

# The Effect of Lenalidomide Ointment on TNF- $\alpha$ Tissue Levels in Mice with Imiquimod-Induced Psoriasis.

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## Abstract:

**Background:** Lenalidomide is an immunomodulatory drug having notable anti-inflammatory, and anti-antineoplastic properties. Lenalidomide suppresses the production of pro-inflammatory cytokines that have been linked to a variety of hematologic malignancies. Lenalidomide enhances the immune system of the host by regulating T cell proliferation, which results in changes in inflammation that are related to the etiology of psoriasis.

**Objectives:** The objectives of this study were to determine the efficacy of lenalidomide as an ointment in treating mouse models of psoriasis as well as how it may affect TNF- $\alpha$  levels in skin tissue in different experimental groups.

**Methods:** The study was carried out between November 2021 and June 2022. 70 healthy male albino mice were randomly divided into 7 groups of 10 animals each. In groups (1, 2, 3, 4, 5 and 6), imiquimod produced psoriasis. Only imiquimod cream was administered to Group 1, after psoriasis induced, Clobetasol ointment was applied to Group 2, placebo ointment was applied to Group 3, and lenalidomide ointment (1%, 2%, and 3%) were applied to Groups (4, 5, and 6), respectively. Healthy mice were utilized as a comparative control in Group 7. SPSS was utilized for the statistical analysis of the data (version 26).

**Results:** Following lenalidomide treatment, the psoriatic region improved. Lenalidomide's effectiveness to treat imiquimod-induced mouse psoriasis was explained by the difference in tissue levels of TNF- $\alpha$  between the examined groups.

**Conclusions:** Findings suggest that different concentrations of lenalidomide ointment can improve mouse models of imiquimod-induced psoriasis. Histopathology and immunohistochemistry assays show that lenalidomide ointment was more effective and had no side effects that were associated with the use of the standard drugs.

**Keywords:** Anti-inflammatory, Imiquimod-induced psoriasis, Immunomodulatory, Lenalidomide, TNF- $\alpha$ .

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## Introduction:

Psoriasis is a common, chronic, noncontagious disease characterized by highly proliferating keratinocytes and extensive leukocyte infiltration (1). People's life may be significantly and detrimentally impacted by this sickness. People of all ages and from all layers of society are affected by psoriasis (2). Numerous factors, including genetic, environmental, viral, and lifestyle-related issues, might contribute to the development of psoriasis (3). Complications with the autoimmune system, genetics, hormones, and psychological health all play a role in how it develops (4). An overactive cellular immune system is a

defining feature (5). In that it is "a clinical condition induced by the activation of T lymphocytes and b lymphocytes, or even both, in the absence of a persistent infection or other recognizable cause (6)." Other types of inflammatory leukocytes may potentially be involved in the pathogenesis of psoriasis. Inflammatory cytokines like tumor necrosis factor (TNF- $\alpha$ ) are known to have a significant pathogenic function in psoriasis (7). Psoriasis is characterized by a variety of factors, including infiltrating leukocytes, resident skin cells, and a variety of proinflammatory cytokines, chemokines, and chemical mediators secreted in the skin under the direction of the cellular immune system (8). Lenalidomide is a thalidomide analogue that was synthesized by altering the chemical structure of thalidomide. On the other hand, it is significantly safer and more powerful than thalidomide, with fewer side effects and toxicities (9). Lenalidomide enhances host immunity in a variety of ways and supports the death of malignant cells (10). Lenalidomide affects the immune

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system's cellular and humoral components (11). The immune system can stop the development of malignancies by removing or blocking oncogenic viral infections, altering the inflammatory environment favorable for tumor genesis (12). Lenalidomide has been shown to inhibit production of pro inflammatory cytokines TNF- $\alpha$ , IL-1, 6, 12 and 17, and elevate the production of anti-inflammatory cytokine IL-10 from Human Peripheral Blood Mononuclear Cells (13). TNF- $\alpha$  is a highly pleiotropic cytokine that is mostly generated by macrophages and monocytes and is crucial for the development of protective immune responses against viral and bacterial infections (14). The etiology of psoriasis is thought to be influenced by increased TNF- $\alpha$  production (15). This study tries to assess the effect of lenalidomide as an ointment in different concentrations in the treatment of psoriasis compared to other treatments such as Clobetasol ointment. Study the histological changes and immunohistochemical (IHC) biomarker such as tumor necrosis factor alpha (TNF- $\alpha$ ) in experimentally induced psoriasis. Investigate the mechanisms underlying the immunomodulatory actions of lenalidomide by investigating their effects on histological changes and IHC biomarkers. Assess the effect of the placebo ointment on the histological changes and the IHC biomarker.

#### **Materials and methods:**

**Chemicals and reagents:** In this experiment, the following substances were used: lenalidomide powder from Hangzhou Hyper Chemicals in China; 5% imiquimod cream from Meda in Sweden; Clobetasol 17-propionate ointment from GSK; Mouse TNF-polyclonal antibody, Abcam from the UK; IHC detection kit - specific HRP/DAB (ABC), Abcam from the UK; castor oil from KTC in Germany; petroleum jelly from Battles, Hayward The chemicals were all of analytical reagent quality.

