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# Effect of Tadalafil in Comparison with Clobetasol ointment in induced Psoriasis in Mice Male

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## Abstract

Psoriasis is an immune-mediated chronic inflammatory skin disease. It is define as a clinical entity, affecting the skin, nails, mucous membranes, and joints. The psoriasis pathogenesis primarily shares the combined influence of several gene susceptibilities and incorrect of the immune system together with pervasive risk factors of environment. Tadalafil is a strong phosphodiesterase- 5 blocker that relaxes smooth muscle and prevents the breakdown of the cyclic guanosine monophosphate. **The aim is** to evaluate the possible beneficial effect of oral tadalafil plus tadalafil gel in comparison with clobetasol in induced psoriasis in mice. Accordingly, forty male BALB/c Albino mice animal experiments in the stage of age 8-11 weeks and body mass ranged 25-40 g; were divided equally into four groups (ten mice/group) after their skin of the dorsal back and right ear being shaved for topical application: Group I (normal control) healthy mice without treatment. Group II (Induction group) in which mice experiment administered topical dose of a limited dose of imiquimod cream (5%) for seven days. The following groups (III and IV), after being received imiquimod cream (5%) as mentioned in the induction group, mice were treated for a further two weeks with either clobetasol ointment (0.05%) topically once daily (clobetasol group), tadalafil gel (0.05%) topically once daily plus oral tadalafil in a dose of 5 mg/kg once daily (tadalafil gel + oral group). This study demonstrated a highly significant reduction in pro-inflammatory tumor necrosis factor-α and interlukin-23 profile, with no significant decrease in the level of interlukin-17 and vascular endothelial growth factor, and significantly elevation in transforming growth factor-β level in tadalafil gel plus oral group in comparison to the induction group.

**Conclusion**

This study conclude the possible effect of anti-inflammatory activity of oral and gel of tadalafil on skin homogenate parameters in comparison with clobetasol in induced psoriasis in mice.

# تأثير عقار التادالافيل الفموي بالمقارنة مع مرهم الكلوبتازاول في الصدفية المستحدثة في ذكور الفئران

**الخلاصة:**
الصدفية هو مرض مناعي يسبب التهاب جلدي مزمن. يُعرَّف بأنه كيان سريري يؤثر على الجلد والأظافر والأغشية المخاطية والمفاصل. السبب في مرض الصدفية في المقام الأول بالتأثير المشترك للعديد من القابليات الجينية، واضطراب جهاز المناعة مع عوامل الخطر البيئية المنتشرة. التادالافيل هو عبارة عن علاج يعمل على تثبيط خميره فسفودايستيراز -٥ يحجب العضلات الملساء ويمنع انهيار أحادي فوسفات الجوانوزين الدوري.

**الهدف من الدراسة:**
الغرض من الدراسة الحالية هو تقييم التأثر العلاجي المحتمل لعقار تادالافيل المستخدم كعلاج موضعي عند دمجه مع صيغته الفموية مقارنة مع عقار كلوبيتازول الموضعي على التهاب الجلد الصدفية الناجم عن كريم الامكيومود في الفئران.

**المواد وطريقه العمل:**
اربعون ذكرًا من الفئران التجريبية ألبينو ج / BALB بمتوسط اعمار يتراوح بين ٨-١١ أسبوعًا واوزان يتراوح بين ٢٥-٤٠ غرامًا تم تقسيمها بالتساوي إلى اربعه مجموعات (عشرة فئران / مجموعة) بعدها تم حلق شعر جلد الظهر والأذن اليمنى من أجل الاستخدام الموضعي احداث التهاب:
المجموعة (الاولى( الفئران السليمة دون علاج. المجموعة (الثانية) التي تلقت فيها الفئران جرعة موضعية من كريم الامكيومود (٥٪) يوميا ولمدة سبعة أيام. المجموعات التالية (الثالثة والرابعة)، بعد تلقي كريم الامكيومود (5 ٪) كما هو مذكور في مجموعة الثانية، تم علاج الفئران لمدة أسبوعين إضافيين باستخدام مرهم كلوبيتازول (٠.٠٥٪) وجل تادالافيل (٠.٠٥٪) موضعيًا يوميًا بالإضافة إلى تادالافيل عن طريق الفم بجرعة ٥ مغم / كغم مرة واحدة.

