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Topical apigenin as a promising therapeutic agent for psoriasis: Evaluating efficacy alone and in combination with clobetasol in an imiquimod-induced model of psoriasis in mice

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ABSTRACT

Psoriasis is an immune-mediated skin disease characterized by excessive growth and abnormal differentiation of keratinocytes that were treated initially with steroids which showed severe side effects for long-term use, so, medicinal plants are being explored as a potential alternative to identify new and effective agents with fewer adverse effects, such as apigenin which is investigated alone and in combination with clobetasol in this study for their anti-psoriatic activity on imiquimod-induced psoriasis on sixty male Albino mice divided into 6 groups. Group I is the control group, while the rest of the groups were induced psoriasis by Imiquimod (IMQ) for 6 consecutive days and administered different interventions for each group for 8 consecutive days including topical apigenin and apigenin/clobetasol combination followed by a measurement of the clinical, histopathological, and laboratory effects and the findings demonstrated that apigenin effectively reduced the PASI score, enhanced the histopathology, downregulated the expression of TNF-α, IL-17, and VEGF, and significantly increased the IL10 level in mice's skin tissue homogenate which indicate that Apigenin alone and in combination with clobetasol hold promise for the management of psoriasis, offering a potential alternative or adjunct to current therapeutic approaches.

Keywords: psoriasis, apigenin, Imiquimod, Clobetasol, IL-10

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INTRODUCTION

Psoriasis is a chronic inflammatory, non-contagious illness characterized by multisystemic inflammation with reddish plaques and white scales that is more common in the knees, feet, hands, elbow, scalp, and sacral regions[1]. It is associated with the activation of the adaptive immune system, particularly T-cells, although its exact cause and development mechanism are unknown. An imbalance between T helper Type 1 and Type 2 cells, as well as cytokine generation, are among the leading potential causes. Epidermal differentiation disruption and impairment of the skin barrier are significant characteristics. Psoriasis-specific Th1/Th17 cytokine milieu impacts epidermal differentiation[2,4].

Imiquimod (IMQ) – which is a Toll-like receptor (TLR) 7/8 ligand dermatological application induces and possibly exacerbates psoriasis, in a treated mouse model it exhibits similarities to human plaque-type psoriasis in terms of skin erythema, thickening, scaling, epidermal alterations (acanthosis, parakeratosis), neo-angiogenesis, and inflammatory infiltrate comprising T cells, neutrophils, and dendritic cells[5]. Prior research has established the significance of various factors in this model, including the psoriasis area severity index (PASI) score, histological staining, and the involvement of inflammatory cytokines[6,9].

Phototherapy, systemic drugs such as methotrexate and cyclosporine, oral medications such as apremilast, and topical therapies are all options for treating moderate-to-severe psoriasis. Yet, even though many therapies are effective and well tolerated, people with psoriasis frequently do not achieve skin clearance[10]. Nevertheless, due to the adverse effects of anti-inflammatory medicines, investigations on natural substances to replace chemical pharmaceuticals have become increasingly active. As a result, it is critical to identify novel candidates for topical administration to decrease side effects and obtain improved therapeutic efficacy[10].

Apigenin, a flavone subclass, is one of the most frequent flavonoids discovered in plants[12]. The bioactive natural chemical is scientifically referred to as 4′,5,7 trihydroxy flavone (4′,5,7-trihydroxy flavone). It is a 270 Da flavonoid that is found in numerous fruits and vegetables, as well as fragrant plants such as chamomile (Figure 1)[13].

Figure 1. Chamomile flower Apigenin powder extract

Apigenin has a variety of physiological effects[14], including anti-inflammatory[15], antioxidant[16], anticancer[17], anti-viral properties[18] and hypoglycemic effects[19,20].

The current study attempts to investigate topical Apigenin's potential therapeutic effects as a new modality for the treatment of psoriasis symptoms alone and in combination with clobetasol on an imiquimod-induced model.

METHODOLOGY

Study design

Male BALB/c albino mice, weighing between 24 and 30 g and aged between 8 and 12 weeks, were split into six groups of ten mice each, for a total of 60 mice. Several body parts were marked to help identify the animals. The mice were acquired from the Al-Nahrain University – Biotechnology Research Center in Baghdad, Iraq. They were kept in polypropylene cages with a temperature control system (15–21°C) and an inverted light–dark cycle (12–12 hours). The mice were given seven days to acclimate before the experiment began at the same facility. The animals were fed a regular diet and were allowed unrestricted access to water. Prior the start of the experiment, the mice were checked for the presence of any skin lesions, and only mice with seemingly healthy skin and coats were included in the study. All of the study animals were shaved from the dorsal region to reveal an area of the back skin measuring approximately 1x2 cm using an electric razor followed by a hair removal cream application (Veet®, Reckitt Benckiser Pvt. Ltd., India). The experiment lasted 14 days in total from the first day. About the distribution of mice:

- G-1 (Apparently healthy group) remained with no intervention during the whole experimental duration.

