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Research Article

Histopathological and Immunological Effects of Nebivolol 5% Topical Cream in Mice Model of Imiquimod-Induced Psoriasis

Zainab Azal Mahdi[1]*, Anwer Mahmood Hussain[2], Hasan Adnan Alblesh[2]

[1]*Department of Pharmacology and Toxicology, College of Pharmacy, University of Kirkuk, Kirkuk, Iraq;* [2]*Department of Pharmacology and Toxicology, College of Pharmacy, Tikrit University, Saladin, Iraq*

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Abstract

Background*:* Psoriasis is a chronic inflammatory skin condition that affects multiple systems. Topical therapy is one of the most important modalities in the treatment of this disease, and efforts are directed toward developing more effective topical therapies. ***Objective****: To* investigate the possible anti-psoriatic effect of Nebivolol 5% topical cream in mice based on observational, histopathological, and biochemical outcomes. ***Methods****:* Forty-five male Swiss Albino mice were divided into five groups; each group contained nine mice with shaved dorsal skin. Group I remained as the control group while the rest of the groups were induced psoriasis by Imiquimod (IMQ) for six consecutive days and underwent different interventions for each group for eight consecutive days, including administering Nebivolol 5% topical cream. The clinical, pathological and laboratory effects were then measured. ***Results***: Topical nebivolol significantly reduced the inflammatory signs of the psoriatic lesions, and these findings were supported by the histopathological examination. Topical Nebivolol also significantly decreased IL-17 levels, as well as Tumor Necrosis Factor-alpha (TNF-α) levels and Vascular Endothelial Growth Factor (VEGF) levels, in comparison with the non-treated Imiquimod-induced psoriatic mice group. ***Conclusions****:* Nebivolol has a comparable anti-psoriatic effect to the effect of clobetasol due to its anti-inflammatory and antioxidant effects. It could be a promising future treatment for psoriasis as an alternative to steroids.

Keywords: IL-10, IL-17, Imiquimod, Nebivolol, Psoriasis, TNF-α, VEGF.

التأثيرات النسيجية المرضية والمناعية لكريم نيبيفولول 5% الموضعي في نموذج الفئران للصدفية المستحدثة بالأميكويمود

الخلاصة

الخلفية: الصدفية هي حالة جلدية التهابية مزمنة تؤثر على أنظمة متعددة. العلاج الموضعي هو واحد من أهم الطرق في علاج هذا المرض ، ويتم توجيه الجهود نحو تطوير علاجات موضعية أكثر فعالية. **الهدف**: التحقيق في التأثير المحتمل المضاد للصدفية لكريم نيبيفولول 5% ٪5 الموضعي في الفئران بناء على النتائج الرصدية والنسيجية المرضية والكيميائية الحيوية. **الطريقة**: تم تقسيم خمسة وأربعين من ذكور الفئران البيضاء السويسرية إلى خمس مجموعات. احتوت كل مجموعة على تسعة فئران ذات جلد ظهري محلوق. بقيت المجموعة الأولى كمجموعة ضابطة بينما تم تحفيز بقية المجموعات بالصدفية بواسطة الأيميكويمود (IMQ) لمدة ستة أيام متتالية وخضعت لتدخلات مختلفة لكل مجموعة لمدة ثمانية أيام متتالية، بما في ذلك إعطاء نيبيفولول 5% كريم موضعي. ثم تم قياس التأثيرات السريرية والمرضية والمخبرية. **النتائج**: قلل النيبيفولول الموضعي بشكل كبير من العلامات الالتهابية لأعراض الصدفية، وكانت هذه النتائج مدعومة بالفحص النسيجي المرضي. كما قلل بشكل ملحوظ من مستويات IL-17، بالإضافة إلى مستويات عامل نخر الورم ألفا ومستويات عامل النمو البطاني الوعائي، مقارنة بمجموعة الفئران الصدفية غير المعالجة التي يسببها الأيميكويمود. **الاستنتاجات**: للنيبيفولول الموضعي تأثير مضاد للصدفية مماثل لتأثير كلوبيتاسول بسبب آثاره المضادة للالتهابات ومضادات الأكسدة. يمكن أن يكون علاجا مستقبليا واعدا للصدفية كبديل للستيرويدات.