**نتائج**
أظهرت الدراسة الحالية انخفاضًا كبيرًا في مستوى IL-23، وانخفاضًا مهم في مستوى TNF-α، وانخفاض غير فعال في مستوى VEGF بالإضافة إلى زيادة كبيرة في مستوى TGF-في كل من جل تادالافيل بالإضافة إلى المجموعة الفموية عند مقارنة بمجموعة الامكيومود.

**الاستنتاج:**
جل تادالافيل بالإضافة إلى الفم يحسن الأعراض الشبيهة بالصدفية التي يسببها التهاب الجلد الناجم عن الامكيومود في الفئران بسبب التأثير المحتمل للنشاط المضاد للالتهابات. إلى جانب ذلك، كان تادالافيل جل بالإضافة إلى الفموي تأثير مخفف على التغيرات النسيجية المرضية عند مقارنتها مع مرهم كلوبيتازول على التهاب الجلد الناجم عن الامكيومود في الفئران.

## Introduction

Psoriasis is an immune-mediated chronic inflammatory skin disease [1]. It is define as a clinical entity, affecting the skin, nails, mucous membranes, and joints. It is a public disorder, influence 2–4 % of the people in worlds, with incidence rates influenced by age, geographic position, and with hereditary family affection [2].

Psoriasis is a chronic disease of living symbols and signs categorized by scaly, erythematous injuries with rigidly demarcated margins [3]. There has been a huge concentration on connection of this diseases to predictable heart threat elements including metabolic disorder, overweight, decrease the body exercise, cigarette consumption, drinking of alcohol, and increase of blood pressure [4].

The psoriasis pathogenesis primarily shares the joint effect of several gene susceptibilities, the incorrect stimulation of the immune body together with pervasive risk factors [5].

Environmental factors generate the reply of immune form, through antigen-presenting cells (APC) inside the epidermis, was stimulated -T cells production. The organ lymph nodes triggered T lymphocytes cells to the skin, with sufficient cytokines formation. The inflammatory mediators such as interleukin IL-12 and IL-23 are discharge by, that encourage naive T cells to separate into Th1 and Th17 cells [6].

Consequently, epidermal and dermal occupant this inflammatory mediator, and reason for variations, containing keratinocyte explosion and epidermal thickness [7].

Inflammatory cytokines such as IFN-γ, IL-2, and TNF-α formed by Th1 cells and others that are produced by dendritic cells (IL-18, IL-20, TNF-α, and IL-23) were essential factors in the pathogenesis of psoriasis, especially [8].

In addition, many drugs correlated to the beginning and exacerbation of psoriasis, such as imiquimod, viral inhibitor agents, lithium compound, beta-receptor inhibitors, and other biological substances [9].

The topical emollients form has the mainstay of treatment for psoriasis. They are respected first-line management because dry skin is public in psoriasis and increases its irritability [10]. In addition, anthralin has presented to be one of the greatest active topical managements of stable plaque psoriasis [11]. The management of all types of psoriasis by corticosteroids, which remain the first-line, as monotherapy or as a supplement to systemic therapy [12].

Overall vitamin D restores healthy structure and skin function, and active in treating psoriasis symptoms. Vitamin D types are prescribed for the controlling of psoriasis, as alone therapy or in grouping with topical corticosteroids [13].

In addition, coal tar established on chronic plaque psoriasis, palmoplantar psoriasis, and scalp psoriasis [14]. Phototherapy comprises frequent contact of the skin structure to ultra-violet (UV) light to treat numerous inflammatory skin situations such as psoriasis, eczema, and vitiligo [15].

The general systemic management for psoriasis such as methotrexate (MTX), an anti-metabolite, which is generally use in psoriasis. The U.S. FDA permitted systemic retinoid in early 1972 for the control of psoriasis [16], which essential for the pustular type [17]. In addition, cyclosporine management for psoriasis is incomplete to small sequences (>1-2 years avoided) due to fears about side effects, such as nephrotoxicity and hypertension [18].

