

“EXPLORING LEPA AS A TOPICAL ANTI-PSORIATIC APPROACH: AN ASSESSMENT IN IMIQUIMOD-INDUCED PSORIASIS IN RATS”

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Abstract:

Background: Psoriasis is a chronic immune-mediated inflammatory skin disorder characterized by erythematous, scaly plaques resulting from abnormal keratinocyte proliferation, oxidative stress, and immune dysregulation. Although conventional therapies are effective, their prolonged use is associated with adverse effects and frequent relapse. Therefore, there is growing interest in developing safer herbal formulations with anti-inflammatory, antioxidant, and anti-psoriatic properties. The present study was undertaken to prepare and evaluate an Ayurvedic Lepa formulation fortified with vitamins for the management of imiquimod-induced psoriasis in rats.

Aim and Objectives: The present study aimed to prepare, standardize, and evaluate an Ayurvedic Lepa (Lepagutti) formulation containing hydroalcoholic extracts of *Vitex negundo* and *Achyranthes aspera* fortified with Vitamin B3 and Vitamin B12. The objectives included phytochemical characterization, physicochemical standardization, evaluation of in-vitro antioxidant activity, acute dermal toxicity assessment, and in-vivo anti-psoriatic activity in an imiquimod-induced psoriasis rat model.

Materials and Methods: Hydroalcoholic extracts were prepared by the maceration method and subjected to qualitative and quantitative phytochemical screening. The Lepagutti formulation was prepared by the Bhavana method and evaluated for physicochemical parameters including pH, water-soluble extractive value, alcohol-soluble extractive value, total ash, and acid-insoluble ash. Antioxidant activity was determined using the DPPH free radical scavenging assay. Acute dermal toxicity was performed according to OECD Guideline 402. Anti-psoriatic activity was evaluated in imiquimod-induced Wistar rats by assessing body weight, PASI score, ear thickness, spleen weight, antioxidant enzymes (SOD and CAT), and histopathological changes.

Results: Phytochemical analysis confirmed the presence of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, triterpenoids, carbohydrates, proteins, and amino acids. Total phenolic and flavonoid contents were found to be 229 mg GAE/g and 289 mg QE/g of extract, respectively. The Lepagutti formulation exhibited acceptable physicochemical properties with pH 5.66 ± 0.10 , water-soluble extractive value $32 \pm 0.09\%$, alcohol-soluble extractive value $8 \pm 0.72\%$, total ash $15 \pm 0.40\%$, and acid-insoluble ash $6.5 \pm 0.40\%$. The hydroalcoholic extract showed concentration-dependent antioxidant activity in the DPPH assay. Acute dermal toxicity studies revealed no signs of toxicity up to 2000 mg/kg. In vivo, treatment with the high-dose Lepa significantly reduced PASI score, ear thickness, and spleen weight while improving body weight, SOD, CAT, and histopathological alterations compared with the disease control group. Histopathological examination demonstrated marked restoration of normal skin architecture with reduced epidermal hyperplasia, hyperkeratosis, and inflammatory cell infiltration.

Conclusion: The fortified Ayurvedic Lepa demonstrated significant antioxidant and anti-psoriatic activities in the imiquimod-induced psoriasis model. The formulation effectively improved clinical, biochemical, antioxidant, and histopathological parameters, with efficacy comparable to the standard drug Clobetasol. These findings suggest that the developed Lepagutti formulation is a safe and promising topical herbal therapy for psoriasis and warrants further clinical investigation.

Keywords: Psoriasis, Ayurvedic Lepa, Lepagutti, *Vitex negundo*, *Achyranthes aspera*, Imiquimod, Antioxidant Activity, Anti-psoriatic Activity, Histopathology.

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Introduction

Psoriasis is a chronic, immune mediated inflammatory skin disease, consisting of red, scaly plaques occurring most commonly on the elbows, knees, scalp, and lower back, but any skin surface can be involved.^[1] The global prevalence of psoriasis is estimated at 2–3%, affecting approximately 125 million people worldwide, with some variability based on geographic and ethnic factors.^[2]

The disease typically begins with a broad spectrum of symptoms including The symptoms differ on the basis of type of psoriasis. The most commonly found type of the psoriasis is plaque psoriasis in which red coloured plaques, often covered with silver coloured scales mostly appear. The plaques are itchy and may be painful can crack and bleed at certain times. Plaques show growth and merge together in severe cases, covering large regions of the body. Discoloration and pitting are key features in the disorders of toe and fingernails. Additionally, nails might disintegrate or detach from the nail bed. Plaques and crusts on the surface of head have been noted. Psoriatic arthritis is a type of arthritis that can affect people who have psoriasis. It causes joint pain and swelling. According to the National Psoriasis Foundation, around 10% to 30% of individuals who have psoriasis are found with psoriatic arthritis.^[3] Psoriasis is a chronic autoimmune skin disorder influenced by multiple genetic factors that regulate immune responses. The HLA-C*06:02 allele, part of the major histocompatibility complex (MHC) on chromosome 6, is the strongest genetic risk factor and is particularly associated with early-onset psoriasis.^[4] Variants in IL12B and IL23R are also implicated, affecting the Th1 and Th17 immune pathways, which drive inflammation and keratinocyte proliferation.^[5]

Psoriasis is a chronic immune-mediated inflammatory skin disorder presenting in several clinical forms. The most common form is plaque psoriasis (psoriasis vulgaris), characterized by well-demarcated erythematous plaques with silvery scales. Guttate psoriasis is identified by multiple small drop-like lesions, frequently triggered by streptococcal infections and seen mainly in younger individuals. Inverse psoriasis occurs in intertriginous areas such as axillae and groin, presenting as smooth, erythematous lesions with minimal scaling due to moisture. Pustular psoriasis is characterized by sterile pustules on erythematous skin and may occur as localized or generalized forms. Erythrodermic psoriasis represents a severe and rare variant involving widespread erythema, scaling, and systemic complications. Scalp psoriasis commonly affects the hairline and scalp region, sometimes extending beyond it. Nail psoriasis involves nail pitting, onycholysis, and subungual

hyperkeratosis. Palmoplantar psoriasis affects palms and soles, often leading to hyperkeratosis and fissures. Psoriatic arthritis is a related inflammatory arthropathy associated with cutaneous psoriasis. Each type differs in clinical presentation, severity, and management strategies, highlighting the heterogeneity of psoriasis as a disease entity

