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Therapeutic Potential of Piper Betle Gel in the Management of Imiquimod-Induced Psoriasis: A Focus on Inflammatory Pathway Modulation

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ABSTRACT

Psoriasis, especially plaque psoriasis, is a chronic inflammatory skin condition characterized by red, scaly plaques that significantly impact patients' quality of life. The imiquimod (IMQ)-induced psoriasis model in mice has become crucial for studying the disease's underlying mechanisms and evaluating new treatments. IMQ, a synthetic immune-response modifier, induces psoriasis-like symptoms by activating inflammatory pathways, particularly IL-17 and IL-23, which are central to the disease's progression. Piper betle, a medicinal plant known for its anti-inflammatory, antioxidant, and antimicrobial properties, presents a natural therapeutic option. Recent studies indicate that Piper betle gel effectively reduces erythema, scaling, and inflammation in IMQ-induced psoriasis models. The bioactive compounds in the gel, such as flavonoids, alkaloids, and polyphenols, are thought to modulate inflammatory pathways like the TLR7/8-NF-κB axis, potentially reducing cytokine activity and alleviating psoriasis symptoms. This study examines the effectiveness of Piper betle gel for IMQ-induced psoriasis, proposing it as a promising alternative to conventional treatments. Findings underscore the need for further clinical trials to confirm its potential for managing human psoriasis, which could broaden therapeutic options for this chronic condition.

Keywords: Psoriasis, Imiquimod, Inflammasomes, IL-17, Piper betle

Introduction

Psoriasis is a chronic, immune-mediated skin disorder affecting roughly 2-3% of the global population, with plaque psoriasis being the most common form [1]. It typically appears as raised, red patches covered in silvery scales, often located on the elbows, knees, scalp, and lower back. These lesions are not only physically uncomfortable—causing itching, pain, and even cracking or bleeding—but also take a toll on patients' emotional and psychological well-being, significantly impacting quality of life [1,2].

Psoriasis likely results from a combination of genetic, environmental, and immune system factors. Family history can increase the risk, pointing to a genetic predisposition, while external triggers like stress, infections, injuries, and some medications can exacerbate symptoms [3,4]. The pathogenesis of psoriasis involves complex interactions between these factors, with T cells becoming hyperactive and producing inflammatory cytokines—particularly IL-17, IL-23, TNF-α, and IFN-γ. This cascade promotes rapid keratinocyte proliferation, thickening the skin and forming psoriatic plaques. The IL-17/IL-23 axis plays a particularly central role, making it a target for recent therapeutic developments [3,5].

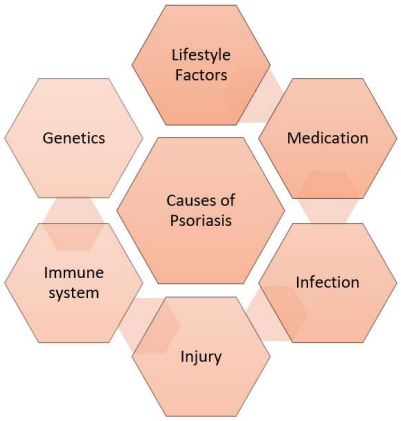


Figure No: 1 Causes of Psoriasis

Imiquimod (IMQ), a synthetic immune-response modifier, is commonly used to induce psoriasis-like symptoms in animal studies, especially in rats. This model closely replicates the immune and histological features of human plaque psoriasis, making it a valuable tool for understanding the disease and evaluating new treatments. When applied topically, IMQ activates Toll-like receptors (TLR7/8), triggering the NF-κB pathway and releasing pro-inflammatory cytokines, including IL-17 and IL-23, which contribute to the inflammation and keratinocyte hyperproliferation characteristic of psoriatic lesions. The IMQ-induced model is particularly useful for testing anti-inflammatory agents and investigating the mechanisms behind psoriasis [6–8].

There is growing interest in natural treatments for psoriasis, especially those derived from medicinal plants. Piper betle, also known as betel leaf, has been used in traditional medicine across various cultures for its anti-inflammatory, antioxidant, and antimicrobial properties. Rich in bioactive compounds like flavonoids, alkaloids, and polyphenols, Piper betle shows promise in treating inflammatory skin conditions, including psoriasis [9–12].

Studies have highlighted the potential of Piper betle gel in managing IMQ-induced psoriasis. Its bioactive compounds may inhibit key inflammatory pathways, like NF-κB activation, thus reducing the release of cytokines such as IL-17 and IL-23. The gel's antioxidant properties also help reduce oxidative stress, which is known to worsen skin inflammation in psoriasis. By neutralizing free radicals, Piper betle gel protects keratinocytes from oxidative damage, maintaining skin barrier integrity and supporting healing of psoriatic lesions.

Preclinical studies reveal that Piper betle gel can significantly reduce erythema, scaling, and lesion thickness in IMQ-treated rats, pointing to its potential in relieving psoriasis symptoms. The gel's multifaceted effects—targeting inflammation, oxidative stress, and keratinocyte proliferation—suggest it may become a valuable addition to available psoriasis therapies.

However, more research is needed to fully understand Piper betle gel's mechanisms and verify its efficacy and safety in humans. Clinical trials are crucial for determining the appropriate dosage, formulation, and treatment duration to achieve optimal results. Long-term studies are also warranted to explore its potential interactions with other psoriasis treatments and any effects of prolonged use.

In conclusion, Piper betle gel represents a promising natural treatment for plaque psoriasis, particularly for cases induced by imiquimod. Its anti-inflammatory, antioxidant, and antimicrobial effects address several key aspects of psoriasis pathophysiology. Continued research may establish Piper betle gel as a holistic, safer alternative for managing psoriasis, potentially improving patient outcomes and quality of life.

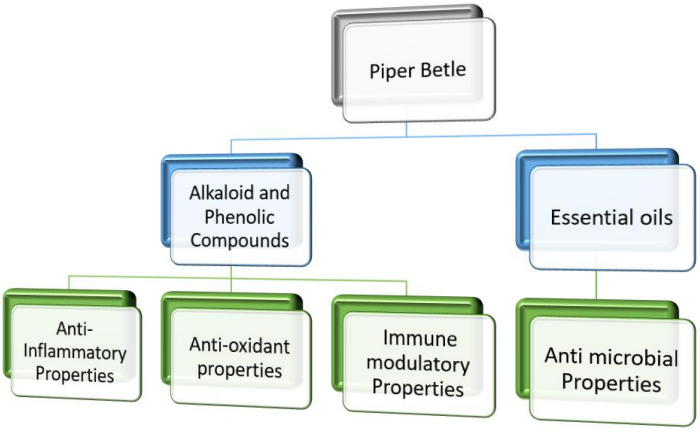


Figure No: 2 Active Components and Therapeutic Properties of Piper betle

Pathogenesis of Psoriasis

Psoriasis is a persistent inflammatory skin disorder that results from the immune system's overactivity, causing abnormal keratinocyte growth and disrupting the skin's natural balance. The origins of psoriasis are rooted in a complex interplay between genetic factors, immune system malfunctions, and environmental factors. Central to this process is the role of inflammasomes—critical elements of the innate immune response involved in recognizing harmful stimuli and triggering inflammation [2,13].

Inflammatory Response in Psoriasis

Innate and Adaptive Immunity Interaction

In psoriasis, the inflammatory process is sustained by both the innate and adaptive arms of the immune system. The initial response is primarily driven by the innate immune system, involving cells like epithelial cells, dendritic cells, macrophages, and polymorphonuclear leukocytes. These cells detect danger signals via pattern recognition receptors (PRRs), setting off an inflammatory response. Meanwhile, T cells—key players in the adaptive immune system—are also deeply involved in psoriasis, contributing to a persistent inflammatory state through complex interactions with innate immunity [14,15,58].

Inflammasome Activation

Inflammasomes are multi-protein complexes that sense inflammatory signals and promote the release of pro-inflammatory cytokines. Their activation is significant in psoriasis, influencing immune response dynamics in both innate and adaptive immunity [16,17,59].

1. **NLRP3 Inflammasome**: Reacts to stimuli like pathogens, ATP, and danger signals. Its activation leads to caspase-1, which converts pro-IL-1β and pro-IL-18 into active forms IL-1β and IL-18, thus promoting inflammation and immune cell migration [18].
2. **NLRP1 Inflammasome**: Genetic variants of NLRP1 have been linked to psoriasis, suggesting a role in keratinocyte activation and disease susceptibility [19].
3. **AIM2 Inflammasome**: Recognizes cytosolic DNA from pathogens or damaged cells, triggering IL-1β and IL-18 production. This inflammasome is active in psoriasis, particularly within keratinocytes [20,21].

Cytokine Production and Keratinocyte Proliferation

Cytokines like IL-1β and IL-18, produced via inflammasome activation, amplify inflammation by promoting keratinocyte proliferation and attracting immune cells, especially Th17 cells. Th17 cells produce IL-17, which further intensifies the inflammatory response and keratinocyte growth abnormalities.

Signaling Pathways

- **TLR7/8 Signaling**: Toll-like receptors (TLRs) like TLR7 and TLR8 recognize microbial and endogenous signals, activating the MyD88 pathway. This cascade ultimately leads to NF-κB activation and cytokine production, contributing to psoriatic inflammation [22,23,55,56].
- **NLRP3 and NF-κB Pathways**: NLRP3 inflammasome activation is interconnected with TLR signaling, where TLR7/8 enhances NLRP3 activity, fueling the persistent inflammation in psoriasis [24,57].

Psoriasis pathogenesis, therefore, involves a complex relationship between the immune system's two branches, with inflammasomes playing a vital role in initiating and sustaining inflammation. Further research is warranted to fully explore inflammasome dysregulation and optimize therapeutic approaches targeting these pathways.