**Lenalidomide ointment preparation:** At the University of Baghdad's College of Pharmacy, lenalidomide powder was formulated into an ointment after being obtained from Hangzhou Hyper Chemicals-China Limited as a white powder. In order to produce three different concentrations of lenalidomide ointment, lenalidomide powder was first dissolved in castor oil (16), which functions as a levigating agent (17), and then combined with petroleum jelly using geometric mixing (1%, 2%, and 3%) (18). **Mice and treatments:** From the Iraqi center for cancer research and medical genetics, 70 healthy albino mice, aged 6 to 8 weeks, were purchased. All of the procedures, which conformed to national guidelines for the care and usage of experimental animals (1697, 6/12/2021), were approved by the College of Medicine-University of Baghdad's Experiment Animal Ethics Committee. The animals were kept in germ-free environments, Food and water are available at all times (18). Seven groups of mice were randomly selected, each of 10 mice. In all the

groups, psoriasis was induced by applying imiquimod on the shaved back, except group 7 (the control group) (18). In Group 1, only imiquimod cream 5% was applied once daily for 6 days to induce psoriasis-like inflammation (19). After imiquimod-induced psoriasis, Group 2 mice were given Clobetasol ointment topically once daily for 6 days on their shaved backs (20). Group 3: Placebo ointment (castor oil and petroleum jelly, which was free of drugs) was administered to (G3). For 6 days, lenalidomide ointment (1%, 2%, and 3%, respectively) was applied topically (18). Only mice who had not had any treatment (and appeared to be in perfect health) were utilized in Group 7 as a comparison control group (18). Plastic cages with a size of 20x25x35 cm were used to house one animal in each cage. Prior to the study procedure, the animals were housed for two weeks in a controlled environment with a temperature of  $(22 \pm 1^{\circ}\text{C})$ , a light schedule of 12 hours of light and dark, and an air vacuum to adapt to the animal house's environment (18).

#### **Histological analysis**

A. Each skin tissue sample was sliced into sections and preserved in neutral buffered formaldehyde (NBF) 10% for histopathological and immunohistochemical examination. After that, the sample underwent formalin-fixed, paraffin-embedded tissue preparation (FFPE), fixation of tissue, dehydration, impregnation, and tissue embedding into paraffin blocks (21).

B. Preparing tissue sections and slides (21).

C. Hematoxylin and eosin is used to stain paraffin sections (H&E) (22).

D. Assessment of the histopathological analysis of the skin tissue: A light microscope (Genx®/USA) was used to capture photographs of a slide at magnifications of X 20, X 10, and X 4. The psoriatic skin changes were evaluated using Barker's scoring system.

**Immunohistochemical (IHC) Analysis (23):** TNF- $\alpha$  (IHC) kits were used to detect TNF- $\alpha$ .

A. New section of (5  $\mu\text{m}$ ) thickness was formed from each of the paraffin-embedded blocks that had been previously prepared. They were set up on positively charged slides for TNF- $\alpha$  immunohistochemistry detection (24).

B. Immunohistochemical (IHC) Procedure (25).

#### **Statistical analysis:**

SPSS was used to perform the statistical analysis (version 26, IBM Corp. 2019) (18). One-way ANOVA, and means comparisons using one-way ANOVA with postHOC = LSD ALPHA (0.05) were used (least significant difference) (18). Results were presented as the mean  $\pm$  SE. Statistics showed that differences were statistically significant when the P value was less than 0.05 (18).

#### **Results:**

The characteristics of psoriasis, such as round, red papules or plaques with a grey or silvery-white dry

scale, arise on the treated area after 6 days after IMQ administration on the shaved back skin of the mice (Fig.1.A). On day 12 (day 6 following induction), the results of psoriasis were completely improved following the application of Clobetasol ointment (Fig.1.C) and completely improved following the application of lenalidomide ointment 1% (Fig. 1.D). Lenalidomide ointment 2% and 3% treated mice did not perform significantly better, while animals given a placebo performed similarly to the 2% and 3% ointment treated mice (Fig.1.G, E, and F respectively). The mouse in (Fig.1.B) appears to be in good health and is presented without any applications

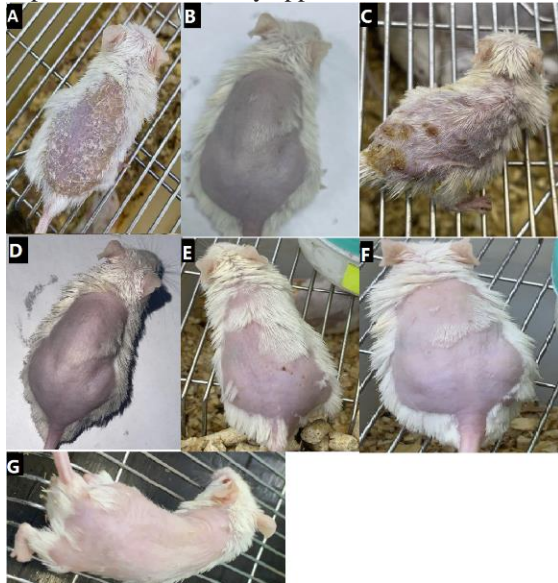


Figure 1: Mice experienced several treatments.

### Histopathological assessment

In comparison to the control group, the stained IMQ-treated skin showed thicker epidermis (hyperkeratosis) and subcutaneous tissue. Additionally, nuclei in the stratum corneum with abnormal keratinocyte differentiation and parakeratosis were seen. In addition, acanthosis, thinning above the papillae, lengthening and clubbing of the rete ridges, as well as the absence of a granular layer, are seen (Fig.2.A). There is a significant lymphocytic infiltrate and papillary congestion in the dermis layer (Fig.2.A). The thickness rise was entirely inhibited by Clobetasol ointment treatment. Skin that had received IMQ treatment had stratum corneum dropouts. Although the layer is thin above the papillae, the rete ridges extend and club, the acanthosis disappears, and the granular layer reappears. There is no papillary congestion or lymphocytic infiltration (Fig.2.B). When the same mice are treated with lenalidomide ointment 1%, histological analysis reveals roughly comparable results (Fig.2.D). Lenalidomide ointment 1% and Clobetasol ointment both produced results that were roughly comparable, therefore there was little noticeable difference between the two groups. Lenalidomide ointment 2% mice exhibited acanthosis,