Psoriasis management is based on disease severity, patient comorbidities, and prior treatment response. For mild psoriasis, topical corticosteroids and vitamin D analogs remain first-line therapies and are highly effective in controlling localized lesions. In moderate-to-severe cases, phototherapy (narrowband UV-B) and systemic non-biologic agents such as methotrexate, cyclosporine, acitretin, or apremilast are recommended. The introduction of biologic agents targeting TNF- α , IL-12/23, IL-17, and IL-23 has revolutionized treatment, providing higher efficacy and improved quality of life. Combination strategies—such as topical therapy with phototherapy or systemic agents—are often used to enhance outcomes. Treatment choice must consider safety, cost, and patient preference to ensure long-term adherence and disease control.^[6]

Traditional Ayurvedic Delivery Methods Unique Ayurvedic approaches include Lepa (herbal paste application), Parisheka (decoction pouring), and Abhyanga (oil massage) to remove scaling, reduce inflammation, and restore dosha balance. Herbal preparations like Vidangadi Lepa, Mahatikta Ghrita, Panchatikta Ghrita Guggulu, and Neem-based Kwath are commonly used.^[8] Plant-based remedies are widely used in herbal medicine to manage chronic skin conditions such as psoriasis. However, the primary challenges with these formulations arise from their incomplete evaluation using modern pharmacological parameters. Ayurvedic Lepa (Lepagutti) with fortified ingredient is one of the well-known traditional topical formulations, widely adapted by Ayurvedic practitioners in their routine clinical practice for the treatment of psoriasis and other inflammatory skin diseases. Several studies demonstrate that the ingredients of Lepagutti, a carefully prepared herbal paste, effectively alleviate psoriatic symptoms by reducing inflammation, scaling, and itching. Lepa is a kind of medicinal preparation in which one or more powdered natural ingredients are mixed with a suitable liquid medium (such as water, oil, ghee, or Gomutra) to form a smooth paste. This paste is applied externally over the affected area for a specified duration. After drying, it is washed off with lukewarm water or wiped with a soft cloth. The applied herbal paste works through local absorption and penetration, providing targeted therapeutic effects on psoriatic

lesions.^[9]

One of the most effective Ayurvedic preparation for psoriasis is Ayurvedic lepa this bioactive phytoconstituents of *Vitex negundo* leaves contain flavonoids (luteolin, vitexin, casticin), phenolic acids, terpenes, and phytosterols. Flavonoids and phenolic acids are mainly responsible for reducing psoriasis markers by inhibiting TNF- α , IL-17, and oxidative stress.^[10] and *Achyranthes aspera* leaves phytoconstituents

Flavonoids (quercetin, kaempferol), alkaloids (achyranthine, betaine), saponins (oleanolic acid, ecdysterone), sterols (β -sitosterol), triterpenoids (lupeol), phenolics (gallic acid, caffeic acid).^[11] In investigations associated to psoriasis, there are very few available applicable animal models so far. Epidermal hyperproliferation, papillomatosis, altered differentiation, presence of inflammatory cells, and abnormal vascularity are the ideal features of a psoriatic model. Histological characteristics related to psoriasis typically include epidermal hyperplasia along with elongated rete ridges, hypogranulosis, parakeratosis, dermis and epidermis leukocytic infiltration. At present, there are several animal models which were quite similar to psoriasis; however, clinical and pathological resemblance was observed in very few models. Many genetically engineered models which includes transgenic/knockout or induced type model such as xenotransplant a murine psoriasis models have been developed up to date. Xenograft model is one of the psoriatic mouse models, where psoriatic skin (human) is transplanted on immunodeficient mice that closely resemble of humans psoriasis with respect to its genetic, phenotypic, and immunological characteristics. However, it has several drawbacks such as it is expensive, requires specialized laboratory skills, and time consuming. Ideal psoriatic animal models must have clinical hallmarks and must represent particular histomorphological patterns of psoriasis, easily reproducible, inexpensive, and ethical Imiquimod (IMQ), a well-known immune activator, is said to be a ligand for toll-like receptor 7 (TLR7) and TLR8 and has also recently been reported for its psoriasis-like inflammatory effects. The immunomodulatory action of IMQ in inducing psoriasis is observed due to TLR7 stimulation on plasmacytoid dendritic cells along with upregulation of Type I interferon pathway. IMQ-mouse model is said to be a suitable experimental model for psoriasis-related studies that presents a typical psoriatic phenotype, which interfere with adenosine receptor signaling pathway and augment inflammation. A study depicted the role of interleukin (IL)-23/IL-17 axis, in IMQ-induced skin inflammation in mice, which was also confirmed through histopathological examination showing similar characteristics to psoriasis. In the present study, rat model for psoriatic-like skin inflammation using IMQ as an inducing agent, which

may prove to be quite important for researchers working in the field of psoriasis.^[12]

Materials and Methods

Plant Material

The leaves of *Vitex Negundo*, *Achyranthes Aspera* required for preparing Ayurvedic Lepa. Leaves of *Achyranthes* and *Vitex Negundo* Local *aspera* was collected. The authenticity of the plant material was verified by a botanist from Matoshri Bayabai Shripatrao Kadam Mhavidhyalay, Kadegaon. The collected plant material was appropriately washed and dried under the shade. The plant material was converted into coarse powder through the use of a grinder after appropriate drying. The maceration method was adopted for preparation of the hydroalcoholic extract. An accurate Wt of powdered plant material was taken and placed in a clean and dry glass vessel having a tightly fitting lid. The plant material was thoroughly soaked in hydroalcoholic solvent system containing ethanol and distilled water in the proportion of 70:30 (v/v). The extraction was performed at room temperature (around 37°C) along with occasional stirring between 200-250 rpm to increase the efficiency of phytoconstituents extraction. Maceration was done for 48-72 hours. the resulting macerate was filtered with the help of muslin cloth and subsequently through Whatman filter paper to get clear filtrate. The filtrate was evaporated to obtain semi-solid crude extract.^[13]

Phytochemical Standardization

Preliminary phytochemical screening of the hydroalcoholic polyherbal extract was initially carried out to confirm the presence of various secondary metabolites ^[14]. Further hydroalcoholic polyherbal extract was subjected to quantification of identified phytoconstituents where total phenolic ^[14] and total flavonoid content^[15]. Total phenolic content in hydroalcoholic polyherbal extract was quantified by using Folin Ciocalteu Reagent^[14]. Aluminium chloride method was determining the total flavonoid content^[15].

