

FORMULATION AND EVALUATION OF POLY HERBAL OINTMENT FOR THE MANAGEMENT OF PSORIASIS

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Abstract

Background: Traditional system describes a vast array of herbs and herbal mixtures that are been demonstrated to possess efficacy in research investigations and this is termed as holistic system of medicine. *Annona squamosa*, *Solanum nigrum*, *Azadirachta indica* are the crude drugs evaluated for its role in anti-microbial and various skin diseases.

Objective: In the current study, to screen selected Indian herbs *Solanum nigrum*, *Annona squamosa*, *Azadirachta indica* for its cytotoxic effect against immortalized HacaT cell lines. To design and develop the new poly herbal formulation for the treatment of psoriasis and to minimize the occurrence of side effects.

Materials and method: Coarsely powdered plant material weighed accurately 500gm of individual plants were extracted with 50% v/v methanol for 24 hours by cold maceration process. Mixing all individual extracts of herbs in a glass beaker in 1:1:1 ratio for the preparation of Poly herbal extract. Mixed well with sufficient quantity of alcohol, concentrated, evaporated and stored. The resultant poly herbal extract subjected to preliminary phytochemical evaluation was done and further the poly herbal extract was screened by chromatographic analysis using TLC and HPTLC methods. The poly herbal extract free radical scavenging capacity was determined by DPPH assay method and the *In vitro* antipsoriatic activity of polyherbal formulation was carried out by MTT assay using HacaT cell lines. The weighed quantity of the poly herbal extracts were incorporated in the ointment base by trituration as per the requirement of the different formulations. Formulations PHF I (5%) and PHF II (10% w/w) showed a significant improvement in the control of keratinocytes in ultraviolet B induced photodermatitis rat model and the significant anti psoriatic activity of these formulations were also confirmed by the histometrical investigation.

Results and conclusion: The poly herbal extract showed the presence of alkaloids, terpenoids, tannins etc.. By TLC and HPTLC analysis the poly herbal extract showed the distinct spots of active constituents. The acceptable physico-chemical properties were observed in formulated polyherbal extract ointment formulations PHF I and PHF II and the formulations were non irritant, anti proliferative activity in HaCaT cell lines and significant suppression of the keratinocytes in light induced psoriasis rat model as compared to marketed preparation. The formulations were showed the promising antipsoriatic activity.

Keywords: Anti-psoriatic activity, Poly herbal extract, HacaT, keratinocytes, Photodermatitis.

1. INTRODUCTION

Psoriasis is an immunological disorder manifesting as localized or widespread erythematous scaling lesions or plaque. On skin of the elbows, knees, scalp, lumbosacral areas, intergluteal clefts, and glands penis we can find the manifestation of psoriasis disease. Mostly in the joints we can observe more this disease.

In the psoriasis treatment, the therapy has to be prolonged and adjusted to the surface of the body as well as severity of the disease. Variable symptomatic relief was given by the topically applied emollients, keratinolytics and antifungal agents and also topical corticosteroids are act as a primary drugs². The primary drugs may effective in mild to moderate diseases and even in severe cases initially. Generally psoriasis treatments started with a high potency steroid further based on improvement either

by weakly or by milder preparation.

According to NIH (National Institutes of Health), United States population has approximately 2.2% of psoriasis³. Internationally, the incidence of psoriasis varies dramatically. Psoriasis can begin at any age. Systemic therapy with corticosteroids and /or immunosuppressants is reserved for severe and refractory cases. Other topically used agents are Calcipotriol, tazarotene and coaltar. Severity of psoriasis can be classified as Mild - Less than 2%, moderate - 3-10%, More than 10% of the body is affected².