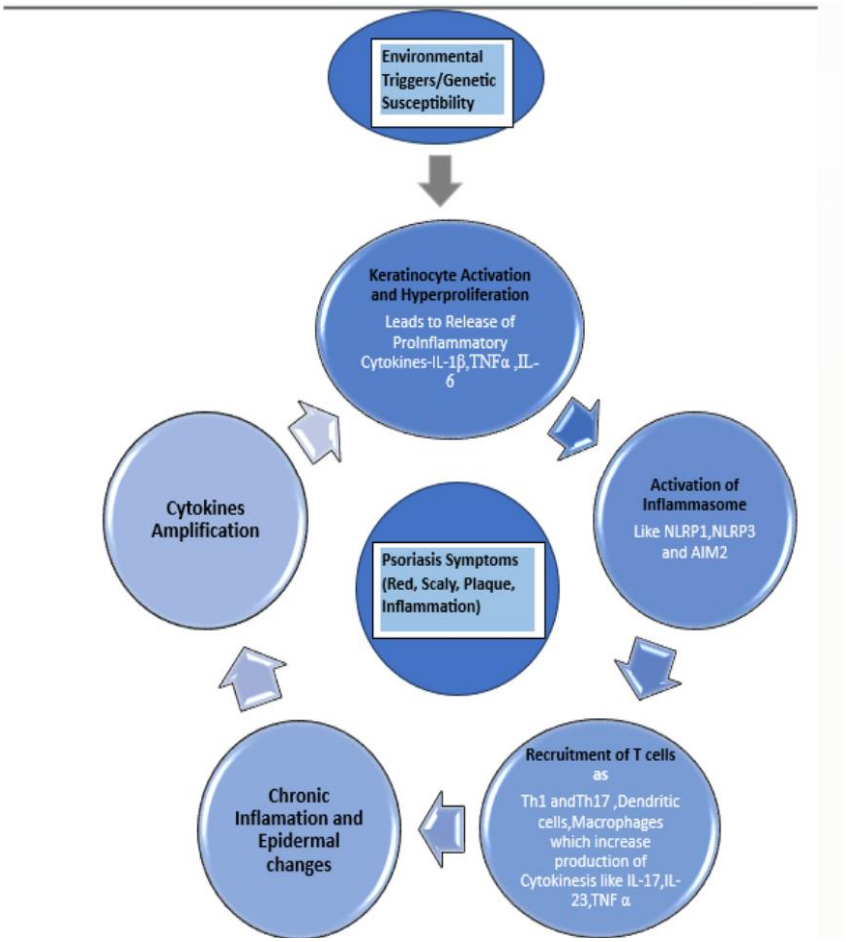


Figure No: 3 Cycle of Psoriasis Pathogenesis: Keratinocyte and Inflammasome Activation

PLANT PROFILE *(Piper Betle)*

Figure No: 4 *Piper betle*

Piper betle

Piper betle, commonly known as Betel or Sireh, is a vine with distinctive heart-shaped leaves. Widely cultivated for its leaves, the plant is used in traditional medicine, religious rituals, and often chewed with slaked lime in certain cultures [25,26].

Taxonomical Classification

- Family: Piperaceae

- Synonyms: Piper siriboa L.
- Common Names: Betel, Betel Vine, Betel Pepper, Pan, Sireh [27]
- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Piperales
- Genus: Piper
- Species: Piper betle L.

Common Names Across Languages

- English: Betel leaf
- Hindi: Paan
- Tamil: Vetrilai
- Malayalam: Vettila
- Bengali: Paan

Description

- **Habit**: Perennial, evergreen vine, growing up to 1–4 meters, mainly in tropical and subtropical regions.
- **Leaves**: Glossy, heart-shaped, aromatic leaves ranging from dark green to yellowish-green.
- **Stems**: Woody at the base, green and flexible at the tips.
- **Flowers**: Small, white to yellowish inflorescences.
- **Roots**: Adventitious roots develop when stems contact the soil [25].

Habitat and Distribution

- **Geographical**: Indigenous to Southeast Asia, widely grown in India, Sri Lanka, Thailand, Malaysia, and Indonesia.
- **Climate**: Thrives in warm, humid conditions, typically in shaded areas with well-drained soil [9,10].

Chemical Constituents

- **Phenolic Compounds**: Hydroxychavicol, chavibetol, eugenol.
- **Flavonoids**: Quercetin, catechin, luteolin.
- **Alkaloids**: Piperine.
- **Essential Oils**: Terpenes, safrole, betel oil.
- **Other Components**: Carotene, thiamine, riboflavin, niacin, ascorbic acid [10,11].

Medicinal Properties

Piper betle is valued for its therapeutic benefits:

- **Antimicrobial**: Strong antibacterial, antifungal, and antiviral actions.
- **Antioxidant**: High in free radical scavengers.
- **Anti-inflammatory**: Reduces inflammation and swelling.
- **Antidiabetic**: Shown to lower blood glucose.
- **Anti-cancer**: Potential for anticancer effects, particularly in oral cancers.
- **Gastroprotective**: Treats digestive issues like bloating.
- **Wound Healing**: Promotes healing [11,28].

Traditional Uses

- **Chewing with Areca Nut**: Commonly chewed with areca nut and lime as a mouth freshener.
- **Folk Medicine**: Treats respiratory issues, wounds, and skin conditions.
- **Cultural Role**: Used in religious and social ceremonies, especially in South Asia [9,10,54].

Pharmacological Activities

1. **Antimicrobial**: Inhibits pathogenic bacteria and fungi.
2. **Anti-inflammatory & Analgesic**: Useful for inflammatory conditions like arthritis.
3. **Antioxidant**: Protects against oxidative stress.
4. **Wound Healing**: Enhances wound recovery.
5. **Anti-cancer Potential**: Induces apoptosis in cancer cells.

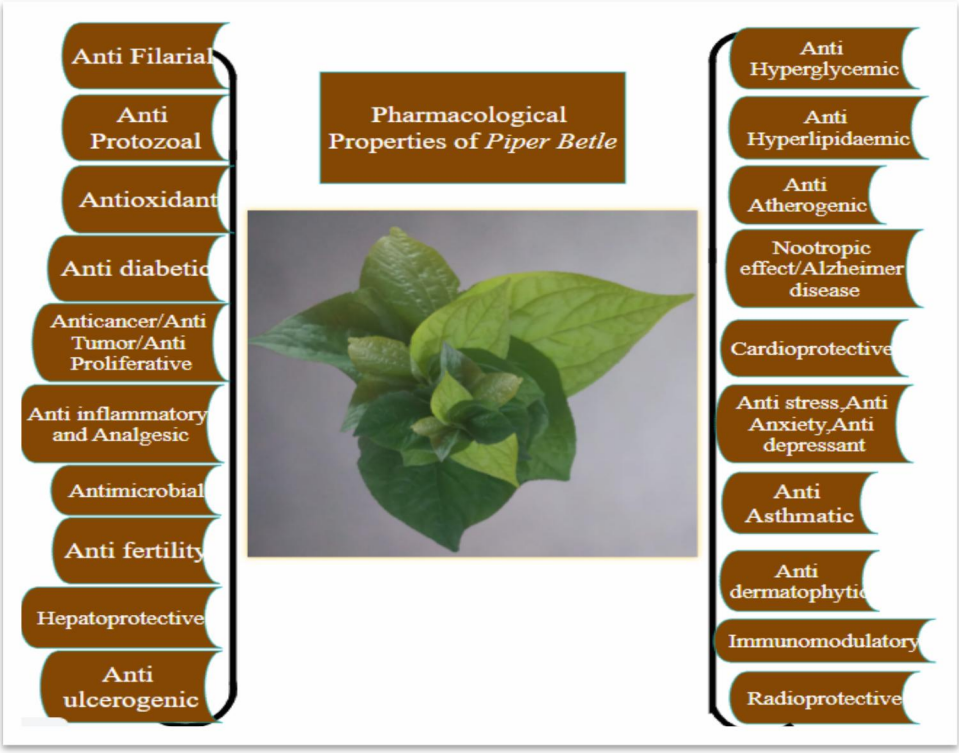


Figure No: 5 Summary of recently reported Pharmacological activities of *Piper betle* in animal models

Recent Research

- **Diabetes Management**: Evidence supports its potential for blood glucose regulation and insulin sensitivity improvement.

Safety and Toxicity

- **General Safety**: Safe in moderate amounts; chronic chewing with areca nut/tobacco raises oral cancer risks.
- **Toxicity**: Overuse or adulterated leaves may cause oral submucous fibrosis, a pre-cancerous condition [9–11,29].

Antioxidants and the Piperaceae Family

Antioxidants delay or prevent cell damage from free radicals by inhibiting oxidation. The Piperaceae family, with its rich antioxidant compounds, has shown promise in managing free radical activity [27,52].

With over 1,500 species, the Piperaceae family has traditional medicinal uses for ailments like infections and diabetes. These plants contain diverse bioactive compounds, including phenolics, flavonoids, alkaloids, tannins, and terpenoids, which contribute to their therapeutic potential in drug discovery [9,53].

MATERIAL AND METHODOLOGY

Figure No: 6 Collection of *Piper betle*

Plant Material

Piper betle, a tropical plant recognized for its medicinal benefits, is abundant in bioactive compounds, including essential oils, polyphenols, flavonoids, and alkaloids. For this study, mature, healthy leaves were selected from Kanda village, Dewas district, known for its rich medicinal flora.

Collection and Preparation

The Piper betle leaves were collected in the morning to help retain their volatile compounds. After thorough rinsing with distilled water to remove surface impurities, the leaves were air-dried in a shaded area to preserve sensitive phytochemicals. Drying continued until the leaves were crisp, ensuring optimal retention of bioactive constituents.