Preparation of Ayurvedic Lepagutti

Achyranthes aspera leaf powder, *Vitex negundo* leaf powder, vitamin B12, and vitamin B3 were used in the preparation of the Lepagutti formulation utilising a typical trituration and Bhavana method. To create homogeneous and fine powders, the dried leaves were ground individually using a grinder and then sieved through an 80-mesh screen. Both herbal powders were precisely weighed and combined in equal amounts. The herbal mixture was then mixed with calculated amounts of vitamin B12 and vitamin B3 to ensure even distribution. A separate starch binder was made by dissolving starch in distilled water, heating it gradually until a translucent paste formed, and then letting it cool to room temperature. In order to achieve a smooth and cohesive semi-solid mass, the Bhavana technique involved progressively adding distilled water to the powder combination and triturating. To enhance

binding qualities, the produced starch paste was applied gradually during trituration. To stop the active chemicals from degrading, the resultant mass was then rolled into tiny, uniform gutti (pellets) and allowed to dry at room temperature in the shade. The Lepagutti were kept in airtight containers for future experimental or medicinal usage after they had completely dried and been shielded from light, moisture, and contamination.^[9]

18 hours. Rapid filtration was performed without allowing the solvent loss. In a china dish, 25 ml of filtrate was dried using evaporation process. It was dried in constant temperature of 105°C until a constant weight is achieved. Percentage of extractive value of alcohol soluble was calculated and presented.

Alcohol Soluble extractive values=



Figure 1:

Lepagutti

Evaluation Of Formulation

1)Determination of pH

A pH meter which had been calibrated previously was used for measuring the pH of the solution. The electrode was cleaned, rinsed with distilled water and calibrated through the use of buffer solutions having pH levels of 4.0, 7.0, and 10.0. One gram of powder was measured and dissolved in one hundred milliliters of distilled water in order to produce a one percent solution of Lepaguti in water. After acquiring a constant reading, the electrode was then immersed into the solution and the pH was measured.

2)Determination of Extractive Values

The substances formed when crude drugs are exhausted by a variety of solvents are termed as "extractive values". In determining the extractives, a number of solvents are used based on the differences in chemical structure of components comprising the drug.

The following are several methods of determining extractive values.

a) Water soluble extractive value ^[16]

Precisely weighed 5 grams of the powdered sample was extracted by maceration in 100 ml of water kept in a closed flask for 24 hours with shaking during the first six hours and resting the other 18 hours. The mixture was filtered rapidly taking into account the prevention of evaporation of the solvent. 25 ml of the filtered extract were then dried in a China dish at 105°C until a constant weight is obtained.

$$\text{Water soluble extractive values} = \frac{\text{Total Extract}}{\text{Wt.of Crude Drug}} * 100$$

b) Alcohol soluble extractive value^[16]

In a close flask, 5g of powder is accurately weighed, extracted using ethanol for 24 hours, shaking periodically for six hours, and allowing standing for

$$\frac{\text{Total Extract}}{\text{Wt.of Crude Drug}} * 100$$

3) Total ash (%):^[17]

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450⁰ until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450⁰. Calculate the percentage of ash with reference to the air-dried drug..

$$\text{Total ash (\%)} = \frac{\text{weight of Total Aqsh}}{\text{Wt.of Crude Drug}} * 100$$

4) Acid-insoluble ash (%)^[18]

To the crucible containing total ash, add 25 ml of dilute hydrochloric acid. Collect the insoluble matter on an ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and weigh without delay. Calculate the content of acid-insoluble ash with reference to the air-dried drug

$$\text{Acid-insoluble ash (\%)} = \frac{\text{Weight of Acid insoluble Ash}}{\text{Wt.of air dried Crude Drug}} * 100$$

IN-VITRO STUDIES:[19]

DPPH free radical scavenging assay:

The antioxidant activity was determined by using free radicals of 2,2-diphenyl-1-picrylhydrazyl. The antioxidant activity was estimated on the basis of scavenging of DPPH free radicals. DPPH solution was prepared by dissolving 3.9 mg of DPPH in 100 ml methanol with stirring overnight at refrigeration temperature of 4 °C. It led to the preparation of 0.1

mM DPPH free radicals. Violet-colored DPPH free radicals were preserved in frozen (-20°C) form for further experiments. Methanolic solutions of five different concentrations, i.e., 100, 200, 300, 400, and 500 µg/ml were prepared by serial dilution of stock solution of each extract. In each case, 2.5 ml of methanolic solution of DPPH was mixed with 0.5 ml of each extract solution. For control experiment, 2.5 ml of 0.1 mM methanolic solution of DPPH was mixed with 0.5 ml distilled water. All samples were incubated in darkness for half an hour. Absorbance was measured at wavelength of 517 nm, using blank consisting of 0.5 ml distilled water + 2.5 ml MeOH. 8 13 8

Here AS = absorbance of sample, Ab = absorbance of blank and AC = absorbance of control..

$$\% \text{ Scavenging} = \left[\frac{(A_s - A_b)}{A_c} \right] \times 100$$

The concentration of samples needed to scavenge 50% of DPPH radicals is referred to as IC50 values, which were obtained from the graph of the radical scavenging ability in relation to extract concentration.

IN-VIVO STUDIES

Experimental Animals

The study was conducted using Wistar albino rats weighing 180–220 g of either sex. Animals were housed in standard polypropylene cages with stainless steel grill tops, and corn cob bedding was used. The animals were maintained in the animal house under controlled environmental conditions with temperature maintained at $22 \pm 3^\circ\text{C}$, relative humidity between 30–70%, and a photoperiod of 12-hour light and 12-hour dark cycle. Standard laboratory pellet diet and water were provided ad libitum. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), recognized by CPCSEA (CCSEA), under protocol number IAEC/SKCP/04/2025-26.