* ***Corresponding author***: Zainab A. Mahdi, Department of Pharmacology and Toxicology, College of Pharmacy, University of Kirkuk, Kirkuk, Iraq; Email: zainabazal@uokirkuk.edu.iq

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INTRODUCTION

Psoriasis is a chronic, inflammatory skin condition that affects multiple systems. This condition can be identified by the presence of scaly erythematous plaques, which commonly manifest on the extensor surfaces such as the elbows and knees and infrequently on the intergluteal and umbilical regions [1]. Psoriasis is associated with the activation of the adaptive immune system, particularly T-cells. Although its exact cause and development mechanism are unknown, the disease is hypothesized to begin with the activation of T-lymphocytes triggered by an unidentified antigen. This activation results in the release of many cytokines and other substances, including IL-17 and TNF-alpha. [2]. Some people think that this condition is caused by the immune system, with too many keratinocytes being made by cytokines like interferon-gamma and tumor necrosis factor (TNF-α) being released by CD4+ and CD8+ T cells and natural killer cells [3]. Imiquimod (IMQ), a ligand for Toll-like receptors (TLR) 7/8 used in dermatology, has been shown to cause and possibly worsen psoriasis in a mouse model that is very similar to human plaque-type psoriasis. This similarity is observed in terms of skin redness, thickening, scaling, changes in the outer layer of the skin (acanthosis, parakeratosis), the formation of new blood vessels, and the presence of inflammatory cells such as T-cells, neutrophils, and dendritic cells [4]. Topical therapy represents one of the attractive treatment modalities for psoriasis, and it functions as an adjuvant to systemic therapy. Topical administration enables the achievement of localized drug action while minimizing the likelihood of systemic adverse effects [5]. Nebivolol is a beta-1 adrenergic receptor blocker that is highly selective and has a long duration of action. It belongs to the third generation of beta blockers [6]. It induces vasodilation by directly increasing the levels of nitric oxide (NO) in the L-arginine-NO pathway through the stimulation of endothelial nitric oxide synthase (eNOS) via its β3 receptor agonistic activity [7]. In addition, nebivolol also demonstrates antioxidant [8] and anti-inflammatory [9] properties. The current study aims to assess the efficacy of Nebivolol in improving the histopathological aspects and immunochemical parameters associated with psoriasis, compared with the efficacy of standard treatment in an Imiquimod-induced mouse model of psoriasis.

METHODS

Study Design

A total of 45 male Swiss Albino mice weighing between 28 and 32 g and aged 2-3 months were randomly assigned to five groups, each with nine animals. Animals were recognized by marking different parts of their bodies. The mice were housed in polypropylene cages in a temperature-controlled setting of 25°C. The mice were subjected to a light-dark cycle that consisted of 12 hours of light and 12 hours of darkness, with the light on at night and turned off during the day. The mice were given seven days to acclimate before the trial began. The animals were fed a conventional pellet diet and had free access to water. Before beginning the trial, the animals were checked for skin lesions, and only mice with seemingly healthy skin and coat were included in the investigation. All animals in the study were shaved from the dorsal region to expose an area of the back skin measuring about 1x2cm for experimental purposes with an electric razor, followed by the application of a hair removal cream (Veet®, Reckitt Benckiser Pvt. Ltd., India). The remaining hair was then cleaned away using gauze. The trial lasted 14 days from the commencement day. Regarding the distribution of mice: Group I (positive control) consisted of 9 mice (n=9) that served as a control group throughout the experiment with no intervention. Group II (psoriatic control) consisted of 9 mice (n=9) that were inducted with psoriasis by a dose of 62.5 mg of Imiquimod cream 5% as a once-daily topical application on the shaved back skin for 6 days until the appearance of a psoriatic lesion, as mentioned in van der Fits *et al*. [4]. This procedure was deemed the standard of induction for the current investigation, and mice in this group got no more treatment for the remainder of the experimental days. Group III (vehicle control) comprised of 9 mice (n=9) which were inducted for 6 days by the previously reported manner [4] and then given Petrolatum gel (Vaseline®) as a twice-daily topical application to the shaved dorsal skin for 8 days. Group IV comprised of 9 mice (n=9) that were inducted for 6 days using the previously established approach [4] and then given Clobetasol propionate 0.05% ointment once daily topical application (at a dosage of 0.25 g/kg) for 8 days. Group V comprised of 9 mice (n=9) that were inducted for 6 days by the previously reported manner [4] and then given a preparation of Nebivolol 5% Cream twice daily topical treatment on the shaved dorsal skin for 8 days. Imiquimod cream was supplied as (Aldara® 5% Cream, Meda Pharmaceuticals, Solna, Sweden), Petrolatum Gel (Vaseline®) was supplied from Sigma-Aldrich, Germany, CAS No. 8009-03-8), Clobetasol propionate was supplied as ointment preparation (Dermovate®, GlaxoSmithKline, Brentford, UK), and Nebivolol Hydrochloride was supplied as powder by (Baoji Guokang Bio-Technology Co., Ltd., Shaanxi, China, CAS No.: 152520-56-4) and later prepared as a cream.

