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Design and *In Vivo* Evaluation of Acitretin Solid Lipid Nanoparticles Loaded Gel for Topical Delivery

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ABSTRACT

Solid lipid nanoparticles (SLNs) are very potential formulations for topical delivery of antifungal drugs. The main objective for this investigation is to develop and optimize the SLNs of Acitretin for the effective topical delivery. Acitretin loaded SLNs were prepared by hot homogenization followed by the ultra sonication using Taguchi's design and based on the results further investigation was made using central composite design (CCD). The lipid Dynasan-116, surfactant poloxomer-188 and co surfactant egg lecithin resulted in better percent drug loading and evaluated for particle size, zeta potential, TEM, drug entrapment efficiency, *in vitro* drug release and stability. All parameters were found to be in an acceptable range. *In vitro* drug release of optimized SLN formulation (F2) was found to be 95.63±1.52%, whereas pure drug release was 30.12 after 60 min. The optimized formulation was incorporated into the gel. The drug content of Acitretin gel formulation was found to 99.86 ± 0.012% and the diameter of gel formulation was 6.9 ± 0.021 cm and that of marketed gel was found to be 5.7 ± 0.06 cm, indicating better spreadability of SLN based gel formulation. The viscosity of gel formulation at 5 rpm was found to be $6.1 \times 10^3 \pm 0.4 \times 10^3$ cp. The release rate (flux) of Acitretin across the membrane and excised skin differs significantly. It is assumed that SLN based gel due to its appropriate physicochemical properties, high skin permeation ability, higher accumulative uptake in skin and improved skin tolerability may be more suitable as a novel regime for topical administration of Acitretin. This topically oriented SLN based gel formulation could be useful in providing site-specific dermal treatment of psoriasis with minimal drug systemic availability and high skin tolerability.

Keywords: Acitretin, Psoriasis, topical gel, skin permeation, histopathological studies.

INTRODUCTION

Psoriasis is one of the most common human skin diseases and is considered to have key genetic underpinnings. It is characterized by excessive growth and aberrant differentiation of keratinocytes [1, 2]. The application of nanotechnology have been extended to many topical preparations for the treatment of cutaneous manifestations of diseases like psoriasis with the intent of containing the pharmacological effect of the drug to the surface of the skin or within the skin [3]. Solid lipid nanoparticles (SLN) are the new generation of nanoparticulate active substance vehicles and are attracting major attention as novel colloidal drug carriers for topical use with advantages such as controlled release, negligible skin irritation and protection of active compounds [4,5].

Improving the permeation of some agents to the dermis layer of skin is desired in the form of gel deeply through the epidermal layers [6]. Thus, topical application of SLNs based gel with increased penetration and retention through skin because of lipid nano formulation will be much promising for the topical treatment of psoriasis.

Acitretin (13- cis- trans retinoic acid), a metabolite of vitamin A, has gained great interest due to its multitude of physiological effects such as regulation of epithelial cell growth and differentiation, sebum production and collagen synthesis [7].

In the present research work, Acitretin loaded solid lipid nanoparticles were prepared by hot homogenization followed by the ultra sonication. The formulation was optimized by using 3- factor, 3- level Central composite design. The optimized SLN preparation of Acitretin was incorporated in gel and the gel preparation was evaluated for in vitro skin permeation study, skin retention, histopathological investigation of skin and skin- irritation testing.

MATERIALS AND METHOD

Materials

Acitretin was obtained as a gift sample from Biocon Limited, Bangalore, India. Trimyristin (Dynasan- 114), tripalmitin (Dynasan116) and tristearin (Dynasan- 118) were purchased from Sigma- Aldrich Chemicals, Hyderabad, India. Egg Lecithin, Carbopol and Poloxamer- 188 were gift samples from Aurobindo Labs, India. All other chemicals and solvents were of analytical grade and used without further purification.

Preparation of Acitretin loaded solid lipid nanoparticles

Acitretin loaded SLNs were prepared by hot homogenization followed by the ultra sonication [8]. Acitretin, lipid and egg lecithin were dissolved in 5 ml of 1:1 mixture of chloroform and methanol. Organic solvents were completely removed using a rota evaporator. The drug embedded lipid layer

was molten by heating to 5°C above melting point of the lipid. Aqueous phase was prepared by dissolving Poloxamer 188 in double distilled water and heated to same temperature (based on lipid melting point) of oil phase. Hot aqueous phase was added to the oil phase, and homogenization was carried out (at 12000 rpm) using homogenizer for 4 min. The coarse hot oil in water emulsion so obtained was ultra sonicated using a 12 T probe Sonicator for 20 min.