Acute Dermal Toxicity Study

The OECD (Organization for Economic Cooperation and Development) Guideline 402 was referred for determination of acute dermal toxicity of Ayurvedic Lepa. The Wistar female rats the shaved skin is treated with a single dermal dose of the lepa. Under semi-occlusive or occlusive dressing, the test material stays in contact with the skin for a predetermined amount of time (often 24 hours). Animals are routinely checked for symptoms of poisoning, altered behaviour, skin responses, and death.^[20]

Imiquimod-induced psoriatic rat model

The rats in the each group were then given 100 mg of IMQ cream (5%w/w) in the dorsal area and 10 mg in the left ear for ten days in a row. A series of tests evaluating biochemical and haemostatic parameters and histology were performed in addition to the grading of erythema, scaling, and thickness as

additional criteria for determining the psoriasis area severity index.^[21]

Grouping of animals

Psoriasis in rats was induced after 10 days of imiquimod application. This was followed by grouping of animals, which included five groups i.e Group 1 as normal control was given vasline. Group 2 was served as Psoriasis induced disease control group and was application of Imiquimod cream. Group 3 was served as standard treated group, which included psoriasis induced rats treated with Clobetasol Cream (30mg/cm² Topical) and Group 4 and 5 included psoriasis induced rats treated with lepa at 200 mg/kg and 400 mg/kg. Except for the normal control group, all animal groups will be subjected to application of Imiquimod Cream for 10 days. The test drug and a standard drug will be application to 3 groups of animals for 13 days. The treatment shall be started at 11th day and continued up to 14th day. The physical parameters of the animals will be assessed on 1st to 14th day. The animals will be sacrificed and the Skin tissue will be removed for biochemical assays. The animals will be randomly divided into six groups of six rats each:

Group I : Control

Group II : Disease control [Imiquimod Cream 37.5mg/cm² (Topical)]

Group III : Imiquimod Cream 37.5mg/cm² (Topical)+Standard (Clobetasol)

Group IV : Imiquimod Cream 37.5mg/cm² (Topical)+ Low Dose (T1) (200mg/kg)

Group V : Imiquimod Cream 37.5mg/cm² (Topical)+ High Dose (T2) (400mg/kg)

The physical parameters of the animals will be assessed on 1st to 14th day. The animals will be sacrificed and the skin tissue will be removed for biochemical assays and Histological analysis.

Sacrifice of rats and sample collection

At the end of study on 14th day the experimental animals were fasted for 16 hours then taken into separate room where they were weighed and sacrifice under co² chamber. Then a portion of skin tissue was excised and 10%w/v homogenate was prepared in ice-cold Tris-Hcl 50mM solution, respectively, and centrifuged at 3000rpm for 25min at 4⁰. The supernatant obtained for the estimation of antioxidant activity (catalase and superoxide dismutase).

Changes in Spleen:

Each rat's spleen was removed and measured in weight. The assessment of splenomegaly as done by determining the spleen weight in relation to the total body weight. A formula is used to determine the Spleen index.

Ear Thickness

Ear thickness is used as an indicator of skin inflammation and psoriasis severity. An increase in thickness reflects edema, immune cell infiltration, and epidermal hyperplasia. Measurement is typically done using a Verner caliper. Reduction in thickness

indicates improvement in inflammatory condition and lesion severity.

PASI Score -

PASI score is a standard method to evaluate overall severity of psoriasis-like lesions. It is based on three parameters: erythema (redness), scaling, and thickness. Each parameter is graded to provide a cumulative severity score with (0-4). Higher PASI values indicate more severe disease, while lower scores reflect improvement.^[22]

Biochemical Estimation

Antioxidant analysis was performed on the homogenate of skin tissue. The supernatant was used for the estimation of superoxide-dismutase (SOD) activity and Catalase activity (CAT).^{[23],[24]}

Histopathology

At the end of the *in vivo* evaluation period, the rats were sacrificed, and a skin sample was collected. The segment was fixed in 10% neutral buffered formalin for 48 h. The samples were rinsed 3 times in 0.1 M phosphate-buffered saline (PBS, pH 7.4), stored in 0.1 M PBS at 4 °C, and embedded in paraffin. Tissue sections of 4 µm were prepared and stained with Hematoxylin and Eosin (HE).

STATISTICAL ANALYSIS:

The presented value are denoted as Mean ± SEM. The statistical assessment involved employing a one-way ANOVA by Dunnett's test (Graph Pad Prism 11). This study chose level of statistical analysis as $P < 0.05$.

RESULTS

Phytochemical Screening of Hydro-Alcoholic Extract

The results of the phytochemical screening of the hydro-alcoholic extract are summarized in Table 1. A secondary metabolites were identified, including alkaloids, flavonoids, tannins, lignans, cardiac glycosides, triterpenoids, carbohydrates, phenolic compounds, proteins and amino acids is performed.

Table 1: Phytochemical Evaluation of Hydroalcoholic Polyherbal Extract

Phytochemicals	Test	Observations Presence (+) / Absence (-)
Alkaloids	Mayer's test	+
	Wagner's test	+
	Dragendroff's test	+
	Hager's test	-
Flavonoids		+
		+
		+
		-
Tannin	Gelatin test	+
		-
		-
		+

Phenolic compounds		+
		+
		+
Phytosterols		+
		+
Triterpenoids		+
Proteins and amino acid		+
		-
		-
Carbohydrates		+
		+
		+
Glycosides		+
		-

Quantitative analysis:

In Quantitative analysis, Total phenolic content and total flavonoid content were determined and it was found that the total phenolic content was in the range of 100-500 µg/mL. The extract at 200 µg/mL showed an absorbance of 0.488 ± 0.0052 , corresponding to **229 mg GAE/gram** of extract, indicating a significant presence of phenolic compounds. The extract at 300 µg/mL showed an absorbance of 0.568 ± 0.0052 , corresponding to **289 mg QE/gram** of extract, indicating a significant presence of flavonoid compounds. These results suggest that formulation is rich in compounds essential for reducing oxidative stress, inflammation and Skin pathology, epidermal thickness, hyperkeratinocytes and psoriasis