Induction of psoriasis

Psoriasis was induced by applying 5% Imiquimod cream topically onto the back of the shaved epidermis once daily for six days, or until a psoriatic lesion appeared [4]. The efficacy of this model was assessed utilizing the Psoriasis Area Severity Index (PASI) score [10] in mice. Throughout the induction phase, the following modifications were noted in the animals: skin erythema, increased skin thickness, and scaling. These manifestations were specifically noted in animals that had

progressed past day 6 of induction. This is regarded as an effective induction model [11].

Psoriasis Area Severity Index (PASI) Score

The assessment of treatment efficacy and the success of the induction models in this investigation incorporated the utilization of the PASI clinical scoring system to determine the severity of inflammation on the dorsal skin of the mice. The methodology involved the visual evaluation of three distinct characteristics located on the dorsal surface of every mouse: desquamation (scale), erythema (redness), and induration (thickness). A numerical value between zero and four was allocated to each attribute: zero denoted absence, one represented mild presence, two moderate presence, three marked presence, and four highly pronounced presence. The cumulative score that ensued was capable of fluctuating between 0 and 12 [10]. A solitary researcher conducted the evaluation in this investigation.

Preparation of Nebivolol 5% cream

Nebivolol Cream 5% was prepared by accurately measuring 5 grams of Nebivolol Hydrochloride powder (Baoji Guokang Bio-Technology Co., Ltd., Shaanxi, China, CAS No.: 152520-56-4) and dissolving it in 1 mL of oleic acid (Sigma-Aldrich, Germany, CAS No. 112-80-1, O1008). The preparation was subsequently combined with a cream base consisting of a 1:1 ratio of Vaseline ® (Sigma-Aldrich, Germany, CAS No. 8009-03-8) and Lanoline (Skyrun Industrial Co. Ltd., China, CAS No. 8006-54-0). The mixture was continuously stirred at room temperature until a homogeneous cream was obtained [12].

Outcome measurements

The experiment ended on day 14, and the results were evaluated the following day. The PASI Score [10] was used to assess treatment efficacy in the test groups, with the mean of all three features calculated after observation. Following the determination of the PASI score, all mice were anesthetized intraperitoneally (IP) with 80 mg/kg ketamine and 10 mg/kg xylazine. Following total anesthesia, the mice were euthanized using exsanguination, a technique suitable for tissue collection and preservation [13]. Tissue samples were taken from the dorsal shaved skin (1cm) and cut into two pieces. The first piece was prepared for histological analysis by dehydration followed by immersion in liquid paraffin at 55-60 °C, and the slide was made using the method described in earlier publications [14]. Histopathological investigation was subsequently performed using Baker's grading method [15]. Biochemical analysis was performed on the second piece of skin tissue to assess TNF-α, IL-17, and VEGF levels. To prepare the tissue, it was thoroughly rinsed in ice-cold phosphate-buffered saline (PBS) to remove excess blood, weighed before homogenization, and then minced into minute pieces. Homogenization was performed in fresh lysis buffer, with 1 mL of lysis buffer added to the tissue sample with a glass homogenizer on ice, all using the homogenizer machine (Electrical tissue homogenizer, Staruar, England). The homogenates were then centrifuged for 5 min at 10,000 rpm. Supernatants were collected and kept at –20°C until analyzed using the sandwich ELISA method. A histological grading system is employed to assess the intensity of inflammation [15,16]. It was employed in the current investigation to assess the pathological alterations of homogenate tissue on a scale of 0 to 10 [16] using light microscopy. The tissue's morphology was examined using a light microscope (Olympus BX51 Microscope, Olympus Corporation, Japan). An X10 magnification lens was used to randomly observe five zones on a slide, encompassing the corners and center.

Biochemical analyses

The stored samples were thawed and used for the analysis by sandwich enzyme-linked immunosorbent assay (ELISA) technique by 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-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 utilized [17], based on Cohen's principles [18]. A table of random integers was used to construct the groupings at random. The animals were placed in labeled containers and given tail tags to minimize misunderstanding.

Ethical approval

The study was approved by the institutional review board (IRB) of the affiliation faculty of the author (Approval No. 7/26/2792 on 14th of November 2023).

Statistical analysis

Data entry and analysis were performed using Microsoft Excel 2010 and SPSS version 26. Continuous variables were expressed as the mean ± SD. Categorical variables were presented as frequencies and percentages using the Chi-square test. The Test of Normality (Shapiro-Wilk) showed that data was non-normally distributed, so non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used instead of parametric tests (independent t-test and one-way ANOVA). The level of significance was considered when the *p*-value was less than 0.05.