rete ridge lengthening and clubbing, hyperkeratosis, and parakeratosis. Papillary congestion and lymphocytic infiltration are also present (Fig.2.E, F). The 3% lenalidomide ointment treated group showed hyperkeratosis, lengthening and clubbing of rete ridges, and acanthosis. Moreover, a lymphocytic infiltration is present (Fig.2.G, H). The placebo ointment, vehicle of the lenalidomide (drug-free) group, showed rete ridge lengthening and clubbing, as well as acanthosis. There is a lymphocytic infiltration and papillary congestion (Fig.2.C). Lenalidomide ointment 2%, 3 %, and placebo ointment groups show a significant difference from the Clobetasol ointment and lenalidomide ointment 1% groups. The control groups exhibited no aberrant phenotype, as seen in (Fig.2.J). These findings might support the idea that lenalidomide 1% treatment was efficient and roughly equivalent to standard. Both lenalidomide ointment 2% and lenalidomide ointment 3% treatments had no beneficial effects, and the psoriasis-like symptoms persisted.

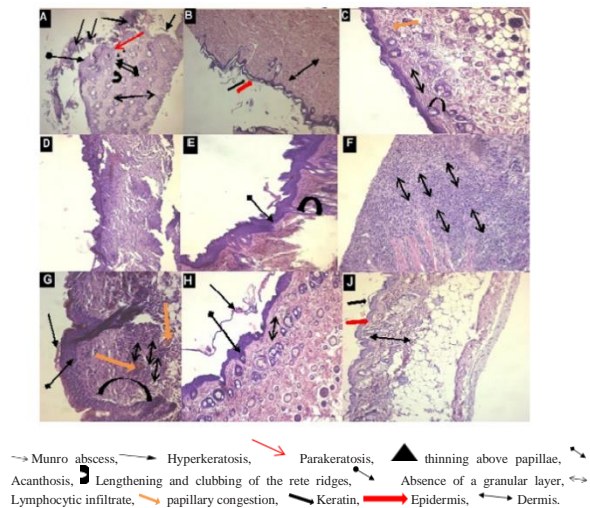
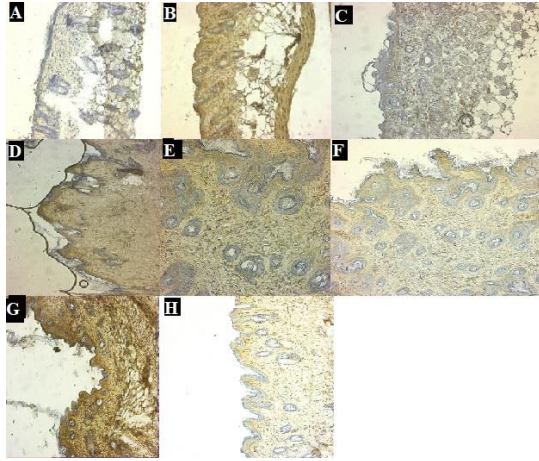


Figure 2: A histological analysis of the skin sections.

### Immunohistochemical assessment of TNF- $\alpha$ :

A brown colored precipitate will form at the antigen site in the tested tissue as a result of a positive reaction. Staining intensity was determined using an arbitrary score ranging from 0 to 3 depending on the detection system that recognizes a particular antibody attached to an antigen in tissue sections (0 = no staining; 1 = mild; 2 = moderate; and 3 = strong staining intensity) (18). After 6 days of IMQ application to the mice's skin, as compared to the control group (G7), which looks to be in good health (Fig.3.A) (negative reaction, so there is no staining), the level of TNF- $\alpha$  increases with strong staining intensity, as shown in Fig.3.B). On day 12, TNF- $\alpha$  levels dropped and the tissue marker turned light (day 6 after induction). This revealed that the psoriasis improved after Clobetasol ointment was applied to the psoriatic area (Fig.3.D), as well as after the application

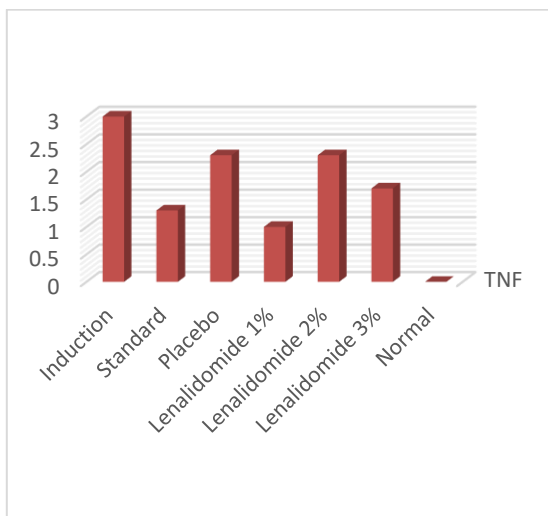
of 1 percent lenalidomide ointment (Fig.3.C). In contrast to G2 and G4, the mice treated with lenalidomide ointment at 2 percent, 3 percent, and placebo ointment displayed moderate to strong staining intensity, indicating a high level of TNF- $\alpha$  (E, F, and H, respectively, in Fig.3.)