Biologic drugs for psoriasis are mainly consider a T-cell mediated disease such as alefacept and efalizumab substantiated the role of T-cells as primary modulators [19]. The binding of etanercept to TNF reduces the bound TNF, resulting in a decrease in inflammatory activity [20]. Other drugs, Ixekizumab is a humanized

IgG4 monoclonal antibody that counteracts IL-17[21].

Tadalafil is an actual selective strong competitive inhibitor of phosphordiesterase type 5 (PDE-5) which particularly inhibits nitric oxide (NO)/GMP pathway that blocking cGMP degradation in smooth muscles inducing vascular dilation [22]. Tadalafil has a prolonged half-life and available for 36hrs after dosing. The drug relaxes smooth muscle by reducing PDE-5 levels and prevent the breakdown of the cyclic guanosine monophosphate [23].

Tadalafil was use in the monitoring of erectile dysfunction, benign prostatic hyperplasia, and of pulmonary arterial hypertension [24]. The chief adverse effect is a headache. Whereas, the rare adverse effects include dyspepsia, back pain, myalgia, nasal congestion, and flushing [25].

**Aims of the study**

1- To evaluate the anti-inflammatory effects of tadalafil in mice models of psoriasis through its effects on serum IL-17, IL-23, VEGF, TGF-β, TNF-α, and histopathology score.

2- To compare the effect of tadalafil drug with that of clobetasol ointment on serum IL-17, IL-23, VEGF, TGF-β, TNF-α in mice model.

3- To compare the histopathological changes induced by oral plus tadalafil gel with those induced by topical clobetasol in mice models.

**Materials and Methods**

The present study done in the Department of Pharmacology in the College of Medicine, Al- Nahrain University between April 2019 and June 2020.

Forty male BALB/c albino mice animal experiments in the stage of 8-11 weeks and weight ranged 25-40 g; divided equally into four groups (ten mice/ group) after their skin of the dorsal back and right ear shaved for topical application:

Group I (normal control) healthy mice without treatment. Group II (Induction group) which the mice experiment administered topical dose of a limited dose of imiquimod cream (5%) for seven days .

The following groups (III and IV), after being received imiquimod cream (5%) as mentioned in the induction group, mice were treated for a further two weeks with either clobetasol ointment (0.05%) topically once daily (clobetasol group), and tadalafil gel (0.05%) topically once daily plus oral tadalafil in a dose of 5 mg/kg once daily (tadalafil gel + oral group). At the end of the experiment, all of the mice were anesthetize by chloroform and then, they were sacrifice. The skin samples were arranged for histopathological examination and assay of biomarkers, i.e., enzyme-linked immunosorbent assay for mouse TNF-α, IL-17, IL-23, TFG-β, and VEGF.

The one-way analysis of variance (ANOVA) independent sample t-test was to determine the statistical significance of differences of laboratory parameters between healthy and treated groups.

**Results**

Table (1) showed a significant elevation of tissue TNF-α and IL17 with a highly significant increase in IL23 and VEGF levels. Beside, a reduction in the level of TGF-β in the induction group as compared to normal control group I. Clobetasol group displayed a highly significant reduction in TNF-α, IL-17, and VEGF levels, beside a reduction in IL-23 and no significant difference in the level of TGF-β when being compared to induction group (table 2).

This study demonstrated, highly significant reduction in TNF-α and IL-23, no significant decrease of IL-17 and VEGF, and a significant elevation in TGF-β level in tadalafil gel plus oral group in comparison to induction group.

**Table (1): Effect of Imiquimod on skin tissues' biomarkers (TNF-α, IL17, IL23, VEGF and TGF-β) in induced psoriasis in mice**

| Biomarkers | **Control** | **Induction** |
|---|---|---|
| TNF-α (ng/ml) | 57.81±17.13 | 94.45±40.72[s] |
| IL-17 (pg/ml) | 29.95±8.03 | 38.64±12.44 |
| IL-23 (pg/ml) | 12.85±4.00 | 19.31±4.85[s] |
| VEGF (pg/ml) | 7.60±2.61 | 12.41±2.08[s] |
| TGF-β (pg/ml) | 77.78±18.92 | 65.08±17.75 |