Authentication

The plant material, collected in India in November, was authenticated by the Department of Botany at A.P.S. University, Rewa, under Dr. S.N. Dwivedi's supervision. Identified as Piper betle through morphological examination, it was assigned the voucher specimen number J/Bot/2024-PB-L/40, with a sample deposited in the herbarium for future reference.

Soxhlet Extraction Process (SEP)

Soxhlet extraction was used to obtain the plant extract. Here, a solvent, heated in a boiling flask, produces vapors that pass-through plant material held in a thimble. This setup, which includes a condenser and siphon arm, was employed with different solvents like methanol, ethanol, hexane, or petroleum ether, allowing precise extraction temperatures for various phytochemicals [34].

Figure No: 7 Soxhlet Extraction Process

Soxhlet Extraction (SE) for Piper betle Using Methanol

The collected leaves were air-dried until brittle, then ground into a fine powder, which was placed in a Soxhlet extraction chamber. Methanol, chosen for its effectiveness, was heated to its boiling point (64.7°C) in the flask below. Methanol vapors condensed, percolating through the plant material and dissolving the bioactives. The solution was siphoned back into the flask, and the cycle repeated for up to 24 hours. After extraction, methanol was removed using a rotary evaporator or by allowing it to evaporate, yielding a concentrated Piper betle extract [27,34].

Percentage Yield Calculation

The yield percentage from Soxhlet extraction was calculated as follows:

$$Percentage\ yield(\%) = \frac{Weight\ of\ Extract}{Initial\ wt\ of\ plant\ material} \times 100$$

Screening for Phytoconstituents in Methanolic Extract of Piper betle

Qualitative Tests

- **Alkaloids**: Dragendorff's reagent indicated alkaloids with a reddish-brown or orange precipitate.
- **Steroids**: Chloroform and sulfuric acid produced a red precipitate, confirming steroids.
- **Flavonoids**: A range of tests, including the ammonia test (yellow), Shinoda test (pink), lead acetate test (yellow precipitate), and sodium hydroxide test (discoloration upon acid addition), identified flavonoids.
- **Terpenoids**: A reddish-brown layer formed with chloroform and sulfuric acid, confirming terpenoids.
- **Carbohydrates**: A brick-red precipitate in Fehling's test confirmed reducing sugars.
- **Glycosides**: Cardiac glycosides were identified through the Keller-Kiliani test, resulting in reddish-brown and bluish-green layers.
- **Saponins**: Foam and Liberman-Burchard tests confirmed saponins with steroidal content.
- **Proteins**: Positive results in the Biuret test (blue) and Xanthoproteic test (yellow precipitate after boiling) indicated proteins.
- **Amino Acids**: A purple/blue reaction in the Ninhydrin test indicated amino acids.
- **Tannins**: FeCl3 and potassium ferricyanide tests confirmed tannins through dark blue/green and deep red colors.
- **Oils and Fats**: Solubility and filter paper tests confirmed the presence of oils and fats [29,35,36].

Quantitative Tests

- **Total Phenolic Content (TPC)**: Determined by the Folin-Ciocalteu method, absorbance was checked at 765 nm and expressed in mg GAE/g [27].
- **Total Alkaloid Content (TAC)**: Alkaloids were measured by mixing the extract with hydrochloric acid, BCG, and a buffer, with absorbance at 470 nm calculated as mg AE/g [37].
- **Total Flavonoid Content (TFC)**: Determined at 415 nm after reaction with aluminum chloride, potassium acetate, and methanol, using a calibration curve [37].

Emulgel Components and Preparation

The emulgel formulation included oils (mineral oil, paraffin), emulsifiers (Tween 80, Span 80), gelling agents (Carbopol 940, HPMC), and pH adjusters. The oil and aqueous phases were mixed separately, then heated to 70-80°C, combined with the active ingredient, and stabilized into a uniform emulgel [38–40].

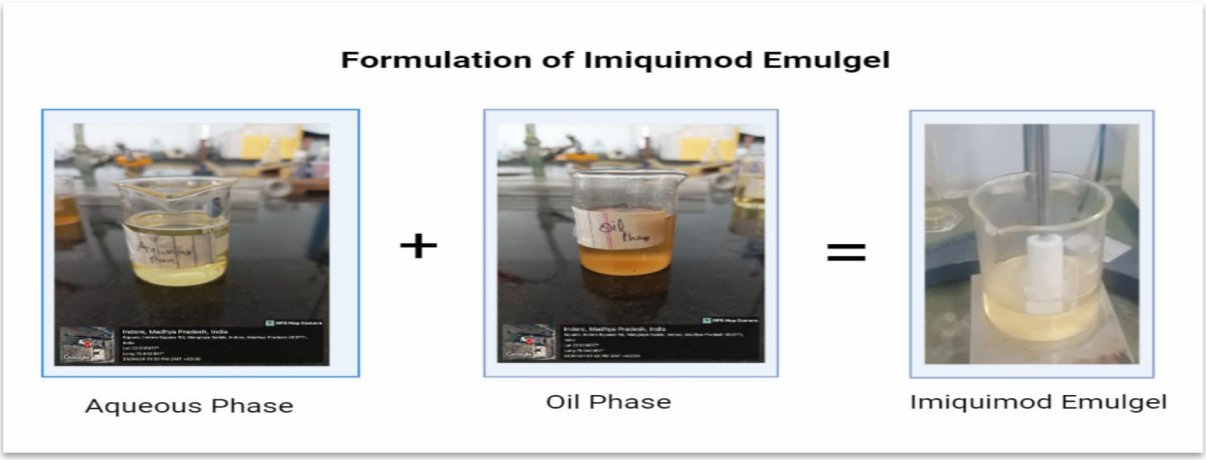


Figure No: 8 Formulation of Imiquimod emulgel

Preparation Method for Piper betle Extract-Containing Gel

The Piper betle gel was prepared by dispersing 1 g of carbopol 934 in 50 mL of water with continuous stirring. Separately, methyl and propyl parabens dissolved in water were combined with propylene glycol and Piper betle extract. The mixture was adjusted to 100 mL with distilled water, blended with the carbopol gel, and pH-adjusted to approximately 6.8-7 for skin compatibility [41].

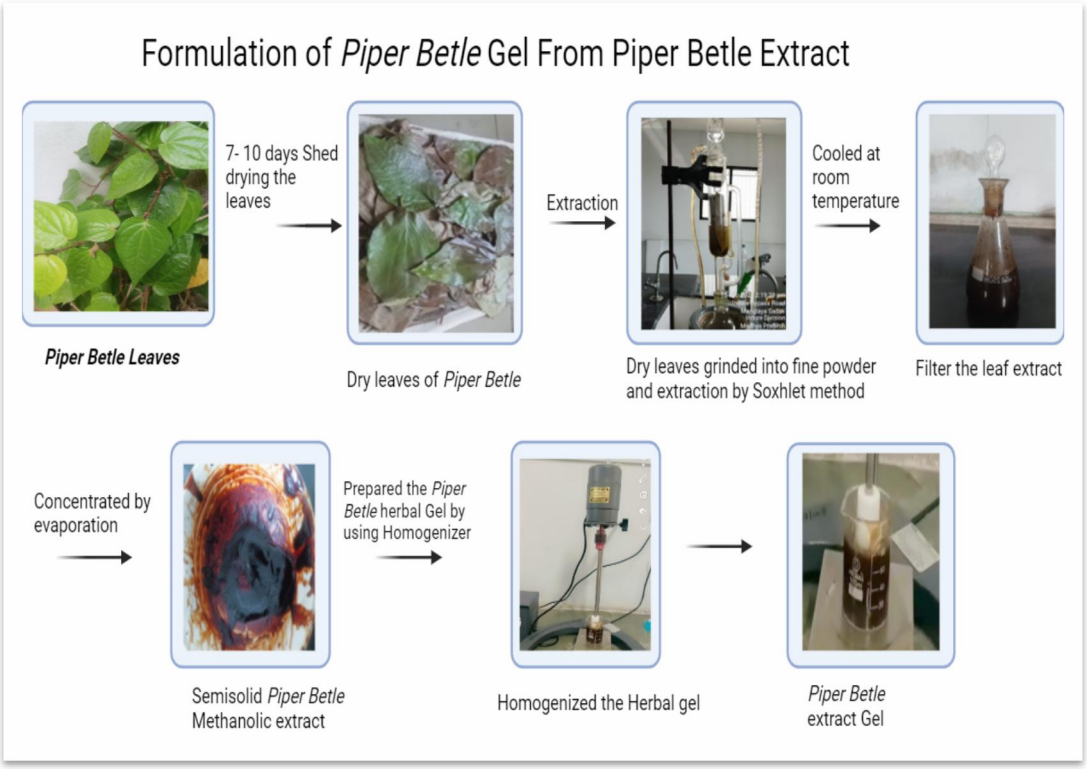


Figure No: 9 Formulation of *Piper betle* Gel from *Piper betle* extract

Evaluation of Imiquimod Emulgel

(a) **Physical Appearance:** The emulgel's consistency, color, and texture were carefully examined through visual inspection, ensuring the formulation was uniform and met the expected standards for appearance.

(b) **pH Measurement:** Using a calibrated pH meter, the pH of the emulgel was assessed to verify its skin compatibility. For this, 1 g of emulgel was dissolved in distilled water, allowed to sit for two hours, and then measured. The pH was ideally around 6.8-7 to ensure it was within a skin-compatible range.

(c) **Rheological Study:** The viscosity of the emulgel was measured using a Brookfield viscometer at 37°C. Approximately 50 mL of the emulgel was tested with spindle numbers 4 or 6, operated at 20 RPM. The viscosity readings were taken after stabilization, with the average value calculated from three trials.

(d) **Drug Content Determination:** To determine the concentration of the drug in the emulgel, a sample was analyzed using UV-Vis spectrophotometry. The sample was first sonicated in methanol, diluted, and its absorbance recorded, with drug content calculated based on a standard calibration curve [39].