Characterization of Acitretin loaded solid lipid nanoparticles

The mean particle size, poly dispersity index and zeta potential of Acitretin nanoparticles were determined by laser light diffractometry using Zetasizer nano series, SM2000K (Malvern Instruments Inc., UK). The SLNs were subjected to lyophilization [9]. Entrapment efficiency was determined by measuring the concentration of un entrapped free drug in aqueous medium containing either PVA or PLX. The free drug concentration measured by the UV absorbance of the supernatant was determined at 353 nm. The morphology of the Acitretin loaded nano formulation was determined by TEM (J EM- 2000 EXI; J EOL, Tokyo, Japan)

Design of Experiment

By adopting Taguchi design, eight parameters (Surfactant type, Type of lipid, Surfactant concentration, Co- surfactant concentration, Lipid- to- drug ratio, Chloroform- Methanol ratio, Organic- aqueous phase ratio and sonication time) with 18 experiments (one 2- level factor and seven 3- level factors) were tested .Based on the results from the analyses of the responses obtained from Taguchi design, three different independent variables including surfactant concentration (%), lipid to drug ratio (w/ w), and sonication time (s) were selected for further investigation using central composite design[10].

Preparation of SLN based gel of Acitretin

The gel of the optimized formulation was developed using a suitable poly mercapable of modifying the rheological behavior. The gel formulation of Acitretin was prepared by mixing the swollen gel matrix with the oily phase at 2% w/w concentration of Carbopol 971P NF.

Characterization of SLN based gel of Acitretin

The SLN based gel of Acitretin was characterized by determination of drug content by UV Spectroscopy, determination of spreadability and pH of SLN gel [11,12].

In vitro drug release studies

The in vitro drug release study of Acitretin loaded nanoparticles was performed using dialysis bag method with PBS (pH 7.4) as dissolution medium at 37°C with the speed of 50 rpm. At predetermined time points, two milliliters of dissolution medium was removed and ultra filtered as

described above and analyzed by UV-Visible absorption measurement at 353 nm. The removed volume was replaced with the same volume of a fresh SIF (37°C). Similarly, in vitro drug release profile of the Acitretin suspension was determined by suspending 2.5 mg of the Acitretin in 25 ml of PBS (pH 7.4) containing 3% w/v SLS.

Rheological studies on the Acitretin loaded SLN gel

Brookfield Viscometer with helipath stand was used for rheological studies. 30 gm of sample was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a T- C spindle at 0.5, 1, 2.5 and 5 rpm.

In vitro Skin Permeation Study

The in vitro skin permeation study was carried out for Acitretin loaded SLN gel as per the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) guidelines. The study protocols were approved by Institutional Animal Ethics Committee (IAEC) with No. 1292/ac/09/CPCSEA-18/A. The abdominal skins obtained from male Wistar rats weighing 250 ± 10 g were used for in vitro permeation experiments of prepared formulations. After hair was shaved carefully with an electric clipper, the skin was excised from the abdominal region of each sacrificed rat and the subcutaneous fat and other extraneous tissues were removed without damaging the epidermal surface. The excise drat skin samples of almost the same thickness were rinsed with physiological saline and placed in refrigerator at 4°C prior to permeation experiments. The skin membranes were first hydrated for 30 min in buffer solution (pH 7.4) at room temperature to remove extraneous debris and leachable enzymes [13].

The rat skin was mounted between the donor and receptor compartment with the stratum corneum facing upper side on the Franz diffusion cell. To maintain sink conditions 30 % (v/v) ethanol in phosphate buffer pH 7.4 was taken in receptor compartment. The temperature was maintained at 37 $\pm$ 1°C. Diffusion media was continuously stirred with Teflon coated magnetic bar at a constant rate, in a way that the rat skin surface just flushes the diffusion fluid.

The formulation (1 g) was gently placed in a donor chamber and then the diffusion cells were covered with an aluminum foil to prevent light exposure. At 1, 2, 4, 6, 8, 10, and 12 h aliquots of 1 ml sample were withdrawn from the receptor compartment and replaced immediately with an equivalent volume of receptor fluid. The samples were analyzed for drug content using UV spectrophotometer at 353 nm. Each experiment was performed in triplicate. The cumulative amount of drug permeated (Q) at different time intervals and various parameters like steady state flux (J ss), permeability coefficient (Kp) and enhancement ratio (ER) were calculated.

