Polyherbal Microemulgels as Potential Therapeutic Agents for Psoriasis: In Vivo and In Vitro Evaluation

Author- Ande S. N.*[1], Kshirsagar M. D. [1], Chandewar A. V.[1], Kochar N. I. [1], Mohale D. S.[1]

[1]*Department of Pharmaceutical Sciences, P. Wadhwani College of Pharmacy, Dhamangoan railway road, Yawatmal-445001, Maharashtra, India.*

Corresponding author- Sagar N. Ande
Research Scholar, Department of Pharmaceutical Sciences, P. Wadhwani College of Pharmacy, Dhamangoan railway road, Yawatmal-445001, Maharashtra, India.
Email: pec99sagar.ande@gmail.com

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Polyherbal Microemulgel, Psoriasis, Inflammation, Cytokine modulation, UV-B and imiquimod-induced model

ABSTRACT

Objective
This research aimed to investigate the therapeutic potential of two polyherbal microemulsion gel formulations, ABC (encompassing Azadirachta indica, Berberis aristata, and Coleus forskohlii) and BCG (comprising Boswellia serrata, Curcuma longa, and Glycyrrhiza glabra), in mitigating inflammation and restoring skin integrity in a psoriasis-like murine model triggered by UV-B irradiation and imiquimod (IMQ) application.

Methods:
Psoriasis-like inflammation was experimentally induced in Balb/c mice using a topical application of 5% imiquimod cream for seven consecutive days and in Wistar rats through UV-B irradiation for ten days. A control group, treatment groups that received 25 mg/kg of ABC or 50 mg/kg of BCG, and a conventional treatment group that received Clobetasol propionate were among the seven groups into which the animals were divided. Disease severity was evaluated using the Psoriasis Area Severity Index (PASI). Biochemical analyses measured pro-inflammatory cytokines such as TNF-α, IFN-γ, and VEGF, while histopathological studies examined epidermal structure and inflammatory cell infiltration, substantiating the anti-psoriatic efficacy of the formulations.

Results:
In vitro analyses demonstrated that both formulations exhibited stability and significant anti-inflammatory effects, with marked inhibition of pro-inflammatory cytokines (TNF-α, VEGF). In vivo, treatment with ABC and BCG substantially reduced PASI scores, with BCG at 50 mg/kg showing the most significant improvement, comparable to the positive control. Histological analysis confirmed reductions in epidermal thickening and inflammatory cell infiltration in treated groups.

Conclusion:
The polyherbal Microemulgel formulations ABC and BCG effectively reduced inflammation, cytokine levels, and epidermal thickening, demonstrating significant anti-psoriatic effects. These findings highlight the potential of these formulations as safe and effective alternatives for psoriasis treatment, warranting further investigation into their molecular mechanisms and clinical applications.

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Introduction

Psoriasis is a persistent, immune-mediated inflammatory condition that impacts several areas of the body., including the skin, scalp, lower back, and joints.[1] It is distinguished by abnormal keratinocyte differentiation and the formation of erythematous plaques with silver-white scales.[2]

Psoriasis affects approximately 2–5% of the global population and presents in various clinical forms, including psoriasis vulgaris, psoriatic arthritis (psoriasis arthropathica), erythrodermic psoriasis, and pustular psoriasis.[3] Beyond physical symptoms, psoriasis profoundly affects mental health, contributing to depression, anxiety, and social stigma.[4]

Psoriasis is categorized into two separate types based on the age of onset: early-onset psoriasis, occurring before the age of 40, and late-onset psoriasis, manifesting after the age of 40. These categories are characterized by unique genetic predispositions and immunological profiles.[5] The disease's pathogenesis is driven by immune dysregulation and keratinocyte hyperproliferation. The activation of T-helper cells, particularly Th17 cells, triggers the release of pro-inflammatory cytokines such as IL-17, TNF-α, and IL-22.[6] These cytokines induce abnormal keratinocyte growth and facilitate the infiltration of inflammatory cells into the skin, leading to the formation of characteristic psoriatic plaques.[7]

Conventional psoriasis treatments encompass topical therapies, systemic drugs, and phototherapy. Topical medications, such as retinoids, corticosteroids, and vitamin D analogues, are commonly employed for managing mild to moderate cases.[8] For more severe cases, systemic drugs like methotrexate, cyclosporine, and biologics targeting cytokines (e.g., TNF-α inhibitors, IL-17 inhibitors) are widely prescribed. Phototherapy, including narrowband UVB and PUVA, is another effective option.[9] Despite their effectiveness, these therapies are often associated with significant limitations, such as high costs, side effects including immunosuppression and organ toxicity, and the risk of relapse upon discontinuation.[10] These limitations underscore the need for safer and more sustainable alternatives.

Genetic predisposition significantly influences psoriasis susceptibility, with loci such as PSORS1 and immune regulation-related genes playing critical roles.[11] Environmental factors, including infections, trauma, and stress, also act as triggers for disease onset and exacerbation. Keratinocytes, the primary cells of the epidermis, are central to the disease's pathology, undergoing hyperproliferation and abnormal differentiation that contribute to the thickened, scaly skin characteristic of psoriasis.[12]

Animal models have become indispensable tools for understanding psoriasis pathogenesis and evaluating potential therapeutic strategies. Common models, including the imiquimod (IMQ)-induced mouse model,[13] UVB-induced psoriasis model,[14] and K5.Stat3C transgenic mice, [15]effectively mimic the inflammatory processes seen in human psoriasis. These models provide a robust platform for validating novel treatments, including polyherbal formulations.

