of Tumor Necrosis

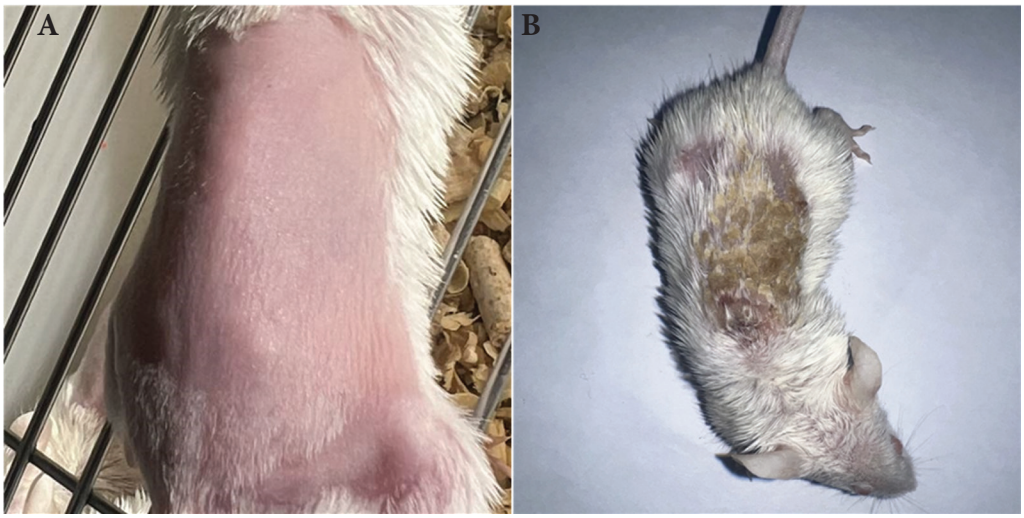

Figure 2. Induction of Psoriasis in mice. **A** Mouse before Induction. **B** Mouse after Induction by Imiquimod cream.

Factor-alpha (TNF-α), Interleukin 17 (IL-17), Interleukin 10 (IL-10), and Vascular Endothelial Growth Factor (VEGF), the preparation was done by rinsing the tissue in ice-cold Phosphate Buffered Saline (PBS) to remove excess blood thoroughly and weighed before homogenization, and then mincing it to small pieces and homogenization was done in fresh lysis buffer where 1 mL of lysis buffer is added to the tissue sample with a glass homogenizer on ice all by using the homogenizer machine (Electrical tissue homogenizer, Staruar®, England.). Then, the homogenates were centrifuged for 5 minutes at 10,000 RPM. The supernates were collected and stored at ≤ -20 °C until used for analysis by sandwich ELISA technique.

Baker's histopathological grading system

A histopathological grading system is used in determining the severity of inflammation (Kang et al. 2016; Mohammed et al. 2022a). It was used in the current study to evaluate the pathological changes of the homogenate tissue on a scale of 0–10 (Mohammed et al. 2022a; Baker and Fry 1992) under light microscopy.

Light microscopy

The tissue morphology was imaged by the light microscope (Olympus BX51 Microscope, Olympus Corporation®, Japan), and five zones of a slide corner and the center were randomly viewed.

Analytic procedure

The stored samples were thawed and used for the analysis by sandwich Enzyme-Linked immunosorbent assay (ELISA) technique using the ELISA Reader (ELISA reader, Diagnostic Automation / Cortez Diagnostics®, California, USA). Regarding the ELISA kits, TNF-α was determined using the mice analytic kit (SCA133Mu, Cloud-Clone Corp.), IL-10 was determined using the mice analytic kit (SEA056Mu, Cloud-Clone Corp.), IL-17 was determined by the mice analytic kit (HEA063Mu, Cloud-Clone Corp.), and VEGF was determined using the mice analytical kit (SEA143Mu, Cloud-Clone Corp.).

Sample size and randomization

For sample size computation, program G Power was utilized (Faul et al. 2007) based on Cohen's principles (Charan and Kantharia 2013). A table of random integers was used to construct the groupings (to minimize misunderstanding), and the animals were placed in labeled containers and given tail tags (Festing 2006).

Statistical analysis

Data entry and analysis were performed using Microsoft Excel 2010 and SPSS version 26. Categorical variables were presented as frequencies and percentages using the Chi-square test. Test of Normality (Shapiro-Wilk) for continuous variables showed that data was non-normally distributed; thus, nonparametric tests (Mann Whitney) were used. Statistical analysis of different parameters in this study was expressed as mean ± standard deviation and P- values were significant when ($P < 0.05$) or highly significant when ($P < 0.001$) (Daniel and Cross 2018).

Results

Scoring for skin Inflammation Severity

On day 4–5 of starting the Experiment, signs of psoriasis induction on mice skin, such as erythema, skin thickening, and scaling, began to show on the skin treated with IMQ and continued in the severity until the last day of Induction as shown in the figure below (Fig. 3).

Then, the mice were treated with petrolatum (group III), topical Clobetasol (group IV), ivermectin (group V), and a combination of ivermectin with Clobetasol (group VI), as shown in Fig. 3 below, which indicates successful Induction of psoriasis-like dermatitis in mice. There was a significant difference between the normal and IMQ-induced groups regarding the psoriasis-like symptoms.

