



RESEARCH ARTICLE

Anti-proliferative, Anti-oxidant and Anti-inflammatory Effects of Topical Rutin on Imiquimod-Induced Psoriasis in Mice

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Received: May 22, 2024

Accepted: Jun 27, 2024

Keywords

Ki-67

MDA

VEGF

TNF- α

Psoriasis

Rutin

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ABSTRACT

Psoriasis is a chronic autoimmune dermatosis characterized by thickened, reddish-brown, and peeling skin lesions. To evaluate the potential Anti-proliferative, Anti-oxidant and Anti-psoriatic effects of Rutin 4% ointment in comparison to clobetasol (0.05%) ointment in imiquimod-induced psoriasis in mice model on the basis of histopathological and observational outcomes, as well as biomarkers is the purpose of this research. Fifty healthy adult Swiss albino mice, weighing 24-30 grams and aged 11 to 15 weeks were randomly divided into five groups (n=10); each group contained ten mice with shaved dorsal skin; apparently healthy mice group (group I) did not receive any medication; induced group (group II) received only topical Imiquimod; vehicle control group (group III) received a topical ointment base only; standard group (group IV) received 0.05% Clobetasol ointment only, received topical Rutin 4% ointment (group V). All treatment groups received topical Imiquimod cream for induction on the shaved back for 6 consecutive days; then received topical treatment for 8 days with induction and scoring for skin inflammation severity (scaling, erythema and thickness) was recorded on daily basis, and the animals were sacrificed on day 14. Psoriasis induction was successful by Imiquimod, and psoriasis like lesions developed on the skin. Topical Rutin treatment groups significantly reduced the inflammatory signs of the psoriatic like lesions and these findings were supported by the histopathological examination. The present study showed a significant ($P<0.001$) decrease in the psoriasis area and severity index score (PASI) and a significant ($P<0.001$) reduction in the tumor necrosis factor- alpha (TNF- α), vascular endothelial growth factor (VEGF), Interleukin-17 (IL-17), oxidative stress marker Malondialdehyde (MDA) and marker of cell proliferation (Ki67) levels in skin tissue of the Clobetasol and Rutin groups as compared to the induction group. Additionally, histopathological outcomes show a significant ($P<0.001$) attenuation in the imiquimod- induced psoriasis in mice. Topical Rutin significantly decreased vascular endothelial growth factor levels. At the same time, Rutin showed a more significant reduction in Interleukin-17 (IL-17) levels ($P<0.001$).

INTRODUCTION

Psoriasis is a chronic autoimmune dermatological condition that is characterized by the presence of red, enlarged, and flaky skin plaques. There are numerous factors that appear to contribute to the

development of psoriasis, such as environmental variables (such as viral diseases and trauma) and immunologic and genetic components (Takeshita et al., 2017, Rajguru et al., 2020). Epidermal hyperplasia, dermal blood vessel proliferation, and inflammatory leukocyte infiltration are the three primary histologic characteristics of psoriasis, which are primarily located in the dermis (Abu-Raghif and Noaman, 2013, Branisteanu et al., 2022; Jam et al., 2017).

Psoriasis frequently occurs in conjunction with metabolic syndromes, which are characterized by high blood pressure, elevated cholesterol levels, diabetes-related complications, and weight gain. These conditions increase the likelihood of atherosclerosis and cardiovascular mortality in patients with psoriasis (Raghif et al., 2017, De Brandt and Hillary, 2022; Farooq et al., 2010). Additionally, there are connections between this dermatosis and other immune-driven inflammatory conditions, including celiac disease, lupus erythematosus, and irritable bowel syndrome. (Daugaard et al., 2022, Oubaid et al., 2023, Jaafar and Abu-Raghif, 2023).

The TNF- α /IL-23/IL-17 axis has a substantial influence on the maintenance stage of psoriasis. IL-17 plays a critical function in the development of psoriasis. Despite the widespread belief that IL-23 generates Th17 cells that produce IL-17, it is also feasible for innate immunity to produce IL-17 without the involvement of IL-23 (Sato et al., 2020, Afonina et al., 2021; Rashid et al., 2023). In numerous cell types, reactive oxygen species (ROS) production and elimination imbalances induce cellular oxidative stress, which is associated with risk factors for the development of autoimmune disorder, Psoriasis is one of the disorders that are characterized by excessive angiogenesis, as a result of disruptions in the processes of normal physiological angiogenesis (Abu-Raghif et al., 2015, Henno et al., 2009). The Ki-67 antigen is a protein complex that is produced at every stage of the cell cycle. As a result, it functions as a marker for cells that are actively involved in cell proliferation (Khairutdinov et al., 2017).