- G-2 (Induction group) that received induction of psoriasis by a dose of 62.5 mg of topical Imiquimod cream 5% (Aldara® 5% Cream, Meda Pharmaceuticals, Solna, Sweden) once daily on the shaved back skin for 6 days until the appearance of a psoriatic lesion as mentioned by van der Fits et al. study[21], mice in this group received no further intervention.
- G-3 (Petrolatum group) received induction of psoriasis by a dose of 62.5 mg of topical Imiquimod cream 5% once daily on the shaved back skin for 6 days as mentioned by van der Fits et al. study and then on the 7th day received medicinal petrolatum jelly (Iraqi Federation of Industries, Baghdad, Iraq) topically twice daily 8 days duration.
- G-4 (Clobetasol group) received induction of psoriasis by a dose of 62.5 mg of topical Imiquimod cream 5% once daily on the shaved back skin for 6 days as mentioned by van der Fits et al. study and then on the 7th day received as standard of care for psoriasis topical Clobetasol propionate 0.05% ointment (Dermovate®, GlaxoSmithKline, Brentford, UK) once daily (at a dosage of 0.25 g/kg)[22] for 8 days duration.
- G-5 (Apigenin 2% group) received induction of psoriasis by a dose of 62.5 mg of topical Imiquimod cream 5% once daily on the shaved back skin for 6 days as mentioned by van der Fits et al. study and then on the 7th day received a preparation of topical apigenin ointment 2% w/w[23,24] (prepared from powder supplied by Hyperchem, Hangzhou, China) twice daily for 8 days duration.
- G-6 (Apigenin-clobetasol combination group) received induction of psoriasis by a dose of 62.5 mg of topical Imiquimod cream 5% once daily on the shaved back skin for 6 days as mentioned by van der Fits et al. study and then on the 7th day received a topical preparation of 0.025% Clobetasol propionate ointment combined with apigenin ointment 1% w/w twice daily for 8 days duration. Figure 2 illustrates the flow chart of the study.