Herbal medicines have gained prominence as alternative or adjunctive treatments for managing inflammatory and dermatological conditions.[16] Natural substances with anti-inflammatory, antibacterial, and wound-healing effects include neem , turmeric, and aloe vera . However, to transform these traditional therapies into clinically viable treatments, solid scientific validation and the development of sophisticated drug delivery methods are required.[17] Recent studies highlight the potential of topical formulations containing anti-inflammatory agents for managing chronic inflammatory skin conditions, as evidenced in the treatment of atopic dermatitis (citation). Such approaches align with the broader shift toward safer and more sustainable treatment options.[18] The efficacy of herbal-based formulations like Sarkaraikolli in promoting wound healing underscores the therapeutic value of natural ingredients in addressing inflammatory and dermatological conditions.[19]

Advancements in nanotechnology and topical delivery systems have paved the way for enhancing the therapeutic potential of herbal medicines. Delivery systems such as

microemulsions, nano-emulsions, and emulgels improve the solubility, stability, and skin permeability of bioactive compounds. Among these, emulgels stand out due to their dual advantage: the structural stability of a gel combined with the enhanced bioavailability of emulsions. This makes them particularly effective for managing chronic skin conditions like psoriasis by targeting both hydrophilic and lipophilic pathways.[20]

This study aims to develop and evaluate a novel polyherbal emulgel formulation enriched with bioactive extracts known for their efficacy against inflammatory and psoriatic-like skin conditions. By bridging traditional medicinal knowledge with advanced therapeutic strategies, this research seeks to deliver a scientifically validated, patient-centric alternative for managing dermatological disorders.

Materials and Methods

Cell Lines and Culture Media

Mouse skin fibroblasts and human keratinocyte (HaCaT) cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. The mouse fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) High Glucose (Gibco, Cat. No. 2120785), while HaCaT cells were maintained in calcium-free Keratinocyte Medium II (PromoCell, Germany, Cat. No. 10175-234).

Instrumentation and Laboratory Supplies

All experiments were conducted using analytical-grade reagents sourced from certified suppliers to ensure reliability. Equipment and consumables used in the study were standardized to align with the experimental requirements.

Chemicals and Reagents Used

This study used analytical-grade chemicals and reagents such as carbopol 940 (gelling agent), light liquid paraffin (oil phase), Tween 80 (emulsifier), and triethanolamine (neutralizer) to generate formulations. Standard drugs for comparative analysis, such as calcipotriol, and cytokine estimation kits (IL-17, IL-22, and TNF-α), were sourced from Sigma-Aldrich and other certified suppliers. Hydroalcoholic solvents (ethanol and water, 80:20 v/v) were utilized for the extraction of herbal materials.

Formulation Development and Herbal Sample Preparation

The formulations were developed using analytical-grade carbopol 940 as a gelling agent, light liquid paraffin as the oil phase, Tween 80 as an emulsifier, and triethanolamine as a neutralizer. Hydroalcoholic solvents comprising ethanol and water (80:20 v/v) were employed both for herbal extraction and formulation development. Standard drugs, such as calcipotriol, were incorporated for comparative analysis to evaluate the efficacy of the developed formulations.

The herbal materials used in the study included neem (*Azadirachta indica*), turmeric (*Curcuma longa*), and aloe vera (*Aloe barbadensis*). These materials were authenticated, cleaned, shade-dried, and finely powdered. Hydroalcoholic extraction was conducted using a Soxhlet apparatus, and the resulting extracts were concentrated as the pressure was lowered. At 4°C, the concentrated extracts were kept in airtight containers until use in formulation development and subsequent pharmacological studies.

By integrating these herbal extracts, the formulations were designed to maximize their therapeutic potential for anti-inflammatory and wound-healing applications.

Physicochemical Characterization

Size of Particles and Zeta Potential:

Using dynamic light scattering (Malvern Zetasizer), the formulations' zeta potential and particle size distribution were determined.

Measurement of pH:

To make sure the formulations would work well on skin, their pH was measured with a calibrated digital pH meter.