The skin of experimental mice was observed for change during all days of the Experiment, and skin condition scoring was based on the PASI scoring system. In the healthy group, where nothing was applied to the skin, there were no signs of erythema, the skin is pink and healthy, and no signs of thickening or scaling appeared.

While on the skin treated with IMQ, the skin shows an increase in redness and inflammation from day three and an increase in severity along the Experiment days. There is also an increase in skin wrinkling and thickness, and scales begin to appear as yellow spots on the skin that advance to large, flaky scales on the last day of Induction.

The vehicle group showed no major changes in the skin after application, and there was only a slight reduction in skin scaling. At the same time, there were significant changes in skin appearance when Clobetasol and Ivermectin were applied. In the Clobetasol-treated group, there was a marked reduction in the skin erythema and the scaling of the skin surface; there was also a reduction in skin thickness noted as a decrease in the skin puckering and wrinkling in the induced areas. This improvement continued until the last day of the Experiment. However, in the group treated with ivermectin, there was a slight decrease in skin redness thickness and a marked reduction of scaling that started after three days of Ivermectin application. The combination group showed a marked reduction in skin erythema, thickness, and scaling, which started 2–3 days upon treatment application and continued until the last day of the Experiment.

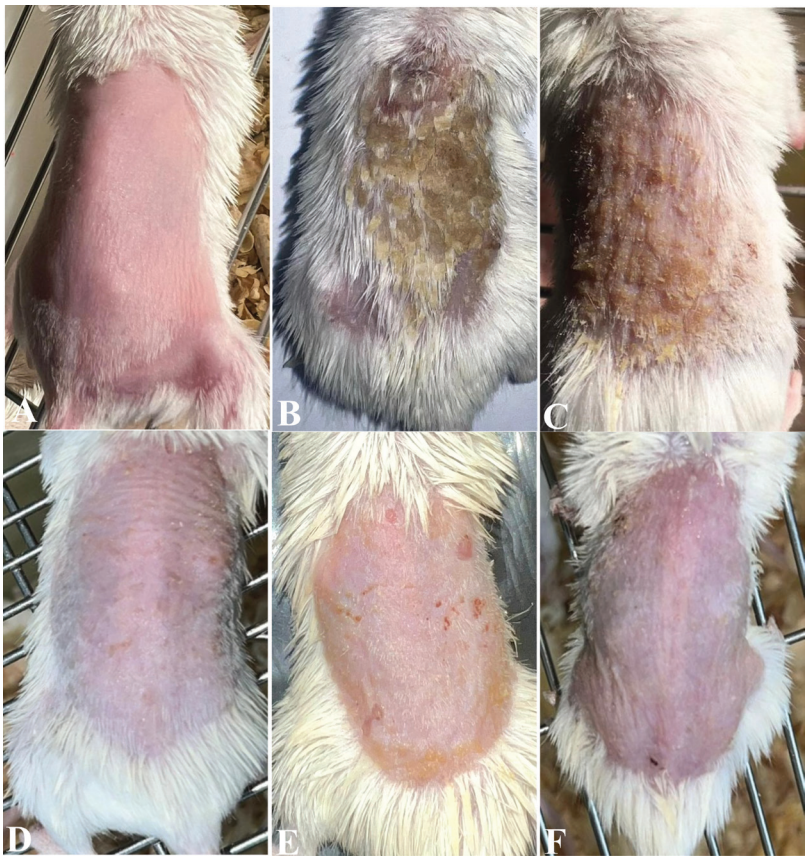

Figure 3. Scoring for skin inflammation severity, the pictures show different inflammation levels of the dorsal skin on which the test substances were applied on day 8 of the Experiment. **A.** Represents the healthy group; **B.** Represents the IMQ-induced group; **C.** Represents the vehicle group; **D.** Represents Clobetasol treated group; **E.** Represents the Ivermectin treated group; **F.** Represents Ivermectin + Clobetasol treated group.

Histological examination

Healthy group

The histopathologic section of healthy groups of skin mice showed normal epidermal, dermal, and subcutaneous tissue layers. In 4×, 10× in (H&E) stain, see Fig. 4.

Induction

In the histopathological section of Induction**,** a group of skin mice showed a multifocal (wide) area of sloughing, severe dense neutrophilic infiltration (the Munro's abscesses), and parakeratosis, hyperkeratosis, with lack of granular layer, acanthosis, increased rete ridges with papillary thinning. The dermis showed severe lymphocytic infiltration and vascular congestion. In 4×, 10×, and 40× H&E stain, see Fig. 5.

