Original Article

Effects of Apremilast on Induced Hypertrophic Scar of Rabbits

Nidhal Ghazy, D¹ *, Rahmah Abu- Raghif, A¹

1. College of Medicine, Al-Nahrain University, Iraq

Received 9 September 2021; Accepted 6 October 2021
Corresponding Author: moon.light64@ymail.com

Abstract
The present study aimed to assess the effect of Apremilast on experimentally induced hypertrophic scars in rabbits. A total of 40 healthy male New Zealand White rabbits between 6 and 12 months of age were assigned to four groups (n=10). Group I consists of apparently healthy control rabbits, in group II, the rabbits with an induced hypertrophic scar received no treatment, except for base gel. In group III, the rabbits with induced hypertrophic scar were treated with triamcinolone acetonide (TAC) 0.1% as standard medication. In group IV, rabbits with induced hypertrophic scars were treated with Apremilast 5%. On the first day, four surgical incisions were made using an 8-mm biopsy punch on the ventral surface of the rabbit ear down to cartilage. The TAC and Apremilast were topically administered to the developed scars on day 31. The results included an examination of skin histopathology, the level of transforming growth factor beta-1 (TGF-β1), and collagen III in skin tissue. In the treatments, the inflammatory score, scar index, as well as immunological scores of TGFβ1 and collagen III, significantly decreased, compared to the hypertrophic induced scar group (P<0.001). Moreover, there was a significant reduction in fibroblast count, compared to the group of induced hypertrophic scars (P<0.05). Apremilast was efficacious in the treatment of hypertrophic scars due to its ability to reduce inflammations and fibroblast counts and scar index. Nonetheless, the reduction of immunological scores was almost comparable to that of topical TAC.

Keywords: Hypertrophic scar, Apremilast, SEI, TGF-β1

1. Introduction
The human body responds to an injury by starting the wound healing process and the formation of scars which are useful neo-formation tissues; nonetheless, they lack the same characteristics and functions as the physiological tissue they replace (1). Wound healing consists of a series of processes that are initiated by intracellular and intercellular biochemical pathways, working together to preserve tissue integrity and homeostasis.

Among the involved cellular components, we can refer to the coagulation cascade and inflammatory pathways. Fibroblasts, keratinocytes, and endothelial cells, as well as immune cells, including neutrophils, monocytes, macrophages, lymphocytes, and dendritic cells, have been implicated (2). Wound healing mechanisms, in their optimal state, result in the formation of generally indeterminate, flat, and thinly lined normotrophic scars. Nevertheless, abnormal scars may form in the event of excessive wound healing (3) which results from a persistent inflammatory process, a prolonged proliferation phase, and reduced remodeling when there is an imbalance between biosynthesis and degradation, driven by apoptosis and ECM deterioration (4).

Hypertrophic scar (HSc) is a form of atypical scar which includes excessive collagen deposition, resulting in elevated scar tissue texture. Following an injury, the
wound healing process begins as it would with normal scarring; however, the buildup of the repair matrix takes longer with growing morphologic and biochemical abnormalities (3). The HSc is a prevalent issue following burn injuries and other skin damages experienced by 67% of Caucasians and 74% of Chinese individuals after severe burns. The HSc causes physical and psychological issues in survivors due to its reddish color, elevated appearance, and poor flexibility. Targeted therapies can be used early on to avoid severe scarring and produce positive functional and aesthetic results (5).

Poor diet, diabetes, obesity, medications, and prior radiation exposure all contribute to impaired tissue repair (6). Hypertrophic scars which are common after damage to the deep dermis (7) are hard red or pink-colored elevations of the skin that are usually pruritic and only extend to the edges of the original lesion. These scars appear 4-8 weeks after the accident and then develop rapidly for up to 6 months before gradually receding over a few years. Histologically, the most typical findings in HSc include flattened epidermis and the replacement of the papillary and reticular dermis by scar tissue which is largely formed of fine well-organized type III collagen orientated parallel to the epidermis (8).