Topical treatments (steroidal medications and vitamin D3 analogs) and systemic treatments (retinoids like acitretin, PDE inhibitors like apremilast, antimetabolites like methotrexate, immunosuppressive agents like cyclosporine, and phototherapies like PUVA) are the primary methods for managing psoriasis (Schön and Wilsmann-Theis, 2023, Raharja et al., 2021; Kanval et al., 2024). Anti-psoriatic biologics include anti-TNF- α (adalimumab, certolizumab, etanercept, and infliximab), anti-IL-17A (ixekizumab, secukinumab), and anti-IL-23 (guselkumab, Risankizumab, and tildrakizumab) (Mahil et al., 2020).

Rutin is a natural glycosylated flavanol-type flavonoid that is polyphenolic and found in a diverse array of plants and fruits. Rutin may be obtained from the fruits of *Dimorphandra mollis* trees, a Brazilian plant that is a significant source of this flavonoid (de Jesus et al., 2024). Rutin, which is frequently referred to as vitamin P, is a yellow substance that is derived from rutinose and quercetin. Its potential as a therapy for diabetes, malignancy, and neurodegenerative diseases has been extensively investigated (Yong et al., 2020, Negahdari et al., 2021). Rutin's actions may be mediated by changes in the generation and reception of gonadotropins, reproductive steroidal hormones, prostaglandin eicosanoids, cytokines, and VEGF, as well as biomarkers of oxidation, inflammation, hyperproliferation, and apoptotic and angiogenic processes (Sirotkin and Kolesarova, 2022, Sirotkin, 2024).

Rutin, in addition to its combined analgesic and anti-nociceptive effects, possesses potent neuroprotective, tranquilizing, and antiepileptic properties. It has the potential to preserve the brain structure, retina, lungs, cardiac system, spleen, hepatocytes, and circulatory system, as well as to promote the overall wound-related repair and healing mechanisms (Yong et al., 2020, Budzynska et al., 2019). Additionally, it might improve sperm fertility, the health of the male genital system, and the function of the skin and hair (Negahdari et al., 2021, Sirotkin and Kolesarova, 2022).

Aim of the Study:

This research assessed the probable anti-psoriatic actions of topical Rutin treatments on an imiquimod-induced psoriasis mouse model while comparing their efficacy to that of the traditional medication clobetasol propionate.

RESEARCH METHOD

Study design

The study was carried out applying a randomized controlled experimental model. The study was conducted at the Pharmacology Department of the College of Medicine, AL-Nahrain University between October 2023 and May 2024. The study was carefully reviewed for ethical and scientific care and received approval from the Institutional Review Board (IRB) of the College of Medicine at AL-Nahrain University (No.20230786).

Drugs and reagents

The petroleum 15% jelly was supplied by the local market (Iraqi Federation of Industries, Baghdad, Iraq). Clobetasol propionate ointment was donated by Dermovate®, GlaxoSmithKline, Brentford, UK. Imiquimod was provided by Meda Pharmaceuticals, Solna, Sweden, under the brand name Aldara® 5% Cream. and Rutin was supplied as powder by (Jinlan pharma-drugs technology, Batch No. L2202262, Hangzhou, China) and later prepared as an ointment.

Pharmaceutical preparation of Rutin (4%) ointment

The levigating method was employed to prepare the ointment by dissolving (4 g) of rutin in a levigating solution of (2 ml) castor oil, which was then blended with (96 g) of Vaseline petroleum jelly to get a uniform and grit-free mixture. Pilot study was conducted to determine the appropriate and effective dosage of the drug (Hassan et al., 2023, Kadhim et al., 2022).

Animal model and experimental design

Experimental mice were purchased from the Animal Facility of the Iraqi Center for Cancer and Medical Genetics Research. They were confined in polypropylene cages and maintained in a controlled environment at a temperature of 25 °C. An inverted light-dark cycle that lasted 12/12 hours regulated the environment. The mice were acclimated at the same facility from which they were obtained for a period of seven days. The animals were granted unrestricted access to water and a conventional pellet diet. The investigation included 50 Swiss albino mice, aged 11–15 weeks, with a weight of 24–30 g. The dorsal aspect of the back skin of all mice was shaved with an electric razor to expose a 2 cm area. Throughout the 14-day experiment, mice were randomly assigned to five distinct groups, each of which contained ten mice.