Viscosity Analysis:
The viscosity of the formulations was evaluated using a Brookfield viscometer at different shear rates.
Thermal Analysis:
The active components' compatibility with the excipients was verified using differential scanning calorimetry (DSC).
Morphological Examination:
Scanning electron microscopy was used to examine the Microemulgels' surface morphology (SEM).
Characterization and Analysis of Extracts
The bioactive constituents of the herbal extracts were analyzed using high-performance liquid chromatography (HPLC). A reverse-phase C18 column was employed, and the samples were scanned at appropriate wavelengths for rutin, curcumin, and aloin. The retention times of the bioactive compounds were compared with those of standards to ensure consistency.
In Vitro Characterization Drug Release Studies:
The release profile of active components was analyzed using Franz diffusion cells equipped with dialysis membranes (20 kDa). Samples were collected at predetermined intervals and analyzed spectrophotometrically.
Experimental Animals
Balb/c mice (25–32 g) and Wistar rats (150–200 g), aged 7–8 weeks, were used following IAEC approval (650/PO/Re/S/2002/CPCSEA/2022/13). The animals had unlimited access to food and drink while being kept in typical laboratory settings, in compliance with CPCSEA regulations. Seven groups (control, induced, standard, and polyherbal formulation treatment) were randomly selected. A total of 42 Wistar rats and 42 Balb/c mice were included, with an additional 8 Wistar rats used for the skin irritation study.
Induction of Psoriasis
Psoriasis-like skin conditions were induced using daily topical 5% IMQ cream applied to the dorsal skin for seven days or by exposing shaved skin to UV-B radiation for 15 minutes daily over ten days.
Experimental Design and Grouping
Acute Toxicity Study
The Acute Dermal Toxicity Study (Fixed Dose Procedure) followed OECD guidelines. The dorsal area of female animals was clipped to expose 10% of the body surface. The test item was applied to the area, covered with gauze, and secured with adhesive tape for 24 hours. After exposure, the remaining test item was removed with moistened cotton.
General Experimental Design
Two distinct animal models, imiquimod-induced psoriasis in Balb/c mice and UVB-induced skin inflammation in Wistar rats, were used to evaluate the anti-inflammatory and anti-psoriatic potential of the formulations. In both models, animals were divided into seven groups. Group I served as the normal control without exposure to any stimulus or treatment. Group II acted as the induced control, receiving the inflammatory stimulus but no therapeutic intervention. Group III animals were treated with Clobetasol propionate at 50 mg/kg body weight, serving as the standard treatment. Groups IV and V received ABC at doses of 25 mg/kg and 50 mg/kg body weight, respectively, while Groups VI and VII were treated with BCG at the same doses. For both models, the severity of inflammation or psoriasis was evaluated using erythema, scaling, and thickness scores on a scale of 0 (none) to 4 (extremely marked). At the end of the experimental period, skin samples were collected for histopathological and biochemical analyses to assess inflammatory markers and confirm the efficacy of the treatments

Imiquimod-Induced Psoriasis in Balb/c Mice

Psoriasis-like inflammation was induced in Balb/c mice by applying 62.5 mg of 5% imiquimod cream daily to the shaved dorsal skin for seven consecutive days. The inflammation was characterized by erythema, scaling, and skin thickening. Treatments with Clobetasol propionate, ABC, or BCG were initiated after induction, and formulations were administered daily until the end of the seven-day period. On day 8, selected animals were euthanized, and skin samples were collected for further analysis.

UVB-Induced Skin Inflammation in Wistar Rats

For the UVB-induced skin inflammation model, Wistar rats were exposed to daily UVB radiation on a 1.5 x 2.5 cm^2 shaved dorsal skin area for 10 consecutive days. The inflammatory response was characterized by erythema, swelling, and microvascular dilation. Following UVB exposure, treatments with Clobetasol propionate, ABC- , or BCG were administered daily until the end of the experiment. On day 11, skin samples were collected post-euthanasia for histopathological and biochemical evaluation to determine the formulations' anti-inflammatory effects.

MTT Assay and HTAC

An MTT test was used on HaCaT cells to determine cytotoxicity and cell viability. The HTAC experiment was undertaken to investigate the immunomodulatory potential of the emulgel, examining its effect on T-cell activation..

Psoriasis Area Severity Index (PASI):

The Psoriasis Area Severity Index (PASI), which ranks skin thickness, erythema, and scales from 0 to 4, was used to assess the severity of psoriasis-like lesions. The cumulative PASI score evaluated the overall severity of the lesions.

Cytokine Quantification:

Skin tissue homogenates were made, and their levels of pro-inflammatory cytokines (VEGF, TNF-α, and IL-17) were measured. In accordance with the manufacturer's instructions, these cytokines' levels were measured using enzyme-linked immunosorbent assays (ELISA) to evaluate the formulations' anti-inflammatory potential.

Histopathological Analysis:

Skin samples were taken from the dorsal and ear regions of the mice and fixed in 10% formalin. Tissue samples were cut into 5 µm thick sections after processing and paraffin embedding. The sections were stained with haematoxylin and eosin (H&E) to evaluate epidermal thickness, inflammatory cell infiltration, and other psoriasis-related histological changes.

Statistical Analysis

All experimental outcomes were expressed as the mean ± SD. To evaluate statistical significance, a one-way ANOVA was performed, followed by Tukey's post hoc test. Statistical significance was considered as P-values less than 0.05.

Results

Physicochemical Characterization

The particle size distribution of the formulations was determined using dynamic light scattering (Malvern Zetasizer), with average particle sizes ranging from 600 to 700 nm, indicating appropriate formulation for topical delivery. The pH values were measured using a calibrated digital pH meter, with ABC and BCG showing 5.62 ± 0.60 and 5.78 ± 0.25, respectively, ensuring compatibility with skin applications. Viscosity measurements, conducted with a Brookfield viscometer, yielded 56,391 ± 0.25 cps for ABC and 42,604 ± 0.35 cps for BCG. Differential scanning calorimetry (DSC) confirmed the compatibility of the active ingredients with the excipients while scanning electron microscopy (SEM) revealed smooth, homogeneous surface morphology for both formulations.

Ex Vivo Drug Release Studies

The cumulative drug release profile of forskolin, berberine, and azadirachtin from the ABC

microemulsion gel was evaluated over 24 hours. Azadirachtin was released more quickly compared to forskolin and berberine. Similarly, glycyrrhizin released faster than boswellic acid and curcumin from the BCG formulation. This indicated different release kinetics for the active ingredients (Table 1).