RESULTS

On days 4-5 of the experiment, indications of psoriasis induction on mice's skin, such as erythema, skin thickness, and scaling, appeared on the skin treated with IMQ and persisted in severity until the last day of induction, as shown in the figure below (Figure 1).

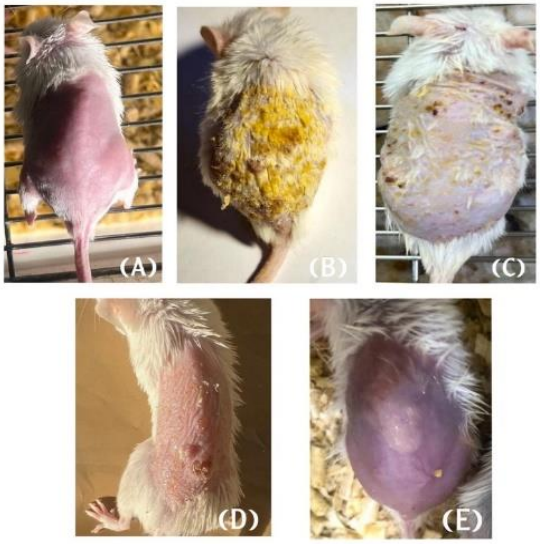

Figure 1: Scoring for skin inflammation severity, the pictures show different inflammation levels of the dorsal skin on which the test substances were applied on day 7 of the experiment and continued for 8 days. (A) the healthy positive control group (Group I), (B) the Psoriatic control IMQ-induced group (Group II), (C) the vehicle control group treated with Vaseline ® (Group III), (D) a clobetasol-treated group (Group IV), (E) the Nebivolol Cream treated group (Group V).

The image then depicts the mice after being administered petrolatum (group III), topical clobetasol (group IV), and Nebivolol Cream (group V). These medications successfully generated psoriasis-like dermatitis in mice, with a significant difference in psoriasis-like symptoms between the control and IMQ groups. The skin of experimental mice was examined for changes during the experiment, and the skin condition was graded using the PASI system. The healthy group (Group I), where nothing was administered to the skin, showed no evidence of erythema; the skin was pink and healthy, with no signs of thickening or scaling. While on the skin treated with IMQ (Group II), the skin showed a rise in redness and inflammation beginning on day 3 and increasing in severity throughout the experiment. Skin wrinkling and thickness increase, and scales emerge as yellow patches on the skin, progressing to big, flaky scales by the last day of induction. The vehicle group (Group III) showed no notable changes in the skin following treatment, with just a minor reduction in skin scaling, whereas Clobetasol and Nebivolol caused considerable changes in skin appearance. Clobetasol treatment significantly reduced skin erythema and surface scaling. Skin thickness decreased, as did puckering and wrinkling in the stimulated areas. This improvement persisted until the final day of the experiment. However, in the Nebivolol group, there was a minor decrease in skin redness and thickness, as well as a significant reduction in scaling, which began three days after Ivermectin application. An H&E stain of a healthy, positive control group of mice revealed that the keratin layer spread appropriately, and the epidermis and granular layer were the appropriate thickness at 10X (Figure 2).

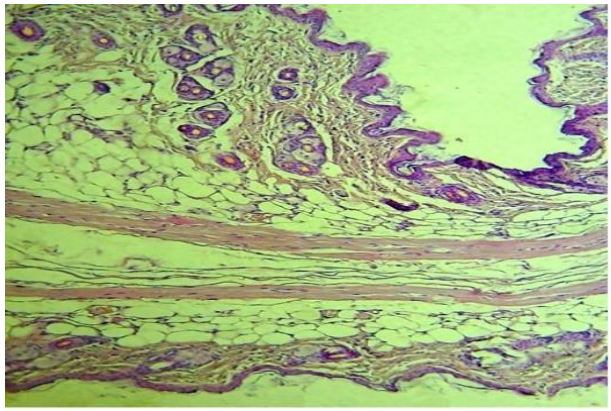

Figure 2: Positive control (Group I) histopathological section showing normal mice skin architecture.

The psoriatic control induction group (Group II) had a lot of sloughing, as well as intense neutrophilic infiltration (the Munro's abscesses), parakeratosis, hyperkeratosis, a lack of granular layer, acanthosis, and more rete ridges with papillary thinning on histopathology. 10X H&E stain (Figure 3).