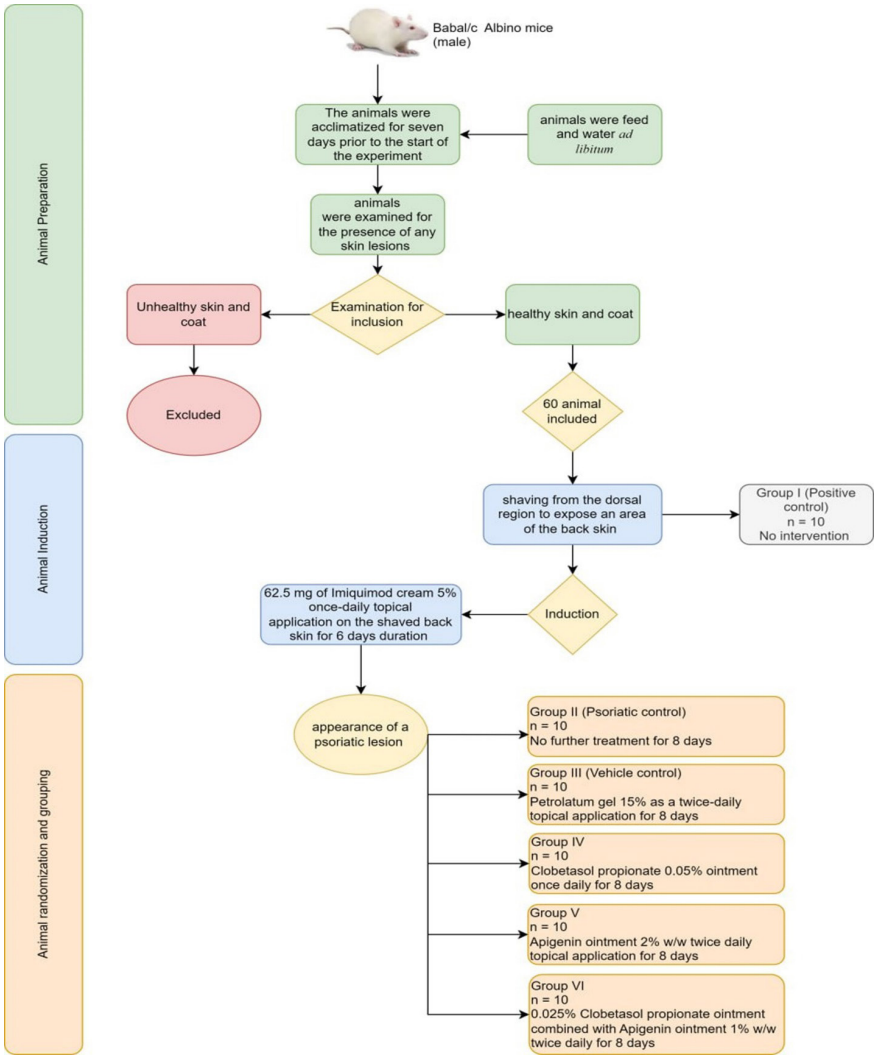

Figure 2. Study design

The Psoriasis Area Severity Index (PASI) score in mice was used to assess the success of the Imiquimod-induced psoriasis model[25]. The skin erythema, increased skin thickness, and scaling that were observed in the animals during the induction phase were considered successful outcomes since they appeared in the animals before day 6 of induction[26]. Each mouse's back skin was examined visually for three different characteristics: induration (thickness), desquamation (scale), and erythema (redness). The total score ranged from 0 to 12[25], with each characteristic being assigned between 0 and 4 (0=None, 1=Slight, 2=Moderate, 3=Marked, and 4=Very marked) (Figure 3).

Figure 3. Induction of Psoriasis in mice. (A) Mouse before induction (B) Mouse after induction by Imiquimod cream

Preparation of apigenin ointment 2% w/w, USP 37 – NF 32

Twenty-five grams of Petrolatum ointment of 2% apigenin was prepared by fusion technique based on USP 37 – NF 32 [27]. 18.75 g of petrolatum was melted in a water bath followed by the addition of the required amount of apigenin (0.5 g) then it was mixed using a stirrer until the mixture was uniform, the weight was completed by petrolatum to reach 25 g with stirring, the combination was then cooled until congealed.

Apigenin and clobetasol combination ointment, USP 37 – NF 32

Commercial clobetasol ointment (Dermovate®) containing 0.05% Clobetasol propionate (CP) was used with the prepared apigenin 2% ointment. An equal amount of both apigenin and clobetasol (half concentration of both) was taken and well mixed by a spatula to obtain the final concentration of 1% apigenin and 0.025% clobetasol combination ointment[27].

Outcome measures

Based on the PASI Score, the effectiveness of the treatments in the tested groups was assessed[25]. Intraperitoneally (IP) anesthesia was administered to all mice using 80 mg/kg of ketamine and 10 mg/kg of xylazine. All mice after being completely anaesthetized were terminated by exsanguination, a procedure appropriate for tissue harvesting and preservation[28]. Tissue samples from the dorsal shaved skin (2 mm) were obtained and split into two parts. The first

part was prepared for histopathological analysis by immersing it in liquid paraffin at a temperature range of 55-60°C after first being dehydrated. The slide was made using this technique, which has been covered in previous research[29].

The pathological changes of the mice's skin tissues on a scale of 0 – 10 were then evaluated under light microscopy (Genix, USA) using Baker's grading system, a histopathological grading system used for assessing the severity of inflammation as illustrated in Table 1. The parts were evaluated in a blinded manner by two distinct investigators, and the average score was recorded[30].

Table 1. Histopathological scoring of the severity of inflammation (Baker scoring system)[31]

Layers	Feature	Score
Keratin	Munro Abscess	2.0
	Hyperkeratosis	0.5
	Parakeratosis	1.0
Epidermis	Thinning over Papillae	0.5
	Rete ridges appearance	1.5
	Acanthosis	0.5
	Lack of granular layer	1.0
Dermis	Lymphocytic infiltrate	
	Mild	0.5
	Moderate	1.0
	Severe	2.0
	Papillary Congestion	0.5

The second piece of skin tissue was ready for biochemical analysis in order to measure TNF-α, IL-17, IL-10, and VEGF. The tissue was first thoroughly rinsed in ice-cold PBS (pH 7.2) to remove any excess blood, and then weighed using an electrical balance and minced into small pieces before being homogenized in fresh lysis buffer. 1mL of lysis buffer was added to the tissue sample using a glass homogenizer on ice, and the tissue was homogenized using an electrical tissue homogenizer machine (Electrical tissue homogenizer, Staruar®, England). Following that, the homogenates were centrifuged at 10,000×g for 5 minutes. Before being used for analysis using the sandwich ELISA technique and the ELISA Reader (ELISA reader, Diagnostic Automation / Cortez Diagnostics®, California, USA), the supernatants were collected and kept at ≤ -20°C. With regard to the ELISA kits, the mice analytical kit (SCA133Mu,

Cloud-Clone Corp.) was used to determine TNF-α, the mice analytical kit (SEA056Mu, Cloud-Clone Corp.) was used to determine IL-10, the mice analytical kit (HEA063Mu, Cloud-Clone Corp.) was used to determine IL-17, and the mice analytical kit (SEA143Mu, Cloud-Clone Corp.) was used to investigate VEGF.