Table 1: Drug content of ABC and BCG formulation

	Extract	Active compound	Mean Concentration (µg/g)
ABC Formulation	*Azadirachta indica*	Azadirachtin	6.754
	Berberis aristata	Berberine	14.860
	Coleus forskohli	Forskolin	15.246
BCG Formulation	*Boswellia sSerrata*	Boswellic acid	28.756
	Curcuma longa	Curcumin	13.592
	Glycyrrhiza glabra	Glycyrrhizin	23.340

Ex Vivo Skin Permeation Studies

Ex vivo skin permeation studies indicated that both ABC and BCG formulations demonstrated effective drug penetration. However, BCG exhibited higher permeation rates compared to ABC, suggesting superior skin penetration potential for the BCG formulation.

Cytotoxic and Immunomodulatory Effects of ABC and BCG on Psoriasis-Induced HaCaT Cells

The cytotoxic effects of ABC and BCG on psoriasis-induced HaCaT cells were evaluated using the MTT assay. Both formulations demonstrated a dose-dependent reduction in cell viability. Among the two, BCG- exhibited greater potency, achieving an IC50 value of 24.98 ± 1.52 µg/ml, compared to ABC 50.35.

± 1.76 µg/ml. This indicates that BCG is more effective in reducing the viability of HaCaT cells at lower concentrations.

The detailed results from the MTT assay (Table No.2) show that at increasing concentrations, the percentage of viable cells decreased significantly. For ABC, the viability dropped from 83.69 ± 2.02% at 12.5 µg/ml to

14.60 ± 0.70% at 200 µg/ml. Similarly, BCG exhibited a sharp decline in cell viability, from 66.33 ± 1.96% at 12.5 µg/ml to 6.01 ± 0.21% at 200 µg/ml.

In addition to the MTT assay, HTAC analysis revealed that both formulations exerted notable immunomodulatory effects, with BCG demonstrating a higher potential for immune activation, particularly at higher concentrations. This suggests its superior efficacy in modulating the immune responses associated with psoriasis.

The data from both assays underline the therapeutic potential of BCG, especially in comparison to ABC, for managing psoriasis-like conditions by targeting keratinocyte activity and modulating immune responses.

Table No. 2: Cytotoxic Effects of Asiaticoside, ABC , and BCG on Cell Viability at Different Concentrations

Treatment	**Concentration (µg/ml)**	**% Cell Viability**	**ABC**	**BCG**
Control	-	100.00%	100.00%	100.00%
Asiaticoside	20	61.75 ± 1.32%	61.75 ± 1.32%	61.75 ± 1.66%
ABC	12.5	83.69 ± 2.02%	83.69 ± 2.02%	-
	25	70.63 ± 1.91%	70.63 ± 1.91%	-
	50	50.44 ± 1.39%	50.44 ± 1.39%	-
	100	31.58 ± 1.10%	31.58 ± 1.10%	-
	200	14.60 ± 0.70%	14.60 ± 0.70%	-
BCG	12.5	66.33 ± 1.96%	-	66.33 ± 1.96%
	25	49.39 ± 1.43%	-	49.39 ± 1.43%
	50	33.29 ± 1.49%	-	33.29 ± 1.49%

	100	19.73 ± 1.67%	-	19.73 ± 1.67%
	200	6.01 ± 0.21%	-	6.01 ± 0.21%

Note: Data are represented as mean ± SEM of three replicates**.**

Evaluation of Imiquimod-Induced Psoriasis Model Using ABC and BCG

In the IMQ-induced psoriasis model, BCG (50 mg/kg/day) showed significant improvements in skin morphology, with reduced erythema, scaling, and epidermal thickening compared to controls. PASI scores were significantly lower in both ABC and BCG groups, with BCG at 50 mg/kg/day showing the most significant changes (Figures 1 (a), (b)). No mortality or adverse clinical signs were observed, but a significant reduction in body weight was noted in all treated groups.

Evaluation of UV-B Psoriasis Model Using ABC and BCG

In the UV-B model, Clobetasol propionate and high-dose treatments showed a significant reduction in PASI scores from Day 4, while other groups showed reductions by Day 12 and 14, though statistically insignificant (Figures 2 (a), (b).

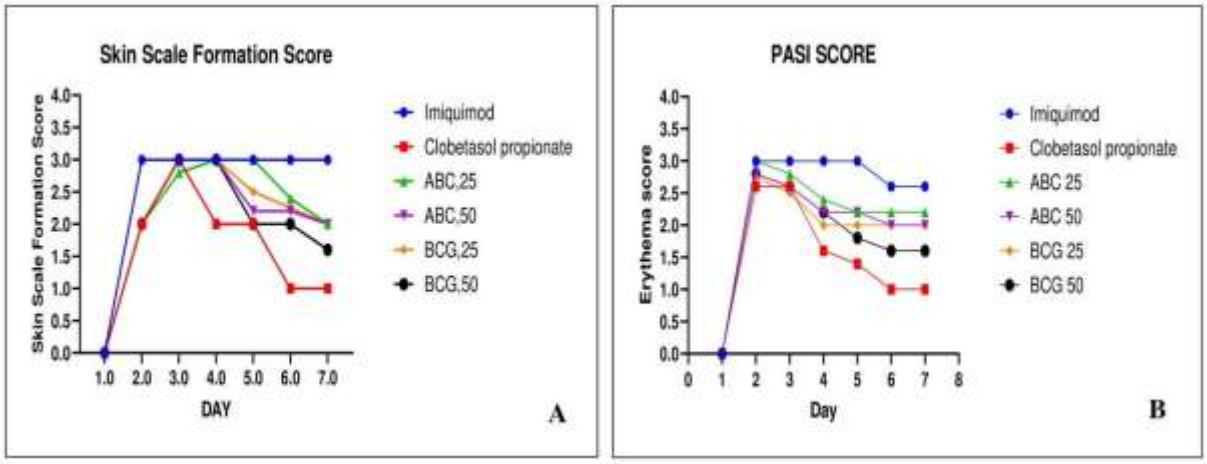

Figure 1: (a) Skin scale formation score and (b) PASI score in the imiquimod-induced psoriasis mouse model.