Evaluation Of Lepagutti

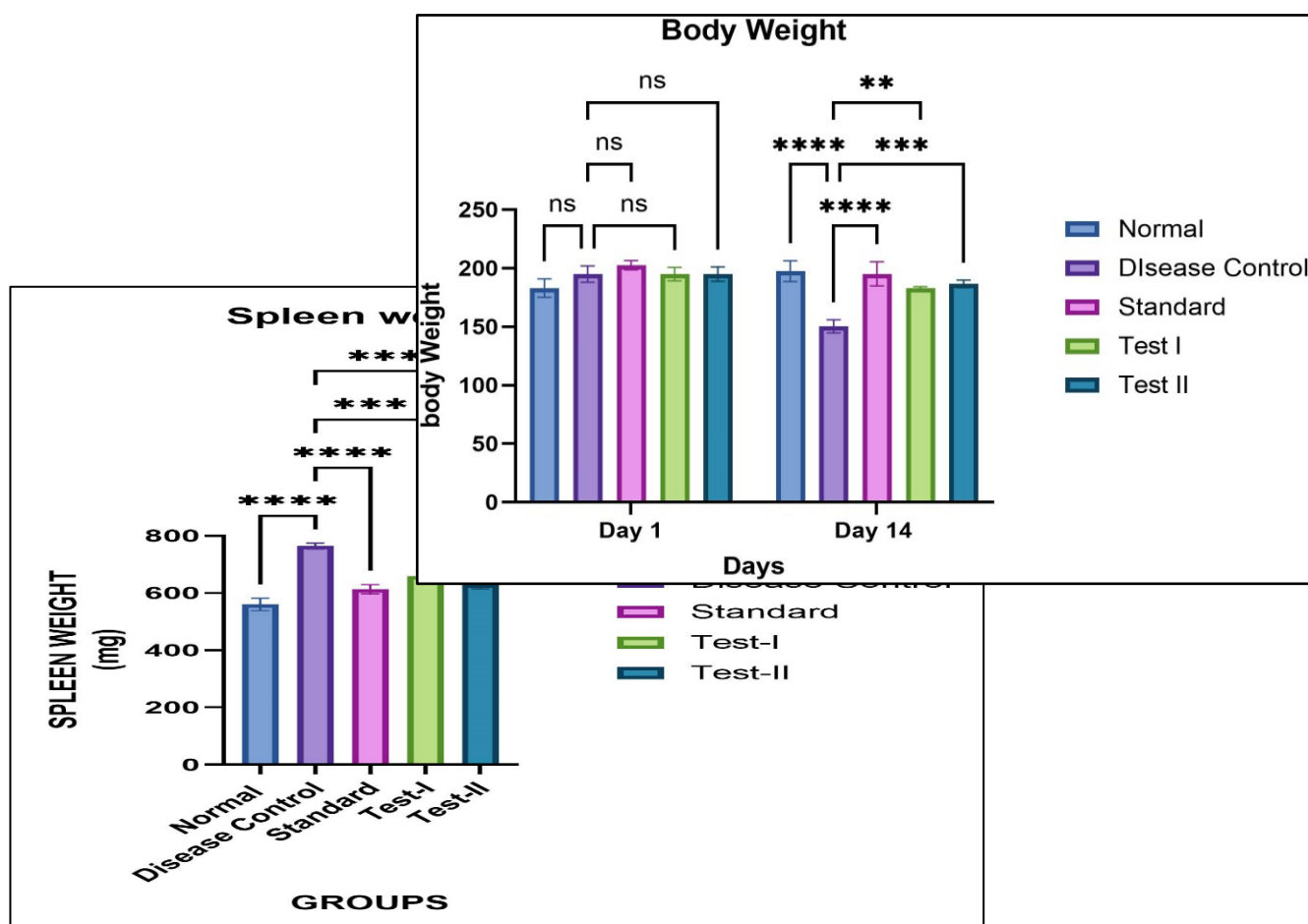
Table 2 : Evaluation parameter of Lepagutti following table:

Sr. No.	Evaluation Parameter	Observations
1	Total Ash Value	15±0.4
2	Acid Insoluble Ash Value	6.5 ±0.4
3	Water Soluble Extractive Value	32 ±0.09
4	Alcohol Soluble Extractive Value	8 ±0.72
5	pH	5.66±0.1

In-Vitro Studies

a) DPPH Assay

It is observed that the hydroalcoholic polyherbal extract possesses concentration-dependent antioxidant activity where the % inhibition increases from 47.04 % at 100 µg/mL to 87.94% % at 500 µg/mL. With an increase in concentration, its activity becomes closer to the standard antioxidant, ascorbic acid.



In-Vivo Studies

Acute dermal toxicity study

From the results of the acute dermal toxicity trial, Aurvedic lepa was found to be safe up to 2000 mg/kg with no evidence of behavioural or dermal toxicity.

Effect of Lepa on body weight

As the disease progressed, the body weight of the disease control group decreased from 210 ± 6.66 g on Day 1 to 140.33 ± 13.6 g on Day 14, showing severe weight loss due to psoriasis induction. The normal control group showed an increase in body weight from 183 ± 7.84 g to 197.5 ± 8.95 g, indicating normal growth. The standard-treated group maintained body weight from 202.66 ± 3.99 g to 195.16 ± 10.29 g, showing protection against weight loss. Similarly, the Test-1 group showed only a slight decrease in body weight from 195 ± 5.65 g to 183.16 ± 1.29 g, indicating moderate improvement. The Test-2 group also near-normal body weight, decreasing from 195 ± 6.12 g to 186.66 ± 3.23 g, suggesting better prevention of disease-induced weight loss.

Figure 3 : Effect Of Treatment on Body Weight in Different Groups
Values are presented as **mean $\pm$ SEM**, with **N = 6 animals per group**. Statistical analysis was performed using **two-way ANOVA**, followed by **post hoc Dunnett's multiple comparisons test**, with **DC group** as the reference control for all comparisons. The statistical significance of differences in body weight

between treatment groups and the DC group is indicated by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.0001$ (****), "ns" denotes non-significant differences ($p > 0.05$).

Effect of Lepa on spleen weight

The spleen weight of the disease control group was increased to 760 ± 21.94 mg compared to the normal control group (560 ± 21.94 mg), indicating severe inflammation and immune activation caused by psoriasis induction. The normal control group maintained normal spleen weight throughout the study. The standard-treated group showed a reduction in spleen weight to 610 ± 21.94 mg, indicating improvement in the inflammatory condition. Similarly, the Test-1 treated group also reduced spleen enlargement, showing a spleen weight of 670 ± 21.94 mg and moderate protection against inflammation. The Test-2 treated group maintained spleen weight near the normal range at 650 ± 21.94 mg, better control of psoriasis-induced systemic inflammation.