Program G Power, which is based on Cohen's principles was used to compute the sample size[32,33]. The groupings were randomly constructed using a table of random integers. The animals were tagged with tails and kept in labelled containers to reduce miscommunication[34] .

Statistical analysis

All analyses were carried out using "GraphPad Prism version 10.0.0. The Kruskal-Wallis's test was applied to verify the significance of the difference between the studied groups, followed by the post hoc "two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (False discovery rate)"[35]. The differences between the groups were considered significant statistically when the p-value was less than or equal to 0.05[36].

RESULTS and DISCUSSION

The results of the current study revealed that the animal model treated with imiquimod exhibited a statistically highly significant increase in PASI score when compared to the healthy control group. The topical application of apigenin ointment and Clobetasol Propionate 0.05% and a combination of them showed a highly significant reduction in the PASI score compared to the induction group and petrolatum group as illustrated in Figure 4 and Table 2. Results also revealed that the same manner of improvement was obtained regarding Baker's score in which G-4, G-5, and G-6 were significantly lower than G-2, and G-3. It was observed that G-4, G-5, and G-6 were non-significantly different from each other regarding both Baker's and PASI scores.

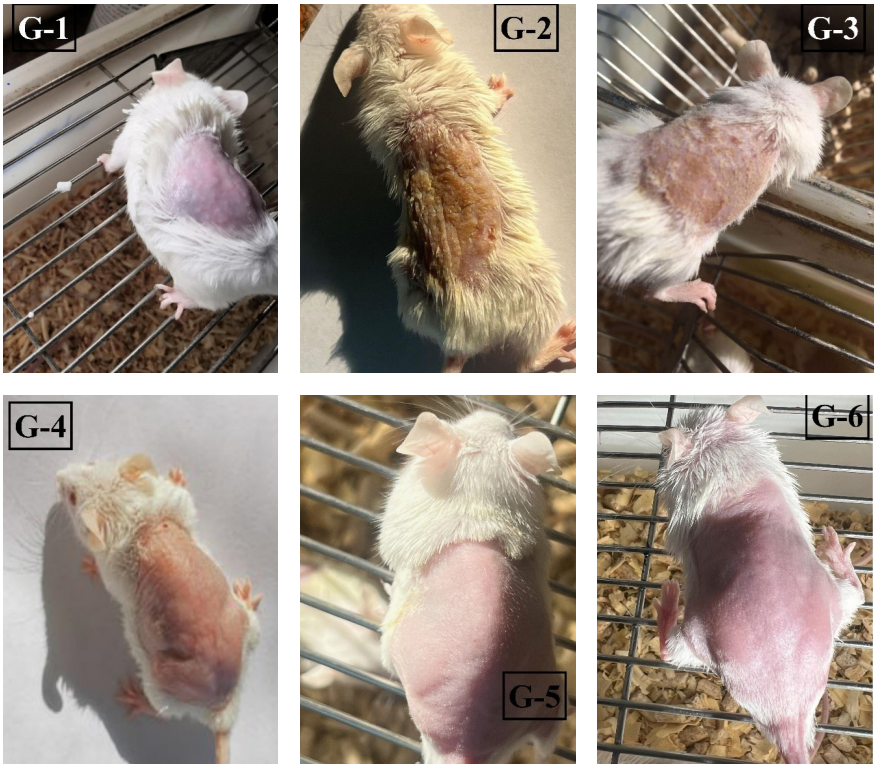

Figure 4. PASI score which includes erythema, thickness, and scales in the course of IMQ induction and treatment of psoriasis-like lesion; control group (G-1), induced non-treated group (G-2), petrolatum group (G-3), clobetasol propionate 0.05% (G-4), apigenin 2% group (G-5), apigenin 1%-clobetasol 0.025% (G-6)

Table 2. Assessment of skin psoriasis scores

Parameters	Baker's score	PASI score
G-1	-	-
G-2	9.00 ± 0.00[a]	11.70 ± 0.48[a]
G-3	7.60 ± 0.84[a]	9.30 ± 0.67[a]
G-4	2.15 ± 1.08[b]	3.00 ± 0.82[b]
G-5	1.85 ± 0.53[b]	1.70 ± 0.67[b]
G-6	1.80 ± 0.42[b]	1.50 ± 0.71[b]
p-value	<0.0001[#]	<0.0001[#]

