RESEARCH PAPER

Ex Vivo and *In Vivo* Study of Nanoemulgel for Psoriasis

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ABSTRACT

Psoriasis, with its complex pathophysiology and the limited efficacy of current treatments, necessitates focused and innovative research.In response, our group has developed a new nanotherapeutic system that uses the natural bioactives Psoralen and Diosgenin. Olive oil was used as the lipid phase in a nanoemulsion gel (NEG-2) to successfully integrate the compounds. This composition is explicitly created to improve therapy results for psoriasis. Diosgenin and Psoralen are recognized for their potential anti-psoriatic effects. Our previously published research outlined the formulation development of NEG-2, whereby we identified and answered several pharmacotechnical issues related to the integration of these two natural chemicals. The optimized NEG-2 underwent *in vitro* testing, exhibiting superior dissolving properties and better transdermal administration of the active substances. This research promotes the advancement of NEG-2 as a dual-action, synergistic nanocarrier for the treatment of psoriasis. *In vivo* assessments using BALB/c mice shown substantial anti-psoriatic effectiveness. Supplementary ex vivo evaluations, including skin contact analysis and antioxidant assessment using the DPPH (1,1-diphenyl-2-picrylhydrazyl) test, corroborated the formulation's efficacy. The combined findings suggest that NEG-2 may be a safe and successful treatment for psoriasis, perhaps lessening the side effects that are frequently connected to conventional drugs.

Keywords: Psoriasis, nanoemulsion, diosgenin, antioxidant, anti-psoriatic, good health and well-being.

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1. INTRODUCTION

Psoriasis is a long-term, immune-mediated inflammatory condition marked by keratinocyte hyperproliferation and aberrant differentiation, as well as inflammatory and angiogenesis in the epidermal layer.About 2-3% of people worldwide suffer with psoriasis, which not only causes physical discomfort in the form of scaly, erythematous plaques but also places a heavy psychological and social burden on sufferers. Despite decades of clinical research and drug development, psoriasis remains a therapeutic challenge due to its complex and multifactorial pathophysiology involving genetic, immunologic, and environmental components[1].

Topical medications (such as corticosteroids and vitamin D analogs), phototherapy, and systemic therapies (such as cyclosporine, methotrexate, and biologics that target TNF-α and IL-6) are being used to treat psoriasis. While these interventions have shown varying degrees of efficacy, they are often associated with limitations such as systemic toxicity, immunosuppression, loss of therapeutic response over time, and high costs particularly in the case of biologics. Additionally, many patients only get partial relief and their symptoms return, underscoring the need for new, safer, and more potent treatment options[2,3].

Natural bioactive substances, many of which have immunomodulatory, anti-inflammatory, and antioxidant qualities, have received more attention in recent years. It has been demonstrated that diosgenin, a steroidal sapogenin obtained from species like Dioscorea villosa, has strong anti-inflammatory and antiproliferative properties[4]. Psoralen, a furocoumarin found in plants like Psoralea corylifolia, is well known for its role in photochemotherapy (PUVA therapy) for psoriasis[5]. Both compounds have been individually studied for their therapeutic effects in various dermatological disorders, including psoriasis. However, their combined application in a single formulation, particularly in a modern nanocarrier system, remains relatively unexplored[6].

The incorporation of nanotechnology into pharmaceutical formulations has opened new avenues in the treatment of skin disorders. Nanocarriers such solid lipid nanoparticles, liposomes, and nanoemulsions[7], and polymeric micelles have demonstrated promise in reducing systemic adverse effects while improving drug solubility, stability, and bioavailability. Because of their high surface area, thermodynamic stability, small droplet size (usually 20–200 nm), and effective penetration of the stratum corneum, nanoemulsions stand out among them. The encapsulation of hydrophobic and photosensitive compounds like Diosgenin and Psoralen in a nanoemulsion gel format offers a strategic advantage by improving their solubility, protecting them from photo-degradation, and enabling controlled and targeted drug delivery[8].

In this context, our research group has developed a novel nanoemulsion-based gel formulation (NEG-2) incorporating Diosgenin and Psoralen as dual bioactive

agents. Olive oil was selected as the oil phase due to its natural emollient properties, antioxidant profile, and compatibility with skin physiology[9]. Olive oil not only serves as a lipid carrier for the lipophilic bioactives but may also contribute synergistically to the overall therapeutic effect through its own anti-inflammatory and skin-repairing actions. The formulation process, as described in our earlier publication, involved overcoming several pharmaco-technical hurdles such as phase separation, instability, and incompatibility of the two bioactives. Through a series of optimization studies, we successfully developed a stable nanoemulsion gel that ensured homogenous dispersion, optimal rheological properties, and desirable skin feelfor good health and well-being. [10].

The *in vitro* characterization of NEG-2 revealed enhanced dissolution rates and significantly improved transdermal permeation profiles for both Diosgenin and Psoralen, compared to their respective conventional formulations. These findings were attributed to the nanoscale droplet size, increased surface area, and the solubilizing effect of the surfactant/co-surfactant system used in the nanoemulsion. The improved skin permeation is particularly crucial in the context of psoriasis, where hyperkeratosis and skin thickening can otherwise impede drug delivery to the targeted epidermal and dermal layers[11].

Building on the promising *in vitro* results, we further assessed the therapeutic efficacy of NEG-2 in vivo using BALB/c mice a well-established animal model for studying psoriatic inflammation. The induction of psoriatic-like lesions in mice was achieved through the application of imiquimod (IMQ), a TLR7/8 agonist known to trigger an immune response mimicking human psoriasis. Topical application of NEG-2 over the psoriatic lesions resulted in a marked reduction in erythema, scaling, and thickness of the affected skin. Histopathological examination confirmed decreased epidermal hyperplasia, reduced inflammatory cell infiltration, and normalized dermal architecture[12]. These findings were consistent with the hypothesis that the combined action of Diosgenin and Psoralen could exert synergistic anti-psoriatic effects by modulating multiple pathogenic pathways simultaneously[13].

A number of *ex vivo* and biochemical tests were carried out to further support the formulation's effectiveness and comprehend its mode of action. The successful penetration and contact of the nanoemulsion with the stratum corneum were verified by skin interaction experiments employing scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). Furthermore, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was used to assess antioxidant activity. NEG-2 demonstrated significant free radical quenching ability, suggesting its potential to lessen oxidative stress, a major factor in the pathophysiology of psoriasis[14].

When combined, the results of *in vitro, in vivo*, and *ex vivo* research highlight NEG-2's therapeutic potential as a dual-targeted nanoemulsion gel for psoriasis treatment.By leveraging the complementary bioactivities of Diosgenin and Psoralen, and utilizing nanotechnology to enhance their delivery and efficacy, our formulation represents a significant advancement in psoriasis therapy. Notably, the use of natural bioactives and a skin-friendly carrier system suggests a favorable safety profile, potentially minimizing the side effects commonly associated with synthetic drugs or immunosuppressive agents[15].