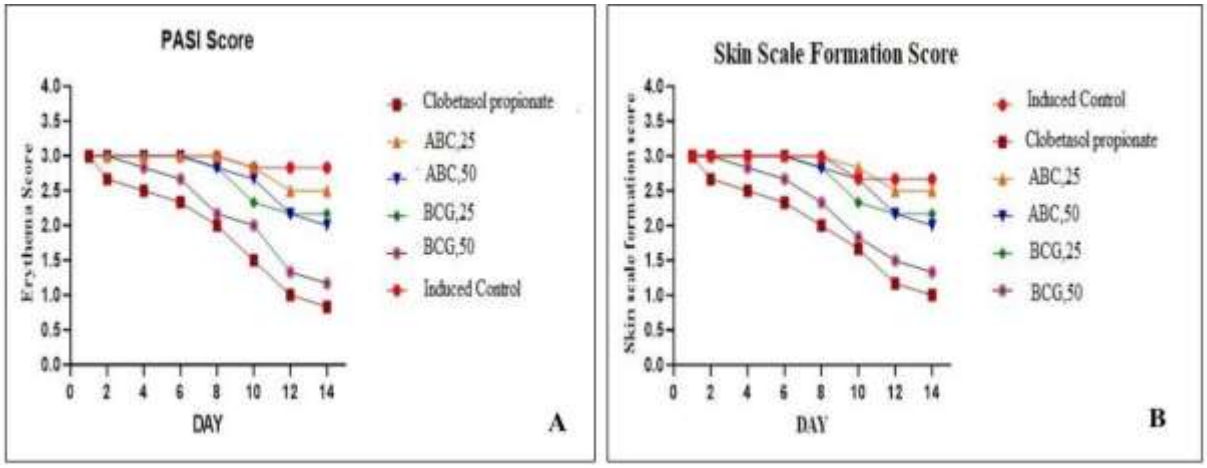

Figure 2: (a) PASI erythema score and (b) skin scale formation score in the UV-B-induced psoriasis model.

Data are represented as mean ± SEM; n = 6. PASI erythema and skin scale formation scores were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. $^{a}P < 0.01$ compared to the Vehicle Control Group; $^{b}P < 0.05$ compared to the Psoriasis Control Group.

Molecular Biomarker Analysis of Psoriasis-Induced Conditions

1. Cytokine Expression Analysis: IL-17 and IL-23

In the imiquimod-induced psoriasis model, the expression levels of IL-17 and IL-23 in skin tissues were significantly elevated in the psoriasis control group compared to the negative control group. Both ABC and BCG formulations effectively reduced these elevated cytokine levels, with results comparable to the standard drug clobetasol (Table 5). However, the lower dose of ABC did not show significant changes, suggesting a dose-dependent efficacy. The negative control group did not exhibit any increase in cytokine levels, confirming the absence of inflammatory conditions.

2. Molecular Biology Analysis of TNF-α, IFN-γ, and VEGF-A

In psoriasis-induced HaCaT cells, the expression levels of TNF-α, IFN-γ, and VEGF-A were significantly elevated in the psoriasis control group compared to the negative control group. Both ABC and BCG formulations effectively reduced these elevated biomarker levels, showing results comparable to the reference drug Asiaticoside(Table 6). The lower dose of ABC exhibited limited inhibitory effects, again indicating dose dependency.

Table 5: Effects of Treatments on IL-17 and IL-23 Levels in Psoriasis-Induced Conditions

Treatment Condition	IL-17 (pg/ml)	IL-23 (pg/ml)
Negative Control	22.66 ± 0.48	11.58 ± 0.97
Psoriasis Control	37.81 ± 2.07 (a)	20.34 ± 0.93 (a)
P+ Clobetasol	22.08 ± 1.36 (b)	14.44 ± 1.19 (b)
P+ ABC-25	37.20 ± 1.65	18.52 ± 0.75
P+ ABC-50	31.02 ± 1.67 (b)	16.34 ± 0.84 (b)
P+ BCG-25	30.61 ± 1.16 (b)	17.53 ± 1.21 (b)
P+ BCG-50	21.43 ± 1.30 (b)	13.76 ± 1.47 (b)

Table 6: Impact of Treatments on IFN-γ, TNF-α, and VEGF-A Levels in Psoriasis-Induced Conditions

Treatment Condition	IFN-gamma (pg/ml)	TNF-alpha (pg/ml)	VEGF-A (pg/ml)
Negative Control	333.01 ± 31	73.11 ± 6.46	65.72 ± 2.37
Psoriasis Control	3659.06 ± 130.5 (a)	1608.42 ± 52.52 (a)	526.64 ± 18.88 (a)
P + Asiaticoside (20 µg/ml)	1102.97 ± 59.63 (b)	916.69 ± 23.18 (b)	156.38 ± 5.52 (b)
P + ABC (50.35 µg/ml)	1347.69 ± 48.98 (b)	761.88 ± 16.27 (b)	122.60 ± 5.01 (b)
P + BCG (24.98 µg/ml)	911.27 ± 64.51 (b)	494.34 ± 20.59 (b)	77.78 ± 4.79 (b)

Data is expressed as mean ± SD; n = 6; Data were analyzed using one-way ANOVA followed by Tukey's multiple comparison test; $^{a}P < 0.01$ compared to Vehicle control group; $^{b}P < 0.05$ compared to the Psoriasis control group.