As compared to the synthetic drugs, herbal medicines are having mild side effects. The herbal medicine is easily to use and readily available in treatment. Herbal resources play a vital role in the management of the inflammatory and skin diseases. Some studies suggest that psoriasis symptoms can be relieved by change in diet and life style. Various plants used in the treatment of psoriasis are *Echinacea angustifolia*, *Echinacea purpurea*, *Lavendula officinalis*, *Achyranthes aspera*, *Sarco asoca*, *Matricaria chamomile*, *Annona squamosa*, *Solanum nigrum*, *Azadirachta indica*, *Aloe vera*³. Hence the present study was planned to prepare a novel poly herbal formulation by combining *Annona squamosa*, *Solanum nigrum*, *Azadirachta indica* and evaluate the anti psoriatic efficacy in Rat ultraviolet ray B model was undertaken.

2. Materials and methods

2.1 COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

The plant materials viz *Annona squamosa*, *Solanum nigrum*, *Azadirachta indica* were collected from the Tindivanam (Villupuram District) Tamil Nadu. Authentication of all crude drugs was done at Plant anatomy research center, Tambaram, Chennai. All the samples are stored in the amber colored plastic container till their experiment use. Dried crude drug samples powdered and used for further studies.

2.2 Extraction

Preparation of methanolic extract

Weighed 500gms of coarsely powdered plant material of individual plants were extracted with 50% v/v methanol by cold maceration method for 72 hours. Then individual plant extracts were filtered with muslin cloth and filtrate was evaporated under reduced pressure. The resultant extract was stored in vacuum desiccators at cold temperature.

Preparation of poly herbal extract

In a glass beaker all the individual plant extracts was mixed in 1:1:1 ratio for the preparation of poly herbal extract with sufficient quantity of alcohol. The poly herbal extract was evaporated under reduced pressure and then vacuum dried. This poly herbal extract was termed as PHE and used for further studies.

2.3 Preliminary Phytochemical evaluation of PHE

Preliminary phytochemical evaluation of the PHE was performed by standard procedure described in Khandalwal et al⁴. The PHE was further analysed presence of phytoconstituents by Thin Layer Chromatography (TLC) was carried out using the mobile phase Toluene:Ethylacetate:Formic acid (5:4:1) and iodine vapor act as detecting agent.

2.3.2. High Performance Thin Layer Chromatography (HPTLC) Analysis of Poly herbal extract 5,6

HPTLC screening was performed on silica gel 60 f 254, 20X10 cm HPTLC precoated plates (Merck, Germany -#5642), with the mobile phase Toluene: Ethyl acetate: Formic acid (5:4:1). The PHE solutions (2.0µl, 4.0 µl and 6.0µl with concentration of 10mg/ml) were applied to the plates as 10mm bands, application of sample done with CAMAG-Linomat IV automated spray on band applicator equipped with a 100 µL syringe and operated with the band length 10 mm, 10 sec/ µL application rate, 4 mm distance between, distance from the plate side edge 1.5 cm and 2 cm distance from the bottom of the plate. CAMAG TLC Scanner IV was used to densitometrically to quantify the bands using WIN CATS software (Version 4 X). The scanner operating parameters were as the Mode is absorption / reflection; Slit dimension is 5 x 0.1 mm; 20 mm/s is the scanning rate and

monochromator band width is 20 nm at an optimized wavelength 254, 366 nm and in visible range).

2.4. In- Vitro Antioxidant studies

2.4.1. Effect of poly herbal extract in DPPH free radical scavenging assay

0.01mM DPPH solution was prepared with the solvent methanol and add 1 ml of this solution to 3ml of different concentrations (25-800 µg/ml) of PHE and control in different test tubes. After shaking the mixture was allowed to stand for 30mts at room temperature. After that the absorbance of the reaction mixture was measured by using double beam spectrophotometer.

$$\% \text{ Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample}) \times 100}{\text{Abs}}$$

Where Abs Control is absorbance of control at time = 0 and Abs Sample is absorbance of test sample. The IC50 Value for PHE was also calculated.

2.5. FORMULATION OF OINTMENT

In the present study, two different formulations were formulated with two different concentrations of prepared PHE of equal concentration of the plants like Solanum nigrum, Annona squamosa, Azadirachta indica.