Vehicle group

The histopathological skin of the induced **vehicle** group also showed epidermal hyperkeratosis and parakeratosis

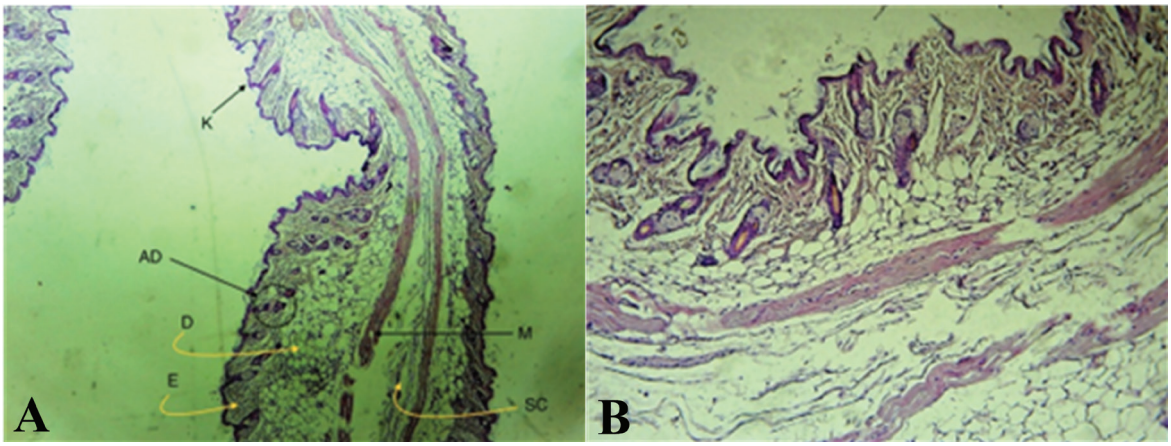

Figure 4. Histopathological section of mice skin (healthy control group) showing normal skin architecture including K = keratin, E = epidermis, D = dermis, AD = adnexa, S.C = subcutaneous tissue, M = muscles, B.V = blood vessels. H&E stain (4×,10×).

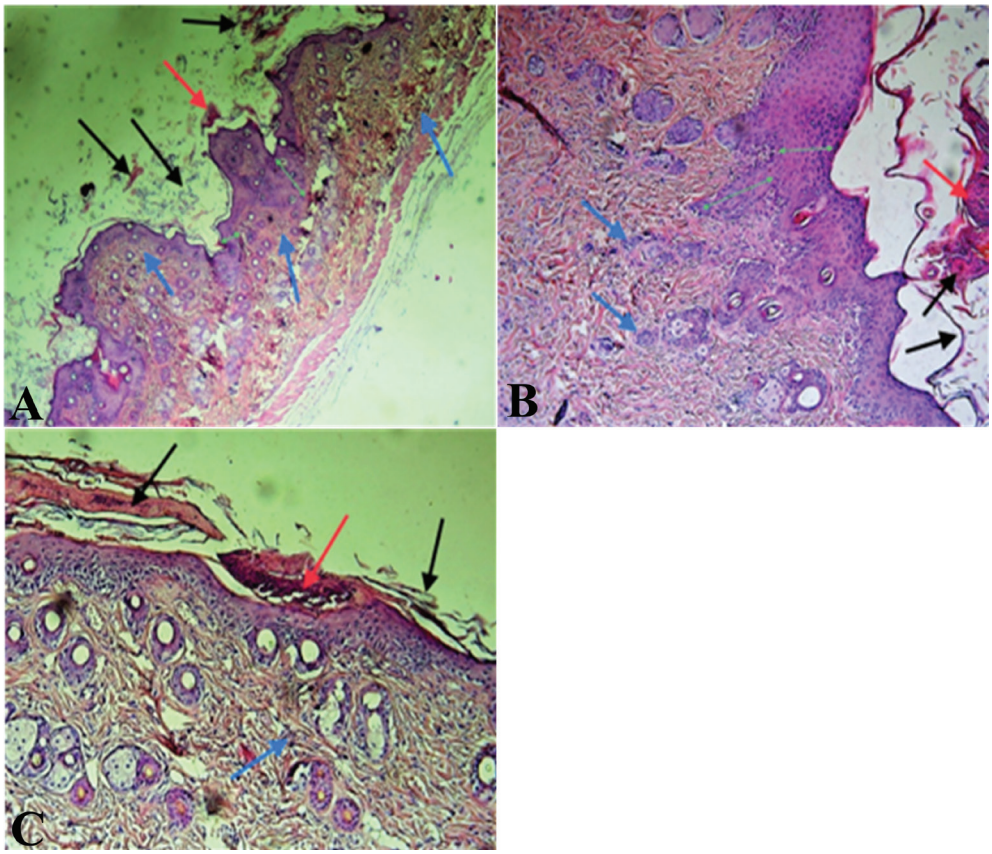

Figure 5. Histopathological section of mice skin (induction group) showing hyperkeratosis, parakeratosis (black arrow), with multifocal dense neutrophilic infiltration (the Munro's abscess) in red arrow, with epidermal acanthosis and thinning papillae and rete ridges appearance (green arrow), and lack of granular layer. The dermis shows moderate to severe inflammatory lymphocytic infiltration (blue arrow). H&E stain (4×,10×, 40×).

