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DEVELOPMENT AND PHARMACOLOGICAL EVALUATION OF A HERBAL CREAM FOR PSORIASIS MANAGEMENT

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ABSTRACT

Psoriasis is a chronic inflammatory skin condition that often necessitates long-term treatment, which can lead to undesirable side effects when using synthetic drugs. This study focuses on the formulation and pharmacological evaluation of herbal cream for the treatment of psoriasis, employing natural excipients to mitigate such side effects. Herbal cream was developed using various natural gums and polymers. Compatibility between the herbal drug and excipients was confirmed through Fourier transform infrared (FTIR) spectroscopy. The formulations were evaluated for parameters such as drug content uniformity, viscosity, pH, and stability. Drug release studies were conducted using a dialysis membrane in phosphate buffer (pH 6.8) at 37°C, with the release kinetics best fitting the Higuchi model. Biological studies on albino mice revealed that formulations containing emulsifying wax and cetostearyl alcohol provided superior drug incorporation, with a drug activity rate of 43.3%. The anti-psoriatic activity was validated through enhanced orthokeratotic cell differentiation in the epidermal scales during mouse tail testing. The findings demonstrate the significant in vitro anti-psoriatic activity of the herbal cream formulation, supporting its efficacy in treating psoriasis while offering a natural and safer alternative to synthetic treatments.

Keywords: Psoriasis; *Azadirachta indica; Pongmia pinnate; Cassia tora*; Cream; Soap; Drug content Uniformity; Biological studies

INTRODUCTION

Psoriasis is an autoimmune skin condition where the immune system mistakenly targets healthy skin cells as harmful, leading to an accelerated skin cell growth cycle. This results in chronic inflammation and thick, scaly patches on the skin. While psoriasis is not contagious, it is linked to an increased risk of stroke. Both genetic and environmental factors play a role in its development. There are five main types of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic **[1-3]**.

The most common type, plaque psoriasis, presents as red patches covered with silvery-white scales, typically on the elbows, knees, scalp, or other areas like the palms and soles. These patches occur due to rapid skin cell buildup. Unlike eczema, psoriasis often affects the outer side of joints. Interestingly, some people with psoriasis may not show visible skin symptoms.

Herbal treatments offer a gentler alternative to synthetic drugs, with fewer side effects and wide availability. Certain herbs are effective in managing inflammatory skin conditions like psoriasis. Plants such as Echinacea, Lavender, *Achyranthes aspera, Saraca asoca*, Chamomile, Neem (*Azadirachta indica*), and *Aloe vera* have been used in traditional remedies. Additionally, lifestyle and dietary changes may help alleviate symptoms, offering a holistic approach to managing this condition **[1, 2, 4]**.

The present study aims to prepare a novel polyherbal cream formulation by combining *Azadirachta indica, Pongmia pinnate, Cassia tora*, and to evaluate its anti-psoriatic efficacy using a rat ultraviolet ray B model.

MATERIALS AND METHODS

Materials

Seeds of *Azadirachta indica & Pongamia pinnata* were collected from Natural Remedies, Dewas, Madhya Pradesh, India and the dried seeds of *Cassia tora* was procured from local market of Ratlam (M.P). The crude drugs were authenticated by Botanist, Govt. Shri*krishna Ji Rao*pawar P. C*ollege Dewas* (M.P.) and were used for the present study. The specimen sample was deposited at the herbarium of the Acropolis Institute of Pharmaceutical Education & Research, Indore, Madhya Pradesh.

Methods

Formulation Development of cream

The cream was formulated using the hot emulsification method. The emulsifier, emollient, and other oil-soluble components were dissolved in the oil phase, while the water-soluble components were dissolved in the aqueous phase. Both phases were heated to 75°C, after which the oil phase was gradually added to the aqueous phase with continuous stirring until a smooth and uniform emulsion was formed. The finished

cream was then transferred into suitable containers and stored in a cool place. A placebo cream was prepared using the same method and ingredients, excluding the herbal oil extracts (as outlined in **Table 1 and Figure 1**).