The current work concludes by highlighting the effective creation and preclinical assessment of a nanoemulsion gel loaded with Diosgenin-Psoralen for the treatment of psoriasis.As a multifunctional therapeutic platform, NEG-2 addresses key limitations of conventional psoriasis treatments, offering improved drug solubility, skin permeation, and localized action with reduced systemic exposure. Future investigations will focus on clinical translation, long-term safety assessment, and mechanistic studies to fully elucidate the molecular pathways modulated by the formulation. The integration of phytochemicals with nanotechnology, as demonstrated in this work, offers a promising strategy for the development of next-generation therapeutics for chronic and refractory skin diseases such as psoriasis[16,17].

2. MATERIALS AND METHODS

2.1 Materials

Diosgenin and Psoralen were procured from Sigma-Aldrich (Darmstadt, Germany). ELISA kits for TNF-α and IL-6 were obtained from Krishgen Biosystems in Mumbai, India. Imiquimod (IMQ) 5% cream (Imiquad®), produced by Glenmark Pharmaceuticals Ltd. (Delhi, India), and was acquired locally. Polyethylene glycol (PEG 200), Tween 80, and Tween 20 were obtained from Sisco Research Laboratories Pvt. Ltd. (SRL, Mumbai, India). All additional reagents and solvents used were of analytical grade.

2.2 Methods

2.2.1 *Ex Vivo* Skin Permeation Study

A Franz diffusion cell setup was used for the *ex vivo* permeation investigation. Subcutaneous fat and other adherent tissues were meticulously removed from excised rat skin before it was placed between the donor and receptor compartments of the Franz cell, which had a receptor volume of 9.0 mL and an effective diffusion area of 1.77 cm^2.The receptor compartment was filled with phosphate buffer saline (PBS, pH 7.4), maintained at 37 ± 1 °C, and continuously stirred at 100 rpm using a magnetic stirrer to simulate physiological conditions. Aeration was maintained by gently bubbling air to ensure adequate oxygenation and uniform mixing throughout the experiment. One gram of each formulation-psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and After 20 minutes of pre-equilibration, each stratum corneum side of the skin received a separate application of their combined formulation (NEG-2). To maintain consistent volume and sink conditions, 1 mL of receptor

fluid was removed and promptly replenished with new buffer solution at predefined intervals (0, 0.5, 1, 2, 4, 6, and 12 hours). The amount of medication that penetrated the skin at each time point was ascertained by analyzing all collected samples using UV-visible spectroscopy. To guarantee that the results could be repeated, each formulation was tested three times[18].

2.3 In-vivo study

2.3.1 *In Vivo* Skin Irritation Test

The potential of skin irritation was evaluated using the Draize scoring method. The test was conducted on Swiss albinoWistar rats by applying the formulations to the previously shaved dorsal region of the skin. The study included three test groups: psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2). A 0.8% v/v formalin solution was used as a positive control to represent standard irritant response, while untreated skin served as the negative control. Each formulation was topically applied (in an appropriate dose) to the designated test site, and the application area was observed over a period of 24 hours. The test sites were assessed for any obvious indications of edema (swelling) and erythema (redness) following the exposure period. A conventional Draize scale, which ranges from 0 (no reaction) to 4 (severe reaction), was used to grade the severity of skin reactions. To evaluate the individual and combination formulations' potential for irritation in comparison to the controls, the scores from each group were compared. Every technique was carried out in accordance with ethical standards for the use and care of animals[18].

2.3.2 Anti- psoriatic activity

The experimental study will be carried out after approval of animals (BALB/c Mice*/Swiss albino* Mice/either sex). Eight-week-old animals weighing an average of twenty to twenty-five grams will be purchased from the institute's Central Animal House Facility. They will be housed in plastic cages for twelve hours at a temperature of 25ºCwith optimal humidity and alternating light and dark cycles. The animals will receive "ad libitum" water and a normal pellet diet. The grouping, treatment schedule, doses and routes of administration are shown below (Table 1). On completion of treatment, the animals will be sacrificed under ether anesthesia to obtain blood samples for biochemical estimation and tissues for histopathology. Further treatment will be given as per the following treatment schedule. Anti-psoriatic activity of different drugs will be evaluated after induction Imiquimod (IMQ). During treatment, each group consist of six animals. The investigation will be conducted for seven days in a row to cause skin inflammation similar to psoriasis[19], [20].

The study will involve six distinct groups, each assigned a specific treatment category.

Group 1 (Normal Control) will be treated with a mixture of 78% olive oil, 20% saline, and 2% Tween 80 for seven consecutive days.

Group 2 (Negative Control) will receive a daily topical dose of 62.5 mg of 5% imiquimod cream for seven consecutive days.

Group 3 (Positive Control) will consist of animals that will be treated with a marketed formulation for the same period.

Group 4(PBF) will be administered a psoralen-based formulation for seven consecutive days.

Group 5(DBF) will receive a diosgenin-based formulation over the same duration. Finally,

Group 6(NEG-2**)** will be treated with a formulation containing both psoralen and diosgenin for seven consecutive days.

***PBF**: Psoralen based formulation: **DBF**: Diosgenin based formulation: **NEG-2**: Psoralen and Diosgenin based combination formulation*

2.4 Scoring severity of skin inflammation

The psoriasis area severity index (PASI) was used to assess the degree of skin irritation. Three distinct PASI parameterserythema, scaling, and thicknesswere each given a value between 0 and 4 (0 none, 1 mild, 2 intermediate, 3 severe, and 4 extremely severe). These scores were then put together to provide a total severity score for the duration of the treatment. On day seven, the mice were put to death. Next, blood samples, major organs, and dorsal skin tissues were collected for additional research [19].

0= no erythema/ edema
1= slight erythema/ edema (light pink)
2= moderate erythema/ edema (dark pink)
3= moderate to severe erythema / edema (light red)
4=severe erythema/ edema (extreme redness).

2.4.1 *In-vivo* Antioxidant Study

The purpose of the in-vivo antioxidant study was to assess how psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2) protected mice from oxidative stress caused by imiquimod (IMQ). For seven days in a row, mice's dorsal surfaces were topically treated with 5% IMQ to induce oxidative stress. Mice were split up into treatment groups that received PBF, DBF, and NEG-2, as well as normal control and IMQ control groups.The mice were killed after seven days, and skin tissue homogenates were made at 4°C using phosphate-buffered saline (PBS). Malondialdehyde (MDA) levels were measured using the thiobarbituric acid reactive substance (TBARS) method, and the absorbance at 532 nm was used to determine lipid peroxidation (LPO). Griess reagent was used to assess nitrite content, and absorbance at 540 nm was used to calculate nitric oxide (NO) levels. The suppression of pyrogallol autoxidation was used to measure superoxide dismutase (SOD) activity, and the change in absorbance was measured at 420 nm. The breakdown of hydrogen peroxide (H2O2) at 240 nm was used to measure catalase (CAT) activity.The efficacy of PBF, DBF, and NEG-2 in reducing oxidative stress was assessed by comparing the antioxidant activity of each

formulation with the IMQ control group[20,21].