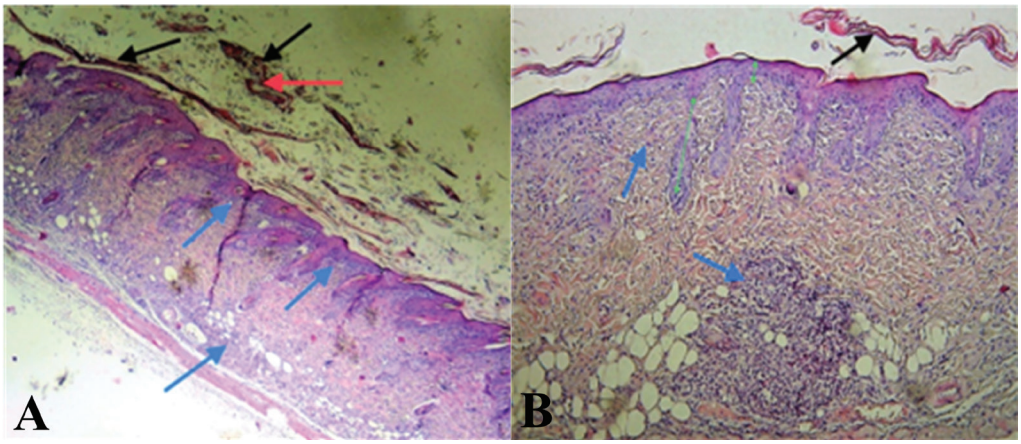

Figure 6. Histopathological section of mice skin (vehicle group) showing hyperkeratosis, parakeratosis (black arrow), with focal neutrophilic infiltration (the Munro's abscess) in red arrow, with epidermal acanthosis and thinning papillae and elongated rete ridges (green arrow), and lack of granular layer. The dermis shows moderate to severe inflammatory lymphocytic infiltration (blue arrow). H&E stain (4×,10×).

with focal Munro's abscesses with acanthosis and elongated rete ridges and papillary thinning with moderate to severe lymphocytic infiltration. In 10× H&E stain, see Fig. 6.

Clobetasol group

The histopathological section of the standard treatment group (Clobetasol) mice skin shows hyperkeratosis, absence of parakeratosis & Munro's abscess. And epidermal granular layer with mild acanthosis, few rete ridges with mild thinning of papillae with mild lymphocytic infiltration of the dermis. In 4×, 10× H&E stain, see Fig. 7.

Ivermectin

As for animals treated with ivermectin, the skin showed mild keratosis without Munro's abscess and parakeratosis and epidermal mild acanthosis with few rete ridges and mild papillary thinning. The dermis was showing severe lymphocytic infiltrate. H&E stain (4×, 10×, 40×), see Fig. 8.

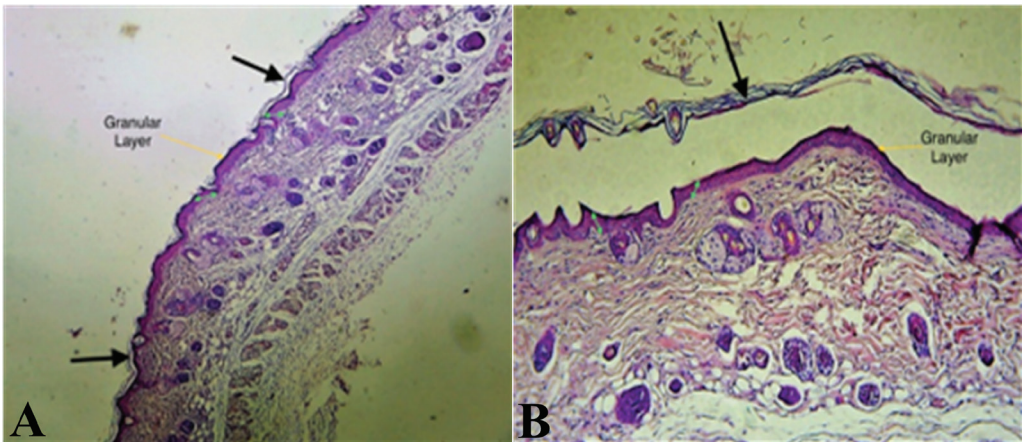

Figure 7. Histopathological section of mice skin (clobetasol control group) showing hyperkeratosis (black arrow), absence of parakeratosis & Munro's abscess. And epidermal granular layer (yellow arrow) with mild acanthosis, mild papillary thinning, and few rete ridges (green arrow). The dermis shows mild lymphocytic infiltrate. H&E stain (4×,10×).