**Figure 3: The TNF- $\alpha$  level is explained by a microphotograph of mouse skin tissue immunohistochemically (IHC) stained.**

**Table 1: TNF- $\alpha$  Means different.**

Groups	Mean (Staining intensity)	Std. Error of Mean
G1	3.0 a	.00000
G2	1.30 d	.15275
G3	2.3 b	.15275
G4	1.0 d	.00000
G5	2.3 b	.15275
G6	1.7 c	.15275
G7	.0 e	.00000
Total	1.6571	.11845



**Figure 4: TNF- $\alpha$  scoring.**

**Table 2: ANOVA table for TNF- $\alpha$  tissue level.**

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59.371	6	9.895	74.214	.000
Within Groups	8.400	63	.133		
Total	67.771	69			

The tissue TNF- $\alpha$  level ANOVA table showed that there was a significant difference in tissue TNF- $\alpha$  level at  $F = 74.214$  ( $P = 0.05$ ). One-way ANOVA with postHOC = LSD ALPHA (0.05) was used to statistically analyze TNF-based on multiple comparisons based on immunohistochemistry stain intensity, as shown in Table 3:

**Table 3: Multiple comparisons of TNF- $\alpha$ , using one-way ANOVA postHOC = LSD ALPHA (0.05).**

(I)CH1	(J)CH1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
G1	G2	1.70000*	.16330	.000	1.3737	2.0263
	G3	.70000*	.16330	.000	.3737	1.0263
	G4	2.00000*	.16330	.000	1.6737	2.3263
	G5	.70000*	.16330	.000	.3737	1.0263
	G6	1.30000*	.16330	.000	.9737	1.6263
	G7	3.00000*	.16330	.000	2.6737	3.3263
	G2	G1	-1.70000*	.16330	.000	-2.0263
G3		-1.00000*	.16330	.000	-1.3263	-.6737
G4		.30000	.16330	.071	-.0263	.6263
G5		-1.00000*	.16330	.000	-1.3263	-.6737
G6		-.40000*	.16330	.017	-.7263	-.0737
G7		1.30000*	.16330	.000	.9737	1.6263
G3		G1	-.70000*	.16330	.000	-1.0263
	G2	1.00000*	.16330	.000	.6737	1.3263
	G4	1.30000*	.16330	.000	.9737	1.6263
	G5	.00000	.16330	1.000	-.3263	.3263
	G6	.60000*	.16330	.000	.2737	.9263
	G7	2.30000*	.16330	.000	1.9737	2.6263
	G4	G1	-2.00000*	.16330	.000	-2.3263
G2		-.30000	.16330	.071	-.6263	.0263
G3		-1.30000*	.16330	.000	-1.6263	-.9737
G5		-1.30000*	.16330	.000	-1.6263	-.9737
G6		-.70000*	.16330	.000	-1.0263	-.3737
G7		1.00000*	.16330	.000	.6737	1.3263
G5		G1	-.70000*	.16330	.000	-1.0263
	G2	1.00000*	.16330	.000	.6737	1.3263
	G3	.00000	.16330	1.000	-.3263	.3263
	G4	1.30000*	.16330	.000	.9737	1.6263
	G6	.60000*	.16330	.000	.2737	.9263
	G7	2.30000*	.16330	.000	1.9737	2.6263
	G6	G1	-1.30000*	.16330	.000	-1.6263
G2		.40000*	.16330	.017	.0737	.7263
G3		-.60000*	.16330	.000	-.9263	-.2737
G4		.70000*	.16330	.000	.3737	1.0263
G5		-.60000*	.16330	.000	-.9263	-.2737
G7		1.70000*	.16330	.000	1.3737	2.0263
G7		G1	-3.00000*	.16330	.000	-3.3263
	G2	-1.30000*	.16330	.000	-1.6263	-.9737
	G3	-2.30000*	.16330	.000	-2.6263	-1.9737
	G4	-1.00000*	.16330	.000	-1.3263	-.6737
	G5	-2.30000*	.16330	.000	-2.6263	-1.9737
	G6	-1.70000*	.16330	.000	-2.0263	-1.3737

\*. The mean difference is significant at the 0.05 level.

**Discussion:**

Although the exact cause of psoriasis is unknown, some characteristics suggest an immune reaction as the likely cause (26). Localized inflammation, leukocyte infiltration, epidermal hyperplasia, and enhanced dermal vascularity are symptoms of the chronic skin problem psoriasis. A dysregulated interaction between immune cells and keratinocytes underlies this immunological-mediated illness (27). Imiquimod is a TLR7/8 agonist (28), it can promote the synthesis of IL-17, IL-6, IL-1, IL-12, IL-18, TNF- $\alpha$ , IFN- $\gamma$ , IFN-R, and other cytokines in the body and activate monocytes, macrophages, and dendritic cells via TLRs, This leads to an immune response (28). In the current study, the application of a 5% imiquimod cream resulted in apparent pathologic alterations like those of psoriasis (28). After accessing the body through the skin, imiquimod may bind to TLR7 on (pDCs) and activate them, causing a significant release of downstream molecules, including TNF- $\alpha$  (29). These cytokines were found in serum and skin lesions at significantly higher levels after receiving imiquimod therapy (29). Additionally, (NF- $\kappa$ B) signaling has been linked to the pathogenesis of psoriasis induced by imiquimod (29). NF- $\kappa$ B protein complex, which regulates cytokine synthesis (30), TLR/MyD88/TRAF6 signaling, specifically on days 4 to 6, can activate NF- $\kappa$ B during chronic inflammation in skin lesions treated with imiquimod (29). They demonstrated that the NF- $\kappa$ B and TLR7 signaling pathways were active throughout the duration of imiquimod-induced psoriasis by significantly increasing synthesis and release of proinflammatory cytokines (29). In the current study, the Barker scoring system was employed to explain the histopathological differences between the groups under investigation. The overall score, which is the sum of the nine histological characteristics, increased statistically significantly when compared to the control group (28). The imiquimod group (G1) had a significantly higher Baker's scoring than the control group (G7) and all other groups, showing psoriasis was developed in group (G1). The findings of this study are consistent with those of a prior study that showed imiquimod treatment can trigger and exacerbate psoriasis in mice (28). The most noticeable abnormalities in the induction group included acanthosis, parakeratosis hyperkeratosis, loss of granular layer, Munro's abscess, and various other changes. There is a noticeable leukocyte infiltrate as well as lengthening and clubbing of the rete ridges. These results support those of Schön 2021, who indicated that histopathological features such as hyperproliferation of epidermal keratinocytes, infiltration of immunocytes, hyperkeratosis, and angiogenesis are what contribute to the typical thickening and scaling of erythematous skin (28). Since it is simple to generate and display the characteristic psoriatic phenotype, the Imiquimod-induced mouse model is thought to be a successful psoriasis model