Kruskal-Wallis's test [p-value≤0.05 indicates significant difference] with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (False discovery rate) to calculate pair-wise p-value between each pair
Data presented as mean ± standard deviation SD: standard deviation, PSAI: psoriasis area and severity index

Histopathologic examination of the skin section of control group showed normal epidermal, dermal, and subcutaneous tissue layers (Figure 5[A]), while the histopathological examination of skin section of Induction group showed a multifocal (wide) area of sloughing, severe dense neutrophilic infiltration (the Munro's abscesses), and parakeratosis, hyperkeratosis, with lack of granular layer, acanthosis, increased rete ridges with papillary thinning. The dermis showed severe lymphocytic infiltration and vascular congestion (Figure 5[B]). The histopathological examination of the skin section of Petrolatum groups also showed epidermal hyperkeratosis and parakeratosis with focal Munro's abscesses with acanthosis and elongated rete ridges and papillary thinning with moderate to severe lymphocytic infiltration (Figure 5[C]).

The histopathological examination of the skin section of the standard treatment group (clobetasol group) shows hyperkeratosis, absence of (parakeratosis & Munro's abscess) and presence of epidermal granular layer with mild acanthosis, few rete ridges with mild thinning of papillae with mild lymphocytic infiltration of the dermis (Figure 5[D]). The skin of mice treated with apigenin 2% showed a mild keratosis with an absence of Munro's abscess and parakeratosis and epidermal mild acanthosis with few rete ridges. The dermis shows mild lymphocytic infiltrate (Figure 5[E]). The histopathological examination of the skin section of a combination treatment group showed mild epidermal thickness, with the absence of Munro's abscess and parakeratosis and epidermal mild acanthosis with absence of rete ridges and few lymphocytic dermal infiltrations (Figure 5[F]).

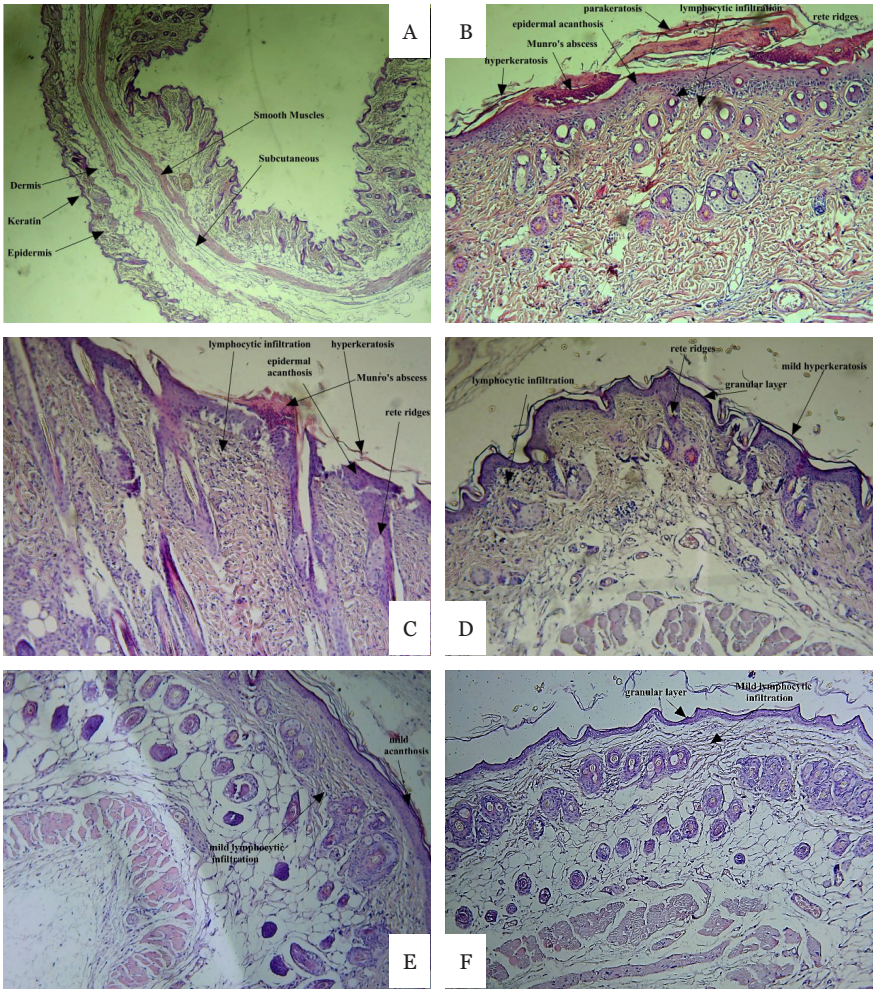

Figure 5. Histopathological section of mice skin: [A] healthy control group [B] induction group, [C] Petrolatum group [D] clobetasol control group [E] Apigenin 2% group [F] Apigenin – Clobetasol combination group; H&E stain (4X, & 10X)

