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Formulation and evaluation of antipsoriatic gel using natural excipients

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ABSTRACT

Objective: To develop topical gel formulations of Psoralen using natural excipients to minimize the side effects of synthetic drugs. **Methods:** The Psoralen gel formulations were prepared using different natural gums and polymers. The physicochemical compatibility between Psoralen and other excipients was confirmed by using Fourier transform infrared spectroscopy. All prepared gel formulations were evaluated for drug content uniformity, viscosity, pH, and stability. The release of psoralen from all formulations using dialysis membrane into a phosphate buffer pH 6.8 at 37 °C was studied. Drug release from the formulations fitted best to the Higuchi model. From the drug release data the best formulation was optimized and the biological studies on albino mice were performed. **Results:** Psoralen gel containing egg albumin and xanthan gum shows better incorporation of the drug. The drug activity was found to be 43.3%. Anti-psoriatic drug enhances the orthokeratotic cell differentiation in the epidermal scales. **Conclusion:** *In vitro* anti-psoriatic activity of F3 showed the significant orthokeratosis in the mouse tail test when compared to control thus indicating that the formulation is effective in treating psoriasis.

1. Introduction

Psoriasis is an auto immune disease that affects the skin. It occurs when the immune system mistakes the skin cells as a pathogen, and sends out faulty signals that speed up the growth cycle of skin cells. It is a chronic inflammatory and proliferative skin disorder involving the interplay of both environmental and genetic factors[1]. Psoriasis is not contagious. However, psoriasis has been linked to an increased risk of stroke. There are five types of psoriasis: plaque, guttate, inverse, pustular and erythrodermic.

The most common form, plaque psoriasis, is commonly seen as red and white hues of scaly patches appearing on the top first layer of the epidermis (skin). Some patients, though, have no dermatological signs or symptoms. In plaque psoriasis, skin rapidly accumulates at these sites, which gives it a silvery-white appearance.

Plaques frequently occur on the skin of the elbows and knees, but can affect any area, including the scalp, palms of hands and soles of feet and genitals. In contrast to eczema, psoriasis is more likely to be found on the outer side of the joint[2].

2. Materials and methods

2.1. Materials

Psoralen and Barbaloin were purchased from Yucca Enterprises, Mumbai. All other materials, solvents and reagents were of analytical grade.

2.2. Identification of drug

2.2.1. Thin layer chromatographic method (TLC)

TLC was performed by dissolving drug in methanol. Mobile phase was prepared. Drug solution was spotted

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the pre-coated TLC plate and was placed in saturation chamber. After the development of the TLC plate, the plate was air dried and the spot was detected by spray reagent. The R_f (Retardation factor) value was calculated using the following formula[3]:

R_f = Distance travelled by solute front/Distance travelled by solvent front

2.2.2. Investigation of physicochemical compatibility of drug and polymer

The physicochemical compatibility between Psoralen and formulation excipients used in the gel formulations was studied by using Fourier transform infrared spectroscopy (FTIR-AT-001, Bruker). Infrared (IR) spectra were recorded using an FTIR in the wavelength region between 4 000 and 400 cm^{-1}. The spectra obtained for Psoralen and physical mixtures of Psoralen with formulation excipients were compared[4].

2.2.3. Scanning electron microscopy (SEM)

SEM is a type of electron microscopy that images a sample by scanning it with a beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface, topography, composition and other properties such as electrical conductivity[2].

2.3. Preparation of formulations

The composition of different gel formulations is shown in Table 1.

Accurately weighed amount of polymer was added to the required quantity of the distilled water which was kept under stirring at 37 °C. Gelling agent was added slowly to the polymer dispersion. After attaining the gel consistency required amount of drug (0.5 g) dissolved in methanol (5 mL) was added. Antioxidant, humectants, preservative were added with continuous stirring until a homogenous gel was formed.

2.4. Analytical method

All samples were analyzed for Psoralen content spectrophotometrically at a wavelength of 246 nm.