Figure 4. Effect Of Treatment on Spleen Weight in Different Groups
Values are presented as **mean $\pm$ SEM**, with **N = 6 animals per group**. Statistical analysis was performed using **two-way ANOVA**, followed by **post hoc Dunnett's multiple comparisons test**, with **DC group** as the reference control for all comparisons. The statistical significance of differences in Spleen weight between treatment groups and the DC group is indicated by

asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.0001$ (****), “ns” denotes non-significant differences ($p > 0.05$).

Evaluation of Psoriasis-like Inflammation Severity Index (PASI)

The PASI score provides a comprehensive measure of both the severity and the extent of psoriasis and is used in both human and animal models as a translational tool to evaluate psoriasis severity [7,12]. It assesses the degree of erythema (redness), induration (thickness), and desquamation (scaling) in different body regions, offering a holistic view of the disease. PASI is a standardized tool used globally, which ensures consistency and comparability across clinical studies, allowing for reliable assessment of disease severity and treatment efficacy. IMQ induced erythema, scaling, and thickening of the skin. After induction of psoriasis, the disease control group showed a high PASI score of 5.166 ± 0.54 , indicating severe psoriasis-like skin inflammation. The standard control group also showed increases PASI score after induction (5.666 ± 0.56), which was significantly reduced to 3.166 ± 0.28 after treatment, indicating improvement in psoriatic symptoms. The Test-1 treated group decrease in PASI score from 5.833 ± 0.72 after induction to 2.50 ± 0.20 after treatment, moderate anti-psoriatic activity. The Test-2 treated group also a marked reduction in PASI score from 5.166 ± 0.49 to 2.0 ± 0.23 after treatment, indicating better protection against psoriasis-induced skin inflammation. Overall, both test treatments reduced the severity of psoriasis, with Test-2 showing comparatively better improvement.

differences in PASI Score between treatment groups and the DC group is indicated by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.0001$ (****), “ns” denotes non-significant differences ($p > 0.05$).

Ear Thickness (mm)

Ear Thickness changes were observed throughout the protocol. Over time, the increase in Ear Thickness in disease group, rat whereas after treatment decrease weight of Ear Thickness and normal size. As the disease progressed, the ear thickness of the disease control group increased from 0.30 ± 0.02 mm on Day 1 to 0.50 ± 0.02 mm on Day 14, indicating severe inflammation and edema caused by psoriasis induction. The normal control group maintained nearly normal ear thickness throughout the study, with values changing from 0.25 ± 0.02 mm to 0.27 ± 0.03 mm. The standard-treated group showed a reduction in ear thickness from 0.27 ± 0.02 mm to 0.26 ± 0.01 mm, indicating effective protection against inflammation. Similarly, the Test-1 treated group showed moderate improvement, with ear thickness increasing from 0.27 ± 0.03 mm to 0.38 ± 0.02 mm. The Test-2 treated group maintained ear thickness near the normal range, with values changing from 0.27 ± 0.01 mm to 0.29 ± 0.01 mm, better prevention of psoriasis-induced ear inflammation.

Figure 6 : Effect Of Treatment on Ear Thickness in Different Groups on Different Day

Values are presented as **mean $\pm$ SEM**, with **N = 6 animals per group**. Statistical analysis was performed using **two-way ANOVA**, followed by

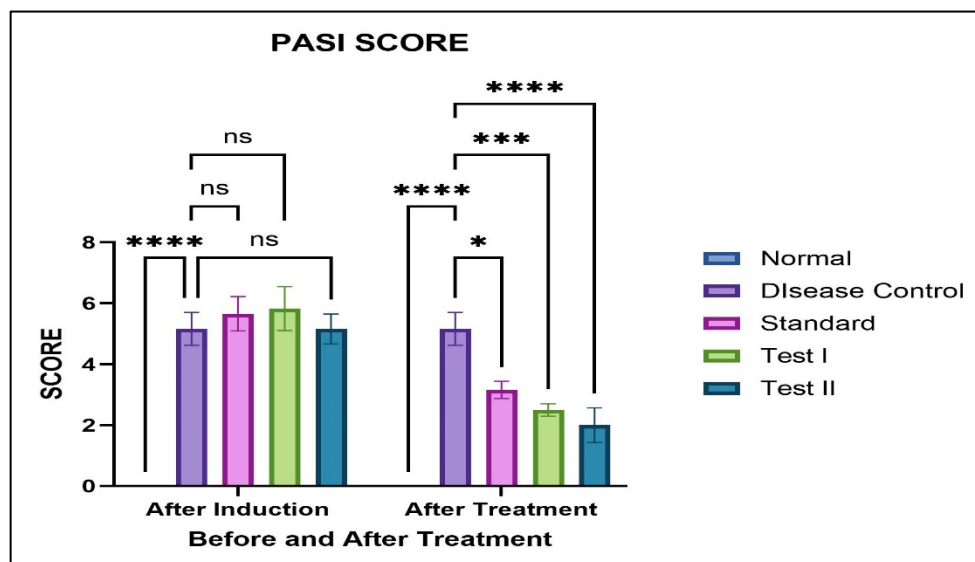


Figure 5: Effect Of Treatment on PASI Score of After Induction and Before Induction Values are presented as **mean $\pm$ SEM**, with **N = 6 animals per group**. Statistical analysis was performed using **two-way ANOVA**, followed by **post hoc Dunnett's multiple comparisons test**, with **DC group** as the reference control for all comparisons. The statistical significance of

post hoc Dunnett's multiple comparisons test, with **DC group** as the reference control for all comparisons. The statistical significance of differences in Ear Thickness between treatment groups and the DC group is indicated by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.0001$ (****), “ns” denotes non-significant differences ($p > 0.05$).