Evaluation of Piper betle Gel Formulation

The Piper betle gel formulation was created by incorporating its methanolic extract into a base gel made of either Carbopol 934 or HPMC. The gelling agent was hydrated over 24 hours to form a smooth base, and the Piper betle extract, dissolved in propylene glycol for better solubility, was gradually added. pH adjustment to 6.0–7.0 was achieved with triethanolamine to ensure the formulation was compatible with skin.

(a) **Physical Appearance:** The gel was visually examined for color, consistency, and any signs of air bubbles or particulate matter. Stability was also monitored after 24 hours to check for phase separation or syneresis.

(b) **Viscosity Measurement:** Viscosity was tested with a Brookfield viscometer using spindle number 6 at 25°C. Roughly 50 mL of the gel was measured at 20 RPM, and the average viscosity was calculated from three readings after stabilization.

(c) **Drug Content Analysis:** The concentration of Piper betle extract in the gel was determined via UV-Vis spectrophotometry. A sample (1 g) was dissolved in ethanol, filtered, and the absorbance measured to calculate the drug content from a standard calibration curve.

(d) **Spreadability:** Spreadability was tested by placing 1 g of gel between two glass slides with a 500 g weight on top. After five minutes, the distance the gel spread was measured in centimeters [41].

Calibration and Permeation Studies

λ Max Calculation and Calibration Curve: A 1000 µg/ml stock solution of Imiquimod was prepared in methanol and further diluted to 10 µg/ml for UV spectroscopic analysis across 200-400 nm. The λ max was recorded, and calibration solutions were created. Absorbance readings enabled construction of a calibration curve by plotting concentration against absorbance [42,47].

In Vitro Drug Permeation: The Franz diffusion cell was used to evaluate drug permeation through a membrane. The formulation was placed in the donor compartment, with a buffer solution in the receptor compartment maintained at 37°C. Periodic samples were analyzed to assess permeation rates [42,43,48].

Animal Selection and Toxicity Studies

Animal Selection: Healthy Wistar rats (150-180 grams) were used in the anti-psoriatic studies, acclimated over seven days in a controlled environment with food and water. Ethical approval was obtained from the Institutional Animal Ethics Committee under CCSEA guidelines. **Acute Dermal Toxicity:** Following OECD guideline no. 402, rats received doses of 2000 mg/kg of various extracts, with observations over 14 days. A skin irritation test with methanolic extract was conducted, helping establish appropriate doses for further study [44,45,49].

Species	Rattus norvegicus (Rat)
Stain	Wistar
Weight	(150gm – 180gm)
Age	8-10 weeks
Sex	Either sex
No. of Animals each group	4/Groups
Total Animals	20

Table No: 1 Details regarding the animal

Groups	Treatments	Dose	No. of Animals
G1	Negative Control	_	4
G2	Positive Control	Placebo Carbopol Gel applied Topically	4

G3	Test Group I PB Extract loaded gel	200mg/kg/rat applied Daily topically *PB* Extract Gel	4
G4	Test Group II PB Extract loaded gel	400mg/kg/rat applied Daily topically *PB* Extract Gel	4
G5	Standard Group	Cyclosporine 3 mg/kg/day by oral route	4

Table No: 2 Grouping of Animals

Experimental Design, Materials, and Methods

In Vivo Studies: Thirty mature female Wistar rats (150-180 grams) were obtained from AIPER's animal facility. Acclimated with unrestricted access to water and food, they were prepared following ARRIVE and NIH standards. Approval was granted by the Acropolis Institute of Pharmaceutical Education & Research (AIPER), Square, Indore, Madhya Pradesh, under Institutional Animal Ethics Committee guidelines (Animal Approval Number: AIPER/IAEC/2024/07).

Study Objective: The study aimed to assess the effects of Imiquimod (IMQ)-induced psoriasis in rats, following a protocol where the dorsal skin was shaved to a consistent size of 2.5 cm × 2 cm as per Sakai et al. (2016). Six rats were randomly allocated to five groups (n = 4 per group):

- **Group 1 (Negative Control):** Topically treated with a neutral base cream (vehicle).
- **Groups 2 to 5:** Induced psoriatic lesions with 125 mg of 5% IMQ emulgel (6.25 mg of IMQ) applied to shaved dorsal skin for seven days, as per Smajlović et al. (2021).
    - **Group 2 (Positive Control):** Treated with vehicle gel.
    - **Group 3 (Test Group I):** Received 200 mg/kg/day of Piper betle (PB) extract gel.
    - **Group 4 (Test Group II):** Received 400 mg/kg/day of PB extract gel.
    - **Group 5 (Standard Group):** Received oral Cyclosporine at 3 mg/kg/day as a reference standard.

Treatments were applied topically for seven days, with photographic documentation capturing changes in skin condition by the study's end [46,50].

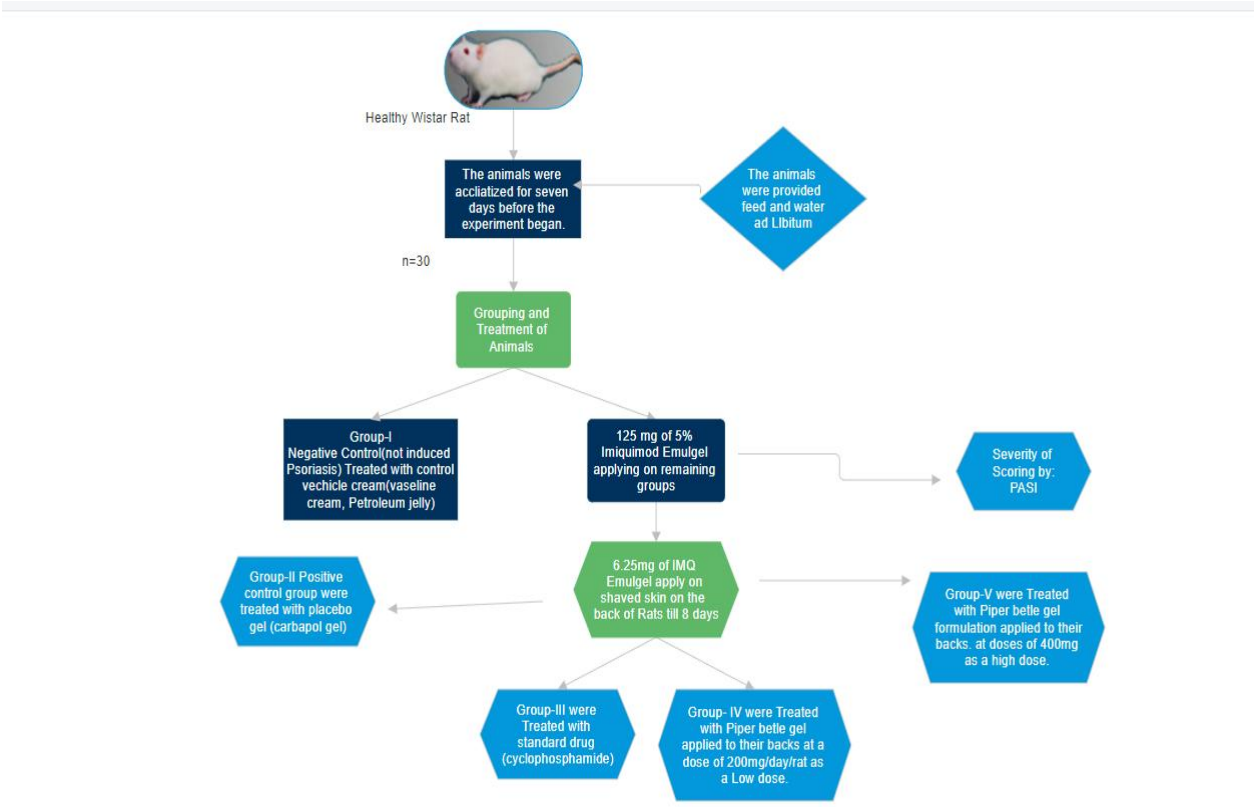
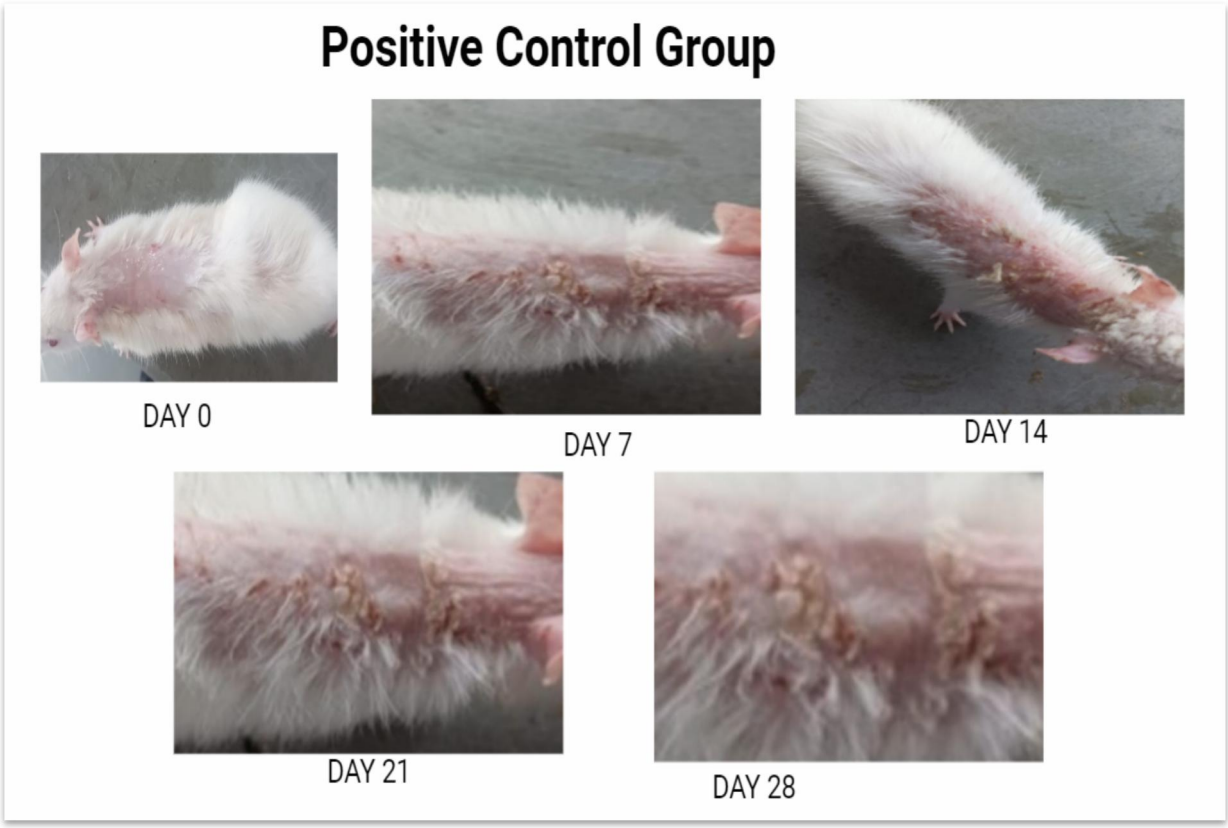
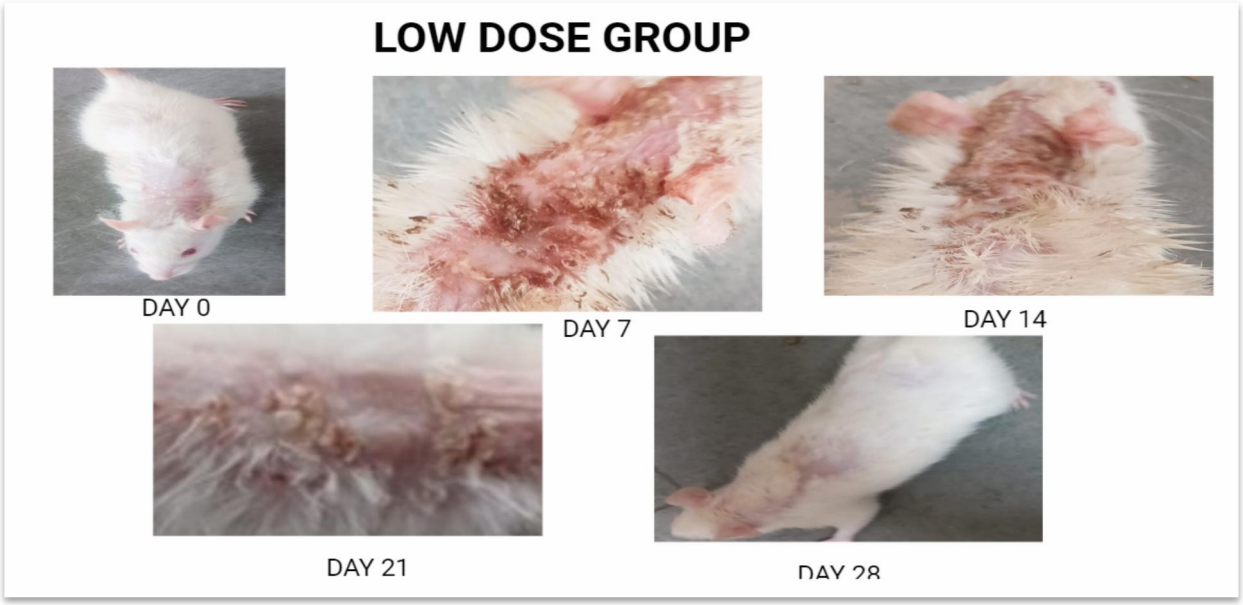