2.5. Evaluation of prepared psoralen gel

2.5.1. pH

To determined pH, 1 g of each gel formulation were transferred into 10 mL beaker and measured by using the digital pH meter[4].

2.5.2. Viscosity measurements

A Brookfield Rotational Digital Viscometer DV II RVTDV- II was used to measure the viscosity (in cps) of gel formulations. The spindle was rotated at 10 r/min and the samples were allowed to settle over 30 min at the temperature [(25±1) °C] before the measurements were taken[5].

2.5.3. Spreadability and consistency

The spreadability of gel formulation was determined by measuring 1 g of gel between horizontal plates for 1 min. Consistency reflects the capacity of the gel to get ejected in uniform and desired quantity when the tube is squeezed.

2.5.4. Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the

Table 1
Composition of psoralen gel formulation (%, w/w).

Materials	Formulation code							
	F1	F2	F3	F4	F5	F6	F7	F8
Psoralen (g)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium alginate (g)	0.75	0.75	–	–	–	–	–	–
Egg albumin (g)	–	–	0.75	0.75	–	–	–	–
Bovine albumin (g)	–	–	–	–	0.75	0.75	–	–
Pectin (g)	–	–	–	–	–	–	4	5
Xanthan gum (g)	0.50	–	0.75	–	0.75	–	–	–
Guar gum (g)	–	0.50	–	0.75	–	1.75	–	–
Menthol (g)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
α-Tocopherol (g)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Barbaloin (g)	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Glycerine (mL)	–	–	–	–	–	–	5	–
Eugenol (mL)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Methanol (mL)	10	10	10	10	10	10	10	10
Distilled water q.s. to make (mL)	50	50	50	50	50	50	50	50

container. They were tested for their appearance and presence of any aggregates.

2.5.5. Drug content

All samples were analyzed for Psoralen content prior to diffusion studies. Drug content of gel formulations (1 g) was determined by dissolving an accurately weighed quantity of formulation in about 50 mL of pH 6.8 phosphate buffer.

The resulting solutions were filtered and subjected to spectrophotometric analysis at λ_{max} 246 nm. If necessary further dilutions were made using the same buffer solution. Drug content was thus calculated.

2.5.6. In-vitro diffusion study

The diffusion studies were performed by applying 1 g of the gel uniformly to the dialysis membrane. The membrane was mounted between the compartments of Franz diffusion cell. Reservoir compartment was filled with 15 mL of 6.8 pH phosphate buffer.

The study was carried out at (37±2) °C and was carried out for 24 h. The sample (1 mL) was withdrawn from reservoir compartment in successive intervals. Each time reservoir compartment was replenished with 1 mL of 6.8 pH phosphate buffer solution to maintain sink condition[2].

2.5.7. Release kinetic study

Data obtained from *in-vitro* release studies of the Psoralen from various gel formulations were fitted to various kinetic equations such as zero order, First order, Higuchi model and Korsmeyer-Peppas model.

2.5.8. Stability studies

Stability testing of drug product being as a part of drug discovery and ends with the commercial product, to assess the drug and formulation stability, stability studies were done.

The stability study was carried out for the most satisfactory formulation. The most satisfactory formulation was kept at 0 °C, (27±2) °C and (40±2) °C. At the end of 1 month, the samples were analyzed for the drug content and *in-vitro* diffusion study[2,5].

2.5.9. Biological evaluation

The anti-psoriatic activity was performed on the mouse-tail. The mouse-tail model is based on the induction of orthokeratosis in those parts of the adult mouse-tail, which have a normally parakeratotic differentiation. The Perry scientific mouse tail method is used and it is accepted as a screening method for measuring anti-psoriatic activity of drugs.

The basis of this method is that topical treatment of a mouse-tail with anti-psoriatic drugs enhances orthokeratotic cell differentiation in the epidermal scales. This characteristic was utilized for direct measurement of drug efficacy in animal model[6]. Drug activity is defined by the increase in percentage of orthokeratotic regions. Drug activity is calculated by using the formula:

DA = (Mean orthokeratosis of treated group-Mean orthokeratosis of control group)/(100-Mean orthokeratosis of controlled group)×100.