Table 1: Formulation of Herbal Cream

S. No.	Ingredient	Quantity(%w/w)
1	Azadirachta indica	4
2	Pongamia pinnate	4
3	Cassia tora	4
4	Stearic acid	5
5	Emulsifying wax	4
6	Cetostearyl alcohol	4
7	Liquid paraffin	2
8	Distilled water	q.s

Evaluation of Cream [5-7]

Physicochemical evaluation of cream

After the formulation the herbal cream was evaluated using the cream was the following parameters:

(i) Formulation properties: The formulation properties of the cream were studied by visual appearance and characteristics like Colour**,** Odour**,** Form of physical state and Appearance

(ii) Determination of pH: pH meter was calibrated firstly with the standard pH solution**.** Then 0.5 gm of the formulated cream was dissolved in 50 ml water and pH was noted using digital pH meter.

(iii) Sensitivity test – It is tested by "Patch test". Apply product on 1cm^2 patch of skin, if no any inflammation or rashes then it considered as free from sensitivity.

(iv) Irritation test – It is carried out by applying product on the skin for 10 minutes. If no irritation is produced then it is considered as non-irritating product.

(v) Grittiness – A pinch of product is rubbed on skin and then observed with magnifying glass; if it is free from rashes or eruption then it is considered as free from grittiness.

(vi) Homogeneity – Prepared cream was tested for homogeneity by visual inspection after the cream has been set in the container. They were tested for their appearance and presence of any aggregates.

(vii) Bleeding test – This evaluation is carried out for semisolid preparation. The products are kept frequently for a period of time alternatively in fridge and at room temperature then bleeding of liquid is observed ,if no liquid phase is omit out then it is considered as stable product for climatic conditions.

(viii) Stability studies - The International Conference on harmonization (ICH) harmonized tripartite guidelines on stability testing of new drug substances & product was issued on October 27,1993.The

formulated cream were filled in the wide mouth container and stored at 40 ± 2^0C / 75%RH ±5%RH for a period of three months.

(ix) In- Vitro Anti-Bacterial Activity

Determination of zone of inhibition – Evaluation of activity was carried out by spread plate method using nutrient agar medium and the antibacterial activity was measured in terms of zone of inhibition. Study was carried out in aseptic area to establish the efficacy of the formulations against *S. aureus* that is most common pathogen causing psoriasis.

Micro-organism used –

Gram-positive: *Staphyllococcus aureus*

Gram- negative: *Escherichia coli*

Preparation of Inoculums – A 24hrs old culture was used for the preparation of bacterial suspension. Suspension of organism was made in sterile isotonic solution of sodium chloride (0.9% w/v).

Procedure – The medium was prepared by dissolving all the ingredient in distilled water and subjected to sterilization in an autoclave at 121^0C for 15 min. The petri plates were washed thoroughly and sterilized in hot air oven at 1100^0C for $1^{1/2}$hrs. 30 ml of sterile molten agar medium was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Then suspension of organism 0.1 ml was transferred on the molten agar plates and with the help of sterile spreader the suspension was uniformly spread on the plate in all direction to form a uniform surface, then plates were kept undisturbed for 5 min. Bore were made on the medium using sterile borer and 0.2ml of the extracts & sample were added to respective bore and 0.2ml of the standard Streptomycin was taken as standard. The petri plates seeded with organisms, containing extracts and the standard were kept in refrigerator at 4^0C for 1 hr. to facilitate the diffusion of extracts and the standard in to the media. After diffusion the petri plates were incubated at 37 ± 1^0C for 24 hour in BOD incubator and zone of inhibition was observed and measured using a scale.