Histological analysis

Iminoquid-induced psoriasis mouse model

In the Imiquimod-induced psoriasis mouse model, severe abscesses and scab formation were observed in the epidermis, along with acanthosis and mononuclear infiltration in IMQ-treated mice. In contrast, normal skin morphology was observed in the BCG (50 mg/kg/day) treatment group, and mild acanthosis and mononuclear cell infiltration were seen in ABC-treated mice (25 mg/kg/day) (Figure 3 a-f).

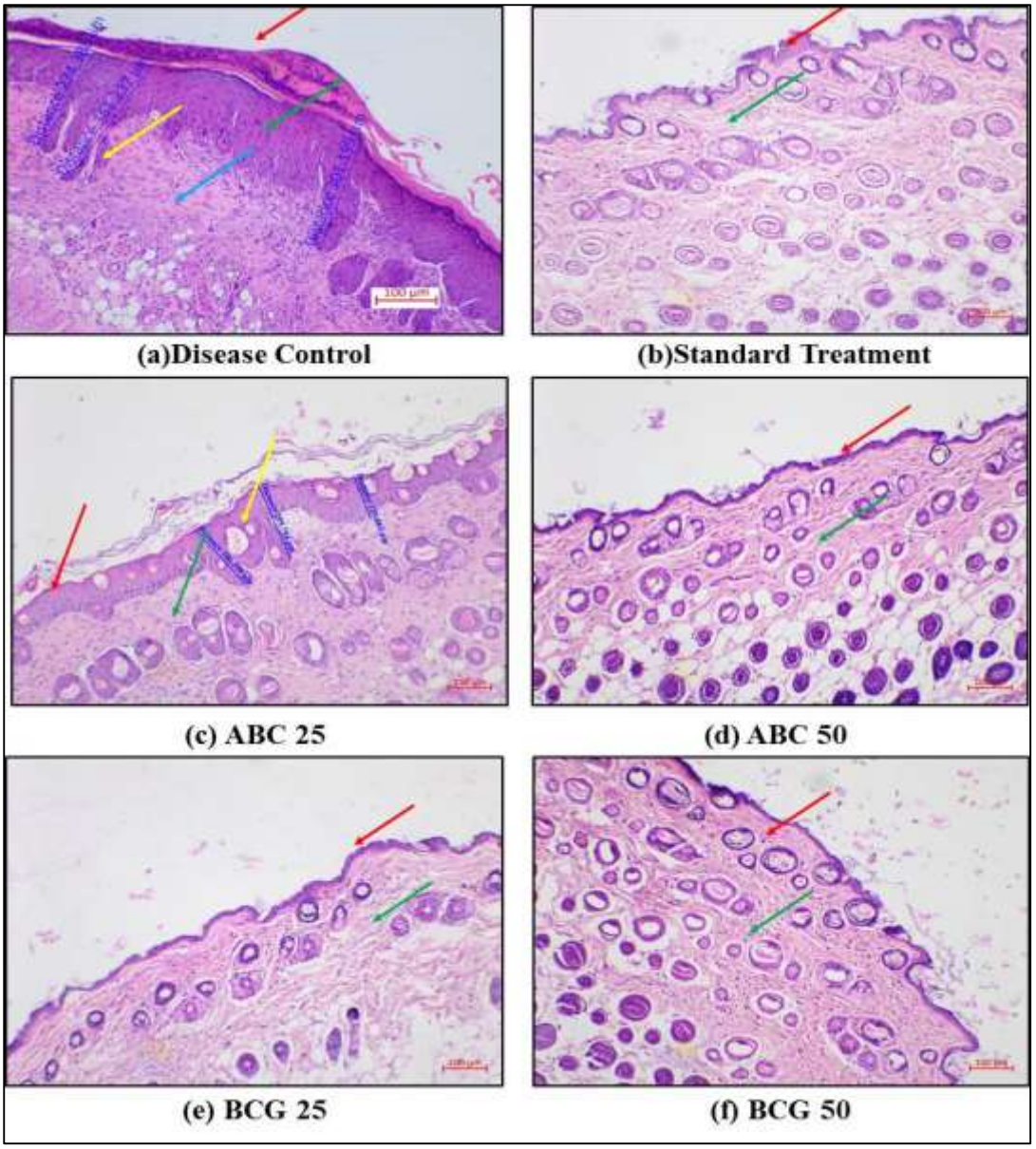

Figure 3. - Histological Images of Skin Sections from Iminoquid-Induced Psoriasis Mouse Model

In histopathological analysis of the Imiquimod-induced psoriasis mouse model, Group 1 (Disease Control) exhibited severe epidermal acanthosis, hyperplasia, and mononuclear infiltration. Group 2 (Standard Treatment) showed normal skin morphology. Group 3 (ABC 25 mg/kg) displayed mild epidermal hyperplasia, while Groups 4 (ABC 50 mg/kg), 5 (BCG 25 mg/kg), and 6 (BCG 50 mg/kg) exhibited normal epidermal and dermal layers. These results suggest that both ABC and BCG treatments maintain skin integrity, with BCG demonstrating the most consistent efficacy (Figure 3).