3. Results

3.1. Preformulation studies

3.1.1. TLC

The mobile phase consisting of ethylacetate: methanol: water (10:1.35:1) with spray reagent 10% methanolic KOH gave the R_f value (0.41) same to that of the reference standard value of Psoralen.

The spot was detected by UV chamber method (Non-Destructive method) at 365 nm. It was observed that the violet colour indicated the Psoralen R_f value. The TLC values are shown in the Figure 1 and Table 2.

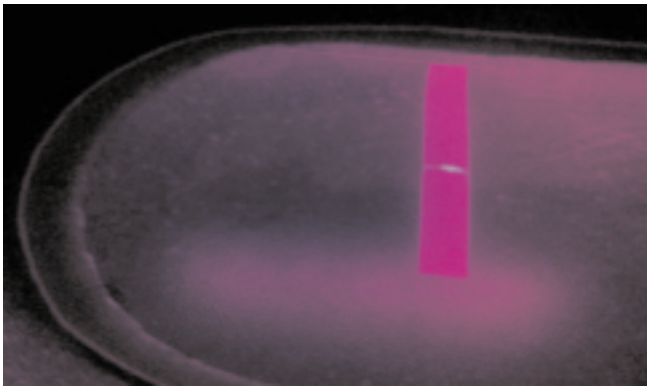

Figure 1. Thin layer chromatography of psoralen.

3.1.2. Drug-excipient compatability studies

The FT-IR spectrum of Psoralen pure drug showed the characteristic peaks at 1 611.97, 1 566.90 due to C=C bond stretching and 1 706.72 due to C=O bond stretching of ketone group and 3 145.55, 3 057.61 due to C-H bond stretching that is due to CH_2 functional group and 1 382.73, 1 318.09 due to C-O bond stretching.

The IR spectrum of the Psoralen formulation clearly shows the retention of these peaks of Psoralen pure drug thus revealing no interaction between selected drug and polymers (Figure 2).

Table 2
Thin layer chromatography for psoralen.

No.	Sample	Mobile phase	Spray reagent	Colour developed	R_f value	Results
1.	0.01 g psoralen+ $CHCl_3$	$CHCl_3$:Methanol (9:1)	Anisaldehyde solution	Violet	0.92	Failed
2.	0.01 g psoralen+ Methanol	Toulene:Ethylacetaete (7.5:2.5)	Anisaldehyde solution	Violet	0.80	Failed
3.	0.01 g psoralen+ Methanol	Toulene:Ethylacetate:Diethylamine (7:2:1)	Dragandroff's reagent	Violet	0.61	Failed
4.	0.01 g psoralen+ Methanol	Ethylacetate:Methanol:Water (10.00:1.35:1.00)	10% methanolic KOH	Violet (at 365 nm)	0.41	Passed

