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Topical Formulation of Allicin Comprising Penetration Enhancer: Evaluation of Anti-psoriatic Potential

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ABSTRACT

Background: Psoriasis was described as a persistent inflammatory skin condition characterized by abnormal epidermal growth and excessive keratinization, both of which impeded effective drug absorption.

Objectives: To enhance transdermal penetration, topical formulations incorporating skin permeability enhancers gained popularity for their ability to facilitate drug penetration and improve therapeutic outcomes.

Methods: In this study, allicin was isolated from *Allium sativum* clove, ointment was developed incorporating eucalyptus, basil, and cardamom oil, and antipsoriatic potential was explored on rat ear and dorsal skin of Wistar rats through the application of imiquimod (IMQ). Allicin 1% and 2% ointment was applied once daily at a dose of 200 and 400 mg/day for 10 days following the development of psoriasis. Evaluation parameters include PASI scoring, ear thickness, body weight and organ weight assessments, antioxidant and anti-inflammatory profiles, and histopathological examinations.

Results: The formulated ointments showed characteristic properties and were found to be stable in the range of 4 to 37°C. Allicin 2% ointment with 0.5% each essential oils significantly alleviated PASI score ($p<0.01$) based on psoriasis-like symptoms and ear thickness ($p<0.001$). Allicin treatment did not show any symptoms of acute topical toxicity and was safe on body weight gain and relative organ index. Allicin treatment significantly ($p<0.05$-0.001) lowered lipid peroxidation, IL-6, TNF-α, keratinocyte hyperproliferation and lymphocytic infiltration in the psoriatic tissue.

Conclusion: Allicin, 2% ointment, applied 400 mg/day dose in combination with essential oil, showed promising elevation of psoriasis symptoms along with antioxidant and anti-inflammatory profile in a lower dose, once a day application.

Keywords: Psoriasis, Inflammation, Allicin, *Allium sativum,* Topical Formulation, Penetration enhancer.

INTRODUCTION

Psoriasis is a persistent, non-infectious inflammatory condition marked by the emergence of red plaques covered with silvery-white scales, often found on extensor surfaces such as the elbows, knees, trunk, scalp, or lumbosacral area. In certain cases, it can also manifest as pustular lesions on mucous membranes, nails, palms, or soles, or as extensive sterile pustulosis. The condition affects an estimated 2-4% of the global population and can develop at any age. Its severity and extent vary significantly, with fluctuations influenced by seasonal changes and individual factors [1]. The condition has a substantial influence on quality of life. It is defined by an aberrant increase in epidermal keratinocyte proliferation combined with decreased differentiation, resulting in an increased turnover rate of epidermal cells. Psoriasis is clinically distinguished by the appearance of isolated to confluent elevated skin plaques with continuous scaling and varying degrees of erythema [2]. Histological analysis of psoriatic lesions typically shows acanthosis, characterized by thickened skin, along with immune cell infiltration, including dendritic cells, macrophages, and T lymphocytes [3].

Psoriasis symptoms are treated using a variety of treatments, including topical therapy, phototherapy, and systemic therapy. Topical treatment is effective for moderate psoriasis, but severe or advanced cases may require phototherapy or systemic medicines [4]. Older topical drugs like coal tar and dithranol are progressively becoming less popular. Various treatment options for psoriasis are available, but challenges persist regarding the cost, accessibility, and potential side effects associated with long-term use of synthetic medications. Herbal formulations generally offer a more affordable alternative and are associated with a reduced risk of adverse effects, making them a promising option for managing psoriasis.

Garlic (Allium sativum L.; Family: Amaryllidaceae) is an aromatic herbaceous annual spice and one of the most ancient and essential herbs used in traditional medicine. Topical application of garlic extract is effective on psoriasis [5]. Garlic can inhibit the nuclear transcription factor kappa B path associated with psoriasis [6]. Allicin [S-(2-propenyl)-2-propene-1-sulfinothioate], garlic's most physiologically active sulfur-containing component, is responsible for its odour and flavour [7]. Allicin is a lipid-soluble sulfenic acid thioester, and its pharmacological impact is linked to its antioxidant activity and interaction with thiol-containing proteins [8]. Allicin is a potential antioxidant by interacting with thiol-containing enzymes on the Fenton oxygen-radical generating system and effectively inhibits lipid peroxidation by reducing the synthesis of conjugated diene hydroperoxides [9]. Allicin demonstrated promising anti-inflammatory properties and regulates the immune system by reducing the levels of TNF-α, IL-6, and IL-8 [10,11].

Zhang et al. [12] reported antipsoriatic effect of allicin induced by imiquimod (IMQ). IMQ induces psoriatic lesions by inhibiting IL-17A expression and excessive keratinocyte proliferation. Allicin inhibits IMQ-induced NF-κB signaling transcriptional activation and down-regulates the expression of chemokines and AMPs in keratinocytes. In this study, we aim to explore the antipsoriatic efficacy of allicin on well-developed psoriatic plaque in the presence of essential oils as a penetration enhancer. Psoriasis causes thickened and hyperkeratotic plaques formation further hindering drug penetration, which results in the ineffective delivery and efficacy of the conventional treatment. Skin penetration enhancers facilitate the permeation of drugs through the skin by disrupting the stratum corneum, modifying the lipid bilayers, or affecting the protein structure within the skin. This enhances the diffusion of drugs into deeper skin layers and systemic circulation.

OBJECTIVES

To develop an effective topical allicin ointment for psoriasis treatment, we aimed to investigate isolated allicin in addition to penetration enhancers against the IMQ-induced psoriasis model emphasizing antioxidant and anti-inflammatory profiles. Eucalyptus oil, basil oil, and cardamom oil are included in allicin ointment to increase the skin permeability of allicin [13]. The present study substantiates the enhanced antipsoriatic potential of allicin by inhibiting oxidative damage and keratinocyte proliferation in the presence of essential oils. Allicin reduces IMQ-induced lipid peroxidation, IL-6, and TNF-α overproduction, enhances the expression of hydroxyproline, and inhibits keratinocyte hyperproliferation. Our data substantiate the hitherto enhanced therapeutic potential of allicin in alleviating psoriasis when coapplied with penetration enhancers.

METHODS

Chemicals

Allicin was procured from Life Technologies (India) Pvt. Ltd., Delhi.

Collection and Authentication of Plant Material

Fresh garlic cloves were collected from Sangamner (Maharashtra) in July - August 2021. The crude drug was identified and authenticated by botanist Dr. S. P. Giri, Department of Botany, Padmashri Vikhe Patil College of Arts, Science and Commerce, Loni-Pravaranagar, Maharashtra, with a herbarium number of PVPC/Bot/2021-22/07.

Extraction of Allicin

Alliinase catalysed the transformation of alliin to allicin in the broken garlic tissues. To catalyse the enzymatic conversion, peeled garlic was chopped and mashed, and then the garlic was incubated with water at room temperature for 20 minutes. The pulp was filtered and subjected to Soxhlet extraction with ethanol (75%) at 40°C for 90 minutes, maximally extracting allicin. The mixture was centrifugated at 6000 rpm/min for 10 min and filtrated to remove the insoluble substance. The supernatant was evaporated with a rotary evaporator to remove the ethanol, and the crude extract of allicin was obtained. Allicin extract was further subjected to freeze-drying for 48 hours, and yield was calculated [14,15]. Allicin was stored below 4°C to protect it from degradation and conversion into other sulfur-containing compounds [16].

Characterization of Isolated Allicin

Organoleptic Properties

The color, odor, and physical nature of the isolated allicin were visually observed. A small amount of allicin was placed on butter paper and viewed in a well-illuminated area.

Melting Point

The melting point of allicin was noted by applying the Capillary technique with a Thieles tube melting apparatus (Oswal Scientific, Pune) [17].