2.4.2 Anti-inflammatory Study

The anti-inflammatory effects of psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2) were assessed by analyzing the levels of pro-inflammatory cytokines, TNF-α and IL-6, in skin tissue homogenates of mice subjected to IMQ-induced inflammation. The experimental groups included normal control, IMQ control, and treatment groups receiving PBF, DBF, and NEG-2. Following the administration of IMQ and the corresponding therapies for seven days, the mice were put to sleep, and skin tissue was removed and homogenized in ice-cold PBS. After centrifuging the homogenates for ten minutes at 10,000 rpm, the supernatant was gathered for cytokine analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure TNF-α and IL-6 levels in accordance with the manufacturer's instructions. A microplate reader was used to quantify the absorbance at 450 nm, and standard curves were used to determine the cytokine concentrations. PBF, DBF, and NEG-2 were shown to have anti-inflammatory properties by lowering TNF-α and IL-6 levels in the treatment groups as compared to the IMQ control group[22–24].

2.4.3 Skin retention study

To evaluate the retention characteristics of psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combination (NEG-2), an in vivo skin retention study was conducted.The formulations were prepared using a water-responsive gel (WRG) base, which enables gelation upon exposure to water. This property increases the residence time of the drug at the siteof application. WRG-gel, a liquid formulation at ambient conditions, was topically applied to the shaved dorsal skin of Swiss Albeno Wistar Rat. Upon application, a fine mist of sterile water was sprayed to induce gelation. The transition from sol to gel occurred rapidly, forming a semi-solid matrix that adhered closely to the skin surface.To measure drug retention, the formulations were allowed to remain on the skin for a predetermined duration (12 hours). Afterwards, the mice were euthanized, and the treated skin sections were carefully excised. The samples were washed to remove surface-adhered drug, then homogenized. The homogenates were analyzed using UV spectrophotometer to quantify the amount of drug retained within the skin tissue.All procedures were performed in triplicate for statistical relevance[25].

2.5 Statistical analysis

Statistical analysis was conducted by Student's t-test and one-way and two-way analysis of variance with Tukey's post hoc test with GraphPad Prism 5.0. (GraphPad, San Diego, California, USA). Every experiment was repeated three times, and the results were displayed as mean ± SD. Statistical significance was defined as $p < 0.05$. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

3. RESULTS

3.1 Ex Vivo Skin Permeation Study

Significant variations in the drug permeation profiles of the test formulationspsoralen-based (PBF)were shown in the ex vivo skin penetration research employing Franz diffusion cells, diosgenin-based (DBF), their combination (NEG-2), and a blank formulation. As shown in both the tabulated data and corresponding graph, drug permeation through the skin increased steadily over time for all active formulations, with the combination formulation (NEG-2) showing superior permeability.At 0.5 hours, the NEG-2 formulation already achieved 8.38% permeation, surpassing PBF (6.38%) and DBF (3.38%). The trend continued as time progressed. By 2 hours, NEG-2had reached 44.24% permeation, compared to 39.38% (PBF) and 27.34% (DBF). The maximum permeation was observed at 12 hours: 78.82% for NEG-2, 64.82% for PBF, and 51.35% for DBF. The blank group showed negligible permeation throughout (0.12–0.30%), confirming the specificity of drug diffusion in the active formulations.

The Cumulative Drug Permeation (CDP) values further emphasized this trend: NEG-2 showed the highest mean permeation (43.61 ± 1.65%), followed by PBF (34.12 ± 1.51%) and DBF (26.32 ± 1.86%). This suggests a synergistic interaction between psoralen and diosgenin in the combined formulation, likely enhancing skin penetration due to modified thermodynamic activity or improved solubility within the carrier base.The NEG-2 formulation may have both short-term and long-term therapeutic benefits, according to the quick early-phase penetration (during the first two hours) and long-term release profile up to 12 hours. These results demonstrate how combining natural active ingredients can enhance transdermal distribution, which could be advantageous for long-term topical treatments that need steady bioavailability.

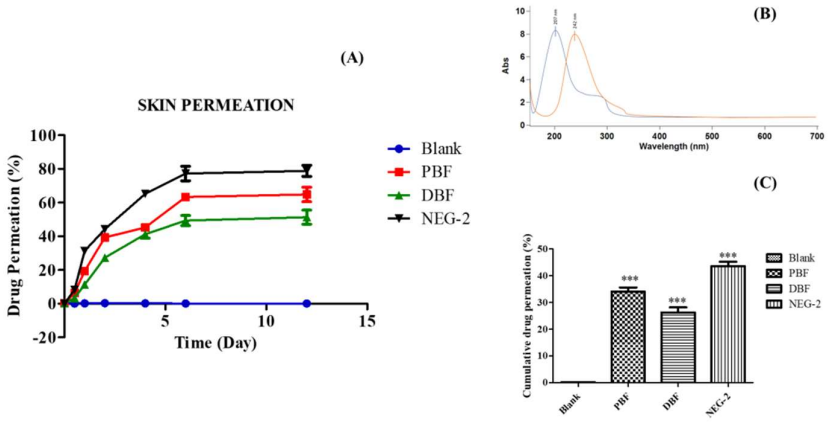

Figure 1: Ex-vivo skin permeation studies. Figure (A) represents the outcome of ex-vivo skin permeation study. Figure (B) represents the UV spectrophotometric examination of both the drug including psoralen and diosgenin and Figure (C) represents cumulative skin permeation index.

Table 1: Ex vivo skin permeation studies.

Time (Hrs)	Blank	PBF	DBF	NEG-2
0.0	0.24±0.02	0.28±0.01	0.24±0.01	0.00±0.01
0.5	0.18±0.03	6.38±0.63	3.38±0.20	8.38±0.26
1.0	0.21±0.03	19.38±0.56	11.35±1.24	31.38±1.05
2.0	0.30±0.02	39.38±1.64	27.34±1.99	44.24±1.12
4.0	0.22±0.03	45.24±1.11	41.23±2.35	65.24±1.56
6.0	0.12±0.01	63.35±2.35	49.38±2.99	77.24±4.29
12.0	0.12±0.02	64.82±4.28	51.35±4.24	78.82±3.28
CDP (%)	0.20±0.024	34.12±1.51	26.32±1.86	43.61±1.65

Cumulative drug permeation (%)

Singh et al., 2024 highlights the therapeutic potential of *Heydotiscorymbosa* (L.) Lam. extract-based nanogel for enhanced treatment of psoriasis-like dermatitis. By incorporating optimisednanophytosomes into a pluronic gel base, the formulation achieved desirable physicochemical properties, including high entrapment efficiency and nanoscale size, which contributed to better skin compatibility and stability. The nanogel demonstrated superior ex vivo skin permeation and dermatokinetic behavior, promoting deeper skin penetration and prolonged retention. In vivo results further supported its efficacy, showing significant improvement in skin condition, reduced inflammation, and effective downregulation of pro-inflammatory cytokines. Compared to conventional formulations, the nanogel offered greater therapeutic outcomes through sustained drug delivery and improved skin deposition. These findings suggest that such a nanocarrier system enhances the bioavailability of phytoconstituents and offers a promising strategy for the topical management of psoriasis. The study confirms the potential of nanogel formulations in delivering natural actives efficiently for chronic dermatological conditions[18].