Control group I: consisted of normal, healthy mice that had not received any sort of treatment. **Induction group II:** Mice were administered topical imiquimod cream (5%) at a dosage of 62.5 mg for up to 6 days (van der Fits et al., 2009). **Vehicle group III:** Mice were administered topical imiquimod application (as in Group II) and then received topical vehicle ointment (petrolatum jelly) twice a day for an additional 8 days (Hasan and Gatea, 2024). **Clobetasol group IV:** Mice were administered imiquimod (as in Group II), followed by topical clobetasol ointment (0.05%) at a dose of 0.25 g/kg twice a day for an extra 8 days (Mohammad et al., 2022). **Rutin group V:** Mice were administered imiquimod (as in Group II), followed by topical rutin (4%) ointment applied twice a day. This cycle of treatment continued for 8 days, figure 1 shows the study design and the animals grouping.

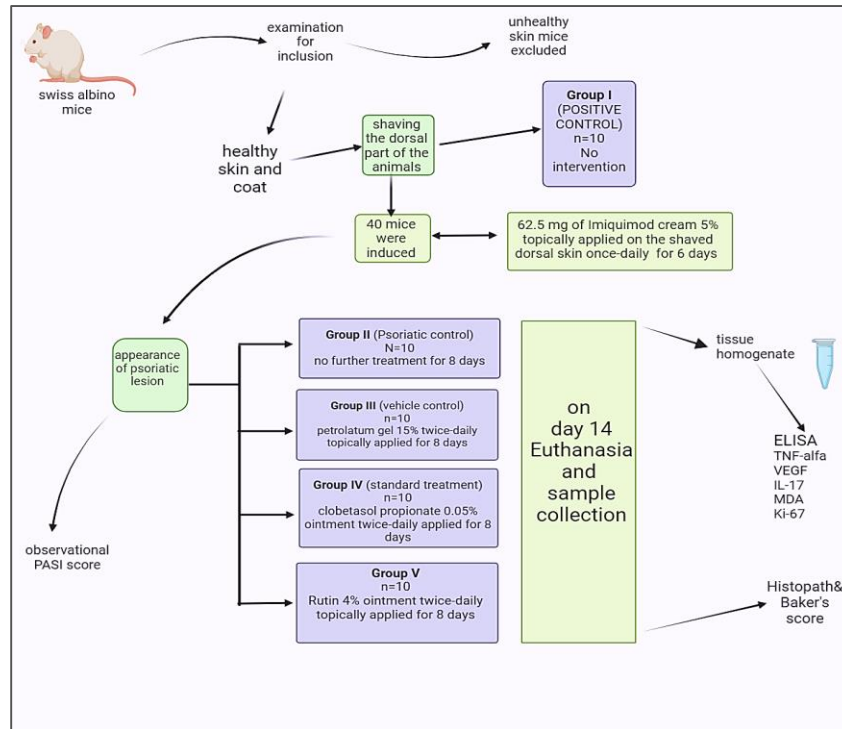


Figure 1: flow chart of the study design

The psoriasis area and severity index (PASI)

Skin erythema, scaling, and thickness were used to evaluate the severity of skin psoriasis and the extent of skin inflammation. Each category's PASI was evaluated on a scale of 0 to 4. The quantities are as follows: "0" denotes "none," "1" indicates "slight," "2" indicates "moderate," "3" indicates "marked," and "4" indicates "severe." The cumulative PASI scores were determined by adding the additive values for erythema, scaling, and thickness to evaluate the severity of the lesion (Fredriksson and Pettersson, 1979, van der Fits et al., 2009).

Preparation and sampling of animals

On day 14, ketamine (80 mg/kg) and xylazine (10 mg/kg) were administered intraperitoneally (IP) to all animals. After undergoing full anesthesia, all mice were terminated by exsanguination, a method suitable for extracting and preserving tissue (Underwood and Anthony, 2020, Pierozan et al., 2017), Skin samples were collected and processed to produce tissue homogenate for biomarker analysis and histopathological examinations (Mekkey et al., 2020, Jabeen et al., 2020).

Preparation of tissue homogenate

One gram of skin from the back that was recently collected has been preserved in a 9-ml buffer solution that is saturated with phosphate and has a pH of 7.2. Utilizing a pestle and mortar, the tissues were homogenized and subsequently centrifuged at 5000 rpm for 10 minutes at a low temperature. In order to facilitate additional investigation, the supernatants were maintained at -80 °C (Jabeen et al., 2020, Manna et al., 2017).

Histological examination

Skin samples from various mouse groups were preserved in 10% neutralized buffered formalin in accordance with established protocols. The tissues were subsequently subjected to paraffin-based fixation (Ali et al., 2021, Yahiya et al., 2023a). The paraffin-embedded specimens were cut into tissue sections and stained with eosin and hematoxylin (Raheem et al., 2023). Barker's scoring system was

employed to assess the presence of any pathological changes in the samples, which were subsequently examined using a microscope and assigned a numerical value between 0 and 10 (Baker and Fry, 1992).