Skin model induced by UV-B rays

Multifocal severe abscess with scab formation was noticed in the keratin layers acanthosis was observed in the epidermis of the skin and severe mono nuclear infiltration was observed in UV-B treated mice, whereas in positive control and test item treated BCG at 50 mg/kg/day mice there was normal morphology was observed. In ABC-treated mice at 25 mg/kg/day, mild acanthosis and monnuclear cell infiltration were observed (Figure 4 a- f).

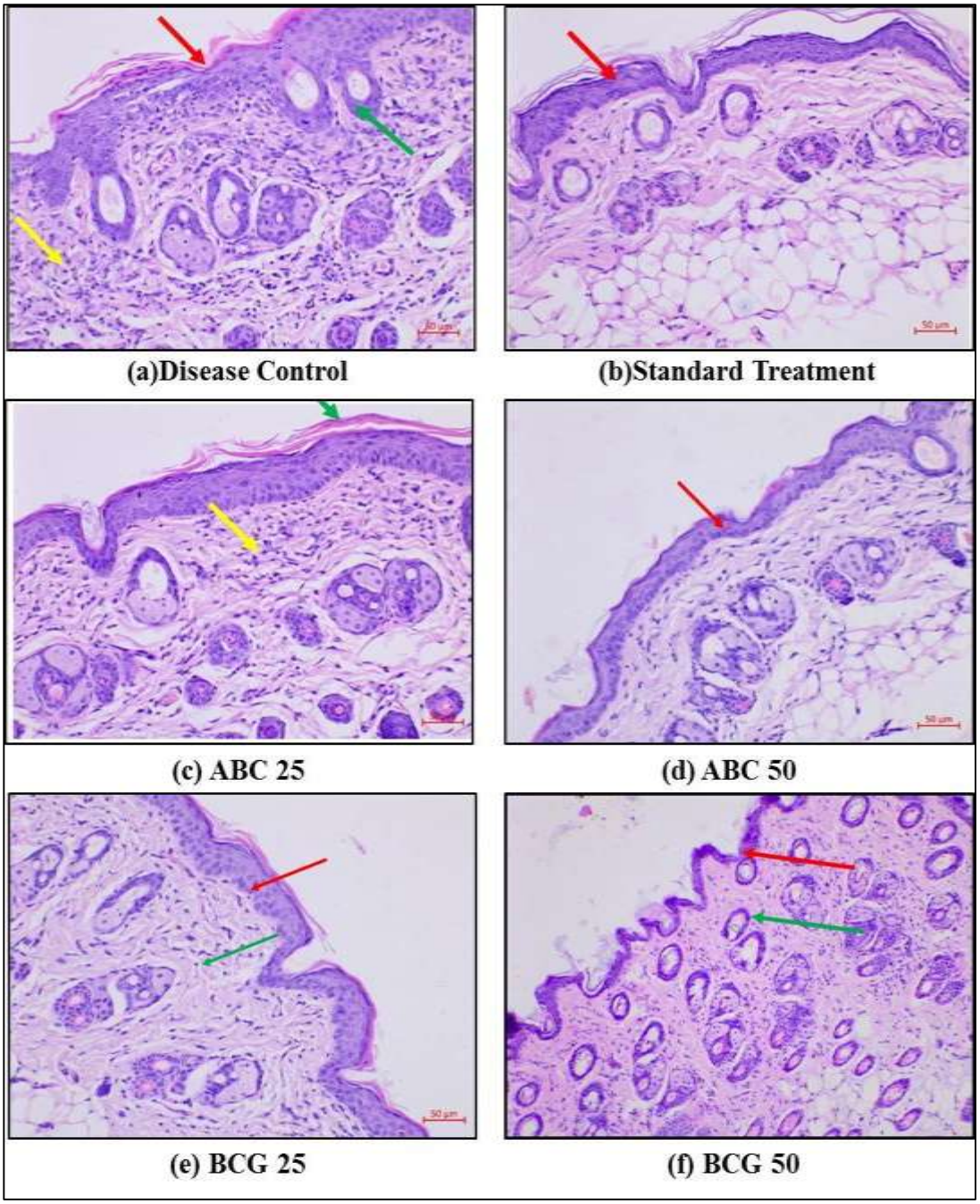

Figure 4.- Histological Images of Skin Sections from UV-B-Induced Psoriasis Model
Histopathological analysis of tissue at 200x magnification, Group 1 (Disease Control) exhibited moderate epidermal hyperplasia, thickening of the stratum basale and stratum spinosum, rete ridge clubbing, and infiltration of neutrophils, eosinophils, and lymphocytes in the dermis. Group 2 (Standard Treatment) showed normal epidermal and dermal morphology, confirming effective recovery. Group 3 (ABC 25 mg/kg) displayed mild acanthosis with parakeratosis and mild fibroblast proliferation, while Group 4 (ABC 50 mg/kg) showed mild hyperplasia. Groups 5 and 6 (BCG 25 and 50 mg/kg) maintained normal skin architecture, emphasizing the superior efficacy of BCG formulations. (Figure 4).