These scars have no hair follicles, sebaceous glands, or sweat glands, and the typically undulating rete ridges between the epidermis and dermis are straightened. The hypocellular dermal layer becomes hypercellular due to the proliferation and migration of fibroblasts, myofibroblasts, endothelial cells, and immune cells. The deposition of extracellular matrix components increases with the growth of cells in number, resulting in an unequal generation of extracellular matrix elements and the existence of many vertically aligned blood vessels (6).

The aspects of wound healing are regulated by the transforming growth factor β (TGF-β). Scarring can result from changes in (TGF) expression or signaling. In comparison with controls, from site-matched normal skin, hypertrophic fibroblasts cells generate substantially more TGF-β, resulting in the overexpression of pro-fibrotic factors. The TGF-β1 mRNA expression is roughly fivefold higher in HSc than in normal skin, and this increase was also found in fibroblasts cells isolated from HSc, compared to normal fibroblasts (9).

Apremilast is a phosphodiesterase-4 (PDE4) inhibitor that acts intracellularly by increasing intracellular cAMP levels by inhibiting the breakdown of cyclic adenosine 3’, 5’-monophosphate (cAMP). This inhibition decreases pro-inflammatory mediator expression while increasing anti-inflammatory mediator expression (10). The PDE-4 inhibitor is implicated in a number of inflammatory processes that result in cutaneous problems; consequently, effective medications in these pathways might have a therapeutic function in dermatology (11).

The majority of dermatologic disorders are rooted in immune and inflammatory dysregulation; accordingly, most of them can be treated using some medicines with immunomodulatory and anti-inflammatory properties. Corticosteroids are one of the most helpful medicines; nonetheless, their long-term adverse effects limit their use (12). The PDE-4 inhibitors have similar effects as corticosteroids, but with fewer side effects; therefore, they can either minimize the need for corticosteroids or improve their efficacy (11).

Apremilast had a wide therapeutic index and exerts noticeable impacts on innate and cellular immunity, particularly the release of inflammatory mediators. In 2014, Apremilast (brand name Otezla®) was approved for the treatment of people with active psoriatic arthritis and moderate-to-severe plaque psoriasis (13). Apremilast is now available in tablet form in doses of 10, 20, and 30 mg for oral use (14). Nevertheless, this method of administration has significant drawbacks in terms of side effects and first-pass metabolism; moreover, it is not suitable for individuals with difficulty swallowing. A topical treatment that targets a particular inflammatory mediator on the skin, in particular, offers a local pharmacological action with fewer adverse effects. This method provides a simple
and painless option in the treatment of dermatological disorders since it permits medicines to pass directly through the afflicted areas within the skin (15). Finally, Apremilast has been shown to improve the appearance and clinical results of inflammatory diseases. In general, Apremilast is a breakthrough and a prize in the field of PDE-4 inhibitor discovery (16).

2. Materials and Methods

2.1. Animals and Experimental Conditions

New Zealand White rabbits were housed under controlled environmental conditions (20±2°C, 14:10 h light: dark cycle) and allowed ad libitum access to food and water. A total of 40 healthy male New Zealand white rabbits between 6 and 12 months of age were randomly assigned to four groups (n=10). Punch biopsy was employed to induce hypertrophic scar in the ears of rabbits. Group I consists of apparently healthy control rabbits, group II, rabbits with an induced hypertrophic scar received no treatment, except for base gel. In group III, the rabbits with induced hypertrophic scar were treated with TAC 0.1% as standard medication. In group IV, rabbits with induced hypertrophic scars were treated with Apremilast 5%. The hypertrophic scar model was described by Caliskan, Gamsizkan (17).