Measurement of inflammatory, oxidative and proliferative biomarkers

TNF- α , IL-17, VEGF, MDA, and KI67 concentrations in mouse skin tissues were measured using sandwich Enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions (Cloud-Clone Corp). The pre-coated micro-ELISA strips plate in this kit was supplemented with anti-marker antibodies. The sample or principle was introduced to the late wells of the micro-ELISA strips prior to the addition of the relevant antibody. Subsequently, fill each micro-ELISA well with an antibody specific to each micro-ELISA strip that has been coupled with horseradish peroxidase (HRP), and then allow it to incubate. After removing all of the free components with water, TMB substrate solution was added. When the stop solution was added, the blue wells containing HRP-conjugated antibodies and markers turned yellow. At a wavelength of 450 nm, the optical density (OD) was determined spectrophotometrically (Wen et al., 2018, Yahiya et al., 2023b).

DATA ANALYSIS

The study found that the induction and vehicle groups experienced significantly higher cumulative PASI and pathological Baker's scores than the presumably healthy control group ($P < 0.001$). Figure 2 shows the degree of psoriasis-like dermatitis among various experimental mouse groups.

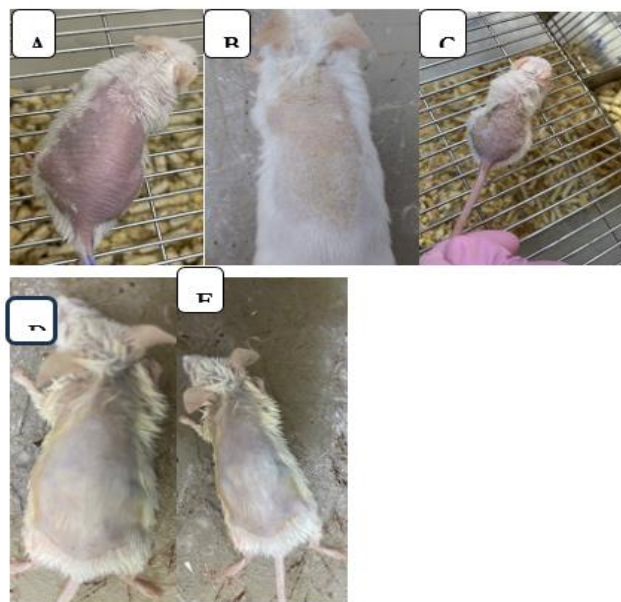


Figure 2: Photographs comparing psoriatic lesions in different experimental mouse groups.

A: healthy group, B: induced non-treated group, C: vehicle group, D: Clobetasol group, E: Rutin group.

Table 1: Comparison between all studied groups (Induced non-treated, Vaseline, Clobetasol, and Rutin groups) in relation to different measured parameters (TNF- α , IL-17, VEGF, MDA, and Ki-67, Baker score, and PASI score) using one-way ANOVA test.

Variables	Induced non-treated Mean \pm SD	Vehicle group Mean \pm SD	Clobetasol group Mean \pm SD	Rutin group Mean \pm SD	P value
TNF- α (ng/L)	454.11 \pm 126.09	414.15 \pm 55.78 a*	127.11 \pm 73.01 a**, b**	100.27 \pm 10.47 a**, b**, cNS	<0.001
IL-17 (pg/ml)	430.90 \pm 69.73	284.82 \pm 62.48 a**	181.60 \pm 27.70 a**, b**	146.35 \pm 12.98 a**, b**, c*	<0.001
VEGF (pg/ml)	1103.81 \pm 1945.08	354.53 \pm 105.87 a*	224.65 \pm 74.09 a**, b**	201.92 \pm 60.65 a**, b**, cNS	<0.001
MDA (nmol/ml)	95.91 \pm 33.18	48.92 \pm 13.40 a**	21.32 \pm 3.83 a**, b**	14.31 \pm 2.64 a**, b**, c*	<0.001
Ki-67 (ng/mL)	17.55 \pm 3.72	12.01 \pm 3.81 a**	5.74 \pm 1.02 a**, b**	5.02 \pm 1.32 a**, b**, cNS	<0.001
Baker score	7.50 \pm 0.0	6.00 \pm 0.00 a**	2.35 \pm 0.24 a**, b**	1.50 \pm 0.53 a**, b**, c**	<0.001
PASI score	9.40 \pm 0.52	6.60 \pm 0.97 a**	3.30 \pm 0.48 a**, b**	2.70 \pm 0.48 a**, b**, c*	<0.001

IL= Interleukin; TNF-a= Tumor necrosis factor alpha; VEGF = Vascular endothelial growth factor; MDA= malondialdehyde. ; a: comparison with induced group; b: comparison with vehicle group; c: comparison with Clobetasol group; d: comparison with Rutin group; *P significant at level <0.05 and **P highly significant at level <0.001; NS=not significant using one way ANOVA test.