***In-Vitro* Drug Release of AE, PE, CE loaded cream [8-10]**

In vitro release studies were conducted using a DBK Franz diffusion cell apparatus to evaluate the release profile of AE, PE, and CE from incorporated cream. A dialysis membrane (Hi-Media, Mumbai, India) with a pore size of 2.4 nm and a molecular weight cutoff of 12,000 was mounted on the Franz diffusion cells. A phosphate buffer solution (PBS) pH 6.8: Methanol (7:3) was used as the receptor medium (20 ml) and stirred at 200 rpm. For the study, 100 mg of the cream was used. During the experiments, the solution in the receptor side was maintained at 37±0.5 °C. At predetermined time intervals, 1 ml samples were withdrawn

from the receiver compartment and replaced with the same volume of freshly prepared PBS (pH 6.8): Methanol (7:3). The samples were analyzed using a UV-Visible spectrophotometer (UV-1800, Shimadzu, Japan) at 214 nm, 285 nm, and 254 nm to determine the release of AE, PE, and CE, respectively.

Stability Studies

The cream formulation was subjected to stability studies for 3 months. The formulations were placed in wide-mouth glass bottles and stored at a temperature of 37±0.5 °C with 75±5% relative humidity. These samples were evaluated for their appearance, pH, viscosity, drug content, spreadability, and in vitro drug release.

Lipid Peroxidation assay of AE, PE, CE loaded cream

The lipid peroxidation assay for cream was determined by measuring the amount of thiobarbituric acid reactive substances (TBARS). $HepG^2$ cells were seeded in 24-well culture plates at an initial density of 2 x 10^5 cells/ml and grown to approximately 80% confluence. Oxidative stress was induced by treating the cells with freshly prepared H_2O_2. The cells were pretreated with samples (AE, PE, and CE loaded cream) at specified concentrations for 24 hours.

Fetal Bovine Serum-free Dulbecco's Modified Eagle's Medium containing H_2O_2 (882 nM) was added to each well, and the cell plate was incubated for 24 hours. The HepG2 cells were lysed using the freeze-thaw method. After analysis, 0.2 ml of the cell suspension was added to the thiobarbituric acid reagent. This mixture was incubated at 90 °C for 1 hour, then cooled. Four milliliters of a mixture of n-butanol and pyridine (15:1, v/v) were added, and the samples were centrifuged for 15 minutes. The absorbance was measured at 532 nm **[11-12]**.

The percentage inhibition of lipid peroxidation was calculated using the following formula:

$$\%\ inhibition = 100 - \frac{\text{OD of cream sample}}{\text{OD of control}} X\ 100$$

DNA fragmentation studies on HaCat cell lines

HaCat cells were seeded at a concentration of $1x10^6$ cells per 35 mm dish and incubated at 37 °C with 5% carbon dioxide for 24 hours. The confluent cells grown after the incubation period were treated with sample concentrations of 160 µg/ml and 320 µg/ml, as well as a control. Following the treatment, cells were trypsinized, and both adherent and floating cells were collected by centrifugation at 2000 rpm for 5 minutes.

The cell pellet was resuspended in 0.5 ml of lysis buffer (pH 7.8) containing a mixture of 10 mmol Tris-Hydrochloric acid (pH 8), 20 mmol Ethylene diamine tetraacetic acid (EDTA, pH 8), 0.2% Triton X-100 or

Sodium-N-lauroyl sarcosinate, and 4M Sodium chloride. The mixture was vortexed vigorously and incubated at 50 °C for 5 minutes. To the lysate, 0.5 ml of phenol chloroform iso-amyl alcohol was added, mixed for 2-3 minutes, and centrifuged at 10000 rpm for 15 minutes at 4 °C.

The upper aqueous layer was transferred to a new tube, to which double the volume of cold 100% ethanol and 3M sodium acetate were added to achieve a final sodium acetate concentration of 0.3 M. The sample was incubated at room temperature for 5-10 minutes and then centrifuged at 10000 rpm for 15 minutes. After removing the supernatant, the DNA pellets were washed with 70% ethanol, centrifuged at 5000 rpm for 10 minutes, and the supernatant was removed. The DNA pellets were air-dried and dissolved in Tris buffer, followed by separation using 2% agarose gel electrophoresis at 100 volts for 50 minutes **[12, 13]**.