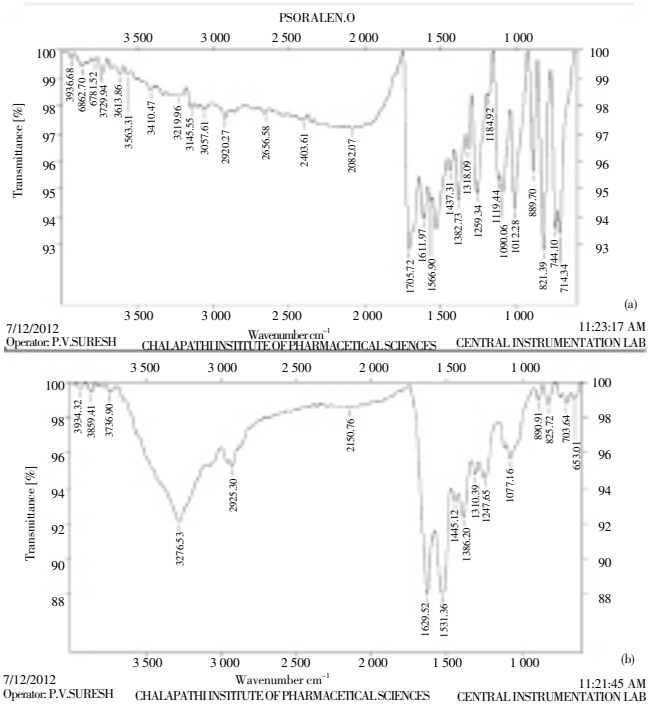

Figure 2. Fourier transform infrared of psoralen (a) and formulation F3 (b).

3.1.3. SEM

Surface topography, particle size, morphology of the gels were investigated with a scanning electron microscope. SEM is one of the common methods used owing to the simplicity of sample preparation and ease of operation.

Even 3D information about macro (0.1-10.0 mm), meso (1-100 μm) and microstructure (10-1 000 nm) is often found in the same micrograph. Scanning electron photomicrographs of placebo gel and formulation F3 are shown in the Figure 3. Psoralen gel containing egg albumin and xanthan gum shows better incorporation of the drug.

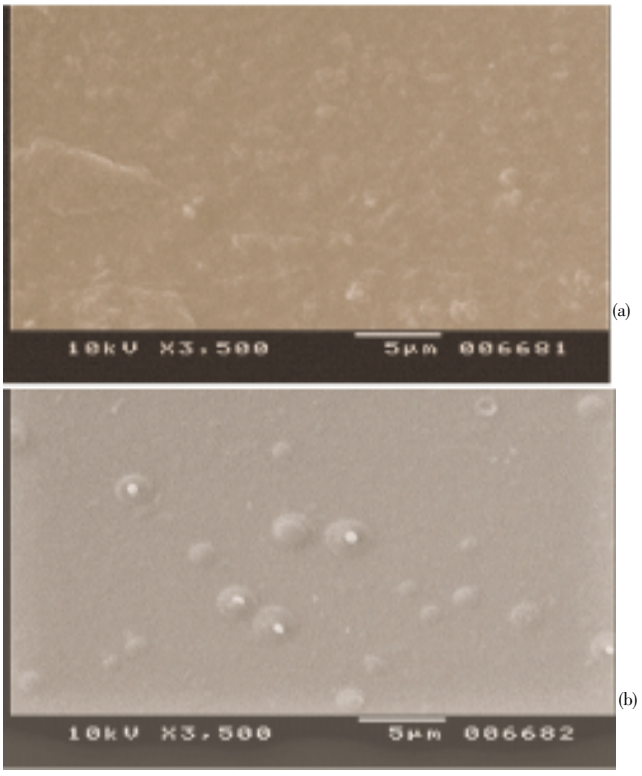

Figure 3. Scanning electron microphotography of placebo gel (a) and formulation 3 (b).

3.2. Evaluation of prepared psoralen gel

3.2.1. Physical examination

F3 formulation consisting of egg albumin and xanthan gum was white in colour, viscous, smooth and homogenous in nature. This may be due to the nature of gelling agent that gives a clear appearance.

Table 3
pH, drug content, viscosity of the prepared formulations.

Formulation code	pH	Viscosity (cps $\times 10^3$)	Consistency	Drug content (%)
F1	6.8	92	Gel consistency	96.1
F2	6.9	94	Gel consistency	99.0
F3	6.8	90	Gel consistency	106.0
F4	6.9	111	Gel consistency	98.5
F5	7.0	110	Gel consistency	95.5
F6	7.2	114	Gel consistency	96.0
F7	6.7	85	Transparent Gel	97.0
F8	6.7	87	Transparent Gel	99.6

3.2.2. pH

pH of all the prepared psoralen gel formulations ranged from 6.7–7.2 is considered acceptable to avoid the risk of irritation upon application to the skin.