On the other hand, mice within the clobetasol and rutin groups revealed substantial reductions in the degree of cumulative PASI and pathological Baker's scores compared to the induction and vehicle groups (P<0.001). Moreover, Rutin group demonstrated statistically significant decreases in both total PASI and histological Baker's, in contrast to the clobetasol group as indicated in Figure 3 (P<0.001).

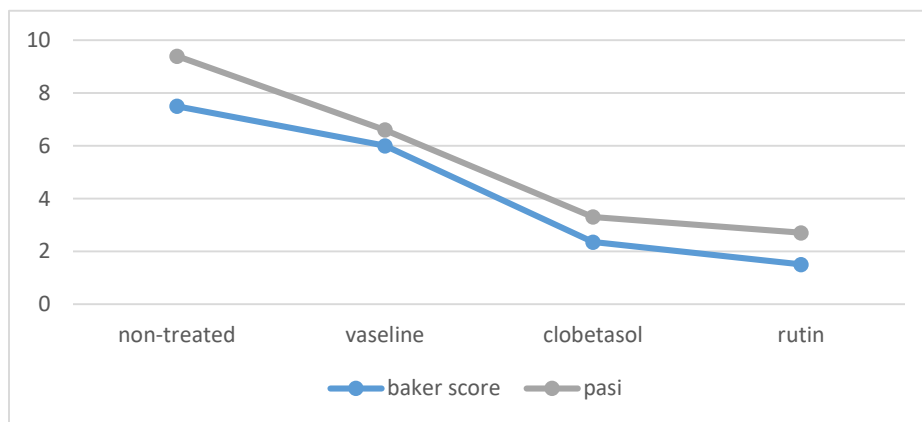


Figure 3: Comparison between all studied groups (Induced non-treated, Vaseline, Clobetasol, and Rutin groups) in relation to (Baker score, and PASI score) using one-way ANOVA test.

Importantly, the induction and vehicle groups reported significantly increased skin levels of inflammatory mediators, including TNF- α , IL-17, and VEGF ($P < 0.001$), compared with the control group. Additionally, clobetasol and Rutin groups exhibited substantially lower levels of TNF- α , IL-17, and VEGF in comparison to the induction and vehicle groups ($P < 0.001$). On the other hand, Rutin group showed observable drop in IL-17 levels compared to the clobetasol group, while there were no significant differences between the clobetasol and rutin groups in terms of TNF- α or VEGF levels ($P < 0.001$), as illustrated in Figure 4.

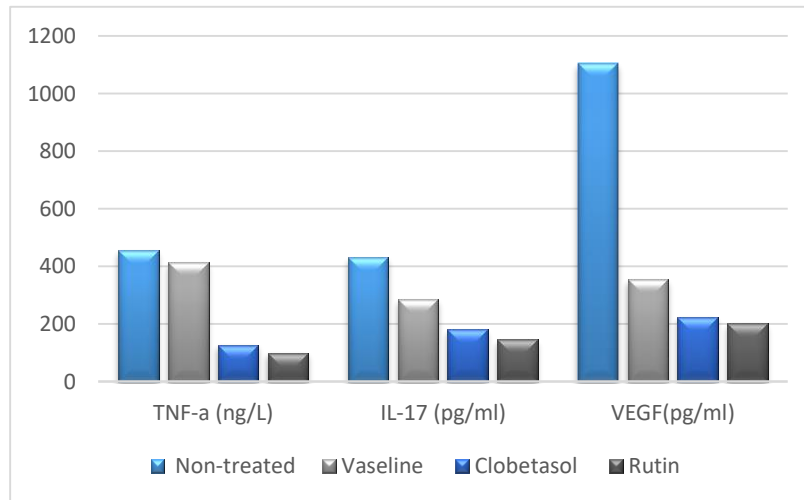


Figure 4: Comparison between all studied groups (Induced non-treated, Vaseline, Clobetasol, and Rutin groups) in relation to (TNF- α , IL-17, VEGF) using one way ANOVA test.