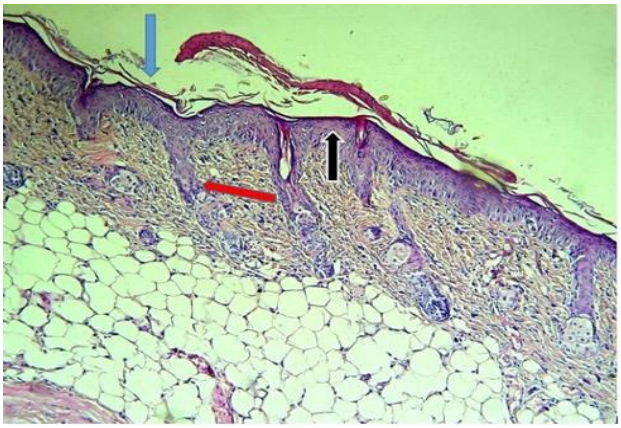

Figure 3: Psoriatic Control (Group II) showing the presence of Monru abscess in the keratin layer, hyperkeratosis, Parakeratosis, and sloughing of the skin, (Black arrow): epidermal layer: the abnormal thickness of the epidermis and thinning above the papilla. (Red arrow): rete ridge and lack of granular layer. (Blue arrow): Hyperkeratosis.

The histological skin of the vehicle control group treated with Vaseline® revealed epidermal hyperkeratosis and parakeratosis, as well as focal Munro's abscesses with acanthosis, elongated rete ridges, and papillary thinning (Figure 4).

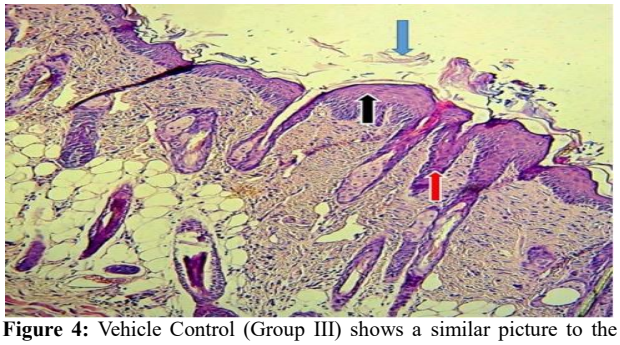

Figure 4: Vehicle Control (Group III) shows a similar picture to the induction group with no significant improvement after treatment. (Black arrow): epidermal layer: the abnormal thickness of the epidermis and thinning above the papilla. (Blue arrow): Hyperkeratosis.

The histological slice of the clobetasol-treated group (Group IV) reveals epidermal and dermal thinning, but no papilla thinning. In the absence of Monru's abscess, parakeratosis, and Rete's ridges, but with hyperkeratosis, the inflammation is mild to moderate, as opposed to the severe inflammation seen in the induction group (Figure 5).

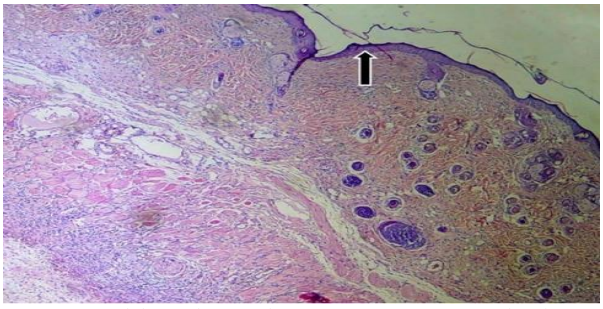

Figure 5: Clobetasol-Treated Group (Group IV), showing an improvement in the dermal and epidermal thickness, absence of Monru's abscess, and parakeratosis with hyperkeratosis still present. (Black arrow): Thinning of the epidermis.

The histopathological section of the Nebivolol-treated group (Group V) shows the disappearance of hyperkeratosis as well as epidermis and dermis thinning; no thinning over the papilla with the absence of Monru's abscess; parakeratosis; and Rete's ridges with only a minimal inflammatory response (Figure 6).

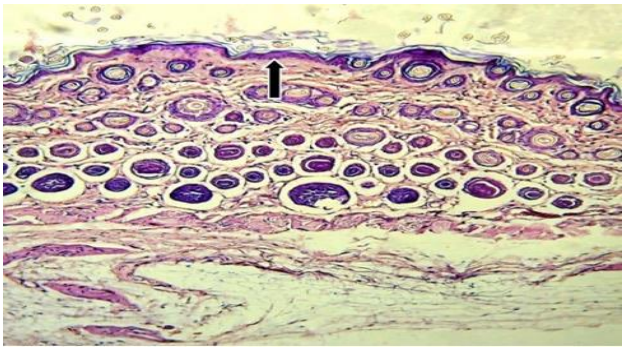

Figure 6: Nebivolol-Treated Group (Group V), showing an improvement in the dermal and epidermal thickness, absence of hyperkeratosis as well as Monru's abscess, and parakeratosis with only mild infiltration of lymphocytes. (Black arrow): Thinning of the epidermis.