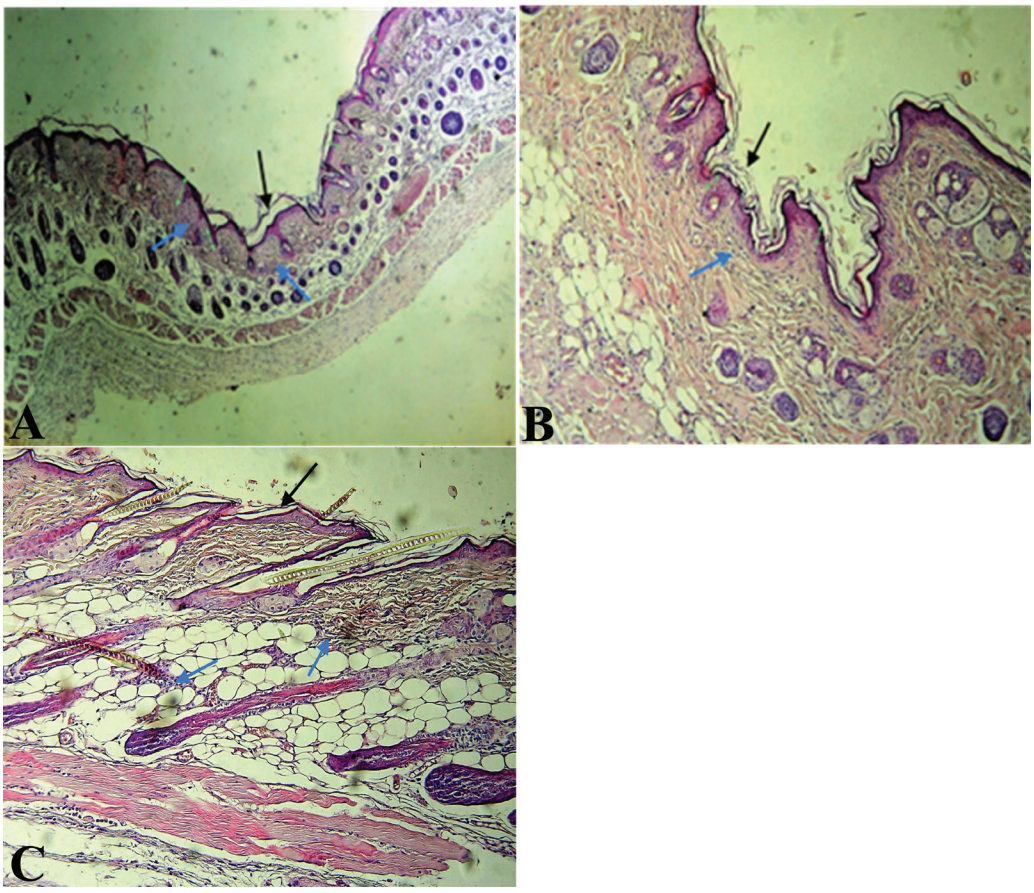

Figure 8. Histopathological section of mice skin (Ivermectin treatment group) showing mild keratosis (black arrow), with the absence of Munro's abscess and parakeratosis and epidermal mild acanthosis with few rete ridges and mild papillary thinning (green arrow). The dermis shows severe lymphocytic infiltrate. H&E stain (4×,10×, 40×).

Ivermectin clobetasol

The histopathological section of mice skin in the combination treatment group showed focal hyperkeratosis, mild epidermal thickness, with the absence of Munro's abscess and parakeratosis and epidermal mild acanthosis with the absence of rete ridges & mild papillary thinning, and few chronic lymphocytic dermal infiltrations. In 4×, 10× H&E stain, see Fig. 9.

Immunochemistry assay

This study included six groups, six mice in each group; these groups were healthy Control, induced non-treated, Petrolatum group, induced treated with Clobetasol group, induced treated with Ivermectin group, and induced treated with Ivermectin - Clobetasol group.

Comparison among the studied groups was made in the levels of tissue biomarkers (IL-10, IL-17, TNF-a, and

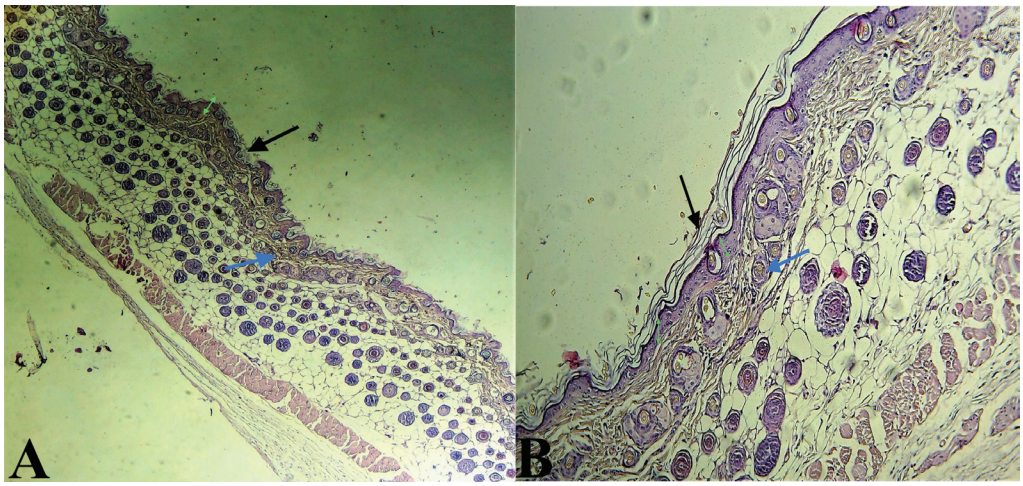

Figure 9. Histopathological section of mice skin (Ivermectin treatment group) showing mild keratosis (black arrow), with the absence of Munro's abscess and parakeratosis and epidermal mild acanthosis with few rete ridges and mild papillary thinning (green arrow). The dermis shows severe lymphocytic infiltrate. H&E stain (4×,10×).

VEGF), histopathological scores (Baker score), and observational scores (PASI score).

Comparison between the healthy group and induced non-treated group in the level of tissue biomarkers (IL-10, IL-17, TNF-a, and VEGF) and histopathological scores (Baker score) and observational score (PASI score)

Tissue homogenate levels of tissue biomarkers (IL-17, TNF-a, and VEGF), histopathological scores (Baker score), and observational score (PASI score) were significantly highly increased, in addition, tissue homogenate level of IL-10 was significantly highly decreased among the induced non treated group after Induction in Comparison with the healthy control group, (P < 0.001)—Table 1.