Additionally, the induction and vehicle groups revealed markedly elevated concentrations of the oxidative marker MDA and the proliferative marker Ki-67 ($P < 0.001$) in comparison with the control group. In addition, MDA and Ki-67 concentrations in the rutin and clobetasol groups were significantly lower than those in the induction and vehicle groups ($P < 0.001$). Of particular importance, Rutin group exhibited significant decreases in MDA levels in comparison to the clobetasol group; nevertheless, there had been statistically insignificant changes among the clobetasol and rutin groups in Ki-67 levels ($P < 0.001$), as shown in Figure 5.

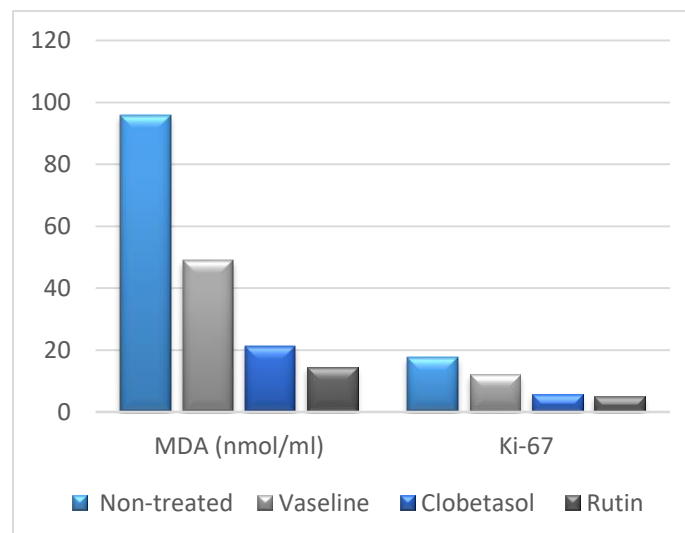
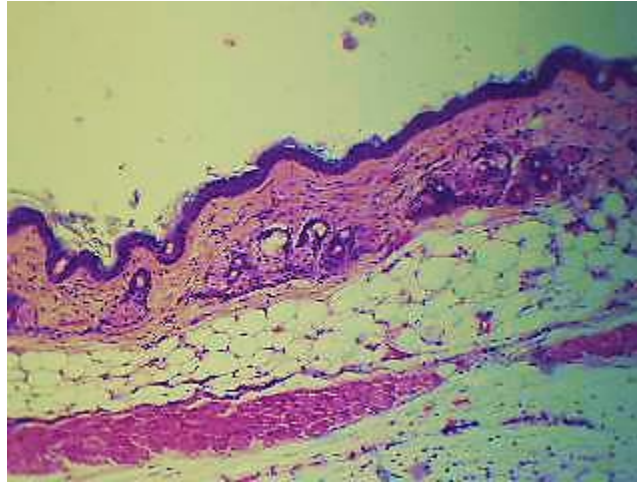
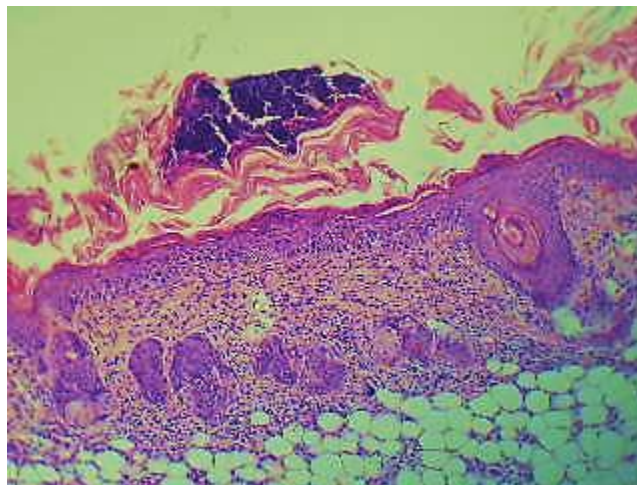


Figure 5: Comparison between all studied groups (Induced non-treated, Vaseline, Clobetasol, and Rutin groups) in relation to (MDA and Ki-67) using one way ANOVA test.