2.5.1 Formulation of ointment

The required amount of ointment bases like hard paraffin and cetostearyl alcohol was melted on a water bath. To this wool fat and white soft paraffin was incorporated, until all the ingredients were melted the mixture was stirred well. Further mixture was stirred continuously until the mixture gets cold. The required quantities of prepared ointment base as weighed and incorporated in to the required concentrations of PHE then triturate well all the semi-solid medicaments on an ointment slab, with the help of stainless steel ointment spatula until a homogeneous ointment was formed. The remaining quantities of the base were added until the appropriate medicaments and uniformly mixed with it.

Table 1. Compositions of The poly herbal extract ointment

Sl.No	Ingredient	Quantity for 100 gm	Quantity for 10gm (1%)	Quantity for 10 gm (5%)
1.	Wool fat	5gm	0.5gm	0.5gm
2.	Hard Paraffin	5gm	0.5gm	0.5gm
3.	Cetostearyl Alcohol	5gm	0.5gm	0.5gm
4.	White Soft Paraffin	80gm	8gm	8gm
5.	Methanol extract	5gm	0.5gm	0.5gm

2.6. Evaluation of ointment

2.6.1 Physiochemical evaluation

1. Colour and Form of physical state

The ointment was observed visually for its physical appearance like colour and form .

2. pH

The 10%w/v ointment solution was prepared for the evaluation of its alkalinity and acidity nature. Digital pH meter was used to check the pH at room temperature condition.

3. Consistency

Ointment checked for its consistency like smoothness and absence of gritty particles.

4. Melting point

A little quantity of the ointment was heated gently and the melting point was determined by using standard melting point apparatus.

5. Solubility

The solubility testing was done with both cooling and warm water.

6. Viscosity measurement of ointment formulation

Viscosity is the important rheological behavior of semisolid dosage forms. This property was studied by keeping PHF in room and elevated temperature (hot air oven) conditions for a period of one month. Brook field viscometer was used to determine the viscosity of the PHF using spindle no.7 at different rpm (1, 5, 10, 50 and 100).

2.6.2 Subjective evaluation of formulated ointment

1. Sensitivity

Sensitivity was tested by using patch test, the PHF was applied in 1cm² patch of the skin of mice.

2. Irritation

It was carried out by applying product on the skin of mice for 10 minutes. After that period the particular area was tested for irritating and non irritating property.

3. Grittiness

A pinch of ointment was rubbed on the skin of mice and grittiness was observed and magnifying glass to observe any rashes or eruption present on the skin.

4. Spreadability

To check easy spreadable nature of PHF, the ointment was applied the skin of mice.

2.7. CYTOTOXICITY ASSAY

Different concentrations of poly herbal extract (12.5, 25, 50, 100 and 200 µg/mL) was prepared by serial dilution method in DMEM to give a volume of 100 µL in each microtitre plate well. Each well was then added with 100 µL of cell lines in complete growth media (DMEM) 5 X 10⁵ cells/mL. Controls that contained only the cells were also prepared for each sample. The assay for each concentration of compounds was performed in triplicate. The plate was then incubated at 37°C, 5% CO₂, 90% humidity for 24&48 hours. Cytotoxicity of the tested compounds was measured using a modified MTT assay (Sigma, USA) (Mosmann, 1983). MTT was dissolved in phosphate buffer saline (PBS) (pH 7.5) at 5 mg/mL. The MTT stock solution was added directly to all appropriate microtitre-plate wells (20 µL for each well). The plate was then incubated for 2 to 4 h at 37°C and 5% CO₂. After incubation, MTT were reduced to insoluble purple formazan crystals by metabolically active cells in the wells. Subsequently, the supernatant was aspirated and 100 µL of Dimethylsulfoxide (Sigma, USA) was added and mix thoroughly to dissolve the dark blue formazan crystals. The optical density (OD) was measured on an automated spectrophotometric EL 340 multiplate/microelisa reader (Bio-Tekinstruments Inc) using test and reference wave length of 570 nm. The cytotoxic dose that kills cells by 50% (IC₅₀) was determined from absorbance versus concentration curve.