*S: means p ≤ 0.05 when being compared control group*

**Table (2): Effect of Clobetasol ointment (0.05%) on skin tissues' biomarkers (TNF-α, IL17, IL23, VEGF and TGF-β) in induced psoriasis in mice**

| **Biomarkers** | **Control** | **Induction** | **Clobetasol ointment (0.05%)** |
|---|---|---|---|
| TNF-α(ng/ml) | 57.81±17.13 | 94.45±40.72[s] | 53.74±25.45[*] |
| IL-17 (pg/ml) | 29.95±8.03 | 38.64±12.44 | 28.36±11.36* |
| IL-23 (pg/ml) | 12.85±4.00 | 19.31±4.85[s] | 15.44±5.28 |
| VEGF(pg/ml) | 7.60±2.61 | 12.41±2.08[s] | 9.62±1.90* |
| TGF-β (pg/ml) | 77.78±18.92 | 65.08±17.75 | 86.52±22.02 |

*S: means p ≤ 0.05 when being compared to group control. * : means p ≤ 0.05 when being compared to induction group*

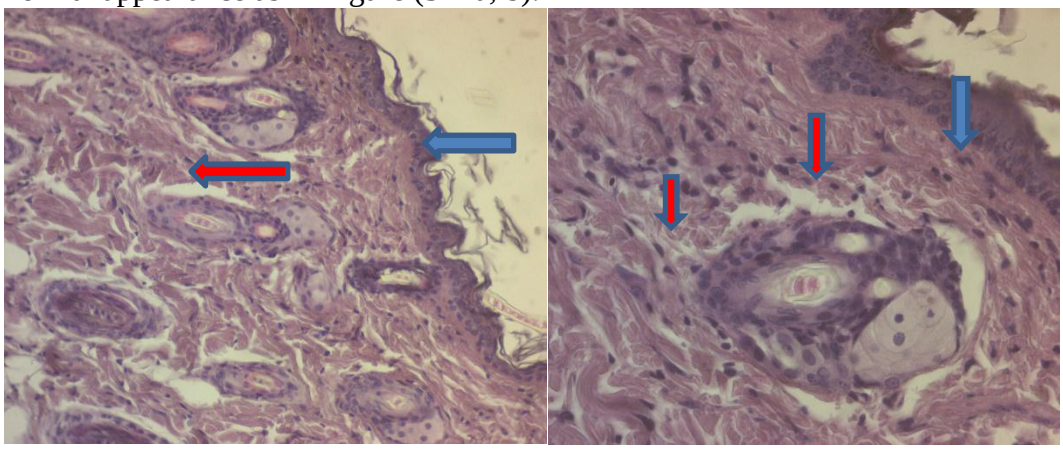
**Table (3): Effect of Tadalafil gel (0.05%) plus oral (5 mg/kg) on TNF-α, IL17, IL23, VEGF, and TGF-β in comparison with clobetasol in induced psoriasis in mice**

| **Biomarkers** | **Induction** | **Clobetasol ointment (0.05%)** | **Tadalafil gel (0.05%) plus oral (5 mg/kg)** |
|---|---|---|---|
| TNF-α(ng/ml) | 94.45±40.72 | 53.74±25.45[*] | 54.06±17.12[*] |
| IL-17 (pg/ml) | 38.64±12.44 | 28.36±11.36[*] | 32.42±12.36 |
| IL-23 (pg/ml) | 19.31±4.85 | 15.44±5.28 | 13.66±4.51[*] |
| VEGF(pg/ml) | 12.41±2.08 | 9.62±1.90[*] | 11.29±4.29 |
| TGF-β(pg/ml) | 65.08±17.75 | 86.52±22.02 | 98.52±37.58[*] |

** : means p ≤ 0.05 when being compared to induction group*

**Histological features of the normal control group**

In the normal control group sections of back area skin, the histological sections showed a normal appearance as in Figure (3-1 a, b).