Thin Layer Chromatography (TLC)

Chromatographic separation was performed on a Camag TLC chamber with 10 × 10 cm silica gel plate (100 µm). Samples were applied utilizing a Camag Linomat applicator, and hexane: isopropanol (92:8) served as mobile phases. TLC plates were dried at 25°C, developed and scanned densitometrically at 254 nm on a UV-detector [16].

Calibration Curve

The maximum absorbance wavelength (λmax) of the allicin was determined using a UV-visible spectrophotometer (1800, Shimadzu, Japan). A stock solution was prepared by dissolving 100 mg of the drug in 100 ml of methanol. Subsequently, 5, 10, 20, 50, and 100 ml of this stock solution were further diluted with methanol to a final volume of 100 ml, resulting in a concentration of 5, 10, 20, 50, and 100 µg/ml, respectively. Absorbance was recorded at 240 nm and values were plotted versus concentration and analysed for regression coefficient [18].

Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra were scanned in the range of 4000 to 400 cm-1 utilizing a Bruker alpha FTIR spectrophotometer (Germany) with an Attenuated Total Reflectance technique. The FTIR spectra of the isolated allicin were then compared with the reported spectrum (Yadav and Swami, 2023).

High Performance Liquid Chromatography (HPLC)

The HPLC separation was performed using an Agilent (1100, Agilent Technologies, Germany) equipped with DAD (G-13148) detector with auto sampler consisting of a G1310A ISO Pump. The separation was carried out on a reversed phase C18 column (Hypersil ODS, 4.6 × 250 mm i.d., 5 µm particle size). The isolated allicin 50 mg and standard 200 mg were precisely weighed and dissolved in 10 ml methanol, which was sonicated for 30 minutes. After cooling, the solution was filtered through a 0.45-millimeter filter and subsequently utilized for HPLC analysis Mobile phase composed with methanol, water, and ethyl acetate (6: 3: 1) was subjected to isocratic elusion at 1.0 ml/min flow rate. The sample, 10 µl was injected at 26°C and detected at wavelength 254 nm for 12 minutes [19].

Development of Topical Formulation

The base formulation consisted of 0.5 gm of wool fat, 0.5 gm of cetostearyl alcohol, 0.5 gm of hard paraffin, 8 gm of yellow soft paraffin, and 0.5 gm of liquid paraffin. The preparation began by melting the grated hard paraffin in an evaporating dish over a water bath. Once fully melted, the remaining ingredients were added and gently stirred to achieve complete dissolution and uniform mixing. The ointment base was then allowed to cool. Ointment formulations were prepared by incorporating finely weighed extracted allicin (as specified in Table 1) for

preparation of A2, A2a, A2b; A3, A3a, A3b, FI, and FI* formulations. Essential oils of eucalyptus, basil, and cardamom were incorporated into the base. The allicin and oils were first mixed with 2 to 3 times the weight of the base to form a smooth paste, followed by the gradual addition of more base until a homogeneous ointment was achieved. The final mixture was then transferred into suitable containers and stored below 4°C [20].

Table 1: Formulation of allicin ointment

Ingredients	Quantity (gm)							
	A2	A2a	A2b	A3	A3a	A3b	FI	FI*
Allicin	0.1	0.1	0.1	0.2	0.2	0.2	**0.1**	**0.2**
Ointment Base q. s.	10	10	10	10	10	10	**10**	**10**
Eucalyptus oil	0.05	----	----	0.05	----	----	**0.05**	**0.05**
Basil oil	----	0.05	----	----	0.05	----	**0.05**	**0.05**
Cardamom oil	----	----	0.05	----	----	0.05	**0.05**	**0.05**

Formulation F1 and FI* respectively 1% (10 mg/gm) and 2% (20 mg/gm) of allicin with 0.5% of each essential oil component.

Evaluation of Ointment

The evaluation of the ointment involved assessing physical parameters in accordance with standard procedures. The organoleptic characteristics of ointment formulations, such as their physical appearance, color, odour, texture, phase separation, and homogeneity [21].

pH

The pH of the prepared ointment was measured using a digital pH meter (Tecnolab Instrument Servises, Gujrat). The ointment solution was prepared using 1 gm formulation in 100 ml of distilled water and kept aside for 2 hours [20].

Spreadability

The spreadability of the ointment was assessed applying the "slip and drag" method, with a device consisting of two glass plates of identical dimensions and a flat wooden block that was propped up by a pulley at one end. To evaluate the drag and slip properties, 2 gm of the ointment was placed on the lower plate and the apparatus was suspended by a hook. In order to release any trapped air and create a uniform film between the slides, 1 kg weight was placed on top of the slides. By utilizing the hook, a 50 gm weight was attached to generate a pulling force, and the time taken for the top slide to move 9 cm was determined [22].

Spreadability was calculated by following the formula: $S = M \times L / T$

Where, S= Spreadability; M= Weight tide to the upper slide; L= Length of glass slide; T= Time taken to separate the slide.

Extrudability

The extrudability test assesses the force needed to extrude a formulation from a collapsible tube after a specific amount of weight has been applied. Allicin ointment of about 5 gm was filled in a clean, lacquered aluminum collapsible tube on a crimped end, and a clamp was applied to avoid any rollback. The extrudability was determined by measuring the amount of 0.5 cm ribbon of ointment extruded in 5 sec through the tip when a certain load was applied. The extruded ointment was weighed, and the percentage of ointment extruded was calculated by using the following formula [21].

% Extrudability = Amount of ointment extruded from the tube / Total amount of ointment filled in the tube × 100.

Viscosity

Rheological studies were conducted using a Brookfield Synchro-Lectric Viscometer (Model RVT, BRK Instruments, India) with a helipath stand. The sample (50 gm) was put in a beaker and allowed to stabilize for 5 minutes before

the dial reading was measured using a T-D spindle at 10 rpm. The dial reading on the viscometer was recorded at room temperature in triplicate. The viscosity in centipoises was obtained by directly multiplying the dial readings by the coefficients listed in the Brookfield viscometer catalogue [21].

Loss in Weight

The Loss on Drying (LOD) was assessed by placing the formulation in a petri dish and drying it in a water bath set at 105°C [23].

Stability Study

The ointment was tested for physical stability as per the ICH guideline Q1C throughout a period of four weeks at various temperatures like 40C, 250C, and 370C [24].

In-vivo Study

Animal

Adult Wistar albino rats weighing approximately 180 - 200 gm at 6-8 weeks of age, both genders, were sourced from the animal house of the BIOCYTE Institute of Research & Development, Sangli, Maharashtra. Prior to the experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions, which included a 12-hour light/dark cycle, an ambient temperature of 25°C ± 1°C, and a relative humidity of 45% – 55%. They were provided with commercially available rat feed and water ad libitum. Ethical guidelines set by CCSEA were strictly followed, with protocol approval from the Institution Animal Ethics Committee under Registration no. (CPCSEA 2114/PO/Re/5/20/CPCSEA), with the approval number IAEC/Sangli/2023-24/11, dated October 11, 2023.

Acute Dermal Toxicity

The acute dermal toxicity of produced ointments was assessed using the Organisation for Economic Cooperation and Development guidelines 402 (Acute Dermal Toxicity: Fixed Dose Procedure). Wistar albino rats were placed into two groups, each consisting of six individuals. Hair was removed by shaving the dorsal portion of each rat (back skin with an area of 2 × 2 cm was shaved). The dose as per body weight was prepared with 2 % (w/w) ointments and applied topically to the shaved area on day 1. Starting at a dosage of 200 mg/kg, 1000 mg/kg, and 2000 mg/kg, stepwise 2 animals per dose were tested for signs and symptoms of acute toxicity. The allergic reactions of the skin were observed at 6 hr, 24 hr, 48 hr, and 72 hr for redness, erythema, and edema. Animals were observed for 14 days for fur changes, sleep patterns, behaviour, and mortality rates [25].