Figure No: 10 Design of IMQ-Induced Psoriasis Model in Rats

PASI Assessment of Skin Inflammation

To gauge inflammation severity, the Psoriasis Area Severity Index (PASI) was employed on day 0 (pre-IMQ) and day 7 (post-IMQ), measuring erythema (redness), scaling, and skin thickness. Each parameter was rated on a 4-point scale (0 = none to 4 = very severe). The area score was excluded, as all rats were treated over a consistent area, resulting in a total PASI score between 0 and 12, indicating inflammation severity [46,51].





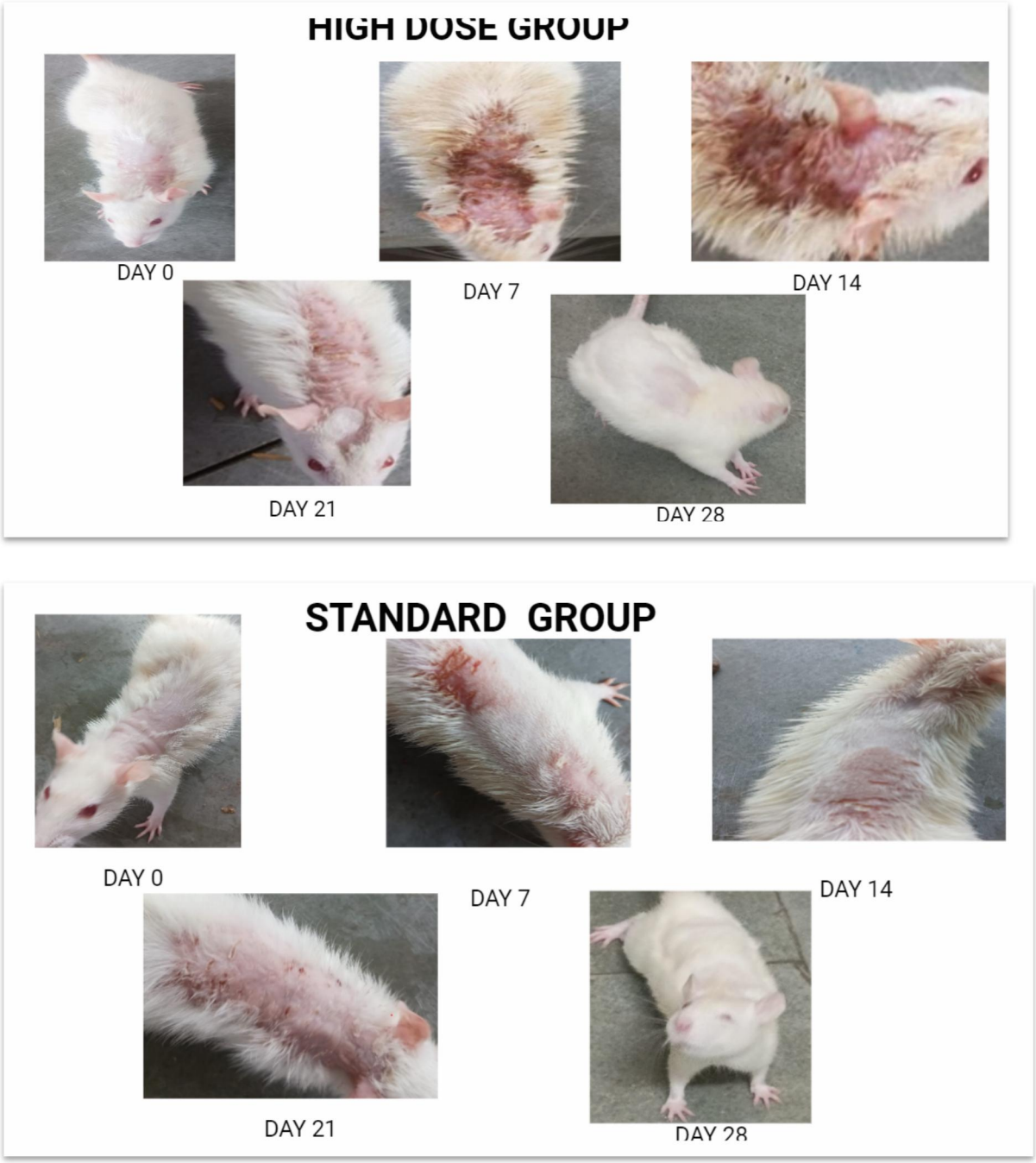


Figure No: 11 Imiquimod Induced Plaque Psoriasis in Different Groups

Results and Discussion

Soxhlet Extraction Process

In this study, Soxhlet extraction was employed to obtain a methanolic extract from the leaves of *Piper betle*, a plant celebrated for its various pharmacological benefits. Methanol was selected as the solvent for its proven effectiveness in extracting a wide range of bioactive compounds. Soxhlet extraction utilizes a cycle of continuous solvent reflux and condensation, which allows for an efficient, exhaustive extraction of soluble compounds. As a polar solvent, methanol is particularly effective at dissolving diverse phytochemicals, including flavonoids, phenolics, and alkaloids. The process yielded approximately 4% extract, underscoring methanol's efficiency in extracting substantial quantities of bioactive compounds.

Property	**Details**	**Observation**	**Implication**
Color	Dark brown to black	The extract exhibits a deep, rich	Indicates the presence of

		color.	concentrated phytochemicals.
Consistency	Viscous liquid	The extract has a thick, syrupy texture.	Suggests a high concentration of soluble compounds.
Odour	Characteristic, herbal aroma	Distinct herbal fragrance.	Reflects the presence of aromatic compounds.
Percentage Yield	4%	The yield of the extract from the starting material.	Demonstrates the efficiency of methanol in extraction
Solvent used	Methanol	Polar solvent used for extraction.	Effective in solubilizing polar bioactive compounds.
Extraction Method	Soxhlet Extraction	Continuous reflux and condensation cycles.	Ensures thorough extraction of soluble compounds.
Bioactive Compounds	Flavonoids, Phenolics, Alkaloids	Key phytochemicals present in the extract.	Indicative of the extract's pharmacological potential.
Purpose of extraction	To extract polar phytochemicals	Targeting compounds with polar properties.	Aims to isolate compounds for pharmacological study.
Application	Pharmacological studies	Utilized for evaluating therapeutic potential	Essential for understanding the medicinal properties.