3.2 In-vivo study

3.2.1 In Vivo Skin Irritation Test

The in vivo skin irritation study was conducted to assess the dermal safety of psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2), with formalin (0.8%) as a positive control and untreated skin as a negative control. The irritation responses were evaluated using the Draize scoring method at 0, 8, 16, and 24 hours post-application based on erythema and edema scores. As expected, the formalin-treated group showed a strong irritant response with progressively increasing erythema and edema over time, reaching maximum scores (4) at 24 hours. The mean erythema and edema scores for formalin were 2.25 and 2.50, respectively, with standard deviations (SD) of 0.17 and 0.09. These results confirm the validity of the test model. In contrast, all treatment groups (PBF, DBF, and NEG-2) showed minimal skin reactions. At 8 and 16 hours, slight erythema and edema (score 1) were observed in all formulations, which diminished or disappeared by 24 hours. The combined formulation (NEG-2) showed mild erythema at 8 hours and slight edema up to 24 hours, but these effects were transient and not severe. The mean irritation scores for all test formulations were 0.75 for both erythema and edema, indicating a very mild irritant potential. Standard deviation values ranged from 0.05 to 1.0, suggesting consistent observations across subjects. The control group showed no signs of erythema or edema throughout the study, as expected. Moreover, the low irritation scores of PBF, DBF, and their combination

suggest excellent dermal tolerance and suitability for topical application. These findings demonstrate that the formulations are non-irritant and safe for further pharmaceutical or cosmetic development.

Table 2: Primary irritation index score of treated rats

Time (h)	Control		Formalin (0.8%)		PBF		DBF		NEG-2	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
0	0	0	0	0	0	0	0	0	0	0
8	0	0	2	2	1	1	1	1	2	1
16	0	0	3	4	1	1	1	1	1	1
24	0	0	4	4	1	1	1	1	0	1
Mean	0	0	2.25	2.50	0.75	0.75	0.75	0.75	0.75	0.75
SD	0.0	0.0	0.17	0.09	0.15	0.15	0.15	0.05	1.0	0.5

Sahu et al., 2024 clearly demonstrate that the SXE-loaded nanotransferosome (SXE-NTF) gel significantly enhances both skin retention and drug permeation compared to the conventional SXE gel. The dermatokinetic analysis revealed that the nanotransferosomal gel not only facilitated deeper penetration of the active phytoconstituents but also ensured their prolonged retention in the skin layers. This improved retention is largely attributed to the nanosized vesicles and optimized lipid composition (Phospholipon 90G, cholesterol, and sodium cholate), This aided in the drug's effective encapsulation and regulated release. These results were further confirmed by using rhodamine-B in CLSM imaging, which demonstrated deeper and more consistent penetration of the SXE-NTF gel in comparison to the control. Moreover, the skin irritation assessment showed that the formulation was well tolerated, with no visible signs of erythema or edema, indicating good biocompatibility of the nanotransferosomal system. This makes it suitable for long-term topical application, especially for conditions like chronic skin inflammation. The enhanced skin deposition and low irritation potential suggest that SXE-NTF gel offers a targeted, sustained, and safe approach to dermal drug delivery. All of these findings point to the creation of SXE nano transferosome gels as a viable method for treating skin conditions locally and effectively while minimizing systemic exposure[26].

3.2.2 Anti- psoriatic activity

The study's findings demonstrated how imiquimod (IMQ) treatment affected mice's psoriasis-like skin inflammation, as indicated by several metrics like erythema, scales, thickness, and overall PASI score. The study included three groups: normal control, IMQ-treated, and a treatment group that appears to mitigate the IMQ-induced inflammation. The data represent the mean values with standard deviation of the mean (SD), with significance denoted by *P < 0.05 versus normal control, indicating statistically significant differences. In the erythema parameter graph, redness levels in the IMQ-treated group increased significantly by day 2, followed by a further elevation on days 4, 6, and 7. This increase reflects a progressive inflammatory response, characteristic of psoriasis-like conditions. Compared to the normal control group, which maintained consistently low erythema levels throughout the observation period, the IMQ-treated group showed marked differences, confirming the induction of inflammation. The treatment group displayed a noticeable reduction in erythema levels when compared to the IMQ group, suggesting that the intervention mitigated inflammation and reduced the severity of redness over time.

Scaling, another characteristic of psoriasis, showed a similar pattern, according to the scale parameter graph. As the disease progressed, the IMQ group's scaling rapidly increased by day 4 and peaked by days 6 and 7. When compared to the IMQ-induced group, the treatment group showed a reduction in scaling severity, indicating that the formulation successfully prevented excessive keratinocyte proliferation and differentiation.The normal control group-maintained baseline values, showing no signs of scaling, which further highlights the severity of the IMQ-induced condition. Thickness measurements indicated that IMQ application led to a significant increase in epidermal thickness, which is another hallmark of psoriasis. By day 4, a substantial increase was observed, which continued to rise through days 6 and 7. The IMQ-treated group consistently displayed higher thickness levels compared to the normal control group. However, the treatment group demonstrated a considerable reduction in epidermal thickness, suggesting a protective effect against excessive hyperplasia and inflammation induced by IMQ.

A thorough evaluation of the psoriasis-like disease is provided by the overall PASI score, which incorporates erythema, scaling, and thickness factors. The IMQ group's total PASI score increased quickly by day 4 and peaked on days 6 and 7, indicating the complete development of skin lesions resembling psoriasis. On the other hand, the therapy group's PASI score significantly decreased, suggesting that the intervention was successful in halting the progression of the illness. The normal control group displayed consistently low PASI scores, further confirming the absence of pathological changes. The outcomes highlight how well the medication works to lessen the intensity of psoriasis-like inflammation caused by IMQ. The capacity to decrease thickness, scaling, and erythema, which eventually results in a lower PASI score, highlights the formulation's potential therapeutic benefits. The reduction in these parameters suggests that the treatment likely modulates the inflammatory response and prevents

excessive keratinocyte proliferation, key features of psoriasis pathophysiology.

The robustness of the induced model was demonstrated by statistical analysis, which showed statistically significant differences (*P < 0.05) between the IMQ-treated group and the normal control group.Similarly, the significant reduction in inflammation and psoriasis severity observed in the treatment group further supports its efficacy. These findings suggest that the intervention effectively mitigated the pathological changes induced by IMQ, restoring skin homeostasis. The progression of erythema, scaling, and thickness in the IMQ group followed a characteristic pattern associated with psoriasis. The initiation of inflammation, evident by increased erythema, was followed by keratinocyte hyperproliferation and differentiation, leading to the accumulation of scales and increased epidermal thickness. This progression mirrors the pathogenesis of psoriasis, where immune dysregulation leads to sustained inflammation and epidermal alterations.

The treatment's ability to inhibit the severity of psoriasis-like lesions suggests that it possesses anti-inflammatory. By downregulating the inflammatory cascade and preventing excessive keratinocyte proliferation, the intervention modulates the critical pathways responsible for psoriasis progression. The substantial decrease in PASI scores seen in the therapy group is probably caused by this dual process. The time-dependent changes observed in all parameters emphasize the progressive nature of IMQ-induced inflammation and the treatment's efficacy in modulating disease progression. By mitigating inflammation and preventing excessive epidermal hyperplasia, the treatment demonstrates potential as a therapeutic option for managing psoriasis and inflammatory skin conditions.