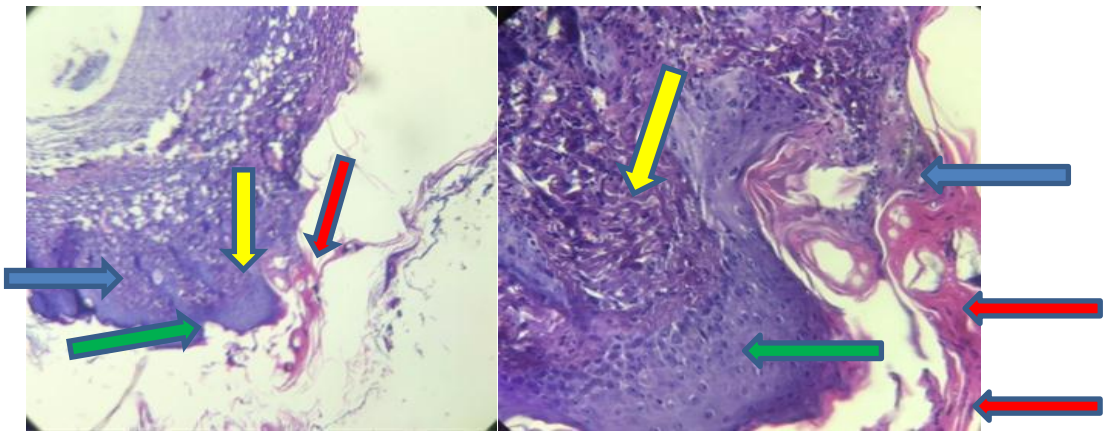


**(a)** **(b)**

**Figure (1 a, b):** Histopathologic sections of skin sections from animals of control group, sharing normal of epidermal (blue arrow) and dermis (red arrow). H&E 20X (a) and 40X (b)

**Effect of Imiquimod on histological features in induced psoriasis in mice**

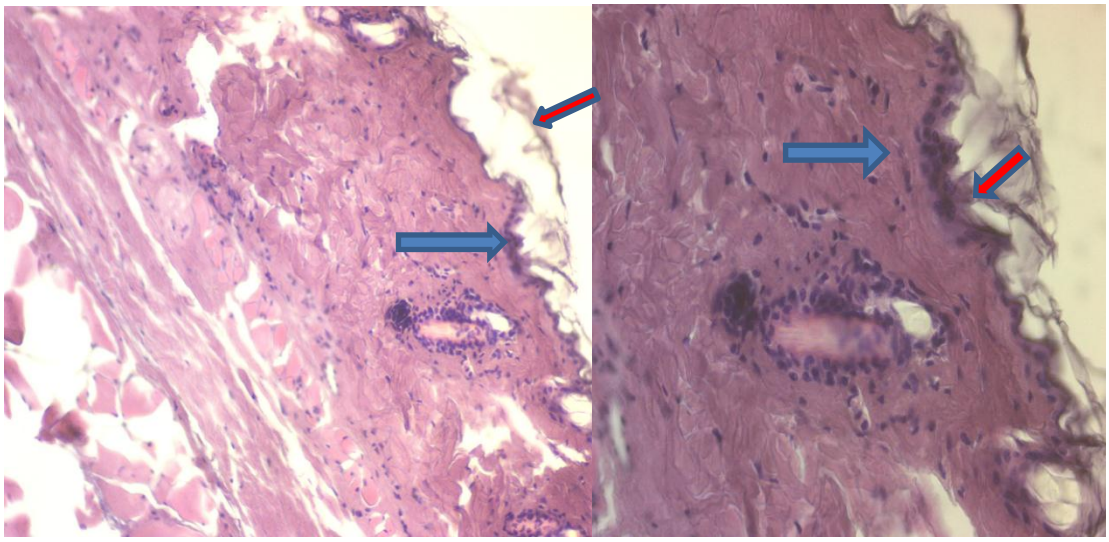
The effects of imiquimod on the dorsal skin of mice characterized by acanthosis, munro abscess, hyperkeratosis, parakeratosis, and mild dermis lymphocytic infiltrate (figures 3-10 a, b).