3.2.3. Viscosity

Gel formulation majorly depends upon its viscosity. Viscosity of the formulation affects the drug release from the gel. If a gel consists of more viscosity the drug release from the formulation is decreased and if the same gel possess less viscosity the drug diffuses immediately into the diffusion medium. Hence for the gel formulation optimum viscosity is necessary to get the maximum drug release.

3.2.4. Spreadability and consistency

All formulations showed good spreadability when applied between the horizontal plates. Consistency of these formulations was acceptable and smooth when applied.

3.2.5. Homogeneity

Formulations showed better homogeneity with the absence of clumps in these formulations.

3.2.6. Drug content

Drug content was performed to estimate the amount of the Psoralen drug present in the prepared gel formulations. The pH values, viscosity, consistency, % drug content were given in the Table 3.

3.2.7. In-vitro diffusion study

Diffusion studies were performed using Franz diffusion cell apparatus. Accurately weighed quantity of sample was placed over the donor compartment. Aliquots of samples were withdrawn at specific intervals and drug release was determined. At the end of 24 h the mean % drug release of each formulation was given in Figure 4.

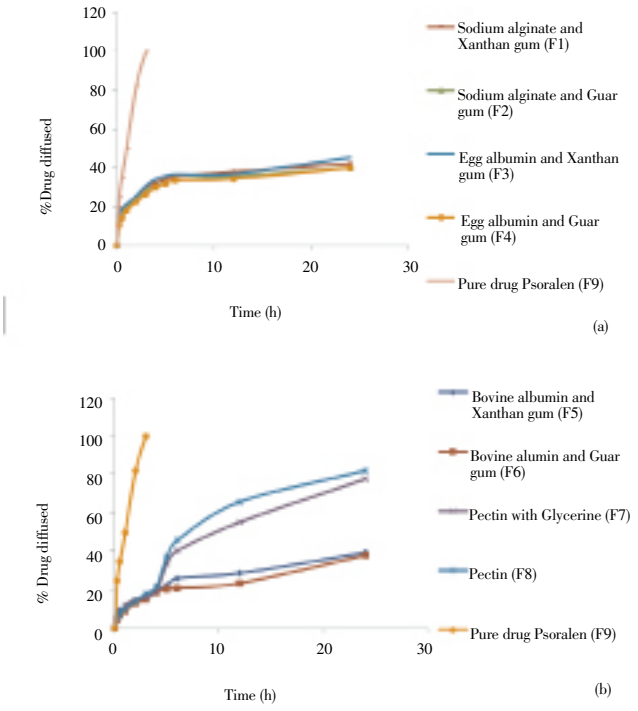

Figure 4. Diffusion profiles for the formulation (F1–F9).

3.2.8. Release kinetics

Data obtained from *in vitro* release studies of the Psoralen from various gel formulations were fitted to various kinetic equations such as Zero order, First order, Higuchi model and Korsmeyer–Peppas model and the results are presented in Table 4 and Figure 5. The release of Psoralen from the gel was First order diffusion as indicated by higher R^2 values in First order kinetics and Higuchi model.

3.2.9. Stability studies

Stability of a drug in a dosage form at different environmental conditions is important as it determines

Table 4
Release kinetics of formulation F1, F3, and F8.

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas model	
	R	R^2	R	R^2	R	R^2	R	R^2
F1	0.981	0.963	−0.991	0.982	0.972	0.945	0.943	0.890
F3	0.940	0.884	−0.988	0.976	0.975	0.951	0.976	0.951
F8	0.957	0.916	−0.993	0.987	0.981	0.963	0.980	0.960

Table 5
Stability studies of F1, F3 and F8 at 0 °C, (27±2) °C, and (40±2) °C (75% RH).