According to Lue et al. (2016), the imiquimod (IMQ)-induced mice model is well known for being a dependable and repeatable method for researching the pathophysiology of psoriasis and assessing possible treatments. Numerous clinical and histological characteristics of human psoriasis, such as erythema, scaling, epidermal thickness, and immune cell infiltration, are replicated in this model. In the current investigation, BALB/c female mice were used to examine the effectiveness of two distinct brands of 5% IMQ creamsLikejie and Aldara—in terms of their capacity to cause skin lesions resembling psoriasis. Three sets of mice were created: Group B (Aldara), Group C (Vaseline, control), and Group A (Likejie). For six days in a row, IMQ or Vaseline was used topically every day. The Psoriasis Area and Severity Index (PASI) was used to evaluate the degree of psoriatic characteristics, Baker's score, and histological examination of epidermal thickness.The Aldara group exhibited significantly more severe psoriatic manifestations than the Likejie group, with a higher PASI score (9.81 ± 0.84 vs. 3.25 ± 1.56), Baker's score (6.47 ± 1.50 vs. 2.93 ± 1.07), and greater epidermal thickness (85.62 ± 17.55 μm vs. 49.79 ± 14.16 μm). The control group showed no pathological changes. Statistically significant differences ($P < 0.005$) between the groups confirmed the variable efficacy of different IMQ brands.These findings emphasize that the choice of IMQ formulation can influence the severity and reproducibility of the psoriasis model. Therefore, standardization of the IMQ source is crucial for consistency in preclinical studies and accurate evaluation of anti-psoriatic therapies[27].

The data further highlight that early intervention with the treatment is crucial for preventing the full development of psoriasis-like lesions. By reducing erythema and inflammation at an early stage, the treatment limits subsequent pathological changes, resulting in better overall outcomes. The ability to maintain lower PASI scores throughout the study duration suggests that continuous application of the treatment provides sustained protection against IMQ-induced skin inflammation. The reduction in PASI parameters observed in the treatment group also implies that the intervention may modulate cytokine expression and immune cell infiltration, which are both essential to the pathophysiology of psoriasis. The treatment probably restores the equilibrium between pro-inflammatory and anti-inflammatory pathways by restricting immune cell infiltration and downregulating pro-inflammatory cytokines, which contributes to the noted benefits.

These results imply that the treatment may work by focusing on important signaling pathways related to the pathophysiology of psoriasis, such as the IL-23/Th17 axis, which is known to cause epidermal hyperplasia and chronic inflammation in psoriasis. By modulating these pathways, the treatment effectively reduces inflammation and restores normal skin architecture. The significant reduction in total PASI scores further underscores the comprehensive efficacy of the treatment in addressing multiple aspects of psoriasis pathology. By simultaneously reducing erythema, scaling, and thickness, the treatment provides a holistic approach to managing psoriasis, highlighting its potential as a therapeutic candidate. In consideration, by decreasing erythema, scaling, and thickness, the treatment successfully reduces IMQ-induced psoriasis-like inflammation, ultimately lowering total PASI scores, as demonstrated by the graphical data. These results highlight the intervention's potential for treating psoriasis and other inflammatory skin disorders[20].

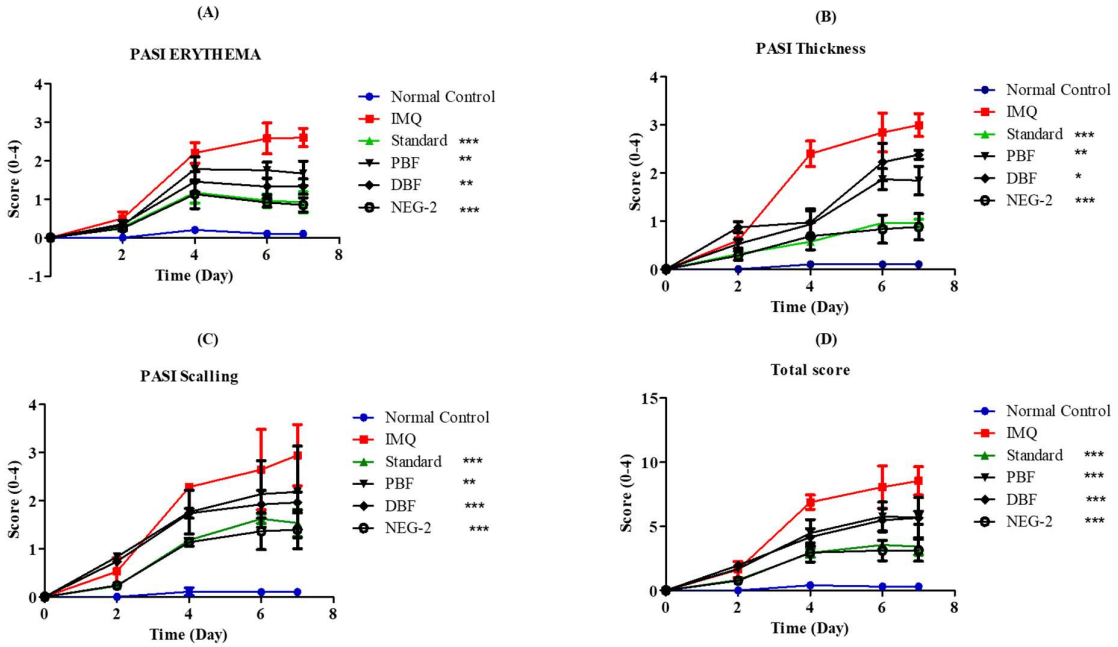

Figure 2: Erythema, scales, thickness, and the overall score of psoriatic skin lesions on the dorsal portion as determined by the psoriasis area severity index score are all affected by the application of imiquimod. The values represent the mean ± the standard error of the mean (n = 6). *P < 0.05 in comparison to the typical control.

Table *3* :Erythema, scales, thickness, and the overall score of psoriatic skin lesions on the dorsal region as determined by the psoriasisarea severity index score are all affected by the administration of imiquimod.

Parameter	**Day**	**Normal Control**	**IMQ**	**Standard**	**PBF**	**DBF**	**NEG-2**						
		Value	**SD**	**Value**	**SD**	**Value**	**SD**	**Value**	**SD**	**Value**	**SD**	**Value**	**SD**
PASI Erythema	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.50	0.17	0.27	0.09	0.31	0.13	0.35	0.09	0.25	0.03
	4	0.20	0.04	2.20	0.26	1.17	0.27	1.78	0.31	1.45	0.26	1.13	0.37
	6	0.10	0.00	2.58	0.40	0.96	0.17	1.75	0.21	1.33	0.22	0.91	0.11
	7	0.10	0.02	2.60	0.24	0.92	0.28	1.67	0.32	1.33	0.20	0.85	0.18
PASI Thickness	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.60	0.17	0.32	0.07	0.53	0.09	0.87	0.12	0.28	0.09
	4	0.10	0.04	2.40	0.26	0.57	0.04	0.93	0.28	0.97	0.28	0.69	0.29
	6	0.10	0.00	2.85	0.40	0.96	0.08	1.87	0.22	2.23	0.39	0.83	0.29
	7	0.10	0.02	3.00	0.24	0.96	0.09	1.85	0.30	2.38	0.09	0.88	0.27
PASI	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	0.0	0.00	0.0