Treatment group

The total duration of the experiment was 20 days, from day 1 to day 10 induction of psoriasis and following that day 11 to 20. The rats were divided into five groups (n= 6) as follows; Group I presented as the vehicle control, uninduced, receiving treatment of ointment base only (200 mg). Rats of groups II to V were subjected to psoriasis-like dermatitis induced by imiquimod (IMQ) application. Group II was disease control left untreated after induction of psoriasis. Group III, designated as positive control, after psoriasis induction was treated with tretinoin 0.05% cream once daily for 10 days; Groups IV and V rats following psoriasis induction were treated with formulation FI (Allicin 1%) and FI* (Allicin 2%) respectively 200 mg ointment containing 2 mg/day and 4 mg/day of allicin. Psoriasis was also induced in the right ear by the application of IMQ as described above, followed by drug treatment.

Imiquimod-Induced Psoriatic Rat Model

Hairs were removed by shaving the dorsal portion of each rat. The remaining hairs were removed with depilatory cream (Veet, Reckitt Benckiser Pvt. Ltd., India). Psoriasis was triggered in the rat through the topical application of a commercially available IMQ 5% w/w cream (Glenmark Pharmaceuticals Pvt. Ltd, Nasik, India), on their shaved dorsal skin. A daily dose of 100 mg of IMQ 5% cream was administered for ten consecutive days a once-daily topical application on the shaved back skin until the appearance of a psoriatic lesion [26]. Psoriasis Area Severity Index (PASI) score showed skin erythema, increased thickness, and scaling, indicating successful induction.

Scoring of Psoriasis Area Severity Index (PASI)

The experiment was terminated on the 20th day, and the outcome evaluation was performed on 21st day. The PASI was used to assess skin inflammation intensity by visual evaluation of scaling (desquamation), erythema (redness), and thickness (induration). Each animal was individually graded for these three characteristics separately on a scale of 0 to 4 by one researcher. On this scale, 0 = none, 1 = slight, 2 = moderate, 3 = marked, and 4 = very marked, resulting in a total score ranging between 0 to 12. The thickness of the right ear was also measured with a Vernier caliper and used to assess skin inflammation before induction (Day 1), after induction (Day 10th) and on 21st day [27].

Body Weight, Thymus and Spleen Weight

The body weight of all the animals was recorded on day 1 and at the end of the study. The thymus and spleen were extracted from each animal, kept in cold saline, blotted dry, and weighed.

Biochemical Analysis

Animals were anesthetized intraperitoneally (IP) with 80 mg/kg of ketamine and 10 mg/kg of xylazine. Following total anesthesia, all rat were euthanised by cardiac puncture. Tissue samples were harvested from the dorsal shaved skin (1 cm) and right ear and divided into two parts. The first specimen of skin and ear was preserved in glass vials containing 10% neutral buffered formalin (Sigma-Aldrich, Singapore) for histological examination. The second part of the psoriatic skin tissues of dorsal and ear regions were promptly rinsed with Tyrode solution. A portion of these tissues was then blotted, dried, weighed, and treated with 6N HCl in a water bath at 130°C for 4 hours in sealed Pyrex-glass tubes to facilitate hydrolysis [28,29].

Protein

A 0.1 ml sample of tissue homogenate was combined with 1.2 ml of distilled water and 6 ml of solution A. The mixture was incubated at room temperature for 10 minutes. Following this, 0.3 mL of solution B was added, and the sample was incubated again at room temperature for 30 minutes. Absorbance was then measured at 680 nm [30].

Hydroxyproline

A 20 µl aliquot of the hydrolysate was combined with 50 µl of chloramine T in citrate buffer (5% citric acid, 7.24% sodium acetate, 3.4% sodium hydroxide and 1.2% glacial acetic acid), incubated at room temperature for 20 minutes, then treated with 50 µl of Ehrlich's reagent and incubated at 65°C for 15 minutes. The absorbance was recorded at 550 nm, and hydroxyproline content was determined using a standard curve (0–10 µg/ml) [29].

Lipid Peroxidation

A homogenate of skin and ear tissue was prepared by combining 0.4 mL of 10% liver homogenate with 1.5 mL of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetate buffer (pH 3.5), and 1.5 ml of 0.8% TBA solution. The mixture was heated at 95°C for 60 minutes and allowed to cool. Afterward, 5 ml of n-butanol-pyridine (15:1) was added, thoroughly vortexed, and left for phase separation. The absorbance of the organic phase was measured at 532 nm using a UV-visible spectrophotometer [31.32].

Cytokines

Cytokines levels were assessed using the enzyme-linked immunosorbent assay (ELISA) method. ELISA kits were utilized to measure pro-inflammatory cytokines, IL-6 and TNF-α. The assays were performed according to the manufacturer's instructions as provided with the commercially available kits (Krishgen Biosystem, Mumbai) [32].

Histopathological Analysis

The tissue sections were prepared for histopathological analysis by dehydration and immersion in liquid paraffin at a 55–60°C temperature range, processed into paraffin blocks and cut into 250 µm thick sections. The resulting slices were stained with hematoxylin and eosin (Sigma-Aldrich, Singapore) and analyzed using a light microscope (Olympus BX51 Microscope, Olympus Corporation, Japan) [33].

Statistical Analysis

The experimental results were presented as mean ± standard error of the mean and were then subjected to one-way analysis of variance. To assess the statistical difference between different groups, the Newman–Keuls multiple comparisons test was utilized. Statistical analysis was carried out using Graph Pad Prism, version 5 software, with a significance level set at $P < 0.05$.

RESULTS

Characterization of Isolated Allicin

Isolated allicin (yield 0.55%) was obtained as brownish-yellow powder with strong pungent odour having melting point between 23-25ºC. TLC of allicin in hexane: isopropanol (92:8) showed Rf at 0.46 as standardized earlier by Gupta et al. [16]. Allicin showed maximum absorbance at 240 nm [18]. The calibration curve of allicin showed linearity between absorbance and concentration with a regression coefficient of 0.9997. The FT-IR spectrum of isolated allicin displayed characteristic peaks at 3210.35 cm^{-1}, corresponding to the $CH=CH_2$ group of the allyl moiety; 2876.48 cm^{-1} for the CH group of aliphatic structures; 1651.77 cm^{-1} for the C=C bond; 1055.8 cm^{-1} associated with the S=O bond; 878.72 cm^{-1} indicative of the C-S bond; and 720.64 cm^{-1} for the S-S single bond. The HPLC indicated a retention time of 3.644 min for the isolated allicin compared to the reported Rt of 3.35 min for allicin Iberl et al. [19] (Figure 1, 2, 3, and 4).