Table 1. Comparison between the healthy control group and non-treated induced group regarding tissue biomarkers.

Parameters	Healthy Control Mean±SD	Induction group Mean±SD	p-value
IL-10 (pg/ml)	286.26±63.80	31.83±3.03	<0.001*
IL-17 (pg/ml)	222.41±64.33	553.04±141.32	<0.001*
TNF-a (ng/L)	83.46±6.02	807.13±500.06	<0.001*
VEGF (pg/ml)	120.99±15.83	552.20±136.63	<0.001*
Baker score	0.50±0.00	9.00±0.00	<0.001*
PASI score	0.00±0.00	11.70±0.48	<0.001*

*P significant at level < 0.05 and highly significant at level < 0.001
**Mann-Whitney U test; IL = Interleukin; TNF-α = Tumor necrosis factor alpha; VEGF = Vascular endothelial growth factor.

Comparison between the induced non-treated group and Petrolatum group in the level of tissue biomarkers (IL-10, IL-17, TNF-a, and VEGF); histopathological scores (Baker score) and observational score (PASI score)

Tissue homogenate levels of tissue biomarkers (IL-17, TNF-a, and VEGF), histopathological scores (Baker score), and observational score (PASI score) were significantly decreased; in addition, tissue homogenate level of IL-10 was significantly increased among Petrolatum group in Comparison with induced non treated group, (P < 0.05), as illustrated in Table 2.

Table 2. Comparison between the induced non-treated group and Petrolatum group regarding tissue biomarkers (IL-10, IL-17, TNF-a, and VEGF); histopathological scores (Baker score) and observational score (PASI score).

Parameters	Induction group Mean±SD	Petrolatum group Mean±SD	p-value
IL-10 (pg/ml)	31.83±3.03	92.50±27.13	<0.001*
IL-17 (pg/ml)	553.04±141.32	278.52±100.27	<0.001*
TNF-a (ng/L)	807.13±500.06	281.79±240.17	0.008*
VEGF (pg/ml)	552.20±136.63	209.56±73.31	<0.001*
Baker score	9.00±0.00	7.60±0.84	0.001*
PASI score	11.70±0.48	9.30±0.67	<0.001*

*P significant at level < 0.05 and highly significant at level < 0.001
**Mann-Whitney U test; IL = Interleukin; TNF-α = Tumor necrosis factor alpha; VEGF = Vascular endothelial growth factor.

Comparison between induced non-treated group and all induced treated groups regarding tissue biomarkers (IL-10, IL-17, TNF-a, and VEGF)

The data obtained has revealed a highly significant reduction in the tissue homogenate levels of tissue biomarkers (IL-17, TNF-a, and VEGF), in addition to a highly significant elevation in the tissue homogenate level of (IL-10) among all treated groups as compared with induction group (P < 0.05). Regarding IL-10 level, in comparison to the induction group, both Ivermectin and Ivermectin combination groups showed a significant elevation in IL-10 level P < 0.05, while ivermectin being more significant when compared with (Ivermectin-Clobetasol group). Regarding IL-17 level, it was significantly decreased among the Clobetasol group in Comparison to other treated groups; P < 0.001. Regarding TNF-a level, it was significantly decreased among the Ivermectin–Clobetasol group in Comparison to other treated groups; P < 0.05. The VEGF level was significantly lower among the Ivermectin

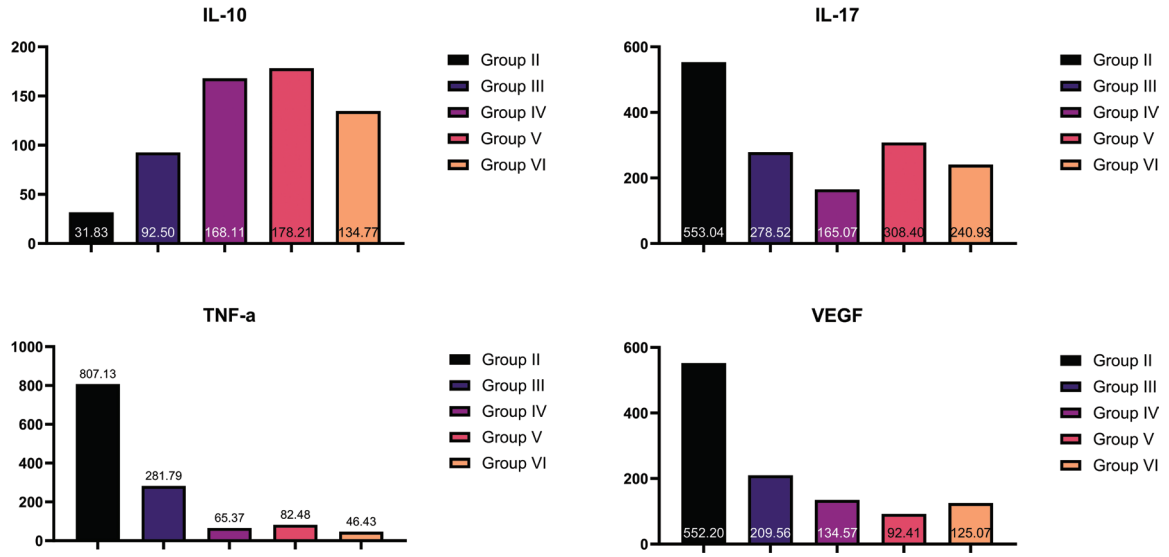

Figure 10. Comparison between induced non-treated group and all induced treated groups regarding tissue biomarkers (IL-10, IL-17, TNF-a, and VEGF).

group compared to the Clobetasol group and the Ivermectin-Clobetasol group; $P < 0.05$, as illustrated in Fig. 10.