This study featured five groups, each with nine mice. The groupings were as follows: Group I was a healthy positive control; Group II was an induced non-treated psoriatic control; Group III received vehicle; Group IV received clobetasol; and Group V received nebivolol. The levels of tissue biomarkers (IL-17, TNF-a, and VEGF) were compared between the study groups, as were histopathological scores (Baker score) and observational scores (PASI score). In comparison to the healthy positive control (Group I), blood levels of tissue biomarkers (IL-17, TNF-a, and VEGF), histopathological scores (Baker score), and observational scores (PASI score) increased considerably after induction (p<0.001) (Table 1).

Table 1: Comparison between Group I and Group II regarding tissue biomarkers (IL-17, TNF-α, and VEGF); and histopathological scores (Baker score) and observational score (PASI score)

Variables	Group I	Group II	*p*-value
IL-17 (pg/ml)	112.84±4.42	266.96±36.00	<0.001
TNF-α (ng/L)	41.83±1.16	153.81±12.42	<0.001
VEGF (pg/ml)	142.07±12.56	462.24±16.46	<0.001
Baker score	0.5±0.00	7.49±0.73	<0.001
PASI score	0.00±0.00	11.67±0.50	<0.001

Values were expressed as mean±SD. IL-17: Interleukin 17; TNF-α: Tumor necrosis factor-alpha; VEGF: Vascular endothelial growth factor.

The vehicle control group (Group III) showed considerably lower serum levels of TNF-α and VEGF, as well as histological and observational scores (Baker score and PASI score) compared to the induced non-treated psoriatic control group (Group II) (p<0.001). There are no significant differences in serum IL-17 levels between Groups II and III; p=0.73 (Table 2).

Table 2: Comparison between Group II and Group III regarding tissue biomarkers (IL-17, TNF-α, and VEGF); histopathological scores (Baker score), and observational score (PASI score)

Variables	Group II	Group III	*p*-value
IL-17 (pg/ml)	266.96±36.0	256.47±26.49	0.73
TNF-α (ng/L)	153.81±12.42	108.17±22.52	<0.001
VEGF (pg/ml)	462.24±16.46	126.24±16.42	<0.001
Baker score	7.49±0.73	6.94±1.04	<0.001
PASI score	11.67±0.50	9.33±0.71	<0.001

Values were expressed as mean±SD. IL-17: Interleukin 17; TNF-α: Tumor necrosis factor-alpha; VEGF: Vascular endothelial growth factor.

Treatment groups (Clobetasol treated Group IV and Nebivolol treated Group V) had significantly lower serum levels of IL-17, TNF-α, and VEGF, as well as histopathological and observational scores (Baker score and PASI score) compared to the psoriatic control group (Group II) (p<0.001) (Table 3).

Table 3: Comparison between Group II with treated groups (Group IV and V) regarding tissue biomarkers (IL-17, TNF-a, and VEGF); and histopathological scores (Baker score) and observational score (PASI score)

Variables	Group II	Group IV	Group V	*p*-value
IL-17 (pg/ml)	266.96±36.0	248.65±27.33	154.87±27.02	<0.001
TNF-a (ng/L)	153.81±12.42	79.29±11.87	101.86±18.68	<0.001
VEGF (pg/ml)	462.24±16.46	50.62±7.63	308.87±12.25	<0.001
Baker score	7.49±0.73	2.39±1.17	1.44±0.39	<0.001
PASI score	11.67±0.5	2.89±0.78	4.78±0.44	<0.001

Values were expressed as mean±SD. IL-17: Interleukin 17; TNF-α: Tumor necrosis factor-alpha; VEGF: Vascular endothelial growth factor.

The nebivolol-treated group had significantly lower serum levels of IL-17 than the clobetasol-treated group (Group IV), with a *p*-value <0.001. The clobetasol-treated group (Group IV) had significantly lower serum levels of TNF-α (p=0.009) and VEGF and observational score (PASI score) (p<0.001) than the nebivolol-treated group (Group V). Baker's score was similar in both treatment groups, with no significant difference (p=0.087) (Table 4).