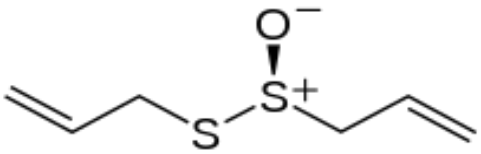

Figure 1: Chemical structure of allicin

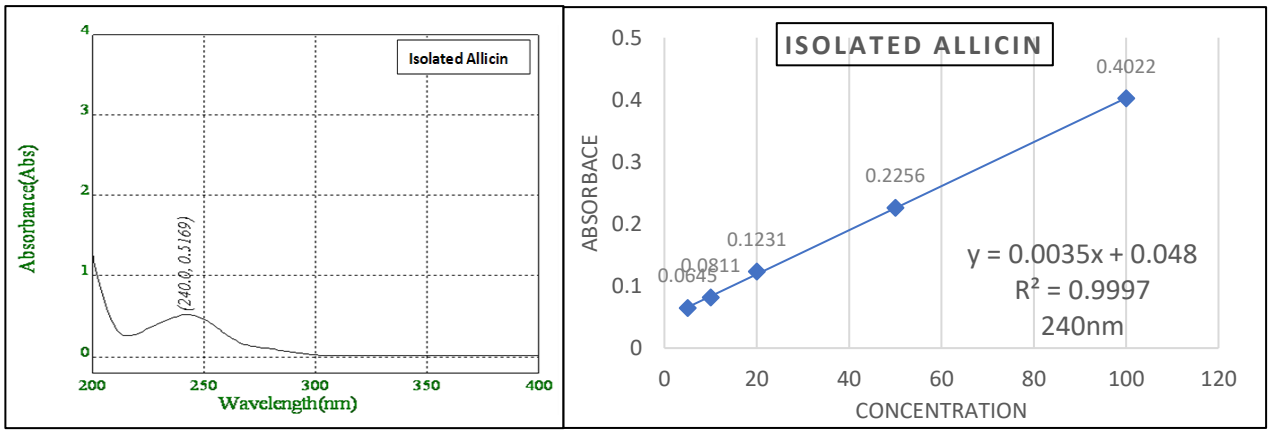

Figure 2: UV spectra of allicin in methanol

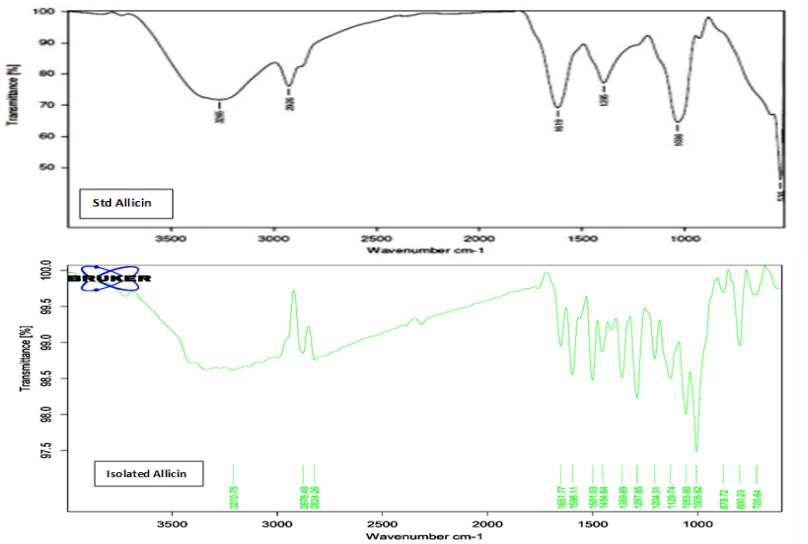

Figure 3: FTIR spectra of standard (Yadav and Swami, 2023) and isolated allicin

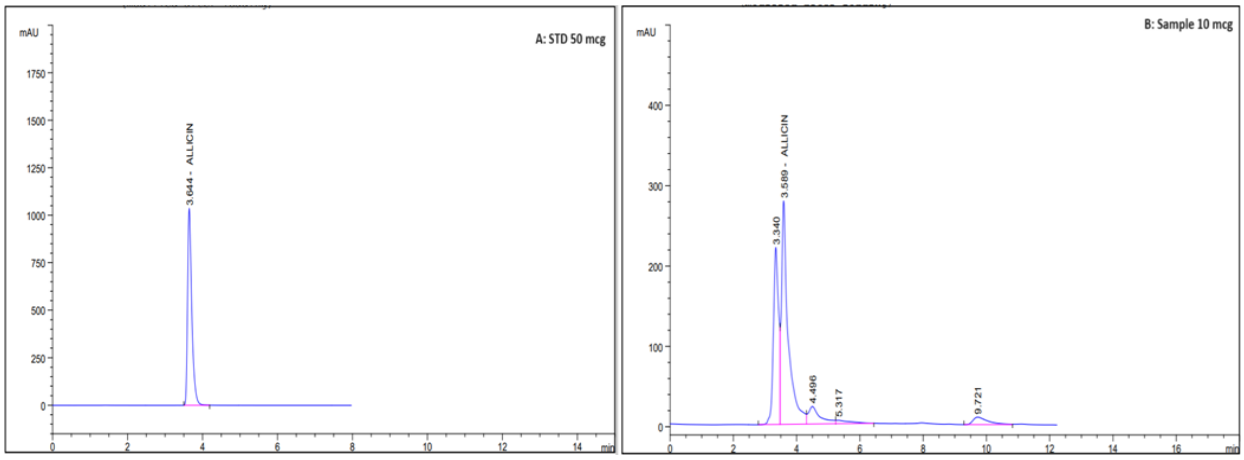

Figure 4: Chromatograms of standard allicin showed Rt at 3.644 min (A) and isolated allicin (sample) showed Rt at 3.589 min (B).

Characterization of Allicin Ointment

Allicin 1% and 2% ointment with eucalyptus, basil, and cardamom oil had a smooth and homogeneous texture, greyish brown colour, and characteristics odour. The pH value of the allicin ointment formulations was in the range of 6.00 to 7.05. The viscosity of the formulations was between 2367.81 and 2608.70 CPS, suitable for ambient dispensing and spreadability. The formulations FI (Allicin 1%) and FI* (Allicin 2%) showed higher spreadability and extrudability (Table 2). Based on the parameters assessed during stability studies of the formulations at three different conditions, products were stable and showed a non-significant difference in stability profile compared to the initial values (Table 3).

Acute Dermal Toxicity

Allicin ointments (2%) were well-tolerated by Wistar albino rats. No skin was noted, and there were no severe adverse effects or mortality, suggesting the absence of acute dermal toxicity.

Table 2: Physical evaluation of the allicin ointment formulations

Formulation	pH	Viscosity at 10 rpm (cps)	Spreadability (g.cm/s)	Extrudability (%)	Loss of drying (%)
A2	6.69 ± 0.78	2412.13 ± 53.34	110.16 ± 1.53	91.74 ± 2.08	26.25
A2a	6.62 ± 0.80	2342.56 ± 82.77	108.48 ± 2.45	93.39 ± 3.14	27.71
A2b	6.60 ± 0.77	2456.02 ± 72.73	97.32 ± 3.53	92.83 ± 3.10	28.82
A3	6.15 ± 0.34	2504.68 ± 58.44	117.03 ± 2.03	93.46 ± 2.29	27.33
A3a	6.02 ± 0.29	2367.81 ± 45.36	107.19 ± 3.79	93.89 ± 4.31	26.94
A3b	6.00 ± 0.21	2470.65 ± 70.08	102.91 ± 2.12	94.02 ± 3.09	26.04
FI	7.05 ± 0.36	2527.59 ± 62.42	119.15 ± 2.10	95.16 ± 2.50	28.68
FI*	6.98 ± 0.51	2608.70 ± 61.46	120.03 ± 3.07	94.98 ± 4.23	28.22

Table 3: Stability of allicin ointment formulations after 3 months of storage at specified temperature