Comparison between induced non-treated group and all induced treated groups regarding histopathological scores (Baker score) and observational score (PASI score)

The data obtained has revealed a highly significant reduction in the histopathological scores (Baker score) and observational score (PASI score) among all treated groups as compared with the induction group ($P < 0.05$). Regarding Baker score, the lowest reduction in Baker score compared to the clobetasol group was shown among the Ivermectin group in Comparison to the ivermectin – clobetasol group with a high significant difference; $P < 0.001$. Regarding the PASI score, a highly significant reduction was shown among the clobetasol group, and the combination group was more significant than the Ivermectin group $P < 0.001$, as illustrated by Fig. 11.

Discussion

Petrolatum is defined as being an oleaginous semisolid, which is one of the commonly used treatment forms as topical formulations, as the oleaginous ointments are less irritant and have superior usability due to their texture and longer adhesion time than other formulas. One of the other advantages they possess is that they display superior skin protection effects, which are well absorbed through the skin and aid in the absorption of incorporated active ingredients in the ointment (Ashizuka et al. 2021). The selection of ivermectin was based on its anti-inflammatory and anti-angiogenic properties (Ōmura and Crump 2014; Liu et al. 2016); Ivermectin topical preparation was made based on the available 1% Ivermectin cream that is approved for use in rosacea in humans (Stein et al. 2014).

This study also investigated ivermectin's topical effect on the mouse psoriasis model. It established IMQ-induced psoriasis and found that ivermectin ameliorated the histopathological changes, inflammatory cell infiltration, and keratinocyte hyper-proliferation.

After IMQ application, the development of psoriasis characteristic signs such as erythema, scaling, skin thickening, and hyperkeratosis, as seen in (Fig. 1), is attributed to the IMQ effect on the toll-like receptor-7/8 (TLR-7/8) in which when it is activated leads to an unwanted inflammatory response that is similar to psoriatic lesions (Horváth et al. 2019). Imiquimod also increases the inflammatory cells in the skin, such as dendritic cells, T lymphocytes, and neutrophils (Lin et al. 2015). This method is reversible and only shortly lived after discontinuing topical Imiquimod because the mouse is not genetically compromised and can revert the inflammatory process (Rodríguez-Martínez et al. 2017).

In this study, the results obtained from IMQ-induced psoriasis showed a significant reduction of the total PASI score of mice after treatment with Ivermectin topical preparation

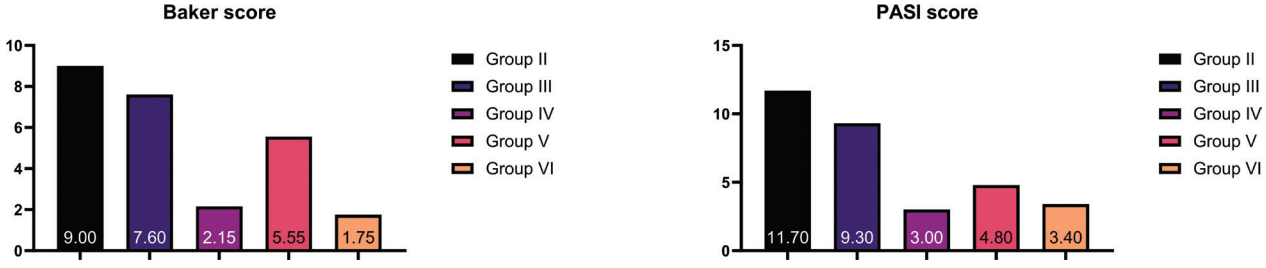

Figure 11. Comparison between induced non-treated group and all induced treated groups regarding histopathological scores (Baker score) and observational score (PASI score).

compared with the induced group and vehicle group, which achieved by a reduction in epidermal thickness and scaling, redness, however, took a longer time to disappear This might also be because of the hair removal cream application which caused a visible irritation and redness on the mice skin shortly upon application (Tsai and Tsai 2022).

In addition, for the histological result, ivermectin showed mild keratosis, with the absence of Munro's abscess and parakeratosis and epidermal mild acanthosis with few rete ridges and mild papillary thinning, the inflammatory cells caused by IMQ, however, were not fully reversed and the dermis was still showing severe lymphocytic infiltrates. The ability of ivermectin to reduce the signs of IMQ-induced psoriasis could be due to its anti-inflammatory properties and ability to inhibit T cells in the skin (Ventre et al. 2017). Ivermectin is also a powerful angiogenesis inhibitor that inhibits the capillary network formation, thus inhibiting proliferation and inducing apoptosis (Liu et al. 2016). This effect was noticed in reducing the VEGF levels in the ELIZA results, which were significantly decreased compared with the induction group.