The animals were anesthetized by an injection of 0.25 ml of ketamine (15 mg/kg b.w.): xylazine (10 mg/kg b.w.) mixture into the marginal ear vein (18). On the first day, surgical wounds were created using an 8-mm biopsy punch. On the ventral surface of one ear, four incisions were carefully made down to cartilage. The removal of the perichondrial layer delayed epithelization, and wounds were bandaged with sterile gauze for 1 day following hemostasis by manual compression. The final scars were produced on day 30 and treated for 21 days.

2.2. Preparation of Gel Formulations

Gel formulations of chemicals were prepared as follows: firstly, to prepare base gel 3 gram of gelling agent, hydroxypropyl methylcellulose (HPMC) was weighted and added to 75 ml of warm distilled water (70°C) then stirred with a magnetic stirrer for 2 h to obtain homogeneous gel (solution A). Secondly, 0.1 gram of triamcinolone acetonide was weighed then dissolved in 10 ml of absolute ethanol alcohol to prepare (solution B) as a standard drug. Thirdly, a solution was prepared from (20 ml of polyethylene glycol 400 and 10 ml of propylene glycol after mix and heated to 50°C). Thereafter, 5 grams of weighted Apremilast was allowed to dissolve in it using a magnetic stirrer for 10 min until the achievement of complete solubility to make solution B. Solution A and B were mixed thoroughly, and the final weight was made up to 100 ml to prepare TAC gel 0.1% and Apremilast gel 5%, respectively (19). The prepared gels were topically administered twice daily for 21 days.

2.3. Sample Collection and Preparation

After the animals were anesthetized at the end of the study (51 days), each animal tissue sample was collected using an 11 mm punch biopsy with 3 mm margins of adjacent skin. Each tissue sample was stored in 10% buffered formaldehyde solution prepared in sections then send for histological and immunohistochemical analysis. The inflammatory degree, fibroblast count, and index of scar were all determined using the hematoxylin-eosin (H & E) technique. The remaining sections were placed on positively charged slides and immunostained with antibodies against collagen III and the TGF-β1 marker. The average (mean±SD) will be calculated for each group (20).

2.4. Assessment of Histopathological Changes

The scar elevation index (SEI) is calculated by dividing the maximum vertical height of the scar section between the perichondrium and the skin surface by the highest vertical height of the normal area around the scar between the perichondrium and the skin surface. A blindfolded examiner used a calibrated ocular reticule to quantify each wound (17, 21). Inflammation levels and fibroblast numbers were assessed semi-quantitatively. The following scores were used to assess the degree of inflammation: 0=no,
1=mild, 2=moderate, and 3=severe. The number of fibroblasts was measured using the following criteria: 0= no fibroblasts, 1=a few fibroblasts, 2=the existence of disorganized fibroblasts, and 3=the presence of fibroblasts parallel to the wound surface.

2.5. Immunohistochemistry

**Detection of Collagen III and TGF-β1**

(I) Anticollagen III antibody: Rabbit polyclonal antibody to collagen III (Code number: SL0549R) (Sunlong biotech). (II) AntiTGFβ1antibody: Rabbit polyclonal antibody to TGFβ1 (code number SL0086R) (Sunlong biotech), (III) Rabbit specific HRP/DAB (ABC) detection IHC kit (Abcam) (code: Ab64261)

2.6. Evaluation of Immunohistochemistry Results

A biotinylated, cross-adsorbed, and affinity-purified secondary anti-rabbit IgG was used to detect primary antibody-antigen complexes. Following reaction with an enhanced detection reagent, the application of a kit resulted in the appearance of a brown precipitate in positive cells on tissue sections using light microscopy at X20. The degree of the immunohistochemistry response of collagen was determined by rating the signal intensities on a scale of 0= undetected, 1= low density, 2= medium density, 3= dense, and 4=very dense (22). The TGF-β1 immunoreactivity was determined and recorded as follows: 0=the absence of immunoreactivity, a=weak immunoreactivity, 2=moderate immunoreactivity, and 3=strong immunoreactivity r (23).