Temp	Parameters	Formulations							
		A2	A2a	A2b	A3	A3a	A3b	FI	FI*
4°C	Viscosity at 10 rpm (CPS)	2589.96 ± 45.56	2674.56 ± 62.08	2603.63 ± 71.67	2684.47 ± 50.16	2645.98 ± 49.11	2622.65 ± 68.15	2583.00 ± 58.49	2602.00 ± 70.03
	pH	6.69 ± 0.54	6.62 ± 0.38	6.60 ± 0.57	6.15 ± 0.24	6.02 ± 0.23	6.00 ± 0.10	6.88 ± 0.22	6.93 ± 0.16
	Spreadability (g.cm/s)	98.93 ± 4.01	102.76 ± 4.38	98.01 ± 4.08	112.03 ± 6.72	105.35 ± 3.18	103.28 ± 4.15	115.20 ± 3.31	117.93 ± 5.10
27°C	Viscosity at 10 rpm (CPS)	2556.67 ± 53.80^{ns}	2681.31 ± 48.64^{ns}	2624.00 ± 69.09^{ns}	2676.65 ± 60.09^{ns}	2636.00 ± 55.02^{ns}	2607.00 ± 65.86^{ns}	2574.00 ± 58.98^{ns}	2602.00 ± 44.16^{ns}
	pH	6.79 ± 0.78^{ns}	6.68 ± 0.81^{ns}	6.67 ± 0.64^{ns}	6.31 ± 0.85^{ns}	6.17 ± 0.19^{ns}	6.23 ± 0.21^{ns}	7.00 ± 0.16^{ns}	7.05 ± 0.42^{ns}
	Spreadability (g.cm/s)	102.03 ± 3.85^{ns}	104.48 ± 5.29^{ns}	98.41 ± 4.05^{ns}	114.01 ± 3.19^{ns}	106.04 ± 4.07^{ns}	102.91 ± 3.94^{ns}	117.36 ± 5.67^{ns}	118.01 ± 4.14^{ns}
37°C	Viscosity at 10 rpm (CPS)	2526.54 ± 68.06^{ns}	2667.36 ± 60.56^{ns}	2616.21 ± 55.54^{ns}	2656.39 ± 62.41^{ns}	2621.47 ± 64.35^{ns}	2593.96 ± 55.61^{ns}	2606.00 ± 47.25^{ns}	2579.00 ± 56.37^{ns}
	pH	6.53 ± 0.77^{ns}	6.76 ± 0.80^{ns}	6.40 ± 0.77^{ns}	6.52 ± 0.34^{ns}	6.00 ± 0.29^{ns}	6.30 ± 0.21^{ns}	6.94 ± 0.43^{ns}	6.99 ± 0.21^{ns}
	Spreadability (g.cm/s)	105.87 ± 3.49^{ns}	105.53 ± 3.62^{ns}	97.81 ± 3.24^{ns}	115.41 ± 4.63^{ns}	106.10 ± 3.58^{ns}	103.70 ± 4.19^{ns}	117.86 ± 5.99^{ns}	118.07 ± 4.62^{ns}

Values are expressed as Mean ± SD (n=3). The term ns = not significant, when compared to stability test parameter values at 4°C temperature.

Table 4: Effect of allicin ointment application on spleen and thymus weight of rats with imiquimod induced psoriasis

Groups	Spleen weight (gm/100 gm body weight)	Thymus weight (gm/100 gm body weight)
Vehicle Control	0.719 ± 0.054	0.293 ± 0.017
Disease Control	0.926 ± 0.062^{ns}	0.387 ± 0.10^{ns}
Tretinoin (0.05%)	0.726 ± 0.049^{ns}	0.290 ± 0.030^{ns}
FI (Allicin 1%)	0.672 ± 0.053^{ns}	0.227 ± 0.012^{ns}
FI* (Allicin 2%)	0.658 ± 0.051^{ns}	0.218 ± 0.040^{ns}

Values are expressed as Mean ± SEM (n=6). The term ns = not significant when compared to vehicle control value.

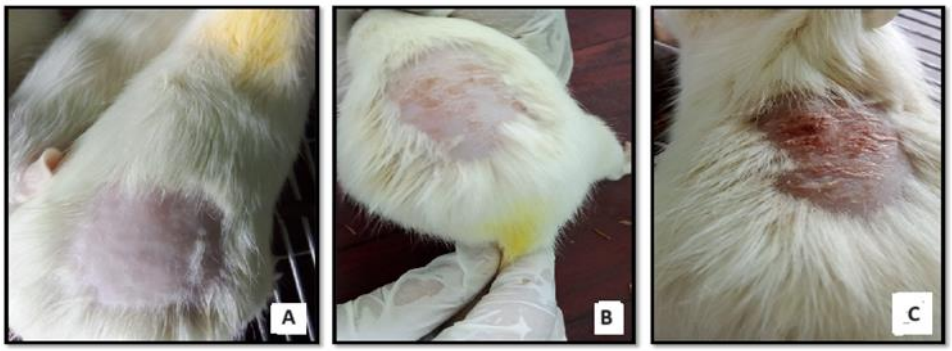

Figure 5: Imiquimod induced psoriasis on rat.

A: rat dorsal portion before induction; B: represent IMQ-induced psoriasis on day 3; C: represent IMQ-induced psoriasis on day 7.

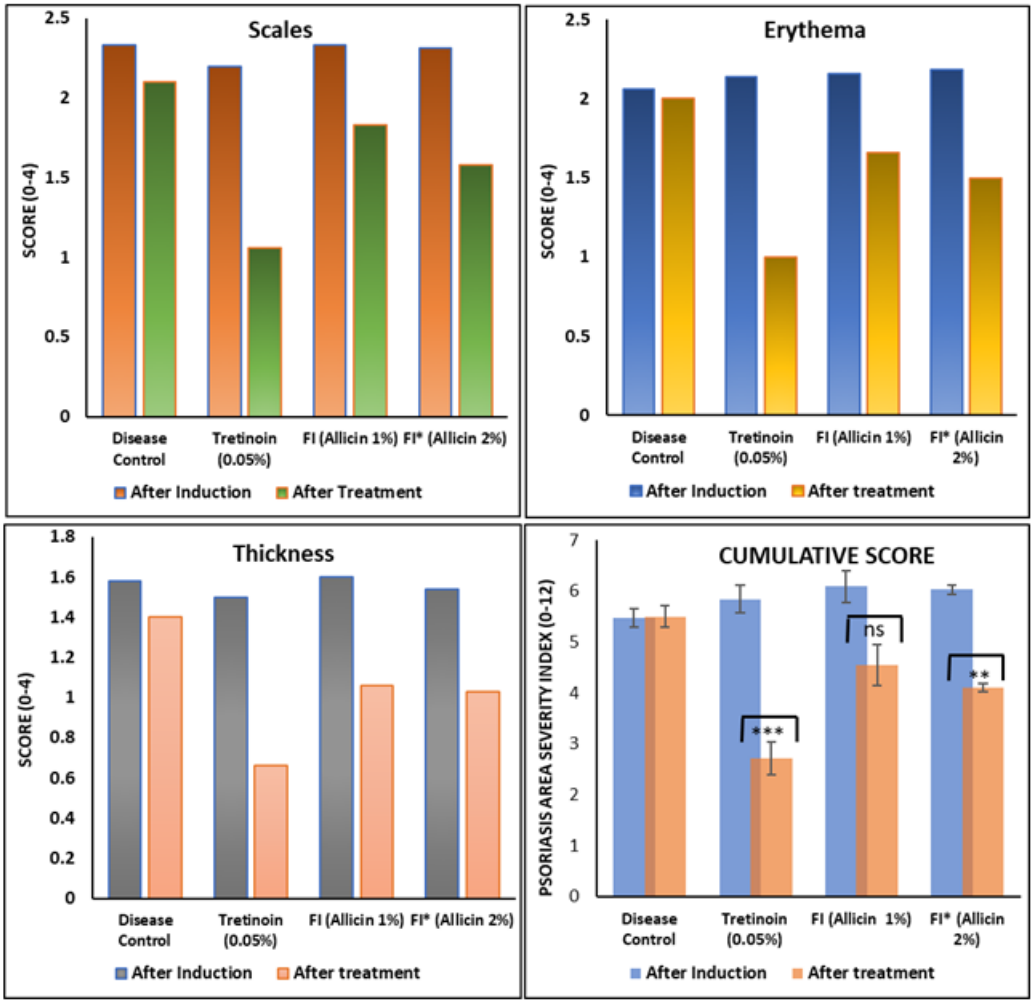

Figure 6: Antipsoriatic effect of allicin ointment against inflammation induced by imiquimod in rats.

Effect on parameters, i.e., erythema, scales, thickness, and cumulative score of psoriatic skin lesions on dorsal portion as indicated by psoriasis area severity index score. Values are expressed as Mean ± SEM (n=6). The $^{**}p<0.01$, $^{***}p<0.001$ and ns = not significant when compared to each respective value after induction.