Formulation code	Physical appearance	pH	Consistency	% Drug content	Result
F1	Change in colour due to interaction	–	–	–	Rejected
F3	No change	No change	No change	No change	Accepted
F8	No change	No change	Change observed	–	Rejected

the expiry date of that particular formulation. Changes in the physical appearance, colour, odor, texture of the formulation indicate the drug stability. The results of stability studies are given in the table. The gels were separately subjected to a temperature of 0 °C, room temperature of (27±2) °C and (40±2) °C for 1 month. Periodically (initially 15 days, up to 30 days interval) samples were removed and characterized by pH, viscosity, *in-vitro* drug release study. Evaluation parameters of the formulations F1, F3 and F8 were given in Table 5 and physical changes along with stability studies are shown in Figures 6 and 7.

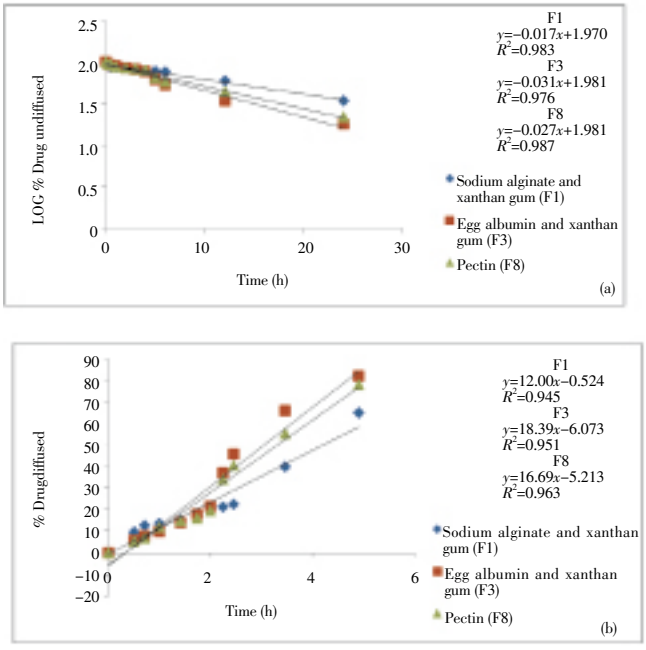

Figure 5. First order (a) and Higuchi model (b) comparative release kinetics of the formulations F1, F3, and F8.

Formulation F3 when subjected to freezer (0 °C) showed a considerable change in drug content and also decreases in release profile. Storage at (40±2) °C also showed change in colour, consistency, pH, viscosity, drug content, and release profile. This may be due to the fact that egg albumin is natural in origin and it gets denatured at high temperatures.

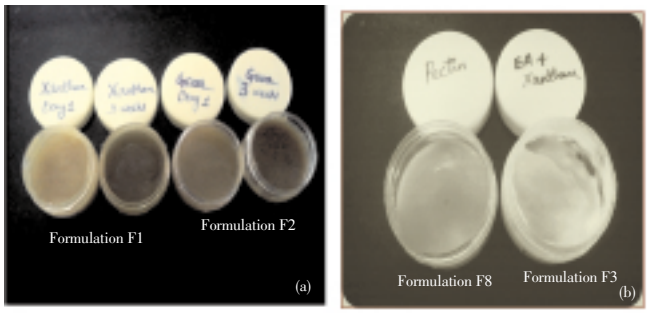

Figure 6. Physical appearance of the formulation F1, F2, F3 and F8 after 3 weeks.

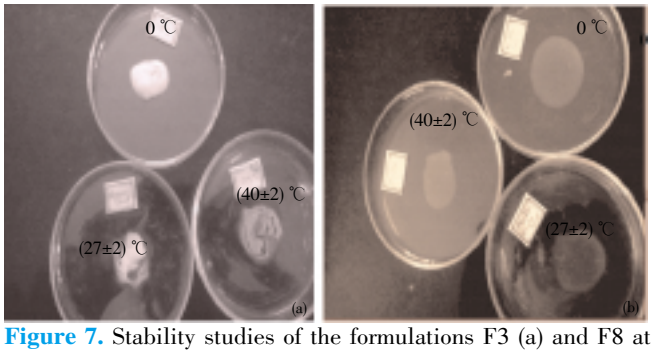

Figure 7. Stability studies of the formulations F3 (a) and F8 at different temperatures (75% RH).