Scales									0		0		0
	2	0.00	0.00	0.53	0.28	0.23	0.04	0.84	0.04	0.74	0.07	0.24	0.04
	4	0.11	0.08	2.28	0.04	1.18	0.04	1.76	0.45	1.74	0.10	1.14	0.08
	6	0.10	0.03	2.65	0.84	1.63	0.10	2.14	0.69	1.93	0.29	1.36	0.38
	7	0.10	0.04	2.95	0.64	1.54	0.28	2.18	0.95	1.96	0.22	1.40	0.40
Total Score	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	1.63	0.63	0.82	0.20	1.68	0.26	1.96	0.28	0.77	0.16
	4	0.41	0.16	6.88	0.56	2.93	0.35	4.48	1.05	4.17	0.65	2.96	0.74
	6	0.30	0.04	8.07	1.64	3.55	0.35	5.77	1.12	5.48	0.90	3.11	0.79
	7	0.30	0.08	8.55	1.11	3.41	0.65	5.70	1.56	5.67	0.51	3.14	0.86

3.3 Biochemical analysis

3.3.1 Oxidative markers study

The results of the study showed the effects of psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2) on lipid peroxidation (LPO) and nitric oxide (NO) levels induced by imiquimod (IMQ) in mice. The data indicates that oxidative stress caused by IMQ led to a significant elevation in both LPO and NO levels, demonstrating cellular damage and inflammation. Treatment with PBF, DBF, and NEG-2 resulted in a notable reduction in these markers compared to the IMQ-treated group. Among the formulations, the combined NEG-2 group exhibited the most pronounced decline in LPO and NO levels, suggesting a synergistic effect in mitigating oxidative stress. The reduction in LPO indicates decreased membrane lipid damage, while the drop in NO levels suggests reduced nitrosative stress and inflammation. PBF and DBF alone also showed considerable antioxidant potential, though their combined application proved more effective in restoring oxidative balance. These findings emphasize the therapeutic potential of using psoralen and diosgenin formulations, particularly in combination, to counteract oxidative damage induced by IMQ[28,29].

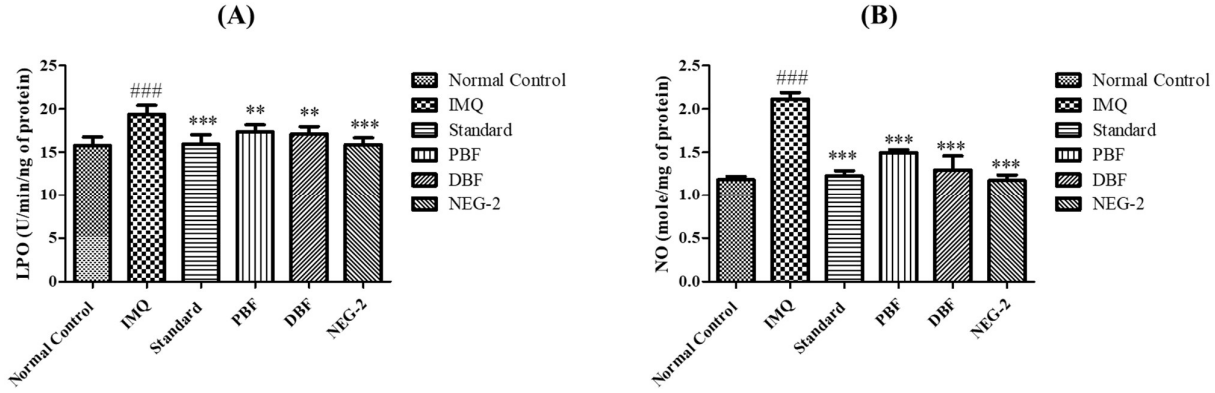

Figure 3: Effect of psoralen-based formulation (PBF) and diosgenin based formulation (DBF) and their combined formulation (NEG-2) on LPO and NO against oxidative stress induced by imiquimod (IMQ) on mice. The data is given as Mean ±SD using Two Way ANOVA and the Bonferroni Posts test to compare the raw mean of replicates and compare each column to all other columns. Summary $p<0.05$ (Low significant difference), $p<0.01$ (Moderate significant difference), and $p<0.001$ (High significant difference) were used to indicate the significance level and difference, which were denoted by (*), (**), and (***), respectively.

The study's findings demonstrated how psoralen-based formulation (PBF), diosgenin-based formulation (DBF),

and their combination formulation (NEG-2) affected the activity of catalase (CAT) and superoxide dismutase (SOD) in mice exposed to oxidative stress brought on by imiquimod (IMQ). SOD and CAT activities were significantly lower in the IMQ-treated group, indicating elevated oxidative stress and compromised antioxidant defense. When compared to the IMQ-treated group, SOD and CAT levels significantly improved after treatment with PBF, DBF, and NEG-2. A better antioxidant response was indicated by the combined NEG-2 formulation, which showed the largest rise in both enzymatic activity. The combination formulation successfully restored SOD activity, which is essential for neutralizing superoxide radicals, indicating improved free radical scavenging.Similarly, CAT activity, which decomposes hydrogen peroxide into water and oxygen, showed a marked improvement, reflecting a reduction in oxidative damage. Although PBF and DBF individually improved antioxidant enzyme levels, the synergistic effect observed with the combined formulation resulted in better protection against oxidative stress. The increase in SOD and CAT levels highlights the potential of NEG-2 in restoring antioxidant balance and mitigating oxidative damage caused by IMQ. These findings suggest that the combined use of psoralen and diosgenin offers a promising strategy to enhance antioxidant defense, thereby reducing oxidative stress and inflammation effectively. The results emphasize the efficacy of using these bioactive formulations in combination to provide superior protection against oxidative damage in pathological conditions.

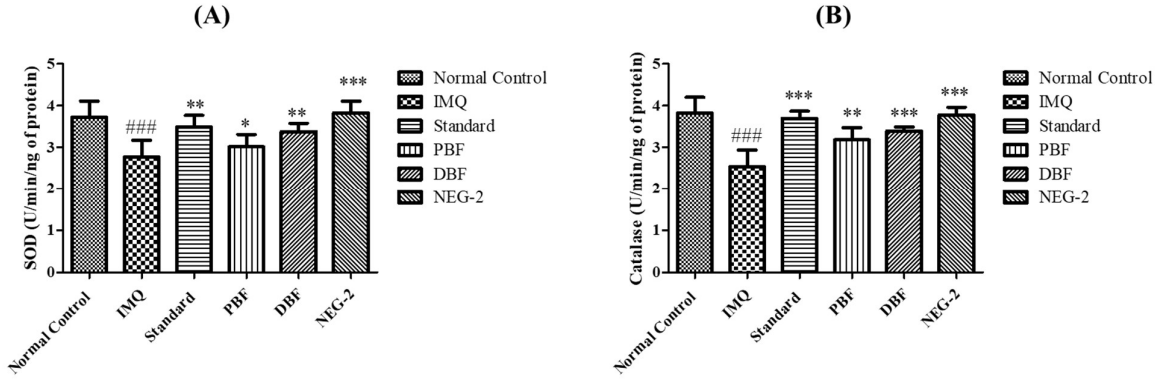

Figure 4: Effect of psoralen-based formulation (PBF) and diosgenin based formulation (DBF) and their combined formulation (NEG-2) on SOD and CAT against oxidative stress induced by imiquimod (IMQ) on mice. The data is given as Mean ±SD using Two Way ANOVA and the Bonferroni Posts test to compare the raw mean of replicates and compare each column to all other columns. Summary $p<0.05$ (Low significant difference), $p<0.01$ (Moderate significant difference), and $p<0.001$ (High significant difference) were used to indicate the significance level and difference, which were denoted by (*), (**), and (***), respectively.