PASI Scoring of Psoriatic Tissue

By the tenth day of treatment, the IMQ application had effectively induced pronounced signs of erythema, swelling, and scaling (Figure 5). The PASI scores confirms successful induction of psoriasis-like dermatitis in the IMQ-treated rats. Beginning on day 11, treatment with allicin 2% ointment showed gradual and consistent reduction in PASI by day 21. On day 21, significant decrease in PASI scores was noted in tretinoin ($p<0.001$) and FI* allicin 2% ointment ($p<0.01$) compared to after induction values of the respective groups. These findings suggest that the positive control and formulation FI* is effective in reducing erythema, scales, and skin thickness (Figure 6).

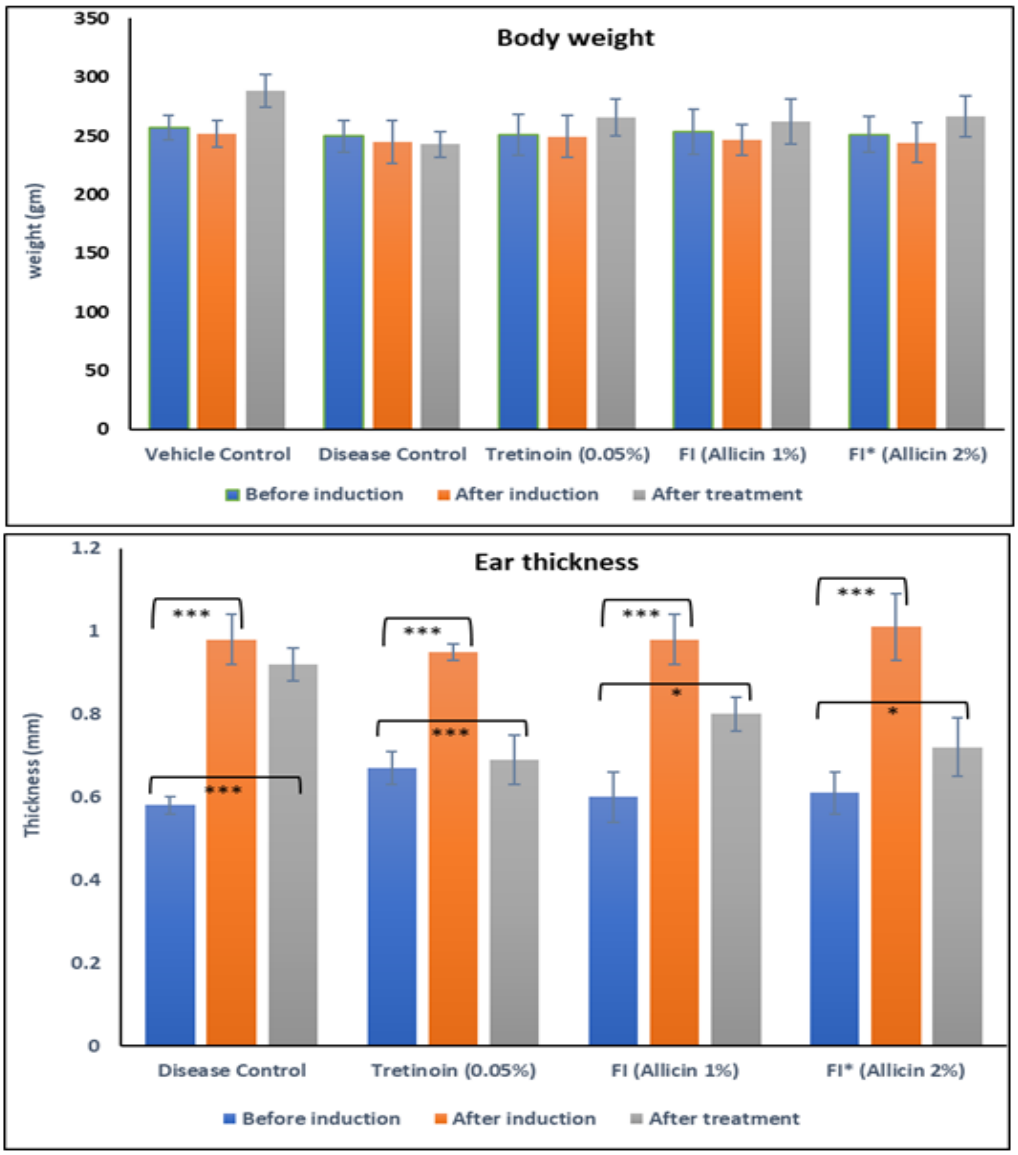

Figure 7: Effect of allicin ointment application on body weight and ear thickness of rats with imiquimod induced psoriasis.

Values are expressed as Mean ± SEM (n=6). The $^{*}p<0.05$, $^{***}p<0.001$ and ns = not significant when compared to each respective value before induction.

Body Weight and Relative Weight of Thymus and Spleen

The body weight changes in different groups during the study period before induction, after induction, and after treatment are depicted in Figure 7. All the groups showed transient increase in body weight of rats for 21 days study period except the disease control group which showed a slight decrease in weight (Figure 7). The spleen and thymus weights across different groups did not show any significant changes following psoriasis induction and treatment course (Table 4).

Ear Thickness

All the groups showed extremely significant ($p<0.001$) increase in ear thickness after psoriasis induction. The disease control group showed a slight reduction after 10 days without any treatment. In the positive control group ear thickness was decreased after treatment and was close to before induction values with non-significant change. Allicin 1% and 2% formulation treatment showed decrease in ear thickness though with a significant ($p<0.05$) change after induction (Figure 7).

Antioxidant and Anti-Inflammatory Parameters

The IMQ-induced rats also showed a significant ($p<0.001$) increase in lipid peroxidation in psoriatic skin and ear with a concomitant increase in the levels of IL-6 and TNF-α as compared to normal rats signifying the induction of psoriasis-like inflammation. Treatment with tretinoin and allicin 2% ointment had significantly decreased lipid peroxidase ($p<0.05$) and conversely increased IL-6 and TNF-α ($p<0.001$) levels. Psoriasis had significantly ($P<0.05$) decreased hydroxyproline content in the tissue and allicin treatment has restored the level to near normal (Table 5).

Histopathological Analysis

The histopathological findings indicated that the dermis of the vehicle control group appeared normal. IMQ treatment-initiated hyperkeratosis, increased and disrupted epidermal proliferation and differentiation. The IMQ treated skin exhibited significant thickening of the epidermis due to hyperproliferation of keratinocytes, a condition referred to as acanthosis along with rete ridges formation. Psoriatic tissue also showed thickening of the cornified layer, and infiltration of inflammatory cell into dermis/epidermis followed by erythematous plaque formation. Tretinoin treatment has significantly reduced hyperkeratosis, keratin flaking, and presence of lymphocytes in the upper dermis. Notably, the granular cell layer was absent, there was no elongation of rete ridges, and polymorphonuclear infiltration. Allicin ointment (2%) application showed thinning of the granular cell layer, indicating a reduction in keratinocyte hyperproliferation and moderate lymphocytic infiltration (Figure 8).

Table 5: Effect of allicin ointment application on oxidative status of psoriatic tissues of rats with imiquimod induced psoriasis

Groups	Lipid peroxidation (nmoles/mg of protein)		Skin		
	Skin	Ear	Hydroxyproline (µg/mg of protein)	IL-6 (pg/mg of protein)	TNF-α (pg/mg of protein)
Vehicle Control	0.93 ± 0.05	1.06 ± 0.08	4.68 ± 0.22	836.14 ± 20.44	496.33 ± 12.87
Disease Control	$1.72 \pm 0.16^{***}$	$1.65 \pm 0.22^{*}$	$2.64 \pm 0.34^{*}$	$4425.87 \pm 189.14^{***}$	$1126.69 \pm 57.14^{***}$
Tretinoin (0.05%)	$1.02 \pm 0.10^{ns,a}$	$1.23 \pm 0.12^{ns,ns}$	$3.15 \pm 0.76^{*,ns}$	$1154.93 \pm 130.07^{ns,c}$	$575.59 \pm 9.36^{ns,c}$
FI (Allicin 1%)	$1.45 \pm 0.11^{**,ns}$	$1.37 \pm 0.05^{*,ns}$	$3.46 \pm 0.21^{ns,ns}$	$3047.76 \pm 250.71^{***,c}$	$819.90 \pm 18.89^{***,c}$
FI* (Allicin 2%)	$1.21 \pm 0.15^{*,a}$	$1.04 \pm 0.06^{ns,a}$	$3.83 \pm 0.33^{ns,a}$	$2987.34 \pm 129.19^{***,c}$	$765.31 \pm 16.42^{***,c}$

Values are expressed as Mean ± SEM (n=6). The $^{*}p<0.05$, $^{***}p<0.001$ and ns = not significant when compared to vehicle control group. The $^{a}p<0.05$, and $^{c}p<0.001$ when compared to disease control group.