2.7. Statistical Analysis

Data were collected and presented using two statistical software programs: the statistical package for social science (SPSS version 23) and Microsoft Office Excel 2016. All results are presented as means±SD. Comparison of mean values between two groups was carried out using an independent t-test. A $P < 0.05$ was considered significant and highly significant when $P < 0.01$ (24).

3. Results

3.1. Visual Remark (Healing Rate)

In group II (induced hypertrophic scar untreated group), all animals showed inflammatory signs from the first day, with partial wound closure starting on the fourth day and excessive fibrosis formation (100% induction) on the 30th day (Figure 1).

![Figure 1](image1.png)

**Figure 1.** Stages of tissue healing and hypertrophic scars formation (duration 30 days)

In group III (treated with TAC), a gradual decrease in inflammatory signs with the closure of the wound and moderate decrease of thickness after 21 days of treatment healing symptoms were visible immediately after treatment (Figure 2).

![Figure 2](image2.png)

**Figure 2.** Treatment with TAC (GIII). A. application of topical TAC gel on the induced model, and B. after 21 days of treatment

In group IV (treated with Apremilast), rapid wound healing signals and a remarkable decrease in inflammatory signs, along with a closure of the wound and a reduction in scar thickness, occurred after starting treatment as illustrated in figure 3.

![Figure 3](image3.png)
3.2. Comparison of Induced Non-Treated Group and Other Studied Groups

In comparison between healthy controls and induced non-treated group, there was a significant increase in inflammation, fibroblast count, SEI, collagen III, and TGF-β in induced non-treated rabbits, in comparison with the control group (P<0.001; Table 1). Moreover, the comparison of non-treated induced hypertrophic scars group with TAC 0.1% and Apremilast treated group revealed a significant reduction in inflammation, fibroblast count, SEI, collagen III, and TGF-β in TAC and Apremilast groups, in comparison with the induced non treated group (P<0.05; Tables 2 and 3, respectively).

Table 1. Comparison between the control group and induced non-treated group

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Induced non-treated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fibroblast count</td>
<td>0</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>SEI</td>
<td>0</td>
<td>0</td>
<td>3.48</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>0.8</td>
<td>0.422</td>
<td>3</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>0.8</td>
<td>0.422</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2. Comparison between non treated induced group and TAC treated group

<table>
<thead>
<tr>
<th></th>
<th>Induced non-treated mean</th>
<th>SD</th>
<th>TAC mean</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>2</td>
<td>0</td>
<td>0.7</td>
<td>0.675</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibroblast count</td>
<td>2.5</td>
<td>0.527</td>
<td>1.7</td>
<td>0.483</td>
<td>0.002</td>
</tr>
<tr>
<td>SEI</td>
<td>3.48</td>
<td>0.989</td>
<td>2.26</td>
<td>0.442</td>
<td>0.004</td>
</tr>
<tr>
<td>Collagen III</td>
<td>3</td>
<td>0</td>
<td>1.1</td>
<td>0.316</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>3</td>
<td>0</td>
<td>1.1</td>
<td>0.316</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3. Comparison between non treated induced group and Apremilast treated group

<table>
<thead>
<tr>
<th></th>
<th>Induced non-treated mean</th>
<th>SD</th>
<th>Apremilast group mean</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>2</td>
<td>0</td>
<td>0.2</td>
<td>0.422</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibroblast count</td>
<td>2.5</td>
<td>0.527</td>
<td>2</td>
<td>0</td>
<td>0.015</td>
</tr>
<tr>
<td>SEI</td>
<td>3.48</td>
<td>0.989</td>
<td>1.42</td>
<td>0.551</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Collagen type III</td>
<td>3</td>
<td>0</td>
<td>1.5</td>
<td>0.527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>3</td>
<td>0</td>
<td>0.7</td>
<td>0.675</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

As depicted in figure 4, healthy control tissue showed no inflammation and no fibroblast cell (IB), induced group illustrated moderate inflammation, as well as the presence of disorganized fibroblast cell and a few organized fibroblast cells (IIB), the TAC group demonstrated mild inflammation and few disorganized fibroblast cells (IIIB), and the Apremilast group displayed no inflammation and few fibroblast cells (IVB). (Yellow arrow signify inflammatory cell, red arrow point to disorganized fibroblast cell, and black arrow indicate organized fibroblast).