Table 4: Comparison between Group IV and Group V regarding tissue biomarkers (IL-17, TNF-α, and VEGF); histopathological scores (Baker score), and observational score (PASI score)

Variables	Group IV	Group V	*p*-value
IL-17	248.65±27.33	154.87±27.02	<0.001
TNF-a	79.29±11.87	101.86±18.68	0.009
VEGF	50.62±7.63	308.87±12.25	<0.001
Baker score	2.39±1.17	1.44±0.39	0.087
PASI score	2.89±0.78	4.78±0.44	<0.001

Values were expressed as mean±SD. IL-17: Interleukin 17; TNF-α: Tumor necrosis factor-alpha; VEGF: Vascular endothelial growth factor.

DISCUSSION

Nebivolol is among the third generation of highly selective beta-1 receptor blockers [19]. Its chemical structure and pharmacological characteristics differ from that of conventional beta-blockers [20]. In addition to selectively blocking beta-1 receptors in the heart, nebivolol dilates blood arteries in the periphery by activating endothelial nitric oxide synthase. This enzyme then produces endogenous nitric oxide, which promotes vasodilation via nebivolol distinct beta-3 agonist activity [21]. This peripheral vasodilation impact may have further benefits by boosting blood perfusion and aiding in the healing and improvement of skin inflammatory lesions such as psoriasis [22]. It has been proven that oxidative stress is associated to a variety of inflammatory disorders, including psoriasis [23]. Previous research on the oxidant/antioxidant involvement in psoriasis yielded conflicting results [24]. However, new research indicates that redox imbalances in the skin and blood, as well as increased reactive oxygen species (ROS) generation, play an essential role in the pathogenesis of psoriasis [25]. Reductions in nitric oxide-dependent pathways cause cutaneous microvascular endothelial dysfunction in psoriasis. Reduced nitric oxide raises the vasoconstrictor tone [26]. Nitric oxide also prevents platelets from activating and inhibits the synthesis of cytokines, growth factors, and cell adhesion molecules, which transport inflammatory chemicals into blood vessels [27]. As a result, nebivolol exerts an antiaggregatory effect. That is why we are looking into a new medicine (Nebivolol), which has antioxidant, anti-inflammatory, and antiaggregatory properties and could potentially be used to treat psoriasis. Furthermore, nebivolol is thought to have an antioxidant effect by counteracting oxidative stress, since one study found that it dramatically reduced the amount of urinary 8-iso-PGF2α, a measure of oxidative stress in volunteers on nebivolol [28-30]. Other stated features of nebivolol include anti-inflammatory and anti-aggregator actions. Although the exact mechanism for nebivolol's antioxidant and anti-inflammatory properties is unknown, this particular element of the drug should be studied [29-31]. Only one study has found that topical nebivolol can promote wound healing in rats. It was discovered that nebivolol had a positive effect on wound healing, particularly over the long term (14 days), regardless of concentration [12]. Many lymphocytes, including CD4+, CD8+, natural killer cells, and Th17, contribute to the development of psoriasis, which is an autoinflammatory, autoimmune, and T-cell-mediated illness [32]. These cells produce a variety of proteins and cytokines, including IL-17, TNF-α, and VEGF, which are likely to help the disease advance. Pro-inflammatory cytokines and interleukins have a crucial role in disease progression [2,3]. It is proposed that cytokine changes could be effective in tracking psoriasis activity. In this investigation, tissue biomarkers such as IL-17, TNF-α, and VEGF were employed to monitor disease progression. The Psoriasis Area and Severity Index (PASI) and Baker's score were used to determine how severe the skin changes were. In this study, we assess nebivolol's anti-inflammatory and antioxidant benefits in mice with IMQ-induced psoriasis, and compare it with clobetasol and a vehicle as a control. As far as we know, there is no study in the PubMed database that examines and compares blood levels of IL-17, TNF-α, and VEGF to the severity of psoriatic illnesses treated with nebivolol cream. This article implies that more research and studies are needed to explore the potential of utilizing clobetasol with nebivolol in topical formulations. It signifies that this