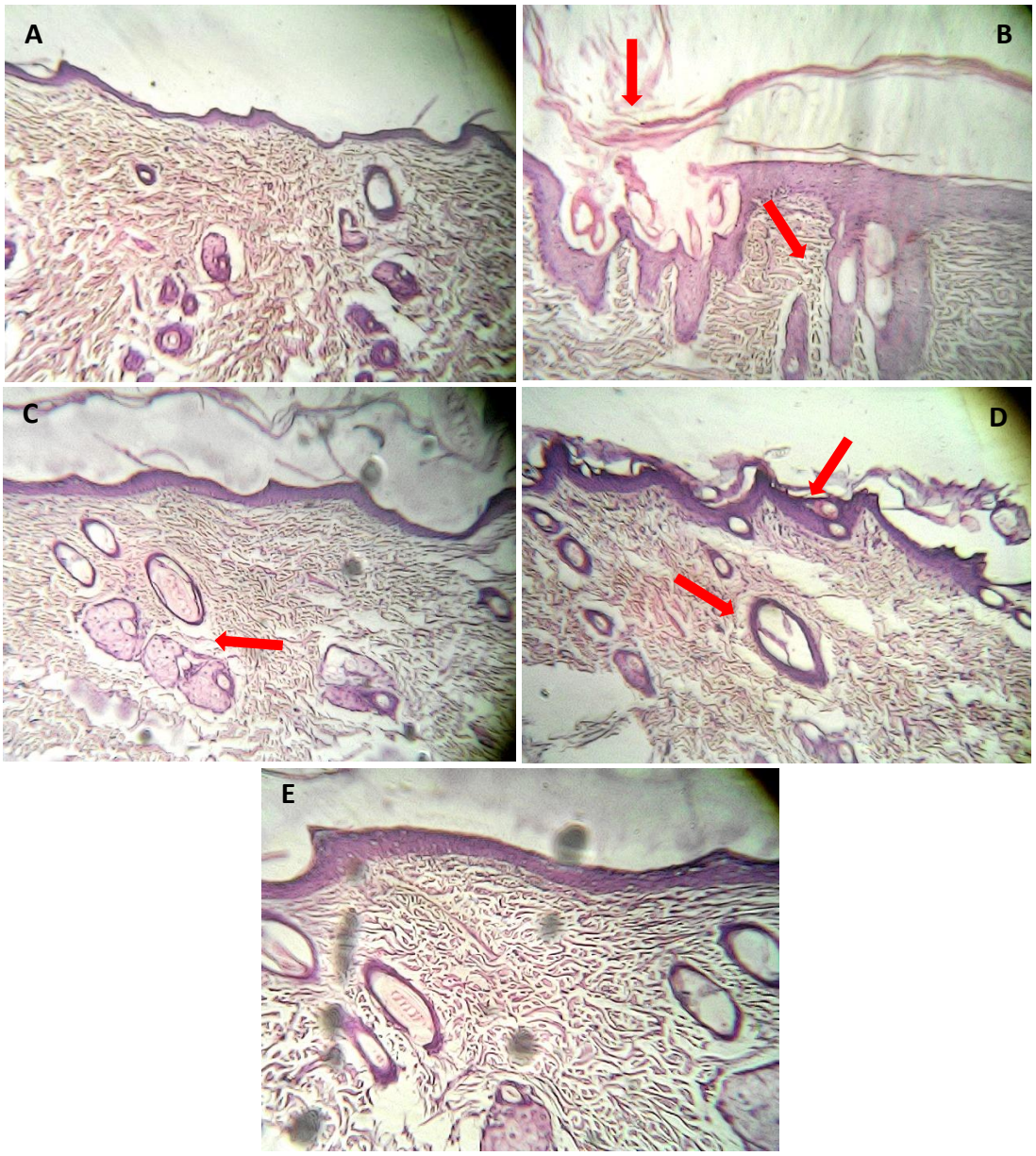

Figure 8: Antipsoriatic effect of allicin ointment on histopathological changes in the skin induced by imiquimod in rats.

A: Vehicle control, B: Disease control, C: Positive control, D: FI (Allicin 1%) and E: FI* (Allicin 2%).

DISCUSSION

This study substantiates the antipsoriatic efficacy of allicin that was potentiated further when applied with penetration enhancer essential oils. Allicin was isolated, authenticated, and formulated into a stable ointment formulation with 0.5% each of eucalyptus, basil, and cardamom oil. Allicin ointment at 2 and 4 mg/day topically exhibited profound antisporiatic potential against fully developed IMQ induced psoriasis along with anti-inflammatory and antioxidant profiles. Although conventional therapies are effective against psoriasis, it is not curable and is often recurrent, emphasizing the importance of long-term, safe, and effective treatment. Comparatively, safe and nontoxic natural products are gaining momentum as a novel strategy for treating psoriasis as a chronic condition. Allicin is effective in treating fungal and bacterial infections having anti-inflammatory, anti-

viral, and anti-cancer activities. Allicin ointment (1% and 2%) formulation was developed with 0.5% each of eucalyptus, basil, and cardamom oil. Allicin 2% with penetration enhancers, eucalyptus oil, basil oil, and cardamom oil. The spreadability, extrudability, and viscosity profiles were found to be satisfactory for allicin topical dosage form. Spreadability and extrudability represent the rheological properties and are the accurate approach to understanding the relationship between viscosity and the force needed to squeeze the ointment out of a tube [34]. Allicin formulations were well-tolerated in rats, suggesting the absence of acute dermal toxicity.

IMQ, is a well-known immune activator that induces psoriasis-like inflammatory effects on rat by acting as ligands for toll-like receptor (TLR) 7 and 8. Though the IMQ-mouse model has been a suitable experimental model for psoriasis-related studies until recently, Parmar et al. [32] successfully induced clinical hallmarks and histomorphological patterns of psoriasis in rats. The application of IMQ for 10 consecutive days in the experimental period resulted in psoriasis development rat skin closely resembling human psoriasis, exhibiting key features such as skin thickening, scaling, epidermal alterations, and erythema. Topical drug therapy is mostly effective in the early phases of psoriatic plaque development, but in the advanced phase, hyperkeratosis blocks drug delivery through the psoriatic epidermal barrier and trans appendageal passage [35]. Essential oils, especially Eucalyptus oil, are known for increasing stratum corneum permeability. Essential oils used together can implore synergistic effects when used together. Essential oils increase penetration by temporarily weakening the subcutaneous barrier without damaging viable cells. Therefore, essential oils mostly do not cause irritation and are safe [36].

Based on an earlier study allicin alleviates psoriasis by suppressing inflammation and hyperproliferation compared to first-line topical vitamin D3 analogue, calcipotriol. Allicin was applied topically concurrently along with IMQ for 10 days. Compared to calcipotriol, allicin showed higher efficacy in improving erythema, and scales of psoriasis [12]. In the current study, allicin topical formulation with penetration enhancers was applied after induction of psoriasis in rats after 10 days of IMQ application compared to tretinoin. The PASI scores confirm the successful induction of skin erythema, edema, and scaling. After proper induction of psoriasis, allicin ointment bolstered with essential oils as a topical penetration enhancer was applied for 10 consecutive days. In this study, allicin ointment was applied after the development of psoriasis scales in a much lower dose and once-a-day protocol to assess antipsoriasis and anti-inflammatory potential in the presence of penetration enhancers.