Results obtained from the inflammatory markers revealed that G-2 showed the highest levels of TNF-α and was significantly higher than groups G-4, G-5, and G-6, whereas non-significant differences between G-2 and G3 and between G4, G5, and G-6 were observed, as illustrated in Table 3 and Figure 6. Results showed that G-2 demonstrated the highest levels of IL-17, which is significantly higher than the other groups (G-3 to G-6). Additionally, G-3 was significantly higher than G-4, G-5, and G-6, with non-significant differences between G-4, G-5, and G-6 as illustrated in Table 3 and Figure 6.

Moreover, G-2 showed the highest levels of VEGF which is significantly higher than all other groups (G-3 to G-6), and G-3 showed to be significantly higher than G-5 and G-6, whereas a non-significant difference between G-3 and G-4 and a non-significant difference between G-4, G5 and G-6 were obtained as illustrated in Table 3 and Figure 6.

G-2 showed the lowest levels of IL-10 which is significantly lower than the other groups (G-3 to G-6). On the other hand, there were non-significant differences between G-4, G-5 and G-6 which are significantly higher than G-2 and G-3, as illustrated in Table 3 and Figure 6.

Table 3. Assessment of biomarkers

Parameters	TNF-α	IL-17	VEGF	IL-10
G-2	807.13 ± 500.06[a]	553.04 ± 141.32[a]	552.20 ± 136.63[a]	31.83 ± 3.03[a]
G-3	281.79 ± 240.17[a]	278.52 ± 100.27[b]	209.56 ± 73.31[b]	92.50 ± 27.13[b]
G-4	65.37 ± 23.12[b]	165.07 ± 43.59[c]	134.57 ± 44.28[bc]	168.11 ± 57.07[c]
G-5	89.25 ± 18.76[b]	149.90 ± 63.30[c]	99.56 ± 17.42[c]	170.4 ± 12.65[c]
G-6	71.45 ± 17.83[b]	150.16 ± 21.19[c]	94.98 ± 7.37[c]	166.11 ± 47.80[c]
p-value	<0.0001[#]	<0.0001[#]	<0.0001[#]	<0.0001[#]

Kruskal-Wallis's test [p-value≤0.05 indicates significant difference] with a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (False discovery rate) to calculate pair-wise p-value between each pair
SD: standard deviation, IL= Interleukin; TNF-α= Tumor necrosis factor alpha; VEGF=Vascular endothelial growth factor

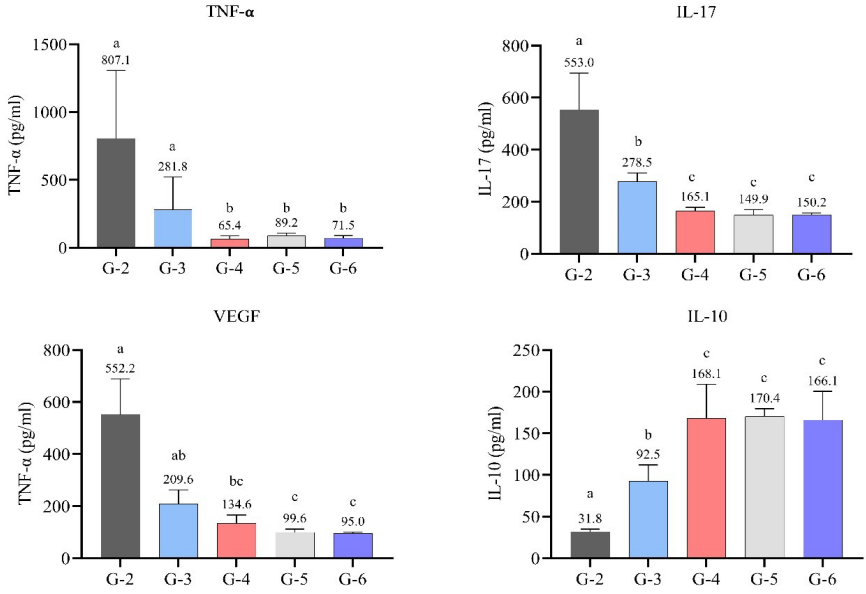

Figure 6. Histogram Comparison between all treated groups in terms of inflammatory markers (TNF-α, IL-17, VEGF and IL10); TNF-α= Tumor necrosis factor-alpha; IL= Interleukin; VEGF=Vascular endothelial growth factor