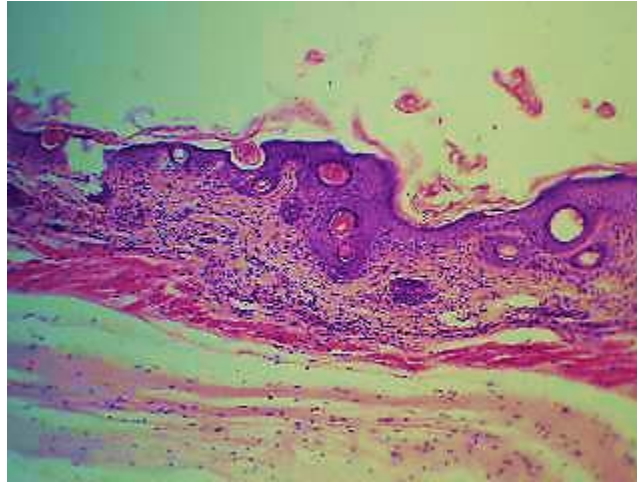
The control group's mouse cutaneous segment exhibits appropriate keratinization, epidermal thickness, and epidermal layer appearance. Additionally, the mouse skin segments from the induction and vehicle groups exhibited significant histological changes in comparison to the control group. These changes included expansion of rete ridges, hyperkeratosis, increased cutaneous thickness, and the invasion of inflammatory cells. In contrast to the induction and vehicle groups, the mouse skin segments of the clobetasol and rutin groups exhibited significant improvements in the skin's histological features. These improvements included a minor influx of inflammatory cells, thinned epidermal layers, and modest hyperkeratosis (H&E 10X) (Figure 6).



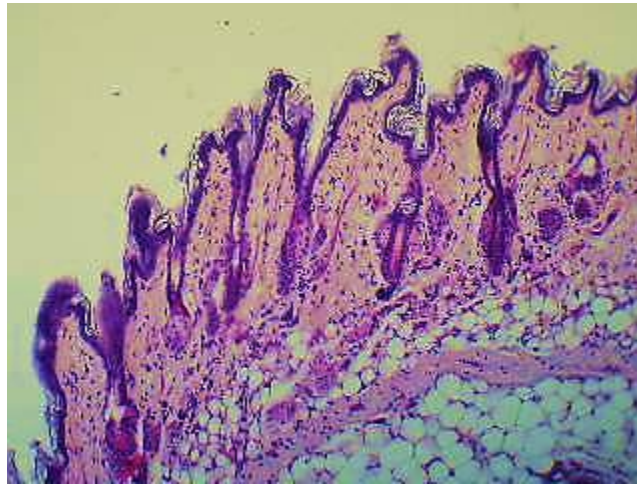
A. The skin mouse segment from the control group (H&E 10X).



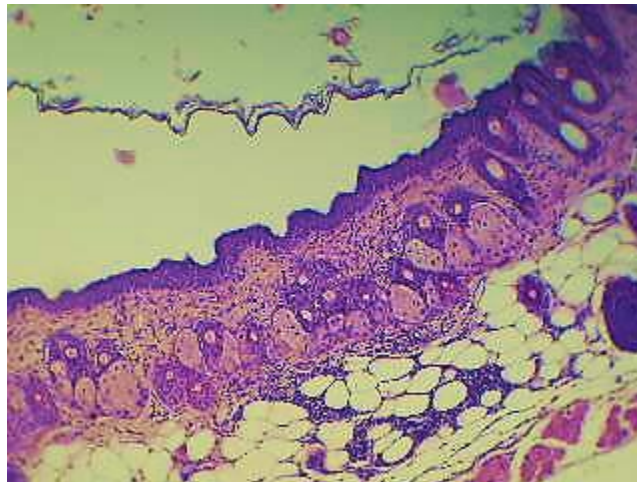
B. The skin mouse segment from the induction group (H&E 10X).



C. The skin mouse segment from vehicle group (H&E 10X).



D. The skin mouse segment from the clobetasol group (H&E 10X).



E. The skin mouse segment from the Rutin group (H&E, 10X).

Figure 6: Effects of the drugs under study on psoriatic histopathological changes. A. The skin mouse segment from the control group (H&E 10X). B. The skin mouse segment from the induction group

(H&E 10X). C. The skin mouse segment from vehicle group (H&E 10X). D. The skin mouse segment from the clobetasol group (H&E 10X). E. The skin mouse segment from rutin group (H&E, 10X).

DISCUSSION

In accordance with prior research, imiquimod resulted in a significant increase in inflammatory, proliferative, and oxidative parameters and the development of skin lesions that resembled psoriasis, including erythema, desquamation, and acanthosis, when applied to the dorsal skin of mice twice daily for 6 days (van der Fits et al., 2009, Moos et al., 2019, Jabeen et al., 2020, Salman et al., 2024, Ahmed et al., 2021b, Mustafa Thamer and Q. Yahya, 2023). However, the standard medication used in this trial, clobetasol, significantly alleviated the symptoms of psoriasis, as evidenced by a substantial decrease in PASI and Bakers scores and a decrease in the concentrations of all biomarkers that were examined. This discovery is in accordance with previous research that has shown that clobetasol has the potential to inhibit the detrimental parameters associated with psoriatic inflammation (Boehncke and Brembilla, 2018, Brembilla and Boehncke, 2023). The antipsoriatic effect of clobetasol may be attributed to its anti-inflammatory and anti-proliferative properties, which alter the adaptive immune response, reduce the invasion of inflammatory cells, prevent the maturation of immune cells, and suppress skin proliferation activity (Dadwal, 2023, Khafaji et al., 2024, Salman et al., 2024). The reduction in melanocyte production, restrictions on keratinocyte growth, and a decrease in mast cell activation may be responsible for the improvements in erythema and desquamation (Mohammed et al., 2022, Ahmed et al., 2021a).