Allicin topical application was totally safe on the systemic immune system with no untoward effect. Allicin treatment did not affect body weight and relative organ index, indicating the safety of allicin treatment. Allicin, 2% ointment, applied 400 mg/day dose in combination with essential oil showed promising elevation of psoriasis symptoms along with antioxidant and anti-inflammatory profile on the tissue. In the present study, we used the allicin 2% ointment, which is effective in reducing erythema, scales, and thickness of dorsal skin and ear. Psoriasis extent and severity are associated with an imbalance between oxidative stress and antioxidant enzyme content. An increase in lipoprotein serum levels has shown a direct correlation with markers of lipid peroxidation and oxidative damage in psoriasis. Psoriatic patients showed higher levels of lipoprotein in the blood compared to normal subjects [37]. The IMQ-induced rats also showed significantly higher lipid peroxidation in psoriatic skin and ear tissue with concomitant elevated levels of IL-6 and TNF-α as compared to normal rats. Topically applied allicin formulation showed transient lowering of lipid peroxidation, IL-6, and TNF-α in the psoriatic tissue.

Lipid peroxidation is one of the major characteristic features of the advanced stage of psoriasis [38]. An increased level of lipid peroxidation was observed along with an abnormal antioxidant status during psoriasis, as reported by Parmar et al. [32]. Our observation also showed a significant elevation in lipid peroxidation level on IMQ application. Therefore, treatment of psoriasis also focuses on reducing the level of lipid peroxidation which ultimately can reduce the expression of inflammatory mediators in psoriasis plaques, and more specifically in keratinocytes [39]. Allicin 1% and 2% ointment with eucalyptus oil, basil oil, and cardamom oil application has shown a significant reduction in lipid peroxidation in the psoriatic tissue. Allicin is well known for its antioxidant free radical scavenging to lower lipid peroxidation [40].

Hydroxyproline is an amino acid found in collagen that plays a crucial role in collagen synthesis and its thermodynamic stability. Hydroxyproline was found to be upregulated in severe psoriasis patients, acting as a marker for tissue collagen degradation. Collagen turnover is reported to be higher in psoriasis patients, along with enhanced levels of collagen breakdown enzymes prolidase and matrix metalloproteases MMP-1 [41.42]. Psoriasis

severity-associated metabolic perturbations of amino acids may stem from increased demand for collagen synthesis and keratinocyte hyperproliferation or potentially the incidence of cachexia. In contrast, in this study, psoriasis-induced rats showed a significant decrease in skin tissue hydroxyproline content whereas allicin treatment does not bring it back to normal. The levels of circulating amino acids are useful for monitoring both the severity of psoriasis as well as therapeutic response to anti-TNFα treatment [43]. TNF-α was reported to promote collagen degradation in ovine cartilage [44]. The high levels of TNF-α in the IMQ-induced rats are likely to promote the degradation of collagen, in turn increasing hydroxyproline levels.

The application of IMQ to rats led to the development of psoriatic-like skin lesions and disruption of normal skin architecture, potentially attributed to reduced levels of collagen (hydroxyproline) and hexosamine. The low level of hydroxyproline found in the psoriatic rats may be due to increased urinary excretion that has not been assessed. Psoriatic patients are reported to have significantly higher urinary excretion of hydroxyproline [45]. Allicin treatment did not show any significant effect on hydroxyproline content in the psoriatic tissue. IL-6 and TNF-α are both proinflammatory cytokines that have important roles in the pathogenesis of psoriasis in genetically susceptible patients [46]. Zhang et al. [12] reported association of NF-κB induced IL-17A expression and chemokine release in psoriatic keratinocytes proliferation. Psoriasis is a T-cell-mediated disease, with a complex role different cytokine.

The serum levels of cytokines TNF-α, IFN-□, IL-6, IL-8, IL-12, and IL-18 were found to be significantly higher in active psoriatic patients that correlated with the clinical severity [47]. Allicin has profound antioxidant efficiency, evident from the attenuation of lipopolysaccharide-induced oxidative degeneration, apoptosis, and lipid peroxidation [48]. Our findings suggest that allicin attenuated IMQ-induced inflammatory response in psoriatic skin by inhibition of lipid peroxidase, IL-6, and TNF-α. Allicin prevents tracheal stenosis via antimicrobial and anti-inflammatory properties in wound healing after tracheal injury [49]. Allicin markedly alleviated spine inflammatory injury in rat by reducing the secretion of IL-6, IL-8, and TNF-α inflammatory factors [10]. Allicin has also been reported to impede the progression of arterial hypertension by regulating pro-inflammatory markers TNF-α, IL-6, TGF-β, IL-1β, and Cd68 in the lung and heart [50].

Psoriasis is primarily characterized by epidermal hyperplasia, leading to the thickening of the epidermis, accompanied by hyperkeratosis or thickening of the cornified layer. This condition is further marked by the infiltration of inflammatory cells into both the dermis and epidermis, which contributes to the development of erythematous plaques, a hallmark of the disease [51]. Imiquimod (IMQ)-treated mouse skin displayed hallmark psoriatic features, including pronounced acanthosis, infiltration of inflammatory cells, and changes in dermal vascularity. IMQ treatment on the rat dorsal skin-initiated hyperkeratosis, thickening of the epidermis along with disruption in epidermal differentiation. The prominent histopathological observation was the formation of acanthosis and rete ridges. Allicin with penetration enhancer treated psoriatic skin tissue showed thinning of the granular cell layer, a reduction in keratinocyte hyperproliferation, and also showed a reduction in lymphocytic infiltration. Allicin significantly improved characteristic pathological changes of psoriasis indicating effective antipsoriatic efficacy [12].

This finding may be attributed to the fact that current first-line drugs are not very effective in reducing inflammation and keratinocyte proliferation associated with psoriasis pathophysiology. Allicin has a multifaceted anti-psoriatic profile as it inhibits inflammatoty activation of chemokines, lipid peroxidase, and cytokines in keratinocytes. Allicin inhibited keratinocyte viability, cell cycle progression and promoted apoptosis, relieved epidermal edema (Zhang et al., 2023). Keratinocytes are actively involved in the pathogenesis and maintenance of psoriasis by producing various pro-inflammatory factors and chemokines [52]. Our study confirmed that allicin coapplied with penetration enhancer effectively inhibited immune cell infiltration in skin lesions, mitigating inflammatory damage in keratinocytes in a much lower dose and once-a-day protocol. This finding highlights allicin as a promising therapeutic strategy for the treatment of psoriasis. Interestingly, it has been reported that allicin inhibits the activation of the MAPK/NF-κB pathway and NLRP3 inflammasome to improve acrylamide-induced hepatotoxicity [53]. Allicin inhibits oxidative damage via the inhibition of NF-κB signaling pathways in the stimulated keratinocytes. however, we did not investigate the in-depth antipsoriatic effects of allicin on the systemic immune system.

CONCLUSION

This study highlights the isolation, formulation, and evaluation of the antipsoriatic efficacy of allicin ointment along with penetration enhancer oils is a promising approach for treating fully developed IMQ-induced psoriasis. The results demonstrated that a 2% allicin ointment applied once daily at a dose of 400 mg/day for 10 consecutive days significantly reduced psoriatic pathology. Comprehensive evaluations, including PASI scoring, body weight and organ weight assessments, antioxidant and anti-inflammatory profiles, and histopathological examinations, confirmed the topical antipsoriatic potential of allicin. Future research should focus on optimizing the formulation, investigating the long-term effects, and elucidating the mechanisms of action in psoriasis management.

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