Table: 3 Characteristics of Methanolic Extract of Piper betle

Phytochemical Screening

The methanolic extract of *Piper betle* was subjected to qualitative analysis to identify the various phytochemical components it contains, many of which have known health benefits. Table 8 presents a summary of these findings, highlighting the presence of several significant compounds:

Figure No: 12 Phytochemical Screening of Methanol Extract of *Piper Betle* (A. Test for Saponins B. Test for Carbohydrate C Test for Flavonoid D. Test for Glycoside E Test for Terpenoids F. Test for Alkaloid)

- **Alkaloids**: These nitrogen-containing compounds serve defensive functions in plants and exhibit a range of pharmacological effects.
- **Flavonoids**: Renowned for their antioxidant properties, flavonoids help shield plants from oxidative stress.
- **Glycosides**: These contribute to energy release via reducing sugars and were detected in the extract.
- **Steroids**: Important for cell membrane structure and metabolism, steroids were also identified.
- **Tannins**: Found in various plant tissues, tannins offer protection against environmental stresses.
- **Terpenoids**: Often used in traditional medicine, terpenoids were detected as well.
- **Carbohydrates**: Essential for energy and metabolic functions, carbohydrates were identified.
- **Volatile Oils**: These compounds, giving *Piper betle* its characteristic aroma, were also present.
- **Saponins**: Known for their surfactant properties, saponins may benefit cholesterol management and immune function.
- **Proteins**: Although detected in smaller quantities, proteins are crucial for diverse biological roles.

Quantitative Phytochemical Analysis of *Piper betle* Methanolic Extract

Quantitative assessments of the methanolic extract provide insights into the concentration of key bioactive compounds, including Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Alkaloid Content (TAC). These measurements are critical to understanding the extract's therapeutic potential.

- **Total Phenolic Content (TPC)**: The TPC of the methanolic extract ranged between 15.1 and 65.2 mg GAE/g, indicating a high presence of phenolic compounds, which are potent antioxidants capable of neutralizing free radicals. The higher end of this range suggests a significant concentration of phenolics, supporting the extract's potential as a rich source of antioxidants.
- **Total Flavonoid Content (TFC)**: The TFC was recorded between 8.3 and 70.4 mg QE/g, suggesting a high flavonoid concentration. Known for their diverse health benefits, including anti-inflammatory and cardioprotective effects, the significant presence of flavonoids enhances the extract's value in therapeutic applications.

- **Total Alkaloid Content (TAC)**: The TAC ranged from 0.5 to 20.4 mg AE/g, reflecting a notable concentration of alkaloids, which are known for their analgesic, antimicrobial, and anti-inflammatory properties.

• S.NO	Phytoconstituents	Methanolic Extract of *Piper betle*
01	Total Alkaloid Content	17.46±0.48mg AE/g
02	Total Flavonoid Content	70.9±0.51mg QE/g
03	Total Phenol Content	65.7±0.48mg GAE/g

Table No: 4 Quantitative assessments of Phytoconstituents

Together, these findings underscore the methanolic extract of *Piper betle* as a promising source of bioactive compounds with strong antioxidant, anti-inflammatory, and therapeutic potential.

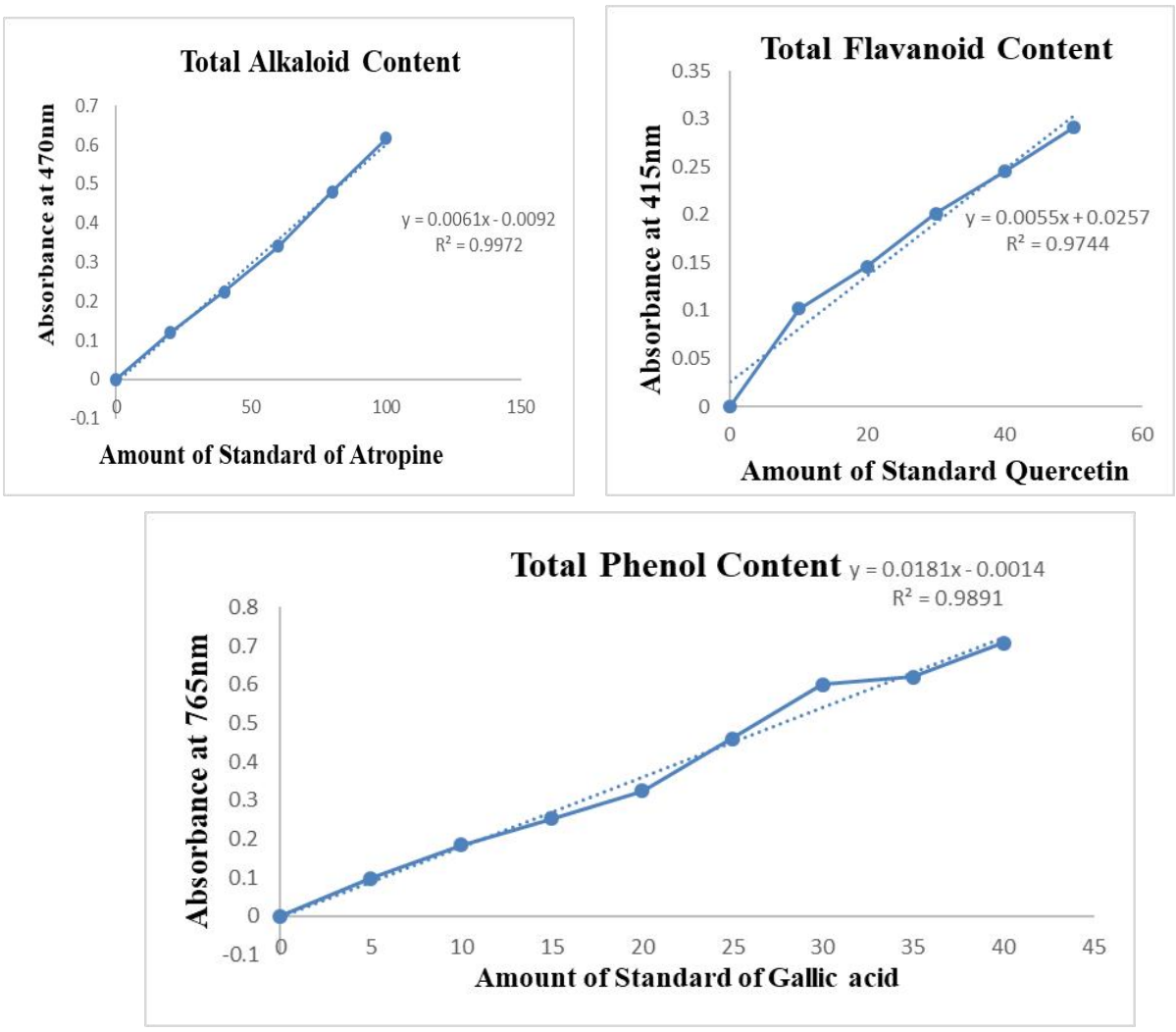


Figure No: 13 Calibration curves for total phenols, total flavonoids, and tannins for the quantification of phytoconstituents.

Evaluation of Imiquimod Emulgel

- **Physical Examination**: Imiquimod emulgel formulations exhibited a range of appearances, from transparent to white opaque, with a consistent, smooth texture and no observable phase separation.
- **pH Determination**: The emulgel formulations had a pH of 6.0–6.5, aligning well with the natural pH of the skin (4.5–7.0), minimizing the potential for irritation.

- **Drug Content**: The drug content measured at 9.46 mg/g indicated a stable concentration of Imiquimod within the emulgel, ensuring effective dosage consistency.
- **Rheological Properties**:
    - **Effect of Gelling Agents**: Gels with higher concentrations of gelling agents (e.g., Carbopol or HPMC) had viscosities between 20,000 and 30,000 cP, beneficial for stability but potentially less spreadable.
    - **Influence of Oil and Surfactant Ratios**: Emulgels with more oil or surfactants such as Tween 80 had lower viscosities (5,000 to 10,000 cP), improving spreadability but possibly reducing control over drug release.
    - **Optimal Viscosity Range**: The ideal viscosity for Imiquimod emulgel is 10,000–20,000 cP, balancing ease of application, skin retention, and drug release.
    - **Temperature Sensitivity**: Viscosity remained stable at 25°C but varied with temperature changes, emphasizing the need for consistent testing conditions.

Evaluation of Piper betle Extract Gel

- **Physical Appearance**: *Piper betle* extract gels had a smooth, uniform texture and displayed a pale green to light brown color, with no phase separation or syneresis over time, indicating good stability.
- **Viscosity**: Viscosities for *Piper betle* gels ranged from 10,000 to 30,000 cP. Higher viscosity gels (25,000–30,000 cP) adhered well to the skin, while lower viscosity gels (10,000–15,000 cP) were easier to spread.
- **Drug Content**: The drug content analysis revealed an average concentration of 10.56 mg/g, indicating uniform distribution and effective preservation of active compounds.
- **Spreadability**: Spreadability values between 5–8 cm, as measured with a parallel plate method, demonstrated that the gels spread easily on the skin, forming an even layer essential for therapeutic effectiveness.