Psoriasis is a persistent autoimmune disorder distinguished by excessive proliferation of epidermal cells, expansion of dermal capillaries, infiltration of inflammatory cells, and anomalous growth of skin cells. The aforementioned factors are responsible for inducing various complications such as the development of erythematous plaques that are characterized by pruritus, inflammation, and discomfort[37]. Apigenin is a flavonoid that has caught attention in this study due to its potential as an immunomodulator. It is particularly noteworthy for its low intrinsic toxicity and lack of mutagenicity, distinguishing it from other flavonoids with similar structures[38] . In the current investigation, as shown in the results apigenin was responsible for a dramatic improvement in phenotypical observation and a highly significant reduction in cumulative (PASI) scores representing erythema, scaling, and thickness compared to the induction group. Present study findings also indicate a noteworthy improvement and a highly significant reduction in Baker score after treatment with topical apigenin in comparison to the IMQ-induced group.

This successful anti-inflammatory effect of apigenin in ameliorating psoriasis observational and histopathologic features attributed to its anti-inflammatory properties by its documented capacity to regulate the production of inflammatory mediators such as interleukin (IL)-1, IL-2, IL-6, IL-8, tumor necrosis

factor (TNF)-a, activator protein-1 (AP-1) factors, and cyclooxygenase (COX)-2. In addition to its antioxidant, anticancer, antiviral, antimutagenic, and antibacterial actions only some of the many therapeutic benefits associated with apigenin as documented in previous studies[39]. In addition, results from ELISA investigation demonstrated that apigenin causes a significant decrease in TNF-α, IL-17, and VEGF levels and an increase in the levels of IL-10, an anti-inflammatory cytokine. Various features of apigenin have been demonstrated in previous investigations to be the underlying mechanisms for its possible anti-psoriatic impact.

Apigenin has been found to mitigate the severity of inflammatory processes significantly through the inhibition of TNF-α and the augmentation of IL-10[40]. According to Kumar et al., apigenin lowers the level of pro-inflammatory cytokines including IL-1 and TNF-α, and increases IL-10 levels at the same time. This makes it abundantly evident that apigenin has a variety of functions in controlling immune response mediators, both pro-inflammatory and anti-inflammatory cytokines[41]. IL-10 is a potent anti-inflammatory cytokine that is essential for immune modulation in psoriasis[42].

Prior research has shown that apigenin enhances the production of IL10 by modulating the balance between Th1 and Th2 immune responses in Experimental Autoimmune Myocarditis (EAM). Apigenin has been found to inhibit the production of Th1-type cytokines, such as TNF-α, IFN-γ, and IL2, while simultaneously enhancing the Th2 response, leading to an upregulation of Th2-type cytokines, including IL10, IL4, and IL5[43] TNF-α is a crucial pro-inflammatory cytokine that promotes inflammation through multiple pathways. TNF-α enhances the expression of adhesion molecules and secondary mediators, which are implicated in the pathogenesis of psoriasis[44].

According to prior research, the anti-inflammatory function of apigenin is partially elucidated by its direct suppression of NF-KB by inhibiting the nuclear translocation of p65. The latter process leads to the deactivation of genes responsible for nitric oxide synthetase (NOS) and cycloxygenase-2 (COX-2), which plays a crucial role in inhibiting the inflammatory process and inducing cell-cycle arrest. Apigenin has been demonstrated to have a significant impact on the inflammatory process through its targeting and down-regulation of TNF-α and NF-kB, as evidenced by previous research[45]. This supports our findings that showed apigenin 2% ointment significantly decreased the level of TNF-α and significantly increased the level of (IL10) in skin tissue homogenate in comparison to the IMQ-induced group.

Apigenin modulated the function of dendritic cells (DCs) to affect naïve T cell differentiation, so, it can improve psoriasis symptoms by regulating inflammatory cytokine transcription through the Toll-like receptor 4 pathway[46]. The utilization of topical apigenin has been observed to enhance psoriasis by impeding the function of dendritic cells, subsequently leading to a reduction in the activation of T-helper cells. Prior research has indicated that apigenin significantly suppresses the activation of Th1 and Th17 cells in individuals with lupus erythematosus. Reduction in the protein expression levels of IL-17A and IFN-g was observed in the dermal tissue of mice treated with apigenin. In addition, the results of an in vitro experiment demonstrated that apigenin can inhibit the production of IL-6 by APCs. This is significant because IL-6 plays a crucial role in the development of Th17 cells and the suppression of Treg cells[47]. This is aligned with our results that showed apigenin significantly reduced the level of skin tissue (IL17) in comparison to the induction group.