In another study (Ventre et al. 2017), ivermectin significantly decreased IL-17 and IL-10 levels; in this study, the IL-17 levels were reduced significantly; however, the level of IL-10 was significantly increased.

Also, TNF is a major inducer of IL-10, as reported by was highly expressed, which may explain why it is increased in the treated groups (Asadullah et al. 2013), which also noted in the ELIZA results as the TNF-levels were decreased as compared to the induction group; and this was consistent with other studies of Ivermectin on TNF-a levels (Jameel et al. 2019), which might be because ivermectin needs more time to reduce the TNF-a levels; and because TNF-a is induced by IL-17, which remained high when measured (Amir Ali et al. 2019).

Ivermectin can also inhibit lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines, including TNF-a**,** interleukin (IL)-1b, and IL-6, mediated by NF-κB pathway suppression, which is one of the main substances in the pathogenesis of psoriasis (Deeks 2015); moreover, ivermectin inhibits a variety of cytokines that has been linked to psoriasis such as IL-8 and all these factors are known to be a cause of psoriasis (Heidenreich et al. 2009; Thibaut de Ménonville et al. 2017).

All these effects of ivermectin can explain its effects on psoriasis-like skin lesions and the reduction of epidermal acanthosis, parakeratosis, and papillary thinning.

Clobetasol, on the other hand, was used as the standard treatment in this study and revealed a substantial improvement in the symptoms of psoriasis, which is explained by its anti-inflammatory and immunosuppressive actions that are linked to the development of psoriasis (Boehncke et al. 2010). Clobetasol works by binding to intracellular corticosteroid receptors and regulating gene transcription genes that code for pro-inflammatory cytokines, thus down-regulating the expression of interleukins and TNF-α (Torsekar and Gautam 2017).

It can also modulate the T-cell response and monocytes (Uva et al. 2012) and enhance the transcription of anti-inflammatory genes (Cruz-Topete et al. 2015). Clobetasol can also inhibit psoriasis inflammation by preventing the gene expression of many cytokines and keratinocytes (Mori et al. 2016). These effects can explain the reduction of keratosis and epidermal acanthosis in skin treated with Clobetasol or in skin treated with a combination of Clobetasol and ivermectin that gives an additional anti-inflammatory and immunosuppressive effect to ivermectin that is reflected by the normal epidermal thickness reduction.

In the current study, ivermectin was combined with clobetasol ointment to investigate whether this combination works better than when using either drug alone or whether it would decrease the side effects of both drugs (Fu and Vender 2011). This combination was applied for the same period as the other treated groups. The result of this study revealed that this combination, which has half the concentrations of both ivermectin and Clobetasol compared to their doses when they were applied separately, has shown a better result in improvement in the psoriatic lesion, showing a significant reduction in IL-17, VEGF, and TNF-α levels and slightly higher levels of the anti-inflammatory cytokine IL-10.

One of the important roles of the used carrier is the texture that helps the medication adhere to the lesion for long periods and provides a lipid-soluble environment for treating Ivermectin, which has high lipid solubility. The effect of petrolatum was also assessed by treating ten psoriasis-induced mice with petrolatum alone, and histologically, there was no obvious improvement in psoriatic lesions. However, IL-17 and TNF-α levels decreased compared to non-treated mice; these findings indicate that petrolatum has no strong anti-psoriatic activity (González Canga et al. 2008).

Furthermore, the histopathologic examination of the skin showed a more normal structure consisting of a keratin layer and a normal-looking Malpighian layer. It showed the absence of rete ridges& mild papillary thinning. These findings were much better than those shown when ivermectin and Clobetasol were used separately, confirming the advantages of combinational therapy mentioned earlier. However, hyperkeratosis took longer to disappear in all groups of treatment, and it may be a sign of skin healing in psoriatic lesions (Farci and Mahabal 2023).

Limitations of this study

While animal models provide valuable insights into human diseases, they may not fully represent the complexity of psoriasis in humans. The study did not include clinical data from human subjects. Although the results in the mouse model are promising, further research is needed to determine the safety and efficacy of ivermectin and its combination with Clobetasol in human subjects.

Conclusion

Topical ivermectin possesses anti-psoriasis activity that increases IL-10 levels and could be used efficiently to

alleviate psoriatic symptoms. It decreases the number of psoriatic lesions and the signs of skin inflammation in all IMQ-induced mice. The cumulative score of ivermectin plus Clobetasol was significantly better than ivermectin alone compared to the IMQ-induced group. These findings suggest that topical Ivermectin/Clobetasol has a promising effect in treating psoriasis and inflammatory skin diseases.

Ethics approval

The "Research Ethics Committee at the College of Medicine, Al-Nahrain University" approved the study (2310, date: 1st December 2022).

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability

Underlying data:

Zenodo: "Ivermectin For Psoriasis". https://doi.org/10.5281/zenodo.8342805 (Almudaris 2023)

The project contains the following underlying data: Data set [Excell sheet]. ARRIVE guidelines 2.0: author checklist.

Author Contributions

All authors contributed to the study conception and design. [Sally Ayad Almudaris] and [Fouad Kadhim Gatea] performed material preparation, data collection, and analysis. The first draft of the manuscript was written by [Sally Ayad Almudaris], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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