3.3. Comparison between TAC Treated Group and Apremilast Group

The comparison between TAC treated group and the Apremilast group suggested that Apremilast treated group was significantly reduced SEI levels, in comparison with TAC treated group (Figures 5 and 6 and P<0.05; Table 4).
Figure 4. Histopathological Comparison between study groups. A. maximum vertical height of the scar section between the perichondrium and the skin surface (x4). B. Inflammation rates and fibroblast presentation(x20). I. Healthy control group. II. Induced non-treated group. III.TAC treated group. IV. Apremilast treated group.

Figure 5. Immunohistochemical (TGF-β1 & collagen III) expression in ECM (x20) A. TGF-β1 (brown color mainly in the epidermis), and B. collagen III (brown color mainly in the dermis) IA.control group showed an absence of TGF-β1 immunoreactivity. IB. control group illustrated a low density of collagen III. IIA.Induce non treated group displayed a strong TGF-β1 immunoreactivity. IIB. Induce hypertrophic scar showed a density of collagen III. IIA. TAC group showed a weak immunoreactivity of TGF-β1. IIIB. TAC group showed a low density of collagen III. IVA. Apremilast group showed weak immunoreactivity of TGF-β1. IVB. Apremilast group showed a medium density of collagen III.
According to the correlation between different variables in the study (e.g., inflammations, fibroblast count, SEI, collagen type III, and TGF-β), there was a positive significant correlation among the studied sample (\(P < 0.001\); Table 5).

### Table 5. Correlation among the study variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fibroblast Count</th>
<th>SEI</th>
<th>Collagen III</th>
<th>TGF-ββ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>(r = 0.561**)</td>
<td>(0.704**)</td>
<td>(0.758**)</td>
<td>(0.747**)</td>
</tr>
<tr>
<td></td>
<td>(P = 0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
<td>(0.000)</td>
</tr>
<tr>
<td>Scar elevation index</td>
<td>(r = 1)</td>
<td>(0.644**)</td>
<td>(0.639**)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(P = 0.000)</td>
<td>(0.000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen type III</td>
<td>(r = 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(P = 0.000)</td>
<td>(0.000)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 4. Discussion

Hypertrophic scar (HSc) is a pathological symptom resulting from excessive deposition of collagen and dermal fibrosis in connective tissue due to skin dermal injury (25). All medications that modulate abnormalities during wound healing could be of benefit in the therapeutic process of scars. Studies and drug manufacturing confirmed that it takes more than 10 years to discover and develop a new drug into the markets, at an average cost exceeding 2.6 billion US dollars. As a result, drug repurposing which is a process of identifying new therapeutic use for the existing medications is cost-effective and promising (26).

Different therapeutic modalities, such as corticosteroids, topical silicone gel sheeting, surgical excision, laser therapy, cryotherapy, and radiotherapy have been used to treat HSc; however, none of these modes of treatments has proven completely effective. Accordingly, the molecular mechanism underlying scar formation still needs to be studied, and successful treatment for scars has remained a challenge since the pathogeneses of HSc have not been elucidated, and current therapeutic approaches have limited effectiveness (27).