Table 3 : *In vivo* Parameters

Gro ups	Normal Control	Disea e Control	Stand ard (Clob etasol)	Test 1	Test 2
Before Induction					
Bod y Wei ght	183±7.84	183±7.84	183±7.84	183±7.84	183±7.84
Ear Thi ckness	0.25±0.02	0.30±0.02	0.27±0.02	0.27±0.03	0.27±0.01
PAS I Sco re	0	5.16±0.54	5.66±0.56	5.83±0.72	5.16±0.49
Gro ups	Normal Control	Disea e Control	Stand ard (Clob etasol)	Test 1	Test 2
After Treatment					
Bod y Wei ght	197.5±8.95	140.33±13.6	195.16±10.29	183.16±1.29	186.66±3.23
Sple en Wei ght	560±21.94	763±10.32	665±11.76	658±17.77	645±21.09
Ear Thi ckness	0.27±0.03	0.5±0.02	0.26±0.01	0.38±0.02	0.29±0.01
PAS I Sco re	0	5.16±0.54	3.16±0.28	2.5±0.20	2±0.23

Estimation of Antioxidant Parameters- a) SOD and CAT of rats in Imiquimod induced Psoriasis

The antioxidant enzyme levels of SOD and CAT were significantly reduced in the disease control group compared to the normal control group, indicating increased oxidative stress caused by psoriasis induction. The normal control group showed normal SOD and CAT levels of 37 ± 0.19 U/mg protein and 0.074 ± 0.0009 U/mg protein, respectively. In contrast, the disease control group showed decreased SOD and CAT levels of 15.9 ± 0.18 U/mg protein and 0.013 ± 0.0005 U/mg protein. The standard-treated group showed improvement in antioxidant status, with SOD and CAT levels increasing to 36.43 ± 0.22 U/mg protein and 0.067 ± 0.0004 U/mg protein, respectively. the Test-1 treated group showed moderate improvement, with SOD

and CAT values of 19.15 ± 0.17 U/mg protein and 0.033 ± 0.0004 U/mg protein. The Test-2 treated group also restored antioxidant enzyme levels near normal range, showing SOD and CAT values of 35.20 ± 0.24 U/mg protein and 0.055 ± 0.00036 U/mg protein, better protection against oxidative stress induced by psoriasis.

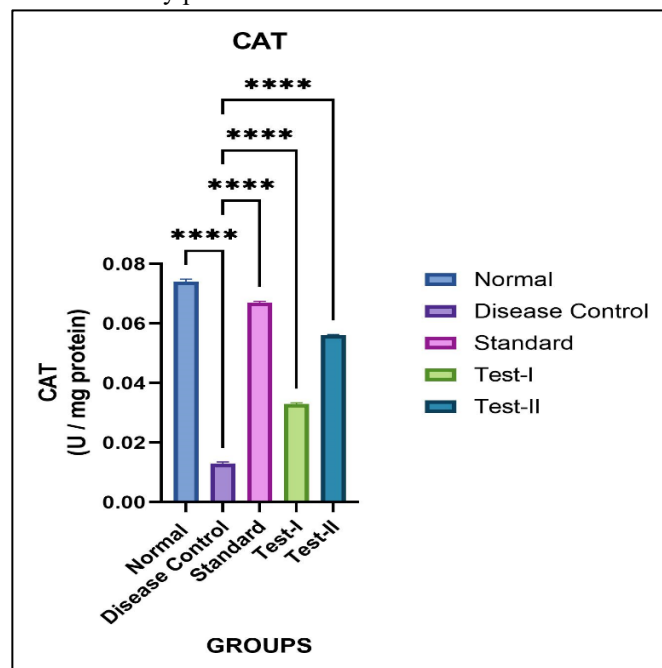


Figure 7 : Effect of treatments on catalase (CAT) And Superoxide dismutase (SOD) activity (U/mg protein) in various experimental groups. SOD, CAT levels were measured in the Normal Control (NC), Disease Control (DC), Standard Drug (STD), Test-1 and Test-2 groups to evaluate cytokine profile. Data are represented as mean ± SD, with N = 6 animals per group. Statistical analysis was conducted using One-way ANOVA followed by Dunnett's multiple comparisons test, with all groups compared against the DC group.

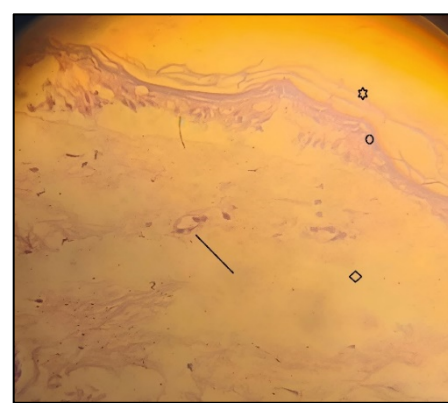
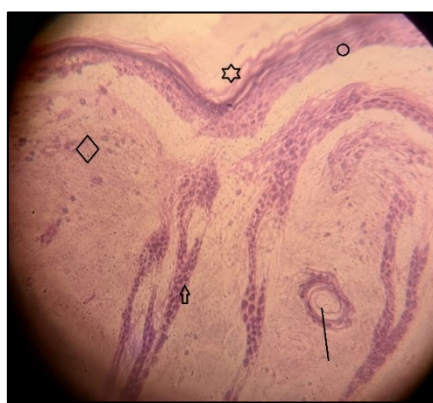
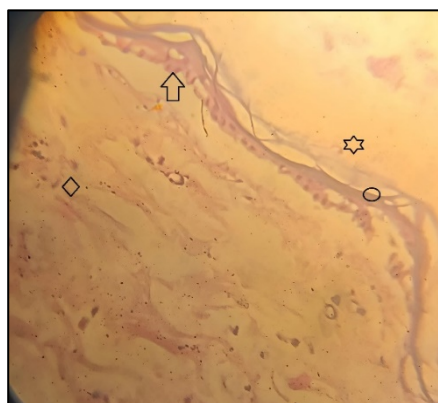
Table 4: Antioxidant parameter of skin tissue homogenate

Gr ou ps	Norm al Contr ol	Disea e Contr ol	Stand ard (Clob etasol)	Test 1	Test 2
SO D	37±0.19	15.9±0.18	36.43±0.22	19.15±0.17	35.20±0.24
C AT	0.074±0.0009	0.013±0.0005	0.067±0.0004	0.033±0.0004	0.055±0.00036

“EXPLORING LEPA AS A TOPICAL ANTI-PSORIATIC APPROACH: AN ASSESSMENT IN IMIQUIMOD-INDUCED PSORIASIS IN RATS”

Evaluation of Epidermal Thickness (Histology):
(A) (B)

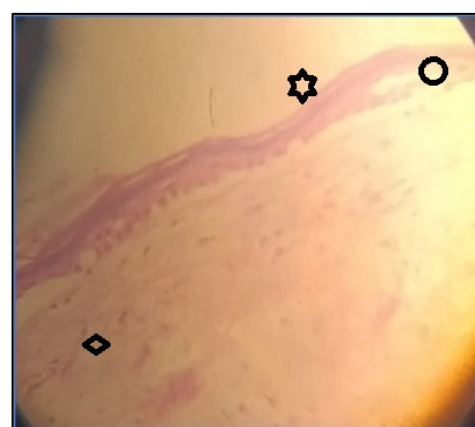
vivo study consisting acute toxicity, Psoriasis parameters, biochemical analysis and histopathological examination.