Mouse-tail Model for Psoriasis

All procedures of the study were conducted in accordance with the guidelines set by the CPCSEA and an approved IAEC protocol number (IAEC-17-019). Fifteen male Swiss albino mice were allowed to acclimatize for 5 days and were randomly assigned to three groups: six mice in Group I (standard group, treated with Clobetasol propionate 0.05% cream), six mice in Group II (treated with AE, LE, and ME NLC-loaded cream), and six mice in Group III (placebo control).

The samples were applied locally to the tails at a rate of 2-5 mg per animal, uniformly to the proximal part of the tail. A plastic cylinder was placed over the tail and fixed with adhesive tape to ensure a contact time of 2 hours. After this period, the cylinders were removed, and the tails were wiped with cotton. The mice were treated once daily for 2 weeks.

Two hours after the last treatment, the animals were sacrificed, and the tails were fixed in 10% buffered formalin and processed for histopathology. Longitudinal sections of about 5 µm thickness were prepared, stained with hematoxylin-eosin, and permanent slides were prepared for evaluation. The sections were examined under a light microscope to observe alterations in epidermal thickness, elongation of ridges, and orthokeratosis. The animals were also observed for mortality, clinical signs, and changes in body weight **[5-9]**.

Rat ultraviolet ray B photo dermatitis model for psoriasis

Healthy male Sprague Dawley rats weighing 150-200 grams were kept in a 12-hour light/12-hour dark cycle at a temperature of 20.4 to 23.8 °C and a relative humidity of 36 to 61%. The rats were provided with food and reverse osmosis water treated with ultraviolet light ad libitum. All procedures

were conducted in accordance with the guidelines set by the CPCSEA and an approved IAEC protocol number (IAEC-17-009).

The study design included three groups: Group I (6 animals) received 2-5 mg/kg of Clobetasol propionate 0.05% cream as the standard treatment, Group II (6 animals) received 2-5 mg/kg of AE, PE, and CE loaded cream, and Group III (3 animals) received 2-5 mg/kg of placebo cream. The hair on the dorsal skin of the rats was clipped and carefully shaved. An area of 1.5 x 2.5 cm on one side of the flank was irradiated for 15 minutes (1.5 J/cm²) at a vertical distance of 20 cm with UV-B lamps, resulting in biphasic erythema.

After 72 hours, the test anti-psoriatic cream, standard cream, and placebo cream were applied topically at 2-5 mg/rat on the irradiated site once daily. The irradiated rats were sacrificed on day 11 after UV-B irradiation using CO2 anesthesia. Skin biopsies were immediately taken, fixed in 10% formalin, and embedded in paraffin. Tissue sections (4 μm thick) were stained with hematoxylin and eosin and examined under a light microscope to observe alterations in epidermal thickness, elongation of ridges, and orthokeratosis. The study also evaluated mortality, clinical signs, and body weight changes. Data were analyzed using one-way ANOVA followed by Dunnett's Multiple Comparison test **[10-14]**.

Figure 1: Herbal formulation of Cream

RESULT AND DISCUSSION

Evaluation of cream

The water in oil cream was prepared for the treatment of Psoriasis, a dreaded skin disease. The developed cream was stored in tightly closed containers and evaluated for their physicochemical properties & antimicrobial activity.

Physicochemical evaluation results of all the formulations are depicted in **Table 2**. Developed formulations showed excellent homogeneity and there were no lumps in the

formulations. Skin irritation study results revealed that all the developed cream was safe for topical application and did not induce any skin reaction. Cream passed the sensitivity test, irritation test, grittiness and bleeding test. Stability of cream (base & formulation) was evaluated on 40 ± 2^0C/75% RH ± 5% RH for the period of three months. No phase separation was observed during the stability study of creams. No liquefaction is observed throughout the study period of three months.

In vitro Antibacterial activity

Spread Plate method – Antibacterial activity was done by Spread plate method and the activity of the cream was measured by zone inhibition of microbes on the plate with agar medium and reports were given in **Table 2 and Figure 2, 3**.

Evaluation of AE, PE and CE loaded Cream

Percent Drug Content

The results for the percentage drug content are illustrated in **Figure 4**, demonstrating a uniform distribution of drugs throughout the cream.