Determination of λmax

To identify the maximum absorbance (λmax) of Imiquimod, a 10 µg/mL methanolic solution was scanned using a UV spectrophotometer over 200–400 nm. The λmax for Imiquimod was identified at 244 nm, as illustrated in Figure 1. This characteristic peak is essential for further analytical assessments of Imiquimod in formulation studies.

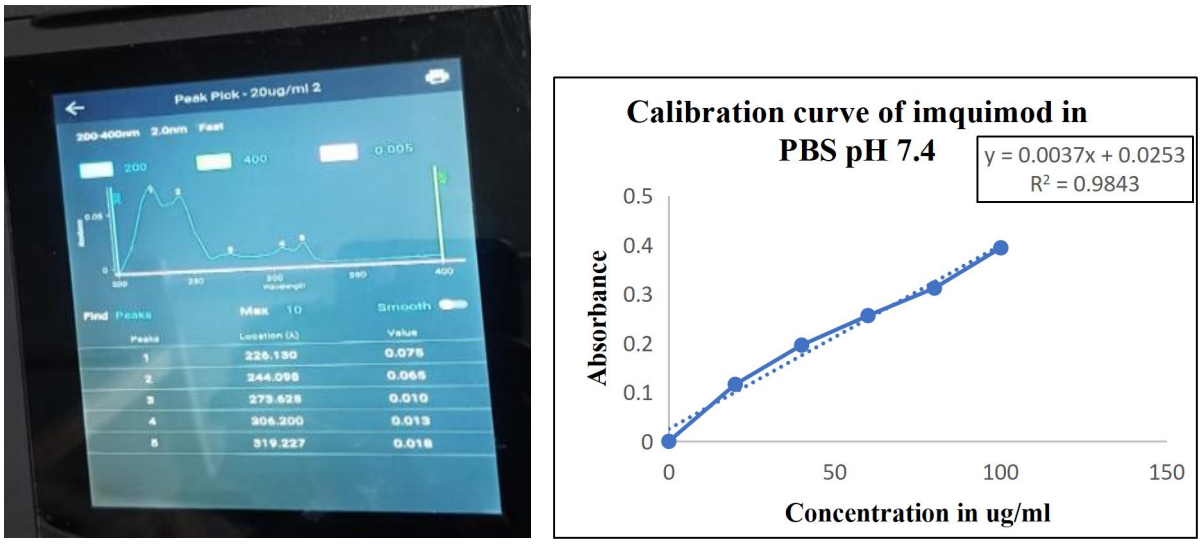


Figure No: 14 Calibration Curve of Imiquimod at pH 7.4

The results reveal that both *Piper betle* extract and Imiquimod emulgel formulations have robust bioactive profiles, and their respective formulations meet key physical, chemical, and therapeutic parameters for potential clinical application in skin treatments

Concentration (µg/ml)	Absorbance(nm)
0	0
20	0.116
40	0.196
60	0.256
80	0.312
100	0.394

Table No: 5 Absorbance of imiquimod at different concentrations

In vitro Permeation Studies

In vitro permeation studies were conducted using the Franz diffusion cell to compare the drug release profiles of three formulations: Imiquimod emulgel, Piper Betle extract-loaded gel, and Imiquimod marketed cream. The study evaluated the performance of each formulation in terms of drug release into the receptor medium over a 24-hour period. The Imiquimod emulgel, which used Carbopol-934 as the gelling agent, exhibited a rapid and significant drug release.

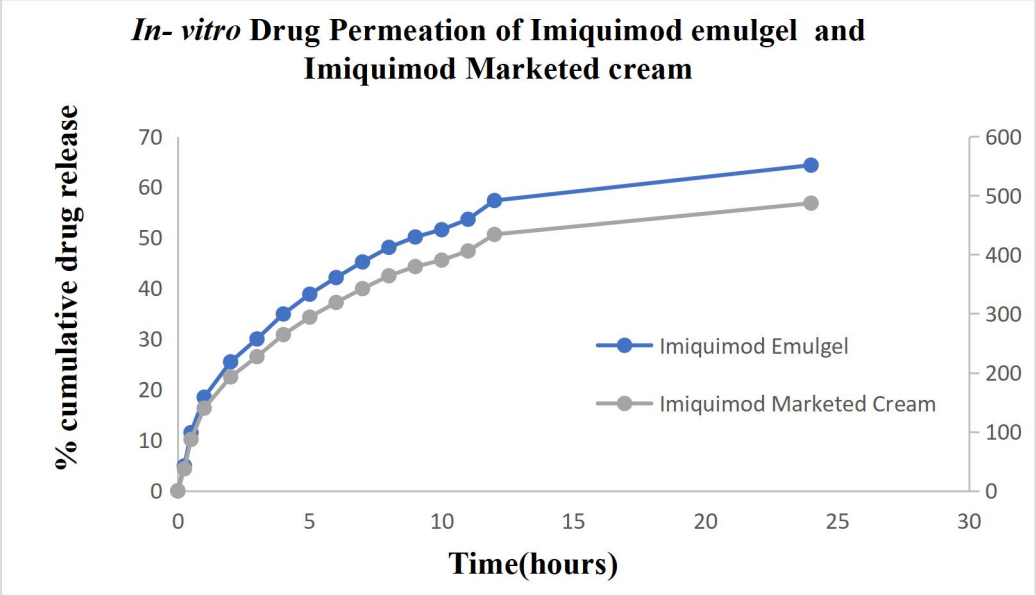


Figure No: 15 Comparative drug release profile

Acute Dermal Toxicity

In an acute dermal toxicity study, the safety of Piper betle leaf methanol extracts were tested by applying a maximum dose of 2,000 mg/kg body weight to the skin of healthy Sprague-Dawley rats, with 5 males and 5 females in the study. The animals were carefully monitored for any signs of mortality, toxicity, or behavioral changes, and body weights were recorded before treatment and at the study's end. Upon completion, all animals underwent necropsies. Results showed that Piper betle leaf methanol extracts were non-toxic at this highest tested dose, as no instances of mortality, toxic reactions, or skin irritation were observed throughout the study or within 72 hours post-application, confirming the extracts' safety at this dosage.

Anti-Psoriasis Activity

Psoriasis was induced in rats (Groups 2 to 5) by applying 125 mg of 5% Imiquimod (IMQ) emulgel to the shaved skin on the dorsal area for seven consecutive days. In Figure 12, which illustrates results from Days 7, 14, 21, and 28, progressive improvements in psoriatic symptoms among the treatment groups are evident over time. Initial reductions in erythema, scaling, and thickening were observed by Day 7, with further improvements continuing consistently through to Day 28. The visual representation in the figure demonstrates this steady alleviation of psoriatic lesions, with treated groups showing notably healthier skin than the untreated control, underscoring the treatment's efficacy across the observation period.

During the psoriasis induction period, symptoms like redness, erythema, and silvery scale formation worsened, as reflected in a cumulative Psoriasis Area Severity Index (PASI) score, which showed a significant increase ($p<0.05$), as shown in Table 11 and Figure 17. Starting on Day 8, test formulations were applied topically to Groups 2 to 5 for up to four weeks. Both visual and histopathological assessments confirmed that rats in Group 5, treated with the standard drug Cyclosporine, experienced a significant reduction ($p<0.05$) in redness, erythema, and scales from Days 7 to 28. In Groups

3 and 4, treated with Piper betle gel at low (200 mg/day/rat) and high (400 mg/day/rat) doses, a dose-dependent reduction in psoriatic symptoms was noted, with significance levels reaching $p<0.05$, though not as strong as $p<0.01$. These results suggest that while the effect is significant, it is moderate. Statistical analysis confirmed that the reduction in severity was significant ($p<0.05$), as shown in Tables 12-14. Comparison between the control group, standard drug, and higher dose Piper betle extract gel indicated that the combination treatment displayed superior therapeutic efficacy against induced psoriasis, as supported by data in Figures 17 and 18.

Days	Thickness	Redness	Scales	Cumulative PASI Score
1	0	0.33±0.18	0	0.33±0.18
2	0.5±0.19	1.16±0.14	0	1.66±0.33
3	1.16±0.14	1±0.22	0.08 ±0.14	2.24±0.50
4	1.66±0.14	1.83 ±0.26	0.25 ±0.12	3.74±0.52
5	1.83±0.26	2.16 ±0.26	0.43 ±0.22	4.42±0.74
6	2.0±0.22	2.5 ±0.19	0.75 ±0.26	5.25±0.67
7	2.5±0.19	3.0 ±0.31	1.16 ±0.19	6.66±0.69
8	3.16±0.18*	3.16 ±0.26*	1.75 ±0.26*	8.07±0.70**

Table No: 11 Evaluation of different parameters in rat with induced Psoriasis

The Psoriasis Area Severity Index (PASI) results are presented as mean ± standard error of the mean (n = 4). The data were analyzed using one-way ANOVA followed by Tukey–Kramer multiple comparisons test. Statistical significance was set at *p<0.05 and **p<0.01.

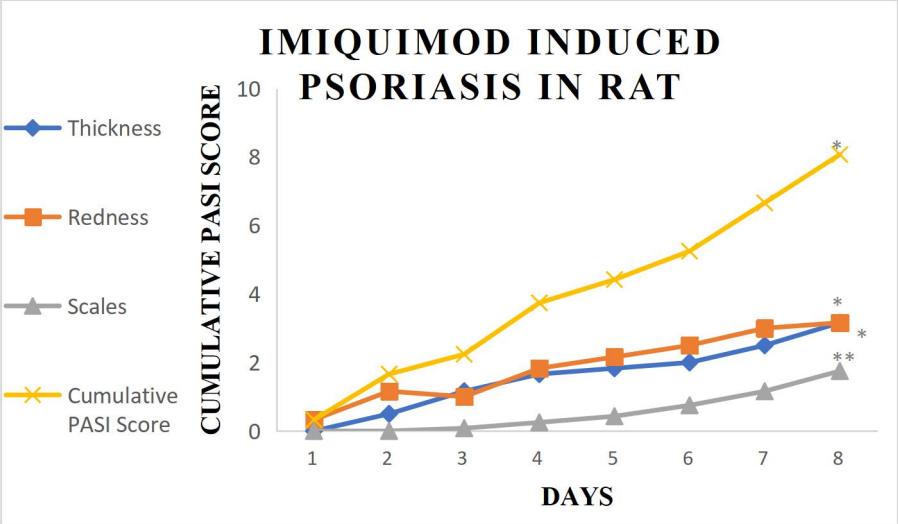


Figure No: 16 Different phenotypic changes observed during Imiquimod-induced Psoriasis.