Consistent with the finding of the study by Zhang, Liu (28), the HSc model of rabbit ears was successful since there were significant differences between the induced non treated group and control healthy group in response to inflammation, fibroblast healthy group in response to inflammation, fibroblast count, SEI, collagen type III, and TGF-β1, pointing to a significant increase (\(P < 0.001\)) (28). Many animal models of HSc, such as the white pig model and mice model, have been utilized to discover the mechanism of scarring and test the novel treatments. The inability of these models to mimic the natural process of HSc formation was their primary limitation. Humans are the only ones who get abnormal scars, such as HSc. The fibromuscular layer under the dermis of laboratory animals is thought to represent the major pathogenic difference between animals and humans (29).
In animal models, healing from injuries and lesions is therefore dependent on wound contraction rather than re-epithelialization. The rabbit ear model of HSc is well-established and frequently utilized in experiments and studies (30). Based on the evidence, scar formation show a marked preference for some locations on the body, such as the anterior chest, that are frequently subjected to tension (31). Moreover, fibroblasts from deeper layers of skin had lower collagenase levels but higher amounts of TGF-β1, collagen, and α-smooth muscle actin. These fibroblasts are thought to be similar to HSc fibroblasts and may play a key role in scar formation and development. All things considered, these facts help to explain why HSc occurs more frequently after deep dermal injuries in some body regions, rather than after superficial lesions (32, 33).

The group treated by TAC had a significant reduction in fibroblast count and SEI, as compared to the group of induced HSc (P≤0.01). In addition, a significant reduction was observed in inflammation, TGF-β1, and collagen III in the TAC group (P≤ 0.001), in comparison with the induced HSc group. These findings were also reported by Pessoa, Melhado (34), and Carroll, Hanasono (35). The TAC is an effective and the most commonly used treatment for HSc. The steroid group was designed since any prospective therapy should outpace the current criterion of steroid as standard therapy with fewer side effects. The TAC significantly suppressed cell proliferation and TGF-β1 expression, leading to the significant induction of matrix metalloproteinase-2 and a down-regulation of production of type III collagen. The lower effect of TAC on apoptosis might be an explanation for the higher recurrence rates in hypertrophic scars treated with TAC alone (36).

In agreement with the results of a study by Uzun, there was a considerable reduction in collagen III in TAC (37). In addition to a drop in TGF-β1 after treatment with triamcinolone acetonide, one possible mechanism for collagen drop in the extracellular matrix is the influence on plasma protease inhibitors, allowing collagenase to break down collagen. In accordance with the findings reported by Caliskan et al. (17), regarding SEI, TAC led to a significant decrease in SEI. Nonetheless, this result disagrees with the finding of the study by Saulis, Mogford (21) who observed an insignificant decrease in SEI after 4 weeks of treatment with onion extract and TAC in rabbits ear model.

The current study found a significant improvement in the Apremilast treated group, in comparison with the non-treated induced group. There was a significant reduction in inflammation, SEI, collagen III, fibroblast count, and TGF-β. In addition, Apremilast demonstrated better anti-inflammatory effects than TAC, and the anti-inflammatory potential of Apremilast was confirmed for its ability to decrease the production of IL-6 and IL-8 in many in vitro models. This effect was confirmed histologically by a reduction in the infiltration of inflammatory cells, and immunologically, by decreasing inflammatory cytokines Interleukin-8 (IL-8), Interleukin 17A (IL-17A), and tumor necrosis factor (TNF-α), suggesting that this formulation could be used as an attractive topical treatment for skin inflammation (38). Furthermore, In vivo, Apremilast significantly reduced epidermal thickness and proliferation, decreased the general histopathological appearance of psoriasis form features, and reduced expression of TNF-α, human leukocyte antigen-DR, in addition to intercellular adhesion molecule-1 in the lesion of that skin (39).