The upregulation of VEGFA, which serves as the primary proangiogenic factor, has been observed in the cutaneous tissue of individuals with psoriasis and is positively associated with the severity of the disease[48,49]. It has been well established through studies that hypoxia is one of the primary stimulators of VEGF expression, which is mediated by the accumulation of HIF-1a[50]. Multiple investigations have demonstrated that apigenin inhibited angiogenesis in rheumatoid arthritis and cell hypoxia-reoxygenation injury by its ability to decrease the expression of various angiogenesis-related factors, such as HIF-1α and VEGF-A, in various types of human cancers, as evidenced by multiple sources[51]. The aforementioned findings indicate that apigenin has an anti-angiogenic effect which is crucial to the pathogenesis of psoriasis[52]. This is in line with our findings, which demonstrated a marked decline in the level of (VEGF) in comparison to the induction group.

Psoriasis is associated with oxidative stress, which triggers the activation of various signalling pathways, such as NF-KB and MAPK. This leads to the activation of Th1 and Th17 cells, secretion of proinflammatory cytokines, hyperproliferation of keratinocytes, infiltration of immune cells into the skin, and changes in blood vessel permeability due to lipid peroxidation. Therefore, the use of antioxidants is crucial in the treatment of psoriasis[53]. Prior studies have indicated that Apigenin exhibits antioxidant properties by enhancing the activity of antioxidant enzymes glutathione-synthase (GSH synthase), catalase (CAT), and superoxide dismutase (SOD). Apigenin exerts an impact on the production of cutaneous barrier components and the entry of calcium ions. Thus, it has the potential to be utilized in the treatment of inflammatory skin conditions and cancer[54]. This antioxidant characteristic has been proposed to contribute to the amelioration of induced psoriasis.

Furthermore, the skin treated with apigenin exhibited observable effects on skin barrier recovery. The application of apigenin ointment in our study resulted in an enhancement of the skin's state through augmentation of the hydration level of the stratum corneum. In mouse models, it was observed that apigenin had an impact on the production of skin structural proteins such as filaggrin, involucrin, and loricrin[55].

Utilizing a combination drug approach represents a viable strategy to enhance the synergistic efficacy of topical therapy. To optimize the therapeutic effect, a multi-target treatment approach utilizing synergistic combinations of two or more therapeutically relevant molecules that act through different mechanisms is recommended[56]. The combination of Apigenin 1% and clobetasol ointment 0.025 was administered in our investigation and the findings indicate that the co-administration of apigenin and clobetasol at reduced concentrations exhibited additive properties in the mitigation of psoriasis lesions. No significant difference in PASI and Baker scores between the combination group and the apigenin and clobetasol treated groups alone, in terms of inflammatory markers there was no significant difference between the combination and when the apigenin 2% ointment or clobetasol were used alone, This could be explained by the mechanisms of action of apigenin, a flavone with anti-inflammatory properties, and clobetasol, a potent corticosteroid that reduces inflammation and has immunosuppressive effects. If both act on the same inflammatory pathways, it could explain why their combination does not have a synergistic effect. Additionally, the concentration of clobetasol and apigenin in the combination preparation might not be optimized for the synergistic effect.

This study demonstrated that Apigenin's anti-inflammatory and antioxidant properties play a crucial role in reducing inflammation and oxidative stress and regulating Psoriasis progression. Topical apigenin improved the animals' anti-inflammatory and anti-oxidant status, maintained skin hemostasis, along with the modulation of key cytokines, reduction in proinflammatory cytokines (TNF-α, IL17) and VEGF, coupled with the increase in the anti-inflammatory cytokine (IL-10). Collectively contribute to the amelioration of psoriasis symptoms and supports the therapeutic potential of apigenin and its anti-psoriatic effect is comparable to that of clobetasol. The combined administration of apigenin and clobetasol exhibited additive properties compared to the individual use of either medication. Thus, the present study indicates that Apigenin alone and in combination with clobetasol hold promise for the management of psoriasis, offering a potential alternative or adjunct to current therapeutic approaches.

STATEMENT OF ETHICS

The study was approved by the "Research Ethics Committee at the College of Medicine, Al-Nahrain University" (Approval number: 2308, date: 1st November 2022).

CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTIONS

Design – Hasan AM, Gatea FK; Acquisition of data – Hasan AM; Analysis of data – Hasan AM; Drafting of the manuscript – Hasan AM; Critical revision of the manuscript– Gatea FK; Statistical analysis– Hasan AM, Gatea FK; Technical or financial support– Hasan AM; supervision – Gatea FK.

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