topic has the potential to become the subject of a unique essay. In this work, we looked at the topical effects of nebivolol on mice psoriasis models. In an IMQ-induced psoriasis research, nebivolol improved tissue biomarker alterations (IL-17, TNF-α, and VEGF), as well as PASI and BSA scores. This was compared to the untreated group. The development of psoriasis-like signs such as erythema, scaling, skin thickening, and hyperkeratosis (Figure 4) is attributed to IMQ's effect on the toll-like receptor-7/8 (TLR-7/8), which, when activated, causes an unwanted inflammatory response similar to psoriatic lesions [33]. Imiquimod also stimulates inflammatory cells in the skin, including dendritic cells, T lymphocytes, and neutrophils [34]. This approach is reversible and only lasts shortly after ceasing topical Imiquimod since the mouse is not genetically damaged and may reverse the inflammatory process [35]. Nebivolol was chosen for this investigation because to its anti-inflammatory, antioxidant, and antiangiogenic characteristics. It will briefly cover the effects of several inflammatory mediators in psoriasis, which are based on this study. IL-17 is a key interleukin in the development of psoriasis [32]. Th17 cells are the primary secretors of IL-17; however, T regulatory cells, natural killer cells, mast cells, and neutrophils all contribute to its synthesis [36]. It exacerbates inflammation by increasing the production of cytokines, colony-stimulating factors (CSF), and chemokines by T cells, neutrophils, dendritic cells, macrophages, and even epithelial cells. It influences the recruitment of these cells that induce inflammation, increases the proliferation of keratinocytes, and inhibits differentiation [37]. People have discovered that producing too many cytokines, such as IL-17, increases the generation of reactive oxygen species (ROS) while decreasing the synthesis of natural vascular nitric oxide [38]. IL-17 is often not detected in the skin; nevertheless, it has been discovered in skin lesions in psoriasis vulgaris [39]. Psoriatic individuals had statistically significant differences in serum IL-17 levels when compared to healthy controls [32]. Michalak *et al*. discovered a link between IL-17 serum levels and PASI and BSA scores [32]. In this study, the results from IMQ-induced psoriasis indicated a substantial reduction in IL-17 after treatment with nebivolol topical preparation when compared to the vehicle and clobetasol-treated groups. The superiority of nebivolol over clobetasol could be explained by its anti-inflammatory properties. TNF-α is another significant element in the pathogenesis of psoriasis, as it is responsible for the maintenance of the majority of the clinical symptoms of the disease [40]. It has the potential to activate immune cells on the skin while also stimulating keratinocyte growth [41]. Furthermore, psoriasis patients had higher serum levels of TNF-α than normal controls, indicating the cytokine's pathogenic function in psoriasis [42]. In this study, TNF-α levels were reduced after nebivolol therapy when compared to the induction and vehicle groups, albeit to a smaller amount than in the clobetasol-treated group. Another pathogenic mechanism in psoriasis is the activation of angiogenesis by VEGF [43]. The significance of VEGF in the initiation of psoriasis is significant since angiogenesis is critical to the disease's development [44]. In this investigation, nebivolol was found to be a mild angiogenesis inhibitor in compared to clobetasol. This effect was observed in the reduction of VEGF levels in the ELISA results, which were lower than in the induction group but considerably less so than in the clobetasol and vehicle-treated groups. Furthermore, the histological results showed that nebivolol reduced Baker score (BSA) more than clobetasol, but the difference was not statistically significant, and was comparable to clobetasol. The efficacy of nebivolol to alleviate the symptoms of IMQ-induced psoriasis may be attributed to its anti-inflammatory and antioxidant capabilities, as well as its peripheral nitric oxide generation effects [30]. Alba *et al*. discovered that BSA was substantially related with decreases in nitric oxide-dependent vasodilation in the psoriatic group, and the greater the nitric oxide reduction, the worse the illness [22]. Nebivolol may have an effect since it increases nitric oxide production. In terms of the observational PASI score, it was proven that nebivolol reduces the PASI score when compared to the induced and vehicle groups, albeit to a smaller extent than clobetasol. This can also be explained by the anti-inflammatory and antioxidant effects of nebivolol, as well as its nitric oxide-dependent peripheral vasodilation capabilities. Clobetasol, on the other hand, was employed as the standard treatment in this trial and resulted in a significant reduction in psoriasis symptoms, which can be attributed to its anti-inflammatory and immunosuppressive properties, which have been associated to psoriasis development. Clobetasol inhibits the production of pro-inflammatory cytokines by binding to corticosteroid receptors and influencing gene transcription [46]. It can also influence the T-cell and monocyte responses [47] and promote the transcription of anti-inflammatory genes [48]. Clobetasol can also reduce inflammation in psoriasis by inhibiting the production of many cytokines and keratinocyte genes [49]. These actions may explain why keratosis and epidermal acanthosis are reduced in clobetasol-treated skin.

Limitations of the study

Additional investigations are required including studies of a combination of nebivolol and clobetasol on psoriatic lesions.

Conclusions

The potential properties of nebivolol as an anti-inflammatory, antioxidant, and peripheral nitric oxide production in psoriasis deems it comparable to the effect of clobetasol treatment. It could be promising in the future treatment of psoriasis as an alternative to steroids or adjuvant treatment to the standard of care.

Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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