In vitro drug release

The data obtained from the in vitro drug release studies also support and indicate complete and prolonged drug release within the cream base. During the preparation of cream, the cooling process from high temperature to room temperature promotes the enrichment of the drug in the outer layers of the cream, resulting in superficial entrapment. This leads to an initial burst release followed by prolonged drug release from the core of the cream for up to 24 hours. The release percentages were found to be 85.14±0.51%, 75.01±0.25%, and 72.13±0.78% for AE, LE, and ME, respectively, as depicted in **Figure 5**.

Lipid Peroxidation assay

Psoriasis has been linked to oxidative stress, which is often characterized by lipid peroxidation, measured through malondialdehyde (MDA) levels in the blood of psoriatic patients. In this study, lipid peroxidation was assessed by quantifying thiobarbituric acid-reactive substances (TBARS) with slight modifications in HepG2 cells.

Oxidative stress was induced by treating the cells with freshly prepared H_2O_2. The results were quantified as the percentage inhibition of lipid peroxidation, expressed as malondialdehyde (MDA) per milligram of protein. The topical cream formulation of the cream exhibited inhibition of lipid peroxidation, with an IC_{50} value of 1487.3 µg/ml. These findings suggest that the prepared incorporated cream, containing a combination of AE, PE, and CE extracts, effectively inhibits lipid peroxidation **(Figure 6)**.

Figure 6 illustrates the anti-lipid peroxidation activity of AE, PE, and CE from the cream formulation.

Nitrogen oxide scavenging assay

Nitric oxide is a gaseous free radical known to react with superoxide anion, forming potent free radicals called peroxynitrite radicals (NOO). The excessive production of peroxynitrite is believed to contribute significantly to tissue damage in chronic inflammation, such as in psoriatic patients. This assay aimed to evaluate the anti-inflammatory effect of selected drugs and formulations in psoriasis treatment.

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which reacts with oxygen to produce nitrite ions. These ions can be quantified using Griess reagent at 546 nm. The percentage inhibition of nitric oxide was calculated, with results expressed as the Nitric Oxide Inhibition of AE, PE, and CE loaded cream. The inhibition increased with the dose, as depicted in **Figure 7**. The AE, PE, and CE based cream demonstrated significant nitric oxide scavenging capability, with an IC_{50} value of 697.9 µg/ml.

Table 2: Antibacterial activity of Herbal cream

Micro organism	Zone of inhibition (mm)			
	Cream	Placebo	Marketed oil	Streptomycin
Staphylococcus aureus	15	-	13	26
Escherichia coli	17	-	15	25