3.3.2 Anti-inflammatory study

The results of the study showed the impact of psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2) on TNF-α and IL-6 levels in mice subjected to inflammation induced by imiquimod (IMQ). IMQ-treated mice exhibited a significant increase in TNF-α and IL-6 levels, indicating heightened inflammation. Treatment with PBF and DBF reduced these inflammatory cytokines, with the combined formulation (NEG-2) demonstrating the most pronounced reduction. TNF-α is a key mediator in the inflammatory response, and its elevated levels contribute to tissue damage and immune dysregulation. IL-6, another crucial cytokine, promotes inflammation and is involved in the progression of inflammatory conditions. The reduction in these cytokines following PBF and DBF treatment highlights their anti-inflammatory properties, while the combined formulation exhibited a synergistic effect, achieving a greater reduction in TNF-α and IL-6 levels. This suggests that the combined approach may provide a more effective strategy for managing inflammation. The significant decrease in inflammatory markers observed with NEG-2 treatment emphasizes its potential in mitigating IMQ-induced inflammation. Compared to individual treatments, the combined formulation effectively modulated the inflammatory response, reflecting its enhanced efficacy. These findings highlight the beneficial impact of combining PBF and DBF in reducing inflammation and restoring immune balance. The ability of the combined formulation to downregulate pro-inflammatory cytokines suggests its potential therapeutic application in managing inflammation-related disorders [30].

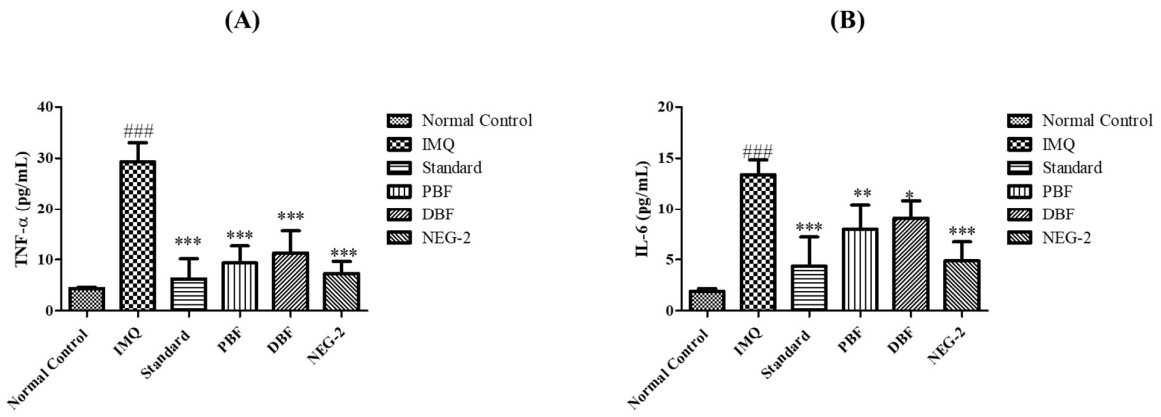

Figure 5: Effect of psoralen-based formulation (PBF) and diosgenin based formulation (DBF) and their combined formulation (NEG-2) on TNF-α and IL-6 against inflammation induced by imiquimod (IMQ) on mice. Two-way ANOVA is used to portray the data as Mean ±SD. The Bonferroni Posts test is then used to compare the raw mean of replicates and each column to all other columns. Summary $p<0.05$ (Low significant difference), $p<0.01$ (Moderate significant difference), and $p<0.001$ (High significant difference) were used to indicate the significance level and difference, which were denoted by (*), (**), and (**), respectively.

3.4 Skin retention study

Using a water-responsive gel (WRG) method, the current study assessed the skin retention capacity of psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combination formulation (NEG-2). The mice's shaved dorsal skin was treated topically with the formulations.Upon spraying water, the WRG transformed from a gel, forming a thin, adhesive layer that adhered firmly to the skin, enhancing localized drug retention.Quantitative analysis conducted 12 hours post-application revealed distinct differences in skin retention among the formulations. The combination formulation (NEG-2) showed the highest retention at 47.141 ± 1.576%, followed by PBF at 33.903 ± 1.238 %, and DBF at 27.350 ± 1.411%. These results suggest that the dual-drug system possesses enhanced retention properties compared to the individual formulations.

The superior performance of the NEG-2 formulation may be attributed to synergistic effects between the two drugs and better gel integrity upon in-situ gelation. The gel formed a semi-occlusive barrier, reducing drug loss and improving skin adherence. Moreover, the formulation ensured sustained contact with the skin, potentially increasing the therapeutic efficacy of the actives.This study highlights the benefits of using WRG-based delivery systems for topical applications, particularly in enhancing skin retention and drug localization. The findings support the development of combination therapies in gel form to improve outcomes in dermatological disorders requiring localized and sustained drug delivery. Further pharmacodynamic and safety evaluations are recommended to confirm long-term benefits.

The goal of topical administration is to minimize systemic exposure while localizing the medication at the site of action.The efficacy of such systems heavily relies on adequate skin retention, which ensures sustained drug availability and action. In this study, WRG-based formulations of PBF, DBF, and their combination were assessed for their ability to retain drugs in the skin over a prolonged period.The results showed that the WRG sol formulation successfully transformed into a gel upon water spraying, forming a thin, adhesive layer on the skin. This gel matrix facilitated prolonged contact with the skin and limited drug loss due to runoff or mechanical disturbance. Among the formulations tested, NEG-2 exhibited the highest skin retention, indicating a synergistic effect of the combined actives when incorporated into a single WRG-based delivery system. There are multiple reasons for the combination formulation's better retention. First, the physicochemical interactions between psoralen and diosgenin may have led to a more cohesive gel network, enhancing viscosity and adherence. Secondly, the gelation mechanism provided a semi-occlusive barrier that reduced drug evaporation and allowed deeper penetration into the stratum corneum. Thirdly, the dual therapeutic agents might have altered skin permeability, facilitating better entrapment within skin layers.

The higher retention of PBF over DBF alone also aligns with their solubility and molecular interaction profiles. However, it is the combination that maximizes skin residence, potentially offering superior therapeutic outcomes in dermatological applications such as vitiligo, psoriasis, or localized inflammation.These findings support the hypothesis that WRG-based dual-drug formulations are effective in enhancing cutaneous retention, which is critical for achieving sustained topical effects. Future studies may explore long-term pharmacokinetics, tissue distribution, and histopathological safety evaluations to further validate these results.