**(a)** **(b)**

**Figure (2 a, b):** Histopathologic sections of skin from animals of induction group, shows acanthosis and elongation of rete ridges (green arrow), Munro's micro abscess (blue arrow), hyperkeratosis and parakeratosis (red arrow), and marked dermis lymphocytic infiltrate (yellow arrow). H&E 10X (a) and 40X (b)

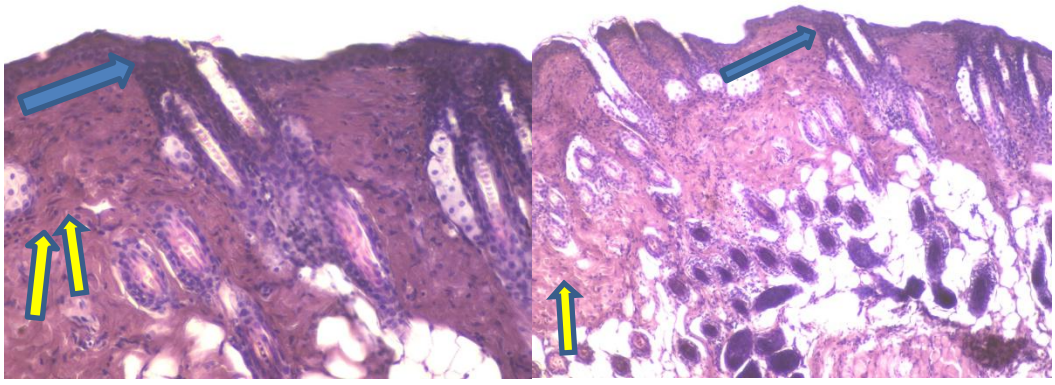
**Effect of Clobetasol ointment on histological features in induced psoriasis in mice**
Section of back area skin showed a thin layer of the epidermis, still, there is hyperkeratosis with mild acanthosis and rete ridge with mild inflammatory cells as in Figure (3-11a,b).



**(a)** **(b)**

**Figure (3 a, b):** Histopathologic sections of skin sections from animals of clobetasol group, shows hyperkeratosis (red arrow) and epidermis thinning (blue arrow) above papillae. H&E 20 X (a) and 40X (b)

**Effect of gel plus oral tadalafil on histological features of back in induced psoriasis in mice**
The effects of oral tadalafil on the skin of mice demonstrated by epidermis lengthening and clubbing of rete ridges, and mild dermis lymphocytic infiltrate (figure 3-5a, b).



**(a)** **(b)**

**Figure (3-15a, b):** Histopathologic sections of skin section, from animals of gel plus oral tadalafil group, shows lengthening and clubbing of rete ridges (blue arrow) and mild dermis lymphocytic infiltrate (yellow arrow) H&E 20(a) and10X (b)

## Discussion

The most common immune-mediated inflammatory skin disease is Psoriasis, which categorized with the enhanced epidermal explosion and enormous permeation of inflammatory cells [26]. Imiquimod induces an immune response that makes the creation of numerous cytokines, such as IL-1, TNF-α, IL-23, and IL-17 [27]. The production of these cytokines further promotes Th17 cell maturation and results in erythema, a mixed inflammatory cell infiltrates and hyperplasia [28].

The effector inflammatory cytokines IL-17 and IL-23 formed by Th17 cells, which a firm study was described that found in the circulation of blood of psoriasis and concerned in pathogenesis of psoriasis [29]. In addition, the production of proinflammatory cytokines such as TNF-α may induce by TGF-β1 [30].

Clobetasol group treated showed a reduction in TNF-α level when compared to the imiquimod group [31]. Clobetasol group displayed a highly significant reduction in IL-17, and VEGF levels besides a reduction in IL-23 and no significant difference in the level of TGF-β when being compared to the induction group. This present study observed that clobetasol was able to decrease the IL-23/IL-17A axis of psoriasis-like inflammation in mice [32].

Moreover, the corticosteroids type is clobetasol decrease of proliferative reaction, reduction in inflammatory mediators, and suppression of the immune system by connecting to intracellular corticosteroid receptors [33].

In the current study, it was a highly significant decrease in tissue TNF-α level in oral plus topical tadalafil as compared to the induction group. On other hand, PDE5 inhibitors reduced TNF-α induced gene that related to inflammation [34]. Our results were showing the anti-inflammatory and anti-oxidative potential of tadalafil [35].

In addition, PDE inhibitors have been to be active in the managing of an autoimmune disease. Modification of pro-inflammatory and anti-inflammatory mediators by specific PDE inhibitors was an advanced plan for the management of inflammatory diseases [36].

In the oral plus topical tadalafil group, there was not reduction in VEGF level as compared to the induction group and a significant increase in TGF beta levels in comparison with induction group.