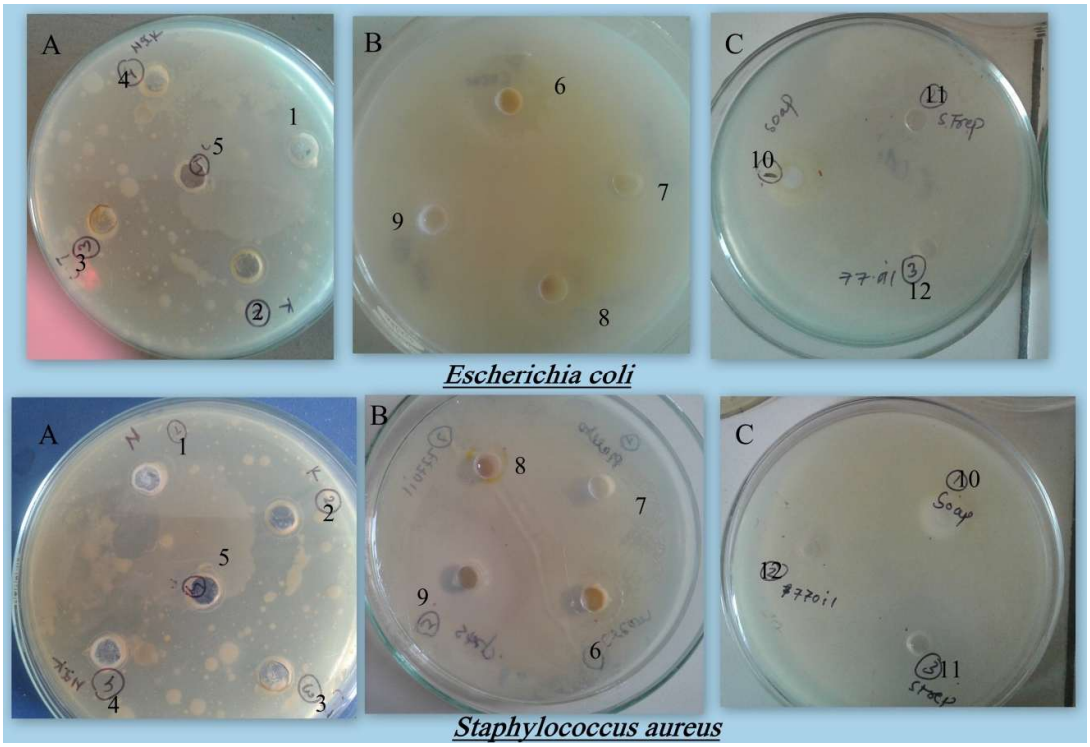

Figure 2: Antibacterial activity of different topical formulation against *Staphylococcus aureus* and *E. coli*
NOTE: (1) *Azadirachta indica;* (2) *Pongamia pinnata;* (3) *Cassia tora;* (4) Combination; (6) Herbal cream; (7) Placebo