(27). It exhibits the majority of psoriasis' histological features, such as cutaneous inflammation, hyperproliferation, and neutrophil and (pDc) infiltration. Effect of 1% lenalidomide ointment compared to standard treatments (Clobetasol ointment) on the histological change of skin tissue (Clobetasol). The results of the experiments revealed a significant decline in scores in the treated groups, particularly the lenalidomide 1% group (G4), although there was no statistically significant difference between the control group G7 and the lenalidomide ointment 1% group (G4). These trials supported earlier research, such as that by Gellad 2019, and demonstrated that the 1% lenalidomide ointment group (G4) experienced successful psoriasis skin healing. However, it is necessary to use medications with long-lasting and safe therapeutic effects. Topical corticosteroids, especially high-potency corticosteroids, have been the standard method for the treatment of psoriasis for many years (30). Numerous modes of action, including anti-inflammatory, immunosuppressive, and anti-proliferative actions, can be attributed to their effectiveness (30). It was observed that clobetasol had a significant effect. It has been demonstrated that clobetasol lowers mice's psoriasis-like inflammation's TNF- $\alpha$  levels (32). The findings of this study were consistent with those of an earlier study that demonstrated effective penetration of Clobetasol ointment (G2) into lesional skin and enhanced skin healing (33) The studies revealed a significant decrease in scores in the treated groups (lenalidomide ointment 1% group (G4) and Clobetasol ointment (G2)); however, there was no difference between the control group (G7), lenalidomide ointment 1% group (G4), and Clobetasol ointment (G2) that could be considered significant. Effect of lenalidomide ointment 2%, lenalidomide ointment 3%, and the placebo ointment in comparison to lenalidomide ointment 1% on the histological change of the skin tissue When compared to the groups treated with placebo ointment (G3), lenalidomide 2% ointment (G5), and lenalidomide 3% ointment (G6), Baker's scoring results in this study reveal a significant difference between these groups and the groups that were treated with lenalidomide ointment 1% (G4), Clobetasol ointment (G2), and the control group (G7). The group receiving placebo ointment (G3) displayed a significant difference from the groups receiving lenalidomide ointment (1%) (G4), Clobetasol ointment (G2), and the control group (G7). The only ingredients in placebo ointment are petroleum jelly and castor oil, which serve as carriers for lenalidomide. Skin integrity loss due to sickness or injury may result in a serious physiological imbalance. (32). The epidermal barrier is necessary in order to maintain the skin's homeostasis and protect the body from various external stimuli (34). Transepidermal water loss is one of the most important elements for determining the condition of the skin barrier (TEWL). Higher TEWL levels are

associated with weakened skin barriers (34). The moisture of the stratum corneum is a crucial additional factor for assessing the performance of the skin barrier (SCH). The stratum corneum's water content is shown by lower SCH readings, which are frequently associated with skin disorders and more serious illnesses. (34). other skin traits associated with the functioning of the skin barrier include PH, elasticity, and temperature. (34). for the skin barrier to be repaired, moisturizing products are essential. Emollients improve the stratum corneum's ability to function as a barrier by supplying lipids and water. With this lipid replacement therapy, inflammation may be decreased and epidermal function may be enhanced (31). They also include emollient ingredients, such as petrolatum, which coat the skin's surface with a water-repellent lipid layer and stop the transport of water in both directions (mostly by minimizing skin water loss) (34) Emollients improve the penetration of other topical treatments while softening fissures, reducing scaling and irritation (34). The results of this study using the baker's scoring system showed how emollients affected the way the epidermal barrier worked in mice with psoriasis. The psoriatic animals showed lower SCH and higher TEWL, temperature, and erythema in comparison to the control group. Vaseline, a petroleum jelly, reduced the TEWL in psoriatic plaques (34). According to the study's findings using Baker's scoring system, lenalidomide ointment 2% (G5) and lenalidomide ointment 3% (G6) had effects that were significantly different from those of lenalidomide ointment 1% (G4), clobetasol ointment (G2), and the control group (G7). T cells are crucial immune response effectors, and activation of these cells is tightly controlled to prevent auto-reactivity (34). APCs transmit peptide fragments to the TCR during T cell activation, and this interaction confers the response's specificity (32). However, a T cell needs more than just this interaction to provide an efficient response against the antigen (34). The T cell surface B7/CTLA4 secondary interaction has a negative signal that controls T-cell activation by suppressing T-cell activation and limiting the availability of B7 to interact with CD-28 (33). Compared to CD-28, CTLA4 has a 20-fold greater affinity for B7. When this interaction is prevented, a co-stimulatory signal is produced, enhancing the T cell response and promoting T cell survival, differentiation, and proliferation. This is followed by a series of cytokine and cellular reactions (34). In some conditions, T cells or B cells that come into contact with their antigens without costimulation may become anergized or eliminated by programmed cell death (35). Lenalidomide is an immunomodulatory medication that affects T cells via the B7/CD28 co-stimulatory pathway by blocking the binding of B7/CTLA4 Ig (36). IMiDs do not increase the expression of CD28 or B7 on T cells or APCs, but they can cause CD28 on T cells to be tyrosine phosphorylated directly, activating downstream targets