In addition, TGF-β produces a vital role in the development of cell growth and immune function. The natural roles of TGF-β in inflammation for tissue repair, and embryonic development [37].

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## Author contribution

Mohammed Abdul Al-Jabbar: collection and analysis of data, interpretation and discussion. Dr. Adeeb and Dr. Ban research reviewer.

## Reference

1. Rachakonda TD, Schupp CW, Armstrong AW. Psoriasis prevalence among adults in the United States. J Am Acad Dermatol.2014;70:512–6.
2. Parisi R, Symmons DP, Griffiths CE, Ashcroft DM, IMPACTproject team. Global epidemiology of psoriasis: a systematicreview of incidence and prevalence. J Invest Dermatol. 2013;133:377–385.
3. Paller AS, Singh R, Cloutier M, et al. Prevalence of psoriasis in children and adolescents in the United States: a

claims-based analysis.J Drugs Dermatol. 2018; 17(2):187-194.
4. Menter A, Griffiths CEM, Tebbey PW, Horn EJ, Sterry W. Exploring the association between cardiovascular and other disease-related risk factors in the psoriasis population: The need for increased understanding across the medical community. J Eur Aacd Dermatol Venereol.2010; 24:1371–7.
5. Raychaudhuri, S. K., Maverakis, E., & Raychaudhuri, S. P.. Diagnosis and classification of psoriasis. Autoimmunity Reviews.2014; 13(4-5), 490–495.
6. Lowes, M. A., Suárez-Fariñas, M., & Krueger, J. G.. Immunology of Psoriasis. Annual Review of Immunology.2014;32(1), 227–255.
7. Zeng, J., Luo, S., Huang, Y., & Lu, Q.. Critical role of environmental factors in the pathogenesis of psoriasis. The Journal of Dermatology.2017;44(8), 863–872.
8. Ogawa, E., Sato, Y., Minagawa, A., & Okuyama, R. 2017. Pathogenesis of psoriasis and development of treatment. The Journal of Dermatology; 45(3), 264–272.
9. Kim GK, Del RJ. Drug-provoked psoriasis: is it drug induced or drug aggravated? Understanding pathophysiology and clinical relevance. J Clin Aesthet Dermatol.2010;3: 32–38
10. Croney, S.. Treating skin conditions in the community: know your emollients. British Journal of Community Nursing. 2016; 21(9), 452–456.
11. 30. Witman, P. M.. Topical Therapies for Localized Psoriasis. Mayo Clinic Proceedings. 2001;76(9), 943–949.
12. Svendsen, M., Ejner Andersen, K., Andersen, F., Hansen, J., Pottegård, A., & Johannessen, H.. Psoriasis patients' experiences concerning medical adherence to treatment with topical corticosteroids. Psoriasis: Targets and Therapy; 2016;6:113-119.
13. Hong SP, Oh Y, Jung M, et al. Topical calcitriol restores the impairment of epidermal permeability and antimicrobial barriers induced by corticosteroids. Br J Dermatol; 2010;162(6):1251-1260.
14. John Koo; Mark Lebwohl. Duration of remission of psoriasis therapies; 1999;41(1), 0–59.
15. Nakamura, M., Farahnik, B., & Bhutani, T.. Recent advances in phototherapy for psoriasis. F1000Research.2016;5, 1684.
16. Wald JM, Klufas DM, Strober BE. The use of methotrexate, alone or in combination with other therapies, for the treatment of palmoplantar psoriasis. J Drugs Dermatol.2015;14: 888-892.
17. Sevrain .M, Richard .M. A, T. Barnetche, Rouzaud. M, Villani .A.P, Paul .C, et al. Treatment for palmoplantar pustular psoriasis: systematic literature review, evidence-based recommendations and expert opinion, J. Eur. Acad. Dermatol.2014;28, 13-16.
18. Griffiths, C., Dubertret, L., Ellis, C., Finlay, A., Finzi, A., Ho, V., Johnston, A., Katsambas, A., Lison, A.E., Naeyaert, J., 2004. Ciclosporin in psoriasis clinical practice: an international consensus statement. British Journal of Dermatology 150, 11-23.
19. Rønholt, K., & Iversen, L. 2017. Old and New Biological Therapies for Psoriasis. International Journal of Molecular Sciences, 18(11), 2297.
20. Schulz, M., Dotzlaw, H., & Neeck, G. 2014. Ankylosing Spondylitis and Rheumatoid Arthritis: Serum Levels of TNF-αand Its Soluble Receptors during the Course of Therapy with Etanercept and Infliximab. BioMed Research International; 1–7.
21. Ren V, Dao H. Potential role of ixekizumab in the treatment of moderate-to-severe plaque psoriasis. Clin Cosmet Investig Dermatol.2013;6:75–80.