Discussion

Psoriasis, a chronic, immune-mediated inflammatory skin illness, affects about 2-3% of the world's population, with Caucasians having a higher frequency. It causes a huge worldwide health burden due to its complex aetiology and impacts the quality of life of the affected. The

increasing interest in plant-based therapies for chronic inflammatory conditions like psoriasis reflects a growing preference for safer, natural alternatives targeting multiple pathways involved in disease pathogenesis. This shift underscores the potential of herbal treatments in managing psoriasis while minimizing side effects.[21] Given the increasing concerns about the side effects of systemic and topical therapies for psoriasis, the use of herbal treatments has gained popularity due to their availability, cost-effectiveness, and lower risk of adverse effects. As noted by the overview of herbal treatments for psoriasis, many plants exhibit promising therapeutic properties, which could offer valuable alternatives for safer psoriasis management.[22]The present study investigated the therapeutic potential of polyherbal formulations ABC and BCG in managing psoriasis-like skin conditions through a comprehensive approach, including in vitro, in vivo, ex vivo, and physicochemical evaluations, highlighting their potential in addressing the multifaceted nature of psoriasis.

The polyherbal formulations were created by selecting plant extracts renowned for their anti-inflammatory, antioxidant, and immunomodulating capabilities. The results of this study not only affirm the therapeutic efficacy of polyherbal formulations ABC and BCG in managing psoriasis but also suggest broader applications of their phytochemical constituents in other chronic and inflammatory conditions. The active compounds, including curcumin, azadirachtin, and berberine, have demonstrated significant potential in addressing various diseases due to their anti-inflammatory, antioxidant, and immunomodulatory properties.[23] Also, these plants have shown efficacy in controlling autoimmune disorders such as rheumatoid arthritis and inflammatory bowel disease, as they target key cytokines like TNF-α and IL-17, which are also implicated in these diseases.[24] Furthermore, the role of these phytochemicals in suppressing VEGF-A and modulating angiogenesis underscores their potential in cancer management, particularly in preventing tumor progression.[25] Their ability to promote wound healing, reduce oxidative stress, and enhance collagen synthesis extends their utility to dermatological applications, including eczema, dermatitis, and wound care.[26] In addition, these plants' anti- microbial activity against fungal and bacterial pathogens highlights their relevance in treating skin infections, such as candidiasis and dermatophytosis.[27]

The physicochemical characterization of the formulations demonstrated their suitability for topical application. The pH values were maintained within the physiological range of 5.5–6.5, optimizing compatibility with the skin and minimizing irritation. Viscosity and Spreadability assessments confirmed the ease of application, a critical factor for patient adherence. Homogeneity studies showed a uniform distribution of active constituents, ensuring consistent therapeutic efficacy, while stability tests under varied conditions validated the formulations' robustness and shelf-life suitability.[28]

In vivo investigations utilized two well-established models to simulate psoriatic-like conditions. The IMQ- induced psoriasis model in mice effectively replicated clinical manifestations of psoriasis, such as erythema, scaling, and epidermal hyperplasia.[29] Treatment with formulations ABC and BCG significantly alleviated these symptoms, as evidenced by reduced PASI scores. Histopathological analyses further corroborated these findings, revealing decreased inflammatory infiltrates, restoration of normal epidermal architecture, and diminished keratinocyte hyperproliferation in the treated groups. Parameters such as epidermal thickness, hyperkeratosis, parakeratosis, and dermal inflammatory cell infiltration were markedly reduced in animals treated with the polyherbal formulations compared to the untreated group. Moreover, the suppression of TNF-α, IFN-γ, and VEGF-A was observed in treated groups, reflecting the formulations' ability to modulate critical inflammatory and angiogenic pathways involved in psoriasis.

The UV-B-induced skin inflammation model in rats provided complementary evidence of the formulations' anti- inflammatory properties.[30] Prolonged UV-B exposure induced pronounced erythema, epidermal thickening, and inflammatory cell infiltration, mimicking psoriasis-

related inflammation. Treatment with the polyherbal formulations resulted in consistent reductions in PASI scores, with significant improvements observed from Day 4 onward. By the conclusion of the study, both formulations exhibited comparable efficacy to the standard treatment with clobetasol propionate, particularly at higher doses of formulation BCG. Histopathological evaluation in the UV-B model confirmed similar improvements, with the treated groups showing a marked reduction in inflammatory markers and restoration of dermal tissue integrity.

Histopathological results across both models demonstrated clear distinctions between treated and untreated groups. Control groups exhibited normal skin architecture with intact stratum corneum and organized epidermal layers, while induced groups displayed significant pathological changes, including hyperkeratosis, parakeratosis, and infiltration of inflammatory cells. In the test groups treated with formulations ABC and BCG, the epidermal and dermal structures were restored, with reduced keratinocyte hyperproliferation, minimal infiltration of inflammatory cells, and normalized angiogenesis. These findings underscore the role of the polyherbal formulations in alleviating inflammation, regulating cytokine expression, and promoting tissue repair.

The combined use of IMQ and UV-B models offered a comprehensive evaluation of the formulations, demonstrating their efficacy in mitigating both structural and inflammatory aspects of psoriatic conditions. The observed therapeutic effects were further supported by sustained drug release profiles, which enhance the bioavailability of active constituents, and the robust physicochemical properties of the formulations, which ensure consistent performance during application and storage.

In conclusion, the findings of this investigation confirm the efficacy, safety, and stability of polyherbal formulations ABC and BCG in treating psoriasis-like skin disorders. By addressing key pathological mechanisms, including inflammatory cytokine modulation and epidermal repair, these formulations present a promising therapeutic option for psoriasis management. Future research, including clinical trials and molecular mechanism studies, is warranted to fully realize their potential and translate these findings into clinical applications.

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