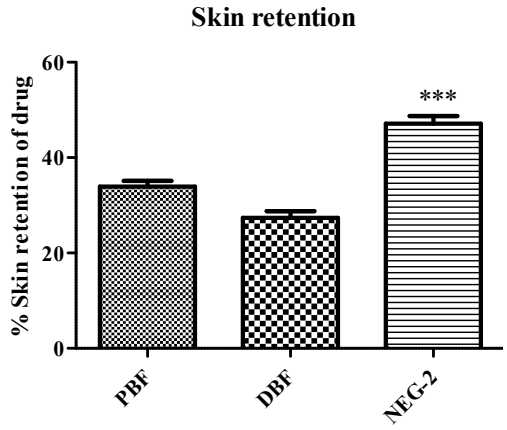

Figure 6: Percentage of skin retention of drug.

Table 2: Skin Retention Percentage of Different Formulations

Formulation	Skin Retention (%)
PBF	33.903 ± 1.238 %
DBF	27.350 ± 1.411%
NEG-2	47.141 ± 1.576%

3.5 Histopathology studies

The histopathological analysis of psoriatic skin lesions induced by imiquimod (IMQ) on the dorsal portion of mice revealed distinct changes in epidermal structure and inflammation. Significant epidermal thickness, hyperkeratosis, parakeratosis, and inflammatory cell infiltration in the dermis were among the hallmarks of psoriasis displayed by the IMQ control group. These histological alterations were in line with disruption of normal skin architecture and severe psoriatic inflammation. Skin histology showed significant improvements after treatment with the psoralen-based formulation (PBF). In comparison to the IMQ control group, the epidermal thickness was decreased, and there was less noticeable parakeratosis and infiltration of inflammatory cells.PBF-treated skin showed a more organized epidermal structure, indicating its potential to mitigate IMQ-induced psoriasis-like lesions.

Diosgenin-based formulation (DBF) treatment also resulted in improvements, but the effect was slightly less pronounced than PBF. DBF-treated samples exhibited moderate reduction in epidermal thickening and partial normalization of skin histology, along with decreased inflammatory infiltration, suggesting its anti-inflammatory and protective role. The combined formulation of PBF and DBF (NEG-2) exhibited the most significant protective effects, restoring the skin's histological architecture to near-normal levels. NEG-2-treated sections demonstrated minimal epidermal thickening, reduced parakeratosis, and marked reduction of inflammatory cell infiltration. The dermal layer appeared more intact with fewer inflammatory changes, suggesting synergistic effects of both formulations in reducing inflammation and oxidative stress caused by IMQ. These histopathological findings correlate with the biochemical and antioxidant results, indicating that PBF, DBF, and their combination effectively mitigate IMQ-induced psoriatic lesions. The combined formulation provided superior protection and tissue repair, suggesting a promising therapeutic approach for psoriasis management[20,27,31].

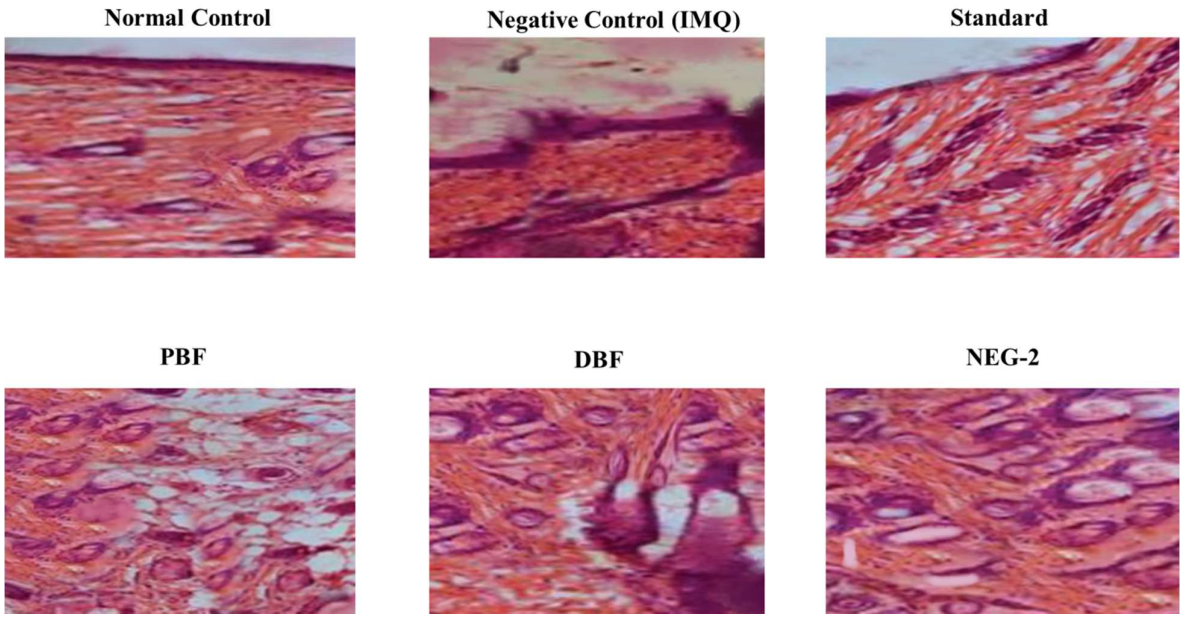

Figure 7: Histopathological evaluation of psoriatic skin lesions on the dorsal portion of mice induced by imiquimod (IMQ) and treated with psoralen-based formulation (PBF), diosgenin-based formulation (DBF), and their combined formulation (NEG-2). The IMQ control group showed significant epidermal thickening, hyperkeratosis, parakeratosis, and inflammatory cell infiltration. PBF treatment reduced epidermal thickness and inflammation, indicating notable improvement. DBF treatment also demonstrated a reduction in parakeratosis and inflammatory cell infiltration, although slightly less effective than PBF. The combined formulation (NEG-2) exhibited the most prominent protective effects, restoring skin histology to near-normal levels with minimal thickening and inflammation. The dermal layer appeared more organized with fewer inflammatory changes, indicating a synergistic effect in reducing inflammation and oxidative stress. The overall histopathological observations suggest that PBF, DBF, and their combination effectively mitigate IMQ-induced psoriatic changes, with NEG-2 offering the highest therapeutic potential for psoriasis treatment.

4. CONCLUSIONS

This study reveals a combined treatment of Diosgenin and Psoralen that effectively penetrates the skin by using Tween 80 as a surfactant and olive oil as the oil phase. Additionally, the skin irritation test examined NEG-2's topical safety profile and found no likelihood of irritation, indicating the feasibility of long-term treatment. The results are encouraging, demonstrating a workable strategy for developing topical treatments for psoriasis and proving that NEG-2 is both safe and efficacious. Topical NEG-2 treatment showed improvement in psoriasis symptoms in the IMQ-produced psoriatic plaque model. PASI scoring, histological examination, and ELISA data suggested that NEG-2 might alleviate and cure psoriasis. In conclusion, NEG-2 outperformed commercially available treatments in the treatment of psoriasis.Both medications' local concentrations were increased by the updated formulation, which decreased systemic adverse effects and allowed for deeper penetration into the epidermal layersa result not possible withconventional formulations. The combination therapy technique enhances psoriasis healing in the present investigation. This approach is less irritating to the skin than the gel-containing free medication. Additionally, there was an improvement in the local topical concentration compared to the other trial groups. This method provides a customized and adequate safety profile. Because of the formulation's lower propensity for irritation, long-term treatment is likely with sufficient patient adherence. This encourages the recipe's future use and commercialization.

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