When compared to healthy volunteers and patients without HSc, the numbers of IL-4+ T cells (Th2), as well as levels of IL-10 and TGF-β, were considerably greater in the blood of patients with HSc in the early stages following burn injuries. Additional Th subsets, called Th17, Th22, Th9, and T follicular helper (Tfh) have been recognized recently (40). Although the function of these subsets in HSc is unknown, both Th17 and Th22 are essential producers of IL-22 which plays a key role in tissue healing. The IL-22 promotes cell survival, epithelial cell proliferation, and barrier function. As a result, IL-22 contributes greatly to the development of extracellular pathogen resistance. Delayed epithelialization and bacterial colonization, as
previously noted, are the key predictors of severe scarring. Consequently, IL-22 has the potential to lower the risk of HSc by encouraging fast epithelialization and reducing bacterial colonization. Apremilast is an oral PDE-4 and this group of drugs has been shown to influence both innate and adaptive responses in a growing body of studies. In macrophages, neutrophils, monocytes, and dendritic cells, inhibiting PDE-4 exhibited regulatory actions (41). Furthermore, PDE4I inhibited T cell receptor (TCR)-induced activation of T cells, resulting in decreased cytokine and chemokine release from T helper-1 (Th1), Th2, and Th17 cells, whereas PDE4 inhibition may have little effect on the phenotype and function of B cells (41). In addition, increased cAMP levels in keratinocytes and epithelial cells may suppress inflammatory responses and control cell development and barrier activities. These facts were implicated also in vivo by Huff and Gottwald (42) who used Apremilast for the treatment of inflammation in a 50-year-old lady with vitiligo in a non-FDA-approved fashion. After six weeks of Apremilast treatment, the patient was starting to re-pigment. Three months after initiating treatment, 60 mg intramuscular TAC was added to Apremilast 30 mg and administered twice a day.

This significant improvement in Apremilast treated group despite its poor water solubility proves the success of the prepared formula to release the drug. This finding is in line with those obtained by Sarango-Granda, Silva-Abreu (38) who prepared Apremilast as a microemulsion and demonstrated its capacity to release the incorporated drug following a first-order kinetic model while also guaranteeing a local anti-inflammatory effect with reduced systemic adverse effects due to the high drug retention in the skin.

Apremilast treated group illustrated a superior reduction in SEI and better TGF-β1, in comparison with TAC. This finding can be attributed to the fact that Apremilast is a PDE-4I which inhibit several profibrotic activities of fibroblasts, and these effects are more pronounced in the presence of TGF-β1. The inhibitory effect of the PDE4I is mediated by potentiating endogenous PGE2 signaling, which in turn, acts, at least in part, by stimulating cAMP and protein kinase-A. In addition to inhibiting the breakdown of cAMP, PDE4 inhibitors potentiate TGF-β1-induced prostaglandin E2 production. By augmenting an endogenous feedback control mechanism, PDE4 inhibitors as therapeutic agents have the potential to limit TGF-β1-driven fibrosis. Furthermore, according to Thangapazham, Sharma (43), TGF-β1 induces angiogenesis; therefore, a reduction in TGF-β1 in all groups was associated with wound closure in the generated hypertrophic scar.

The current study reported a positive significant correlation between the inflammatory response parameters in the studied samples. In Conclusion, Apremilast was efficacious in the treatment of hypertrophic scars due to its ability to reduce inflammations and fibroblast counts and scar index. Nonetheless, the reduction of immunological scores was almost comparable to that of topical TAC.

Authors' Contribution
Study concept and design: D. N. G.
Acquisition of data: A. R. A. R.
Analysis and interpretation of data: A. R. A. R.
Drafting of the manuscript: D. N. G.
Critical revision of the manuscript for important intellectual content: D. N. G.
Statistical analysis: D. N. G.
Administrative, technical, and material support: D. N. G. and A. R. A. R.

Ethics
This cross-sectional study was subjected to the control and supervision of the protocol that was reviewed by the Institutional Review Board (IRB) in Al-Nahrain University/College of Medicine following the approval of the Scientific Committee of Pharmacology Department in the College of Medicine/Al- Nahrain University.
Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors are grateful to the College of Medicine, Al-Naharin University for the provision of opportunity and facilities to achieve this work.

References