such as PI3K, GRB-2-OS, and NF- $\kappa$ B (37). This may be the reason for their limited success in overcoming CTLA4 Ig blockage (37). Lenalidomide promotes Th1 cytokine response upon T cell co-stimulation, leading to enhanced secretion of TNF- $\alpha$ , which in turn stimulates clonal T cell proliferation and NK cell activity (38). T-cell activation requires two independent signals. (39). According to the study's findings, lenalidomide is a T-cell co-stimulatory molecule that inhibits pro-inflammatory cytokines by leading to programmed cell death in the absence of a stimulation signal. As its concentrations rise, lenalidomide stimulates T-cell proliferation and increases TNF- $\alpha$  production in T cells, increasing NK cell cytotoxicity and ADCC. TNF-level effects of lenalidomide ointment at 1% compared to standard treatments (Clobetasol ointment) the current study confirmed the immunomodulatory properties of lenalidomide, which decreased the production of pro-inflammatory cytokines linked to the development of psoriasis. Lenalidomide improves the host's immunity via changing cytokine production, controlling T cell co-stimulation, and increasing natural killer (NK) cell-mediated cytotoxicity (39). The lowest lenalidomide content in ointment (G4, lenalidomide ointment 1%) had the lowest mean for the TNF- $\alpha$  biomarker, with a significant difference from the induction group. This was established by analyzing the anti-psoriatic activity of different doses administered to a mouse model of psoriasis. The findings showed that there was no significant difference in TNF- $\alpha$  levels between the groups receiving lenalidomide 1% ointment (G4) and Clobetasol ointment (G2). These results corroborate those who suggested immunosuppressive drugs like lenalidomide had an anti-psoriatic effect that might be linked to a decrease in cytokine production like TNF- $\alpha$  (39). These findings were consistent with previous research showing that clobetasol reduces cytokine production, including TNF- $\alpha$ , and has an anti-psoriatic effect (39). The effect of the placebo ointment, lenalidomide ointment 2%, and lenalidomide ointment 2% on TNF- $\alpha$  In this study, the effect of placebo ointment (G3) on tissue levels of TNF- $\alpha$  was significantly different from the effects of lenalidomide ointment (G4) at 1%, Clobetasol ointment (G2), and the control group (G7). Additionally, the effects of the placebo ointment (G3) are significantly different from those of the induction group, which is consistent with a prior study that suggested the vasiline ointment may have an effect on inflammatory cytokine levels in tissues or the blood (40). When the epidermal permeability barrier was broken, mouse cutaneous and serum inflammatory cytokines increased (31). Previous research has shown that imiquimod disrupts the epidermal permeability barrier, causing an increase in epidermal cytokine production that results in cutaneous inflammation in animal models (29). Skin-and systemic inflammation are both produced by barrier disturbance (40). Previous

research showed that correction of the epidermal permeability barrier controls cutaneous inflammation by reducing the amounts of epidermal cytokines in the skin when its function is impaired (31). Topical vasiline (petrolatum jelly), a material that has previously been shown to improve the function of the epidermal permeability barrier when applied to the skin, corrects epidermal functional impairment and lowers cytokine levels in the skin and serum (40). Topical vasiline had a significantly different effect than lenalidomide ointment 1% (G4) because it reduces cytokine levels in the skin and serum by correcting ongoing problems with the epidermal permeability barrier's functionality. The current study confirmed the immunomodulatory effects of lenalidomide, which lowers the production of pro-inflammatory cytokines linked to the pathogenesis of psoriasis. Additionally, by modifying T cell co-stimulation and cytokine production (40). Moreover, Vaseline's effect was also noticeably different from that of clobetasol ointment (G2), which has a variety of modalities of action, such as anti-inflammatory, immunosuppressive, and anti-proliferative effects (30). The B7/CTLA4 Ig interaction was blocked by increasing the lenalidomide concentration in the applied ointment (36). Bringing about the co-stimulatory signal that enhances the T cell response and promotes T cell proliferation, differentiation, and survival, which is followed by a cascade of cytokine and cellular responses and activates NF-Kb (34). Lenalidomide increases the Th1 cytokine response, which in turn stimulates NK cell activity and clonal T cell proliferation, leading to a rise in TNF- $\alpha$  production (38). This explains the significant differences between the groups treated with lenalidomide ointment 1% (G4), the Clobetasol ointment group (G2), and the control group (G7) as compared to the groups treated with lenalidomide ointment 2%(G5) and lenalidomide ointment 3% (G6). In our study, shown in table 1, we discovered that the TNF- $\alpha$  staining in the psoriatic mouse skin was significantly ( $p < 0.05$ ) higher than in normal skin. Similar findings were made by Lin et al., who discovered "increased TNF- $\alpha$  expression in psoriatic plaques, which may play a critical role in the pathogenesis of the disease. Our findings demonstrated a substantial reduction in the TNF- $\alpha$  mean staining intensity in groups (G4) lenalidomide ointment 1 % (1.0 d) and G2 Clobetasol ointment (1.30 d), in comparison to the induction group. Lenalidomide's immunomodulatory properties inhibit the production of pro-inflammatory cytokines that have been associated with the pathogenesis of psoriasis. Lenalidomide increases NK cell-mediated cytotoxicity, regulates T cell co-stimulation, and changes cytokine production to increase the host's immunity (40), the same as Clobetasol (32).

### Conclusions:

These results show that imiquimod-induced psoriasis in mice can be treated with lenalidomide ointment at a specific dose. It is efficient and works in a way similar to that of a standard medication, reducing the production of pro-inflammatory cytokines that have been linked to the pathogenesis of psoriasis by altering cytokine production and controlling T cell co-stimulation.