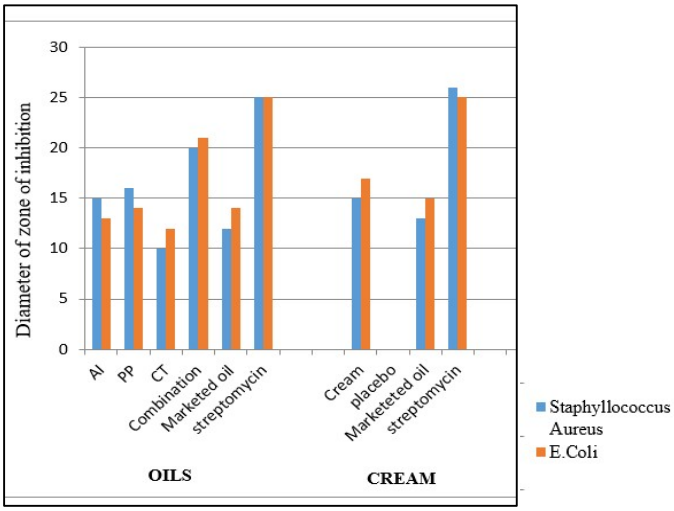

Figure 3: Antibacterial Activity of Different Topical Herbal Formulation

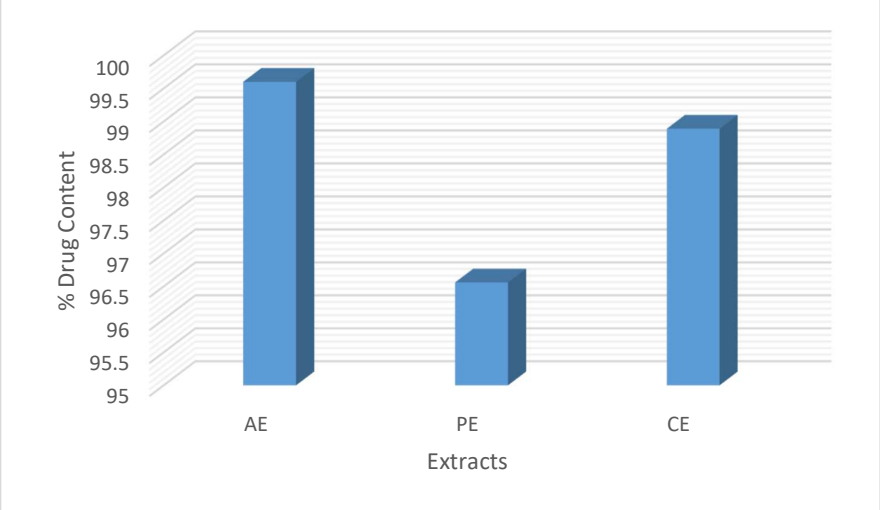

Figure 4: Percentage Drug content of the extracts incorporated in cream

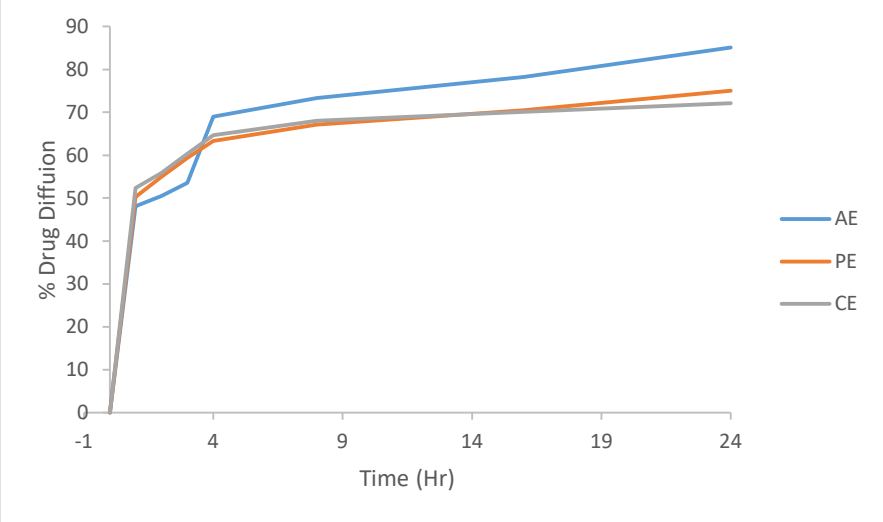

Figure 5: % Drug diffusion of the AE, PE and CE from the cream

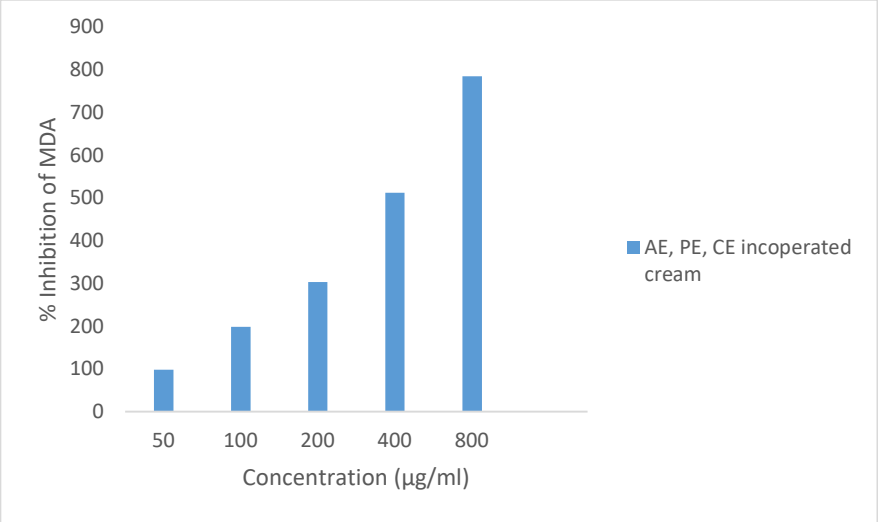

Figure 6: Lipid Peroxidation activity of AE, PE and CE incorporated cream

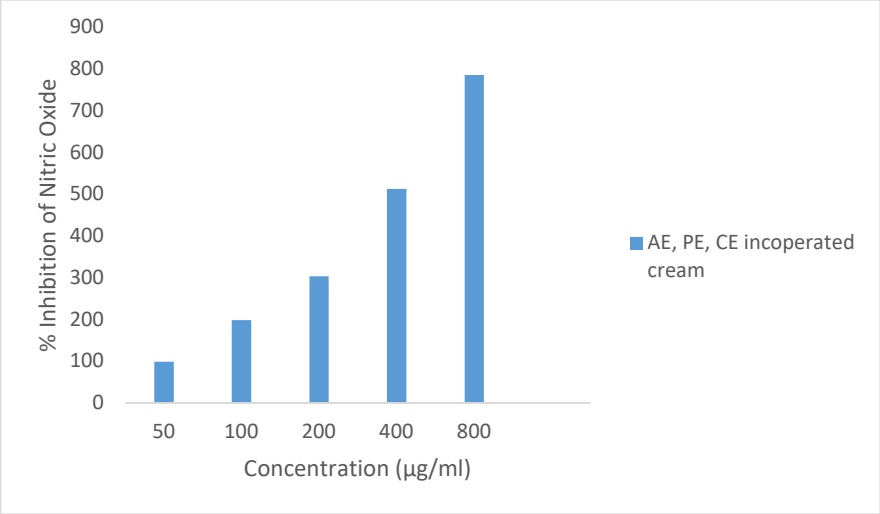

Figure 7: Percent inhibition of nitric oxide AE, PE and CE incorporated cream

Cytotoxicity studies for HaCat cell lines

Psoriasis arises from the excessive proliferation and abnormal differentiation of keratinocytes. The cream loaded with AE, PE, and CE extracts was assessed for its anti-proliferative activities using the MTT assay. The results revealed significant anti-proliferative effects, with an IC50 value of 181.7 µg/ml.

DNA fragmentation studies on HaCat cell lines

In this study, we investigated the tendency of keratinocytes derived from psoriatic plaques to undergo apoptosis, a programmed cell death process. The characteristic feature of apoptosis is the fragmentation of DNA into oligonucleosomal ladders. The DNA fragmentation profiles for the AE, PE, and

CE loaded NLC-based cream formulation were analyzed using gel electrophoresis.

HaCat cells treated with samples of the topical cream formulation of 160 µg/ml and 320 µg/ml exhibited dose-dependent increases in DNA fragmentation compared to the control group. These findings indicate that the DNA fragmentation was induced, suggesting apoptotic activity induced by the cream formulation.

Mousetail model for psoriasis

Advanced anti-psoriatic studies were conducted on animal models to evaluate and compare the safety and efficacy of a nanoparticulate anti-psoriatic cream loaded with AE, PE, and CE cream versus Clobetasol propionate 0.05% cream. These studies utilized the mouse tail method for topical applications in psoriasis.