22. Li Z, Xi X, Gu M, Feil R, Ye RD, Eigenthaler M, et al.. A stimulatory role for cGMP-dependent protein kinase in platelet activation.Cell. 2003;112, P: 77-86.
23. Morelli A, Sarchielli E, Comeglio P, et al. Phosphodiesterase type 5 expression in human and rat lower urinary tract tissues and the effect of tadalafil on prostate gland oxygenation in spontaneously hypertensive rats. J Sex Med.2011;8:2746–2760.
24. Brock GB, et al.. Efficacy and safety of tadalafil for the treatment of erectile dysfunction: results of integrated analyses. J Urol.2002;168: 13321336.
25. Andersson KE, et al.. Tadalafil for the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia: pathophysiology and mechanism(s) of action. Neurourol Urodyn.2011;30:292-301.
26. Cai Y, Fleming C, Yan J. New insights of T cells in the pathogenesis of psoriasis. Cell Mol Immunol.2012;9: 302‑309.
27. Weber, Andreas; Zimmermann, *et al*. Induction of pro-inflammatory cytokine production in thymocytes by the immune response modifiers Imiquimod and Gardiquimod. International Immunopharmacology.2013, 17(2), 427–431.
28. El Malki. K; Karbach. H; Huppert, Jula; Zayoud. M; *et al*. An Alternative Pathway of Imiquimod-Induced Psoriasis-Like Skin Inflammation in the Absence of Interleukin-17 Receptor A Signaling. Journal of Investigative Dermatology.2013; 133(2), 441–451.
29. Rizzo.H. L.; Kagami, S.; Phillips, K. G *et al*. IL-23-Mediated Psoriasis-Like Epidermal Hyperplasia Is Dependent on IL-17A. The Journal of Immunology.2011; 186(3), 1495–1502.
30. Di Fusco.D., Laudisi. F., Dinallo.V, *et al*. Smad7 positively regulates keratinocyte proliferation in psoriasis. British Journal of Dermatology.2017; 177(6), 1633–1643.
31. Boehncke. W.-H. & Brembilla. N. C. Unmet Needs in the Field of Psoriasis: Pathogenesis and Treatment. Clinical Reviews in Allergy & Immunology; 2017.
32. Torsekar R & Gautam MM. Topical therapies in psoriasis. Indian Dermatol Online J.2017; 8(4):235-245.
33. Gresele P, Momi S, Falcinelli E. Anti-platelet therapy: phosphodiesterase inhibitors. Br J Clin Pharmacol. 2011; 72:634e46.
34. Vignozzi, Linda; Gacci, Mauro; Cellai, Ilaria; Morelli, Annamaria; Maneschi, Elena; Comeglio, Paolo et al. PDE5 inhibitors blunt inflammation in human BPH: A potential mechanism of action for PDE5 inhibitors in LUTS. The Prostate.2013;73(13), 1391–1402.
35. Gokakin, Ali Kagan; Deveci, Koksal; Kurt, Atilla; Karakus, Boran Cihat; Duger, Cevdet; Tuzcu, Mehmet; et al. The protective effects of sildenafil in acute lung injury in a rat model of severe scald burn: A biochemical and histopathological study. Burns.2013;39(6), 1193–1199.
36. Padmanabha Shenoy; Vikas Agarwal. Phosphodiesterase inhibitors in the management of autoimmune disease. .2010;9(7), 511–515.
37. Newsted, Daniel; Banerjee, Sunandan; Watt, Kathleen; Nersesian, Sarah; Truesdell, Peter; Blazer, Levi L.et al (2018). Blockade of TGF-β signaling with novel synthetic antibodies limits immune exclusion and improves chemotherapy response in metastatic ovarian cancer models. OncoImmunology, 1–14.