### Authors' contributions:

Sajjad M. Thamer :student

Mohammed Q. Yahya: supervisor

### References:

1. Petit RG, Cano A, Ortiz A, Espina M, Prat J, Muñoz M, et al. Psoriasis: From pathogenesis to pharmacological and nano-technological-based therapeutics. *International Journal of Molecular Sciences*. 2021;22(9):4983.
2. Ciuluvica C, Fulcheri M, Amerio P. Expressive suppression and negative affect, pathways of emotional dysregulation in psoriasis patients. *Front Psychol*. 2019;10:1907.
3. H Adbullah T, I Latif I, Kh Ibrahim K. Role of Tumor Necrosis Factor Alpha and Transforming Growth Factor Beta as Predictive Marker for Psoriasis Patients. *Diyala Journal of Medicine [Internet]*. 2020 Oct 5;19(1):1–7. Available from: <http://djm.uodiyala.edu.iq/index.php/djm/article/view/583>
4. Gautam S, Kumar U, Dada R. Yoga and its impact on chronic inflammatory autoimmune arthritis. *Frontiers in Bioscience-Elite*. 2020;13(1):77–116.
5. Abbas Kareem, Y. A.-T. A. N. (2021). Evaluation of IL-17, IL-18 and IL-22 as Vital indicators of Iraqi Patients with Psoriasis. *Iraqi Journal of Biotechnology*, 20, 103–108.
6. Hu P, Wang M, Gao H, Zheng A, Li J, Mu D, et al. The Role of Helper T Cells in Psoriasis. *Frontiers in Immunology*. 2021;12.
7. Saheb EJ, Al-Issa YAH, Mussa IS, Zghair KH. Incidence of toxoplasmosis in psoriasis patients and possible correlation with tumor necrosis factor- $\alpha$ . *Baghdad Science Journal*. 2020 Mar 1;17(1):214–9.
8. Bahir A.R. Mshimesh. Treatment of Mild to Moderate Plaque Psoriasis with Pimecrolimus, Clobetasol, or Calcipotriol Cream: A comparative study. *J Fac Med Baghdad*. 2016 Apr 3;58(1):42–43.
9. Paumgarten FJR. The tale of lenalidomide clinical superiority over thalidomide and regulatory and cost-effectiveness issues. *Ciência & Saúde Coletiva*. 2019;24:3783–92.
10. Qiu Q, Lin Y, Ma Y, Li X, Liang J, Chen Z, et al. Exploring the emerging role of the gut microbiota and tumor microenvironment in cancer immunotherapy. *Frontiers in Immunology*. 2021;11:612202.

11. McComb S, Thiriot A, Akache B, Krishnan L, Stark F. Introduction to the immune system. In: *Immunoproteomics*. Springer; 2019. p. 1–24.
12. Abbott M, Ustoyev Y. Cancer and the immune system: the history and background of immunotherapy. In: *Seminars in oncology nursing*. Elsevier; 2019. p. 150923.
13. Shallis RM, Chokr N, Stahl M, Pine AB, Zeidan AM. Immunosuppressive therapy in myelodysplastic syndromes: a borrowed therapy in search of the right place. *Expert Review of Hematology*. 2018;11(9):715–26.
14. Yang L, Xie X, Tu Z, Fu J, Xu D, Zhou Y. The signal pathways and treatment of cytokine storm in COVID-19. *Signal Transduct Target Ther*. 2021;6(1):1–20.
15. Sharma RK, Sharma MR, Mahendra A, Kumar S. Role of Inflammatory Cytokines in Pathophysiology of Psoriasis. *Current Pharmacology Reports*. 2022;1–7.
16. Kar M, Chourasiya Y, Maheshwari R, Tekade RK. Current developments in excipient science: implication of quantitative selection of each excipient in product development. In: *Basic Fundamentals of Drug Delivery*. Elsevier; 2019. p. 29–83.
17. Li Y, Yesharim O, Hurvitz I, Karnieli A, Fu S, Porat G, et al. Adiabatic geometric phase in fully nonlinear three-wave mixing. *Physical Review A*. 2020;101(3):033807.
18. Mahmood AM, Kareem A, Abd H, Qasim BJ. NOVEL MITOXANTRONE HCL OINTMENT FORMULA VERSUS CLOBETASOL FOR TREATMENT OF PSORIASIS IN MICE [Internet]. Available from: <https://connectjournals.com/>
19. Amalia SN, Uchiyama A, Baral H, Inoue Y, Yamazaki S, Fujiwara C, et al. Suppression of neuropeptide by botulinum toxin improves imiquimod-induced psoriasis-like dermatitis via the regulation of neuroimmune system. *J Dermatol Sci*. 2021;101(1):58–68.
20. Mohammed SS, Kadhim HM, Al-Sudani IM, Musatafa WW. Study the Topical Effect of Six Days Use of Different Lycopene Doses on Imiquimod-Induce Psoriasis-Like Skin Inflammation in Mice.
21. Farah K NN. Effects of Vitamin D3 on Methotrexate- Induced Jejenum Damage in Rats. *Iraqi Journal of Pharmaceutical Sciences*. 2020 Jun 25;29(1).
22. Sy J, Ang LC. Microtomy: cutting formalin-fixed, paraffin-embedded sections. *Biobanking*. 2019;269–78.
23. Farah K MQ. Gastro Protective Effect of Ethanolic Extract of *Anchus astrigosa* on Indomethacin-Induced Gastric Ulcer in Rats. *Drugs and Cell Therapies in Hematology*. 2021;10(3):1–18.
24. Hermann J, Noels H, Theelen W, Lellig M, Orth-Alampour S, Boor P, et al. Sample preparation of formalin-fixed paraffin-embedded tissue sections for MALDI-mass spectrometry imaging. *Anal Bioanal Chem*. 2020;412(6):1263–75.
25. Wang WQ, WFH, QW, LHY, LB, CC, ZJ, GQ, SW and WC. Joint antiangiogenic effect of ATN-161 and anti-VEGF antibody in a rat model of early wet age-related macular degeneration. *Mol Pharm*. 2016;
26. Tan L, Zhao S, Zhu W, Wu L, Li J, Shen M, et al. The *Akkermansia muciniphila* is a gut microbiota signature in psoriasis. *Exp Dermatol*. 2018;27(2):144–9.
27. Jabeen M, Boisgard AS, Danoy A, el Kholti N, Salvi JP, Boulieu R, et al. Advanced characterization of imiquimod-induced psoriasis-like mouse model. *Pharmaceutics*. 2020;12(9):789.
28. Schön MP, Manzke V, Erpenbeck L. Animal models of psoriasis—highlights and drawbacks. *Journal of Allergy and Clinical Immunology*. 2021;147(2):439–55.
29. Li Y, Zhang G, Chen M, Tong M, Zhao M, Tang F, et al. Rutaecarpine inhibited imiquimod-induced psoriasis-like dermatitis via inhibiting the NF- $\kappa$ B and TLR7 pathways in mice. *Biomedicine & Pharmacotherapy*. 2019;109:1876–83.
30. Heath MS, Kolli SS, Dowling JR, Cline A, Feldman SR. Pharmacotherapeutic strategies for standard treatment-resistant psoriasis. *Expert Opinion on Pharmacotherapy*. 2019;20(4):443–54.
31. Maroto-Morales D, Montero-Vilchez T, Arias-Santiago S. Study of skin barrier function in psoriasis: The impact of emollients. *Life*. 2021;11(7):651.
32. Kim HR, Mun Y, Lee KS, Park YJ, Park JS, Park JH, et al. T cell microvilli constitute immunological synaptosomes that carry messages to antigen-presenting cells. *Nat Commun*. 2018;9(1):1–19.
33. Thokchom SK, Gulati K, Thakur T, Rai N, Ray A. Dendritic Cells and Immunomodulation: Role in Health and Disease. *Current Immunology Reviews*. 2017;13(2):132–43.
34. Liu F, Huang J, Liu X, Cheng Q, Luo C, Liu Z. CTLA-4 correlates with immune and clinical characteristics of glioma. *Cancer Cell Int*. 2020;20(1):1–10.
35. ElTanbouly MA, Noelle RJ. Rethinking peripheral T cell tolerance: checkpoints across a T cell's journey. *Nature Reviews Immunology*. 2021;21(4):257–67.
36. Gudi RR, Karumuthil-Melethil S, Perez N, Li G, Vasu C. Engineered dendritic cell-directed concurrent activation of multiple T cell inhibitory pathways induces robust immune tolerance. *Sci Rep*. 2019;9(1):1–16.
37. Tajeja N. Lenalidomide-current understanding of mechanistic properties. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*. 2011;11(3):315–26.
38. D'Souza C, Prince HM, Neeson PJ. Understanding the role of T-cells in the antimyeloma effect of immunomodulatory drugs. *Frontiers in Immunology*. 2021;12:632399.
39. Costache DO, Feroiu O, Ghilencea A, Georgescu M, Căruntu A, Căruntu C, et al. Skin Inflammation