Table No: 12 Evaluation of Redness (score 0-4) after treatment with extracts

Days	G-2	G-3	G-4	G-5
7	4±0.21	4.11±0.19	4.13±0.18	4.10±0.20
14	3.98±0.25	3.16±0.11	3.04±0.24	2.95±0.10
21	4.10±0.31	2.45±0.04	2.16±0.29	1.75±0.14
28	4.12±0.04	1.85±0.16*	1.51±0.12*	1.22±0.15*

The results represent mean ± standard error of mean (n=4). Data were analyzed by one way ANOVA, followed by Dunnett comparison test against untreated animals. Values were considered significant at *p<0.05.

Days	G-2	G-3	G-4	G-5

7	3.16±0.31	2.83±0.14	3.19±0.26	3.0±0.19
14	3.33±0.22	2.55±0.23	2.45±0.10	2.25±0.11
21	3.36±0.11	2.15±0.25	1.95±0.11	1.75±0.17
28	3.40±0.10	1.85±0.02*	1.65±0.02*	1.55±0.03*

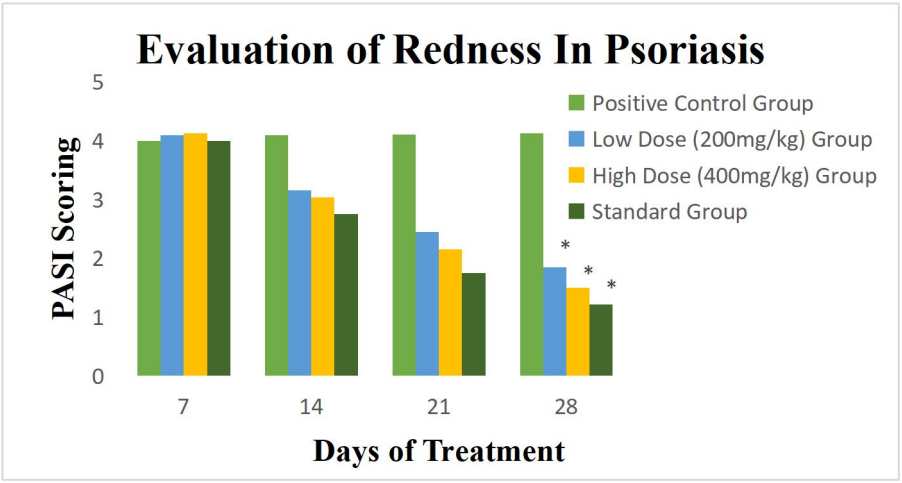
Table No: 13 Evaluation of Thickness (score 0-4) after treatment with extracts

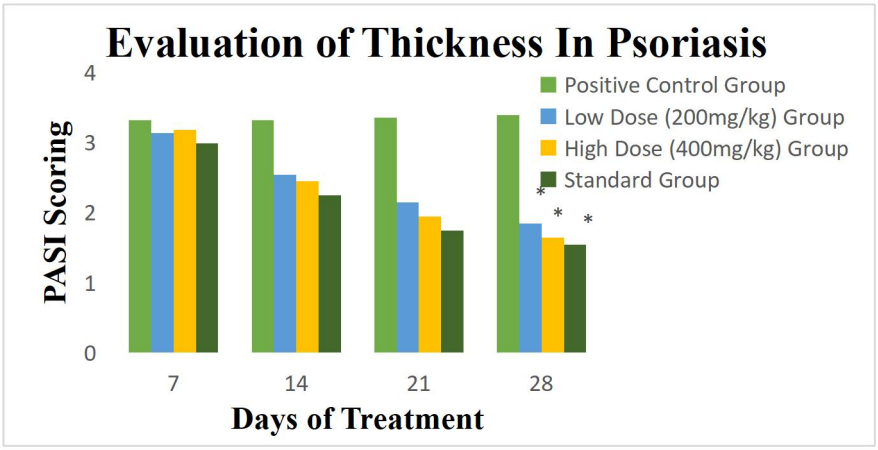
The results represent mean ± standard error of mean (n=4). Data were analyzed by one way ANOVA, followed by Dunnett comparison test against untreated animals. Values were considered significant at *p<0.05.

Table No: 14 Evaluation of scale formation (score 0-4) after treatment with extracts

Days	G-2	G-3	G-4	G-5
7	2.16±0.25	2.25±0.11	2.30±0.08	2.23±0.51
14	2.18±0.28	1.86±0.05	1.75±0.10	1.86±0.11
21	2.18±0.25	1.16±0.15	1.05±0.14	0.96±0.15
28	2.17±0.26	0.96±0.02*	0.87±0.03*	0.79±0.10*

The results represent mean ± standard error of mean (n=4). Data were analyzed by one way ANOVA, followed by Dunnett comparison test against untreated animals. Values were considered significant at *p<0.05.





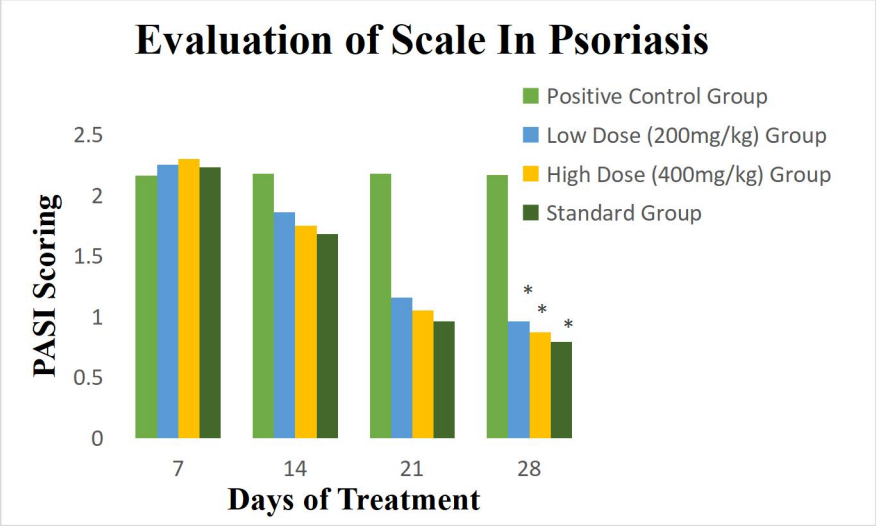


Figure No: 17 Graphical Representation of Phenotypic Changes During Imiquimod-Induced Psoriasis

Statistical analyses were performed in Excel using the Real Statistics add-in for accurate group comparisons. A One-Way ANOVA was initially conducted to assess significant differences among groups. Following the ANOVA, post-hoc tests were applied for specific group comparisons. Dunnett's test compared each treatment group to the control, identifying significant differences, while the Tukey-Kramer test provided pairwise comparisons across all groups, ensuring a thorough assessment while controlling for multiple comparisons. Significance levels were set at $*p < 0.05$, and significance markers were manually added to the graphs to enhance clarity.

Conclusion

This study highlights the promising potential of Piper betle gel as a natural treatment for plaque psoriasis, particularly in Imiquimod-induced models. The bioactive compounds in Piper betle—such as flavonoids, alkaloids, and polyphenols—contribute to its potent anti-inflammatory, antioxidant, and antimicrobial properties. These benefits are especially relevant in managing psoriasis, a condition marked by immune dysregulation, keratinocyte proliferation, and oxidative stress. The repeated application of Piper betle gel significantly reduced psoriatic symptoms, including redness, scaling, and skin thickness, by modulating inflammatory pathways and counteracting oxidative damage.

Statistical analysis underscored the efficacy of Piper betle gel, with One-Way ANOVA showing significant differences among groups. The post-hoc Dunnett's test confirmed notable improvements in each treatment group compared to the control ($*p < 0.05$). The higher dose of Piper betle gel led to approximately 70% inhibition of psoriatic symptoms, while Cyclosporine achieved around 65% inhibition, suggesting that Piper betle gel may sometimes be even more effective. These results underscore the gel's potential as a therapeutic treatment, offering a natural and patient-friendly alternative to conventional psoriasis therapies.

Future Outcomes

Optimizing Piper betle gel for plaque psoriasis treatment could lead to several positive outcomes. First, refining the formulation would enhance its physical properties, such as texture, viscosity, and stability, ensuring consistent efficacy and patient adherence. The gel's anti-inflammatory effects, powered by its bioactive compounds, are expected to significantly reduce psoriasis symptoms, as demonstrated by improvements in PASI scores. Its antioxidant properties would also mitigate oxidative stress, supporting improved skin barrier function and overall skin health.

The gel's stability and uniform drug content promise long-term effectiveness, making it suitable for continuous use. Given its safety profile and ease of application, it is likely to receive positive feedback from patients, especially those seeking natural alternatives. Piper betle gel may also serve as a complementary therapy alongside standard treatments, offering a holistic approach to psoriasis management without added systemic risks. The success of this formulation may expand its application to other inflammatory skin conditions, like eczema or dermatitis, broadening its therapeutic potential. With further research and large-scale clinical trials, Piper betle gel could advance to commercial development, contributing to the growth of phytotherapy in dermatology.

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