2.8. Experimental Animals

The acute toxicity study of PHF was performed as per OECD guidelines 423. For the Swiss Albino mice of either sex,

weighing about 20-30g was selected. Animals were kept in a temperature controlled environment ($23\pm 2^{\circ}\text{C}$) at 24 hours light/dark cycle. All the protocols were performed in accordance with Institutional Animal Ethics Committee of Vels University. Experimental protocol was approved by the Institutional Animal Ethics Committee IAEC Ref no XIII/VELS/PCOL/59/2000/CPCSEA/IAEC/8.8.2012.

2. 9. Evaluation of anti-psoriatic activity

2. 9.1. Rat ultraviolet ray B photodermatitis model for psoriasis

Rat ultraviolet ray photodermatitis has been proposed as an experimental model of psoriasis vulgaris.

Procedure

For this study we selected male wistar rats around 300gm. The animals were grouped in to 6 and each group contains 4 animals. Group I served as a positive control (normal skin), group II served as negative control (Psoriatic animal) , group III treated with ointment base, group 4 treated with standard drug (Soriafit), group V was treated with PHF I and Group VI was treated with PHF II. The rats were carefully shaved on the dorsal skin . An area (1.5×2.5 cm) on one side of the flank is irradiated for 15 min ($1.5\text{J}/\text{cm}^2$) at a vertical distance of 20 cm with UV -B lamps. A biphasic erythema was seen. After irradiation, immediately faint erythma appears and disappeared within 30 mts. After 6 hrs of irradiation the second phase of erythema started and increased gradually, showed the peak at 24-48hrs. The colour is brownish-red, and the reaction is confined to the exposed area with a sharp boundary. By 48-72 hrs after irradiation, erythematous lesion formed with a dark brown scale 9.

Method of screening

The formulated ointments were applied externally, everyday once or 5 times a week for couple of weeks. The treated animals were sacrificed after two hours of drug administration. The irradiated rats were sacrificed after various time intervals by decapitation under ether anesthesia. Immediately Skin biopsies were taken, fixed in 10% formalin and embedded in paraffin. Skin tissue section ($4\mu\text{m}$ thick) was stained with hematoxylin eosin. Level of orthokeratonic region was determined. The results of different concentration ointment against psoriasis are shown in Fig.5.

3. Results and discussion

3.1. Preliminary phytochemical evaluation

In the preliminary phytochemical evaluation the poly herbal extracts showed the presence of various secondary metabolites like alkaloids, tannins, terpenoids, glycosides etc

3.1. TLC Analysis

TLC was accomplished by using a stabilized solvent system and 6 spots were identified with Rf values as follows 0.83, 0.80, 0.77, 0.65, 0.37 and 0.30.

3.2. High Performance Thin Layer Chromatography (HPLC) of poly herbal extract

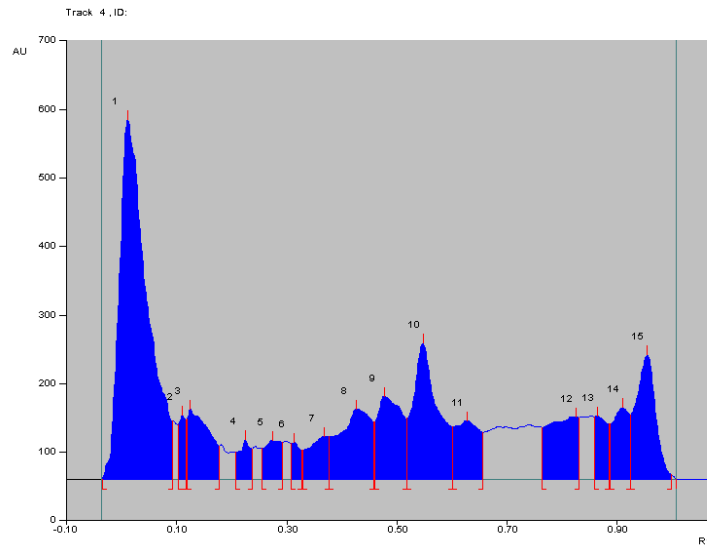
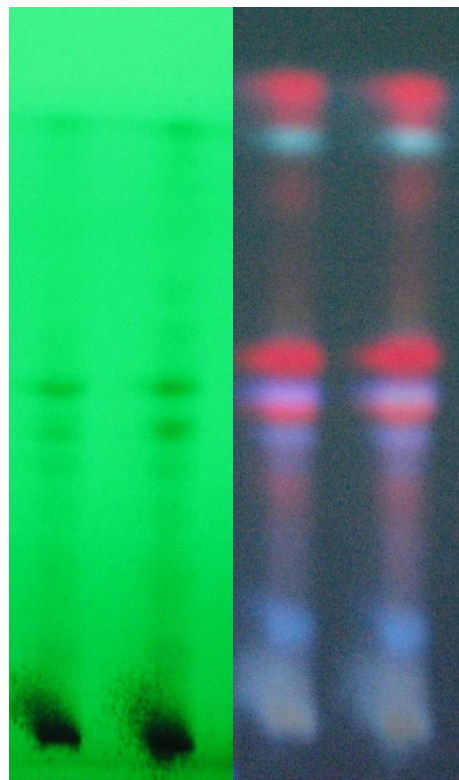