Vitamins and dietary supplements have been found to be beneficial for many psoriasis patients, as they soothe irritation and facilitate skin clearing (Raut and Wairkar, 2018). Furthermore, Vijayapoopathi et al. (2020) It was noted that the treatment of imiquimod-exacerbated psoriasis in mice is enhanced by the combination of multiple nutraceutical supplements, In the present study, Rutin significantly reduced the elevated levels of inflammatory markers (VEGF, IL-17, and TNF- α), oxidative markers (MDA), proliferative markers (Ki-67 protein), histopathological scores (Baker score), and observational scores (PASI score). Our findings agree with those of Alshanwani et al. 2020, who determined that the administration of quercetin flavonoid could have significant implications for the development of a novel therapy regimen for the recovery of hypoxia-induced kidney impairment (Alshanwani et al., 2020). Additionally, Significant downregulation of TNF- α levels was achieved as a result of the anti-inflammatory effect of flavonoids (Al-Rasheed et al., 2016). The agent's advantageous effects may be attributed to its immunomodulatory and anti-inflammatory properties, as evidenced by prior research that indicated that supplementation with either flavonoid could mitigate inflammatory responses (Negi et al., 2011). This may predict the synergistic protective impact of flavonoids against immune-mediated conditions, like psoriasis (Al-Rasheed et al., 2017).

Rutin, a flavonol molecule that is extracted from a variety of plants, primarily reduces inflammation by reducing NF- κ B, TNF- α , COX-2, and IL-6. Additionally, it inhibits caspase-3 and activates B-cell lymphoma 2 (Bcl-2), which contributes to its anti-apoptotic effects (Rahmani et al., 2023). Further, This flavonoid component demonstrates an efficient ROS-scavenging action, which leads to the reinforcement of blood vessel capillaries (Gautam et al., 2016, Kurisawa et al., 2003). Moreover, Yi et al. (2024) found that Rutin, by modulating the activity of peroxisome proliferator-activated receptors (PPAR γ), alleviated the release of inflammatory cytokines such as IL-6, TNF- α , and IL-17, and inhibited the transcriptional activity of NF- κ B and STAT3. Additionally, it prevented the activation of dendritic cells and macrophages (Yi et al., 2024). In addition, Flavonoids significantly reduced the synthesis and gene expression of pro-inflammatory mediators TNF- α , IL-1B, and IL-6. flavonoids inhibit the migration of leukocytes to the site of injury (Abu-Raghif et al., 2015). Additional observations indicated that this flavonol improved the dinitrochlorobenzene-induced atopic dermatitis mouse model by inhibiting lymphocytic proliferation and decreasing the production of

IFN- γ , IL-4, IL-5, IL-10, IL-17, and TNF- α (Choi et al., 2013). Likewise, our outcomes demonstrate that Rutin effectively reduces the levels of VEGF in the epidermis. Previous research has established that rutin are likely to downregulate the expressions of TNF- α and VEGF, which yielded similar results (Gupta et al., 2020, Lin et al., 2023).

However, Psoriasis appears to be associated with elevated levels of proliferative cell nuclear antigen (PCNA) and Ki-67 biomarkers, which are indicative of hyperproliferation of keratinocytes (Yazici et al., 2005, Huang et al., 2019). An earlier study revealed that Rutin significantly reduced the elevated level of PCNA that occurred as a result of lung injury (Li et al., 2014), while our work is the first evidence that Rutin has a reduced impact on Ki-67 levels.

ACKNOWLEDGEMENT:

The research was performed as part of a Master thesis at Al-Nahrain University's College of Medicine, specifically in the Department of Pharmacology.

DECLARATIONS

ETHICS APPROVAL:

The Al-Nahrain University College of Medicine's institutional review board evaluated the study and granted permission for the most recent installment. The Declaration of Helsinki's ethical guidelines were followed when conducting the research project. A local ethics board approved the study after reviewing the consent documents, topic information, and research plan on September 17, 2023, as per Document IRB/133 and approval number UNCOMIRB20240628.

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