Hence it is clear that the gel containing egg albumin and xanthan gum (F3 formulation) is stable at room temperature and stability study data are shown in Table 6 and Figure 7a.

3.2.10. In-vitro anti-psoriatic activity

The mouse-tail model is based on the induction of orthokeratosis in those parts of the adult mouse-tail, which have a normally parakeratotic differentiation. Saline and Formulation 3 (egg albumin and xanthan gum) were applied topically in the form of a gel. In the mouse tail test, Psoralen produced significant

Table 6
Stability studies of formulation F3.

Evaluation parameter	Initial	0 °C		Room temperature	
		15 d of observation	30 d of observation	15 d of observation	30 d of observation
Physical appearance	Clear white in colour	No change	No change	No change	No change
pH	6.87	6.85	6.80	6.86	6.87
% Drug content	81.80	78.00	77.06	80.00	80.30
% Drug diffused	45.32	44.60	44.00	43.10	43.90

Table 7
Effect of psoralen on the degree of orthokeratosis and drug activity in mouse-tail (mean±SEM) (n=6).

Group	Orthokeratosis (%)	Drug activity (%)
Control group (Saline solution)	17.00±2.30	-
Treated group (Formulation F3)	53.00±0.98	43.3

orthokeratosis when compared to control. Parakeratotic condition is seen in the adult mouse tail which is one of the hallmarks of psoriasis.

The presence of nucleus in the control was more in number. The Formulation 3 (egg albumin and xanthan gum) containing Psoralen drug showed decrease in the number of nucleus which shows the increase in orthokeratotic activity. Saline that acts as control showed 20% orthokeratosis whereas the Formulation 3 showed 53% orthokeratosis in mouse tail. The drug activity was found to be 43.3%. Anti-psoriatic drug enhances the orthokeratotic cell differentiation in the epidermal scales. Orthokeratosis and drug activity in the mouse tail are shown in Table 7 and Figure 8.

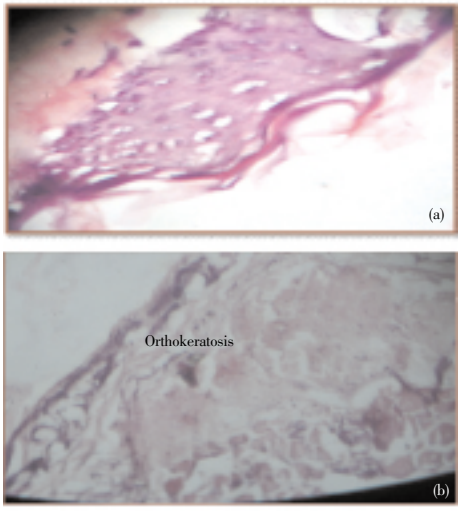

Figure 8. Cross-section through the skin of the mouse tail treated topically with saline solution (Control group, Figure 8a) or with formulation F3 (Treated group, Figure 8b).

4. Discussion

Topical formulations are advantageous over oral formulations as they avoid gastrointestinal drug absorption difficulties caused by gastrointestinal pH and enzymatic activity. Formulations containing egg albumin and xanthan gum showed more release rates when compared to formulations containing egg albumin and Guar gum. Release of drug from the gels was first order diffusion controlled as indicated by higher r^2 values in first order kinetic and Higuchi model and the n values obtained from the Korsmeyer-Peppas model showed that the release mechanism was non-fickian.

F3 formulation showed 82% drug release in 24 h. On application to the skin the gel leaves the cooling effect, no gritty particles on the surface of the skin and also no irritation to the skin. *In vitro* anti-psoriatic activity of F3 showed the significant orthokeratosis in the mouse tail test when compared to control thus indicating that the formulation is effective in treating psoriasis.

Conflict of interest statement

We declare that we have no conflict of interest.

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