(C)



(D)



(E)

Figure 8: Histopathological Evaluation- (A) Normal Control, (B) Disease Control, (C) Standard (Clobetasol), (D) Test-1 (200 mg/kg), (E) Test-2 (400 mg/kg),

We performed a histological analysis using Hematoxylin and Eosin (HE) staining upontreatments in the psoriasis-like skin pathology and IMQ-induced thickening of the epidermis. Histopathological Observation of disease control group shows psoriatic alterations like marked epidermal hyperplasia, hyperkeratosis, elongated rete ridges, dermal structure disruption compared with normal control group, which showed normal skin. Standard treated group showed better improvement like normal skin morphology. Test 1 Group showed moderate reduction in epidermal thickening and inflammatory infiltration and Test 2 better restoration of normal epidermal and dermis with mild inflammatory changes.

4. Discussion

The present study aimed prepare, standardize and evaluate a novel ayurvedic lepa formulation fortified with vitamins for anti-psoriasis activity preclinically. This discussion includes finding across multiple domain including formulations and standardization, physiochemical characteristics, phytochemical profiling. In-vitro study includes anti-oxidant activity as well as *In-*

The Ayurvedic Lepa was prepared using classical

ayurvedic procedure and Modern approach is fortification of vitamins. It was observed that Lepagutti formulation showed pH(5.66±0.1), Water Soluble Extractive Value(32±0.09), Alcohol Soluble Extractive Value(8±0.72), Total Ash Value(15±0.4), Acid Insoluble Ash Value(6.5±0.4) respectively which are within limits for dermal administration as well as appropriate for maintaining their uniformity and physiochemical stability.

Qualitative phytochemical evaluation revealed the presence of important secondary metabolites such as alkaloids, flavonoids, phenolics, glycosides, tannins, triterpenoids, saponins and carbohydrates. These constituent are well documented for anti-inflammatory, antioxidant, wound healing . These properties indicate potential of Lepa formulations for anti-psoriatic activity. Quantitative analysis confirmed the presence of bioactive compounds. Total phenolic content and total flavonoid content were determined and it was found that the total phenolic content was in the 200 g/mL, which corresponds to 229 mg GAE/gram . Total Flavonoid content was found in 200 g/mL of 289 mg QE/gram of extract. These results suggest that formulation is rich in compounds essential for reducing oxidative stress, inflammation and enhancing the anti-psoratic activity. Anti-oxidant activity of the extract and formulations was evaluated using DPPH Scavenging Assay. It showed that IC₅₀ (128.94 µg/mL) of polyherbal extract was lower than Ascorbic Acid (105.17 µg/mL).

Acute dermal toxicity studies conducted according to OECD 402 guidelines revealed no sign of toxicity and mortality at a dose of 2000 mg/kg, establishing safety of formulation for further pharmacological studies. Anti-psoriatic assessment found that ear thickness, spleen weight, PASI score of treated animals (was reduced as compared to disease control. Body weight was also increased in treated animals as compared to disease control.

Biochemical estimation further validated the anti-Psoriatic activity of formulations. There was a marked improvement in catalase activity at 400 mg/kg of lepa dose (0.055 ± 0.00036 U/mg protein) as well as SOD Activity (35.20 ± 0.24 U/mg protein). This indicated reduced oxidative stress and restoration of antioxidant defence systems in the skin region.

Histopathological analysis provided visual confirmation of anti-psoriatic activity. psoriatic alterations like marked epidermal hyperplasia, hyperkeratosis, elongated rete ridges, dermal structure disruption compared with normal control group, which showed normal skin. Standard treated group showed better improvement like normal skin morphology. Test 1 Group showed moderate reduction in epidermal thickening and inflammatory infiltration and Test 2 better restoration of normal epidermal and dermis with mild inflammatory changes. In conclusion, the lepa formulation demonstrated significant antioxidant, and anti-psoriatic activity in both in-vitro and in-vivo models of psoriasis disease. Its effects were comparable to standard treatment with Clobetasol, thereby supporting its potential as an alternative or adjunct therapy in the management of Psoriasis disease

5. Conclusion

The current research aimed to prepare, standardize and evaluate a novel ayurvedic lepa formulation fortified with vitamins for anti-psoriasis activity preclinically using phytochemical analysis, In-vitro antioxidant Assay and In-vivo Imiquimod induced psoriasis model approaches.

The Formulations exhibited acceptable physicochemical characteristics, including appropriate pH, water soluble extractive value, alcohol soluble extractive value, total ash, acid insoluble ash ensuring their quality, stability and suitability for the dermal administration.

DPPH assay showed that hydroalcoholic polyherbal extract had dose dependent free radical scavenging activity.

No toxicity was observed in acute dermal toxicity (OECD 402) up to 2000 mg/kg, confirming the formulation safety.

In-vivo imiquimod induced psoriasis in rats model demonstrated significant improvement in psoriatic condition. The lepa treated groups exhibited highest improvement in histopathological lesions as shown by reduced spleen weight, PASI score, ear thickness, oxidative stress, and body weight is increased as compared to disease control. Biochemical parameters further supported the anti-psoriatic activity of the

formulation, showing elevated catalase and SOD activity.

Phytochemical Analyses revealed the presence of key bioactive compounds such as alkaloids, flavonoids, phenolic acids, glycosides, tannins and carbohydrates which are pharmacologically effective in psoriatic conditions. Quantitative estimation confirmed a high content of total phenolic content and total flavonoid content. In Conclusion the Lepa formulation demonstrated significant antioxidant, and anti-psoriatic effect comparable to standard treatment with Clobetasol Cream. It would be appropriate to conclude that the lepa Formulations containing *Achyarathes aspera* and *Vitex negundo* leaves extract and Vit. B 3, Vit.B12 are a promising candidate for further development as an alternative or complementary treatment for Psoriasis disease and future clinical validation.

CONCLUSION

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Conflict of interest

No conflict of interest.

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