Fig 1: HPTLC chromatogram of poly herbal extract



256 nm

366 nm

Fig 2: HPTLC fingerprint of poly herbal extract

In the present HPTLC chromatographic condition the Polyherbal methanolic Extract of plants leaves *Solanum nigrum*, *Annona squamosa* and *Azadirachta indica* 15 peaks were observed at 254nm and 365nm.

3.4. Effect of poly herbal extract in DPPH free radical scavenging assay

Figure 3 represents the free radical scavenging capacity of PHE (poly herbal extract) using DPPH initiated radical in in-vitro.

It was observed that increase in percentage inhibition of free radicals has observed in raising concentration of poly herbal extract. The IC50 values of poly herbal extract was found to be 85 µg/ml and the extract was compared with the standard Vit C IC50-465 µg/ml.

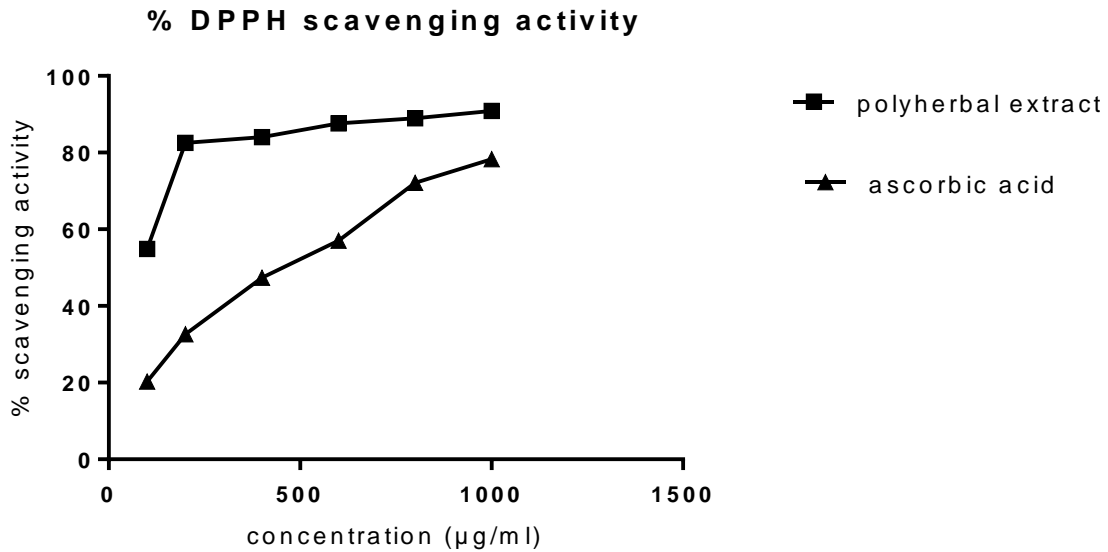


Fig 3: Effect of poly herbal extract in DPPH free radical scavenging assay

3.5. Effect of poly herbal extract in HacaT cell lines using MTT assay

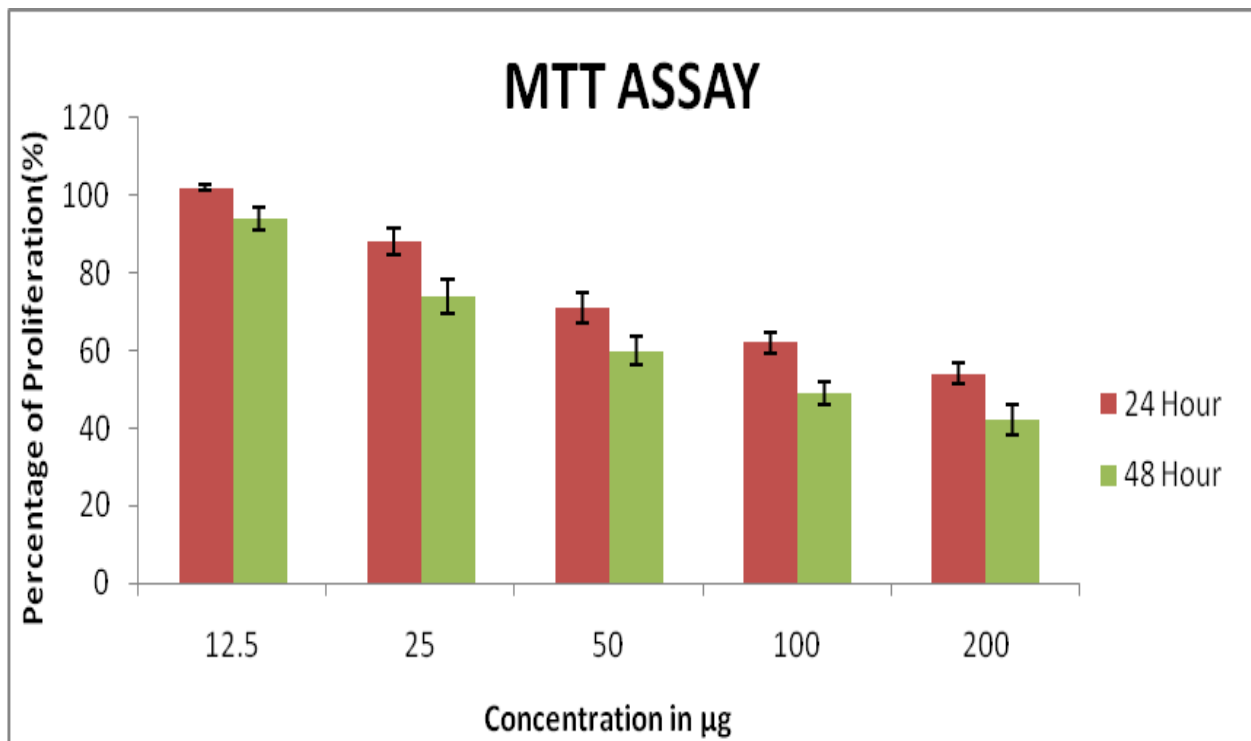


Fig 4: Effect of poly herbal extract in HacaT cell lines using MTT assay

MTT assay results reveals that PHF I and PHF II formulations exhibited appreciable antiproliferant activity in HaCaT cells.

3.6. Evaluation of poly herbal ointment:

Physical evaluation - All the formulated ointments were evaluated for its pH, spreadability, extrudability, loss on drying, stability, rheological behaviours by the Rotational Brookfield viscometer. The results were tabulated.