Modulation via TNF- $\alpha$ , IL-17, and IL-12 Family Inhibitors Therapy and Cancer Control in Patients with Psoriasis. *International Journal of Molecular Sciences*. 2022;23(9):5198.

40. Pervaiz N, Kaur H, Parsad D, Kumar R. Immuno-modulatory effects of lenalidomide inhibited the

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## تأثير مرهم ليناليدوميد على مستويات أنسجة عامل نخر الورم ألفا في الفئران المصابة بالصدفية التي يسببها إيميكيومود.

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#### الخلاصة

**الخلفية:** ليناليدوميد دواء مناعي له خصائص ملحوظة مضادة للالتهابات ومضادة للأورام. يثبط ليناليدوميد إنتاج السيتوكينات المؤيدة للالتهابات التي تم ربطها بمجموعة متنوعة من الأورام الدموية الخبيثة. يعزز ليناليدوميد الجهاز المناعي للمضيف عن طريق تنظيم تكاثر الخلايا التائية، مما يؤدي إلى تغيرات في الالتهاب المرتبط بمسببات الصدفية.

**الأهداف:** كانت أهداف هذه الدراسة هي تحديد فعالية الليناليدوميد كمرهم في علاج نماذج الفئران المصابة بالصدفية وكذلك كيفية تأثيره على مستويات عامل نخر الورم ألفا في أنسجة الجلد في مجموعات الاختبار المختلفة.

**المواد والطرق:** أجريت الدراسة في الفترة ما بين نوفمبر 2021 ويناير 2022. تم تقسيم 70 فأراً من الذكور الأصحاء بشكل عشوائي إلى 7 مجموعات من 10 حيوانات لكل منها. في مجموعات (1 و 2 و 3 و 4 و 5 و 6)، أنتج إيميكيومود الصدفية. تم إعطاء كريم إيميكيومود فقط للمجموعة 1، بعد حدوث الصدفية، تم تطبيق مرهم كلوبيتازول على المجموعة 2، وتم تطبيق مرهم وهمي على المجموعة 3، وتم تطبيق مرهم ليناليدوميد (1، 2، 3٪) على المجموعات (4، 5 و 6) على التوالي. تم استخدام الفئران السليمة كعنصر تحكم مقارن في المجموعة 7. تم استخدام الحزمة الإحصائية للعلوم الاجتماعية للتحليل الإحصائي للبيانات (الإصدار 26).

**النتائج:** بعد العلاج بالليناليدوميد، تحسنت منطقة الصدفية. تم تفسير فعالية ليناليدوميد في علاج صدفية الفئران التي يسببها إيميكيومود من خلال الاختلاف في مستويات الأنسجة لـ عامل نخر الورم ألفا بين المجموعات التي تم فحصها.

**الاستنتاجات:** وفقاً للتجربة، يمكن أن يتسبب الليناليدوميد بتركيزات مختلفة في تفاقم أو تحسن الصدفية لدى الفئران. له فعالية مماثلة للعلاج القياسي عند جرعة معينة.

**الكلمات المفتاحية:** ليناليدوميد، مناعي، مضاد للالتهابات، عامل نخر الورم ألفا، الصدفية التي يسببها إيميكيومود في الفئران.