Compared to the normal control group, the mice treated with the standard drug (Clobetasol propionate 0.05% cream) and the test drug (AE, PE, and CE loaded NLC-based cream) showed a significant reduction in epidermal thickness of the tail skin. Based on these results, it can be concluded that both the standard and test drug formulations may possess potential anti-psoriatic activity.

Rat Ultraviolet ray-B photodermatitis model for psoriasis

The Rat Ultraviolet Ray-B photodermatitis model was used to evaluate the antipsoriatic potential of a anti-psoriatic cream compared to Clobetasol propionate 0.05% cream. Compared to the normal control group, the rats treated with the standard drug (Clobetasol propionate 0.05% cream) and the test drug (AE, PE, and CE loaded cream) exhibited a significant reduction in epidermal thickness and inflammatory changes. Based on these results, it can be concluded that both the standard and test drug formulations have potential anti-psoriatic activity.

CONCLUSION

Psoriasis is a chronic, debilitating inflammatory disease characterized by erythrosquamous scaly skin lesions. There is substantial evidence that the excessive production of pro-inflammatory cytokines by T cells and keratinocytes, particularly tumor necrosis factor (TNF), plays a major role in the disease. The developed polyherbal cream and soap formulations, demonstrated acceptable physicochemical properties, were non-irritant, and exhibited antiproliferative activity in HaCaT cell lines. Additionally, these formulations significantly suppressed keratinocytes in a light-induced psoriasis model in rats, outperforming the marketed preparation.

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Conflict of Interest

Authors have no conflict of interest.

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