Evaluation parameter	Formulation 1 (2%)	Formulation 2 (5%)
Colour	Dark brown	Dark brown
Form of physical state	Viscous semi solid	Viscous semi solid
pH	6.8	7.2
Consistency	Smooth no solid particles	Smooth no solid particles
Melting point (°C)	44	39
Solubility	Soluble in 9 parts of water and 17 parts of boiling water	Soluble in 9 parts of water and 17 parts of boiling water
Sensitivity	No	No
Irritation	No	No
Grittiness	No	No
Spreadability	Easily spreadable	Easily spreadable

Spreadability of the formulations was found in the range of 5-7gm.cm/sec which ensures the better spreadability of the formulations. Force required to extrude the contents from the ointment tubes was evaluated by extrudability test and values were in the acceptable range. Formulations showed 22- 38% (loss on drying) and it is determined to estimate and limit the amount of water and other volatile substances present in the formulations. Viscosity of the formulations was found in the range of 167 to 243 cps. The ointments showed satisfactory stability at 00, 300 and 450 C and the evaluation of stability parameters showed that there was no phase separation, objectionable odour and physical instability.

3.7. Rat ultraviolet ray B model for psoriasis photodermatitis

Poly herbal methanolic extract from *Solanum nigrum*, *Annona squamosa*, *Azadirachta indica* (in two concentration dose) screened for their practicable antipsoriatic activity using ultraviolet ray B photodermatitis model. Formulation was topically applied in the semisolid dosage form of ointment. Drug activity is explained by increase in the numbers of the keratinocytes layers inclusive of the basal layer. Formulations PHF I (5%) and PHF II (10% w/w) showed a significant improvement in the control of keratinocytes in ultraviolet B induced photodermatitis rat model and the significant anti psoriatic activity of these formulations were also confirmed by the histometrical investigation. The effect produced by the formulations was comparable with that of marketed formulations. Illustrative examples of the histological specimens underlying the histometrical observations were shown in fig.5

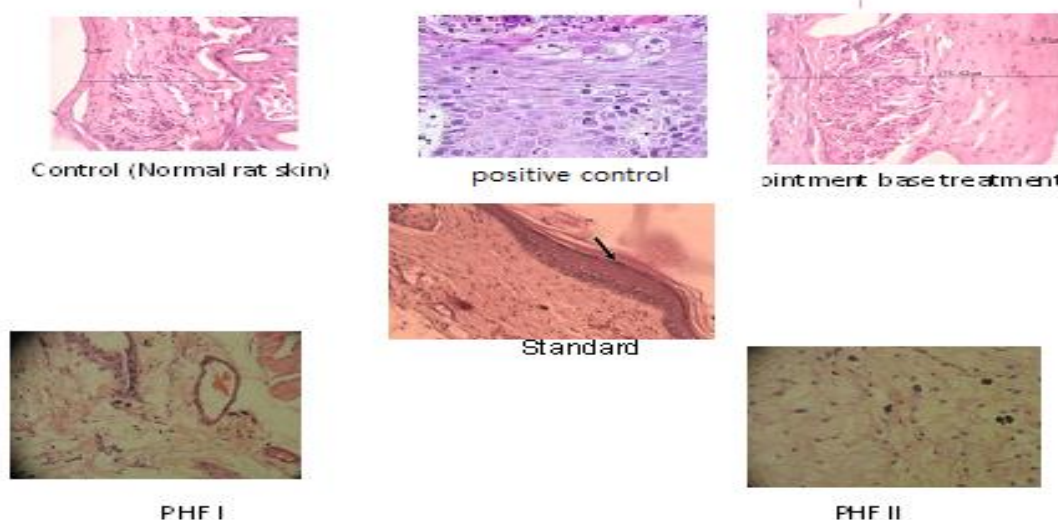


Fig 5: Rat ultraviolet ray B model for psoriasis photodermatitis

4. Conclusion:

Psoriasis is a chronic, devitalizing, inflammatory disease specified by erythroscamous scaly skin lesions. There is cogent evidence that excessive production of pro-inflammatory cytokines by T cells and keratinocytes, including TNF (tumor necrosis factor), plays a very predominant role. The developed polyherbal ointment formulations PHF I and PHF II showed acceptable physico- chemical properties, non irritant, antiproliferant activity in HaCaT cell lines and significant suppression of the keratinocytes in light induced psoriasis model in rats as compared with marketed preparation.

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Declaration of competing interest

None.

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