Skin retention study

Skin retention study was performed for Acitretin loaded SLN gel in order to analyze the content of the drug in the skin. At the end of the in vitro skin permeation study, the skin samples were washed with water and methanol on both sides and carefully dried. Then a defined amount of methanol was added to each piece of skin. The samples were vortexed for 10 min in order to extract its drug content and stirred over night. After centrifugation the samples were analyzed by UV spectrophotometer [14].

Histopathological investigation of skin

The rat abdominal skin was mounted on the Franz diffusion cell. The optimized gel formulation and marketed gel were applied similar to the method of permeation study and the effects were compared against control. A piece of fresh excised untreated skin sample was used as control. The skin was fixed in 10 % neutral formalin for 24 h and then cut vertically against the surface at the central region (4 mm width). Each section was dehydrated using graded solutions of ethanol and then embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. The sections were observed under100x magnifications and photographed [15].

Skin- irritation testing (Draize patch test)

The irritation potential of the SLN based Acitretin gel in comparison with marketed Acitretin gel was evaluated by carrying out the Draize patch test on rabbits. Animal care and handling throughout the experimental procedure were performed in accordance with the CPCSEA guidelines. The experimental protocol was approved by the institutional Animal Ethical Committee (IAEC) with No: 1292/ac/09/CPCSEA-18/A. White New Zealand rabbits weighing 2.5 – 3 kg were acclimatized before the beginning of the study [16].

Animals were divided into four groups (n = 3) as follows:

Group 1 was with No application (control), group 2 with Marketed formulation (Acitretin gel containing 0.05% w/w), group 3 was applied with Gel formulation without Acitretin (placebo gel) and group 4 was treated with SLN based gel containing Acitretin (0.05%, w/w).

The back of the rabbits were clipped free of hair 24 h prior to the application of the formulations. 0.5 g formulations were applied on the hair free skin of rabbits by uniform spreading within the area of 4 cm2. The skin was observed for any visible change such as erythema (redness) at 24, 48 and 72 h after the application of various formulations.

The mean Erythemal scores were recorded (ranging from 0 to 4) depending on the degree of erythema as follows: no erythema = 0, slight erythema (barely perceptible-light pink) = 1, moderate

erythema (dark pink) = 2, moderate to severe erythema (light red) = 3, and severe Erythema (extreme redness) =4.

RESULTS AND DISCUSSION

Optimization and confirmation experiments

All the prepared formulations (Table 1) were analyzed in order to determine their particle size distribution, PDI and zeta potential.

Table 1: Formulations of Acitretin (Taguchi orthogonal array table of L-18)

Experimental conditions								Particle size (nm)			Entrapment efficiency (%)			% Drug loading		
A	B	C	D	F	G	H	I	Actual value	Predicted value	S/N ratio	Actual value	Predicted value	S/N ratio	Actual value	Predicted value	S/N ratio
PLX-188	D-114	150	100	10	1.5:0.5	1.5:0.5	180	165.4	165.82	-49.10	79.6	82.81	-36.72	7.9	7.84	-26.59
PVA	D-118	200	100	30	1.0:1.0	0.5:1.5	180	243.8	242.48	-44.10	76.7	75.43	-45.75	7.8	7.11	-20.70
PLX-188	D-114	100	200	30	1.0:1.0	1.0:1.0	120	266.7	260.87	-37.64	81.2	82.81	-37.06	6.8	7.84	-20.70
PVA	D-116	100	100	20	1.0:1.0	1.5:0.5	240	187.6	185.47	-42.01	74.6	75.43	-38.76	7.6	7.89	-23.33
PVA	D-114	100	100	10	0.5:1.5	0.5:1.5	120	183.1	184.20	-44.88	74.1	75.43	-36.61	5.9	5.76	-20.28
PLX-188	D-118	150	100	30	0.5:1.5	1.0:1.0	240	224.2	227.55	-40.05	79.6	82.81	-39.12	7.6	9.19	-21.08
PVA	D-118	150	200	20	0.5:1.5	1.5:0.5	120	217.4	218.78	-43.89	74.4	75.43	-38.59	6.9	7.11	-23.09
PLX-188	D-118	200	100	10	1.0:1.0	1.5:0.5	120	181.2	184.20	-40.53	83.3	82.81	-37.82	10.3	9.19	-24.77
PLX-188	D-118	100	150	20	1.5:0.5	0.5:1.5	180	200.6	200.40	-52.29	83.8	82.81	-36.94	9.5	9.19	-19.64
PVA	D-118	100	150	10	1.5:0.5	1.0:1.0	240	152.3	150.88	-43.79	76.3	75.43	-35.89	6.8	7.11	-17.85
PVA	D-116	150	150	30	1.5:0.5	0.5:1.5	120	264.7	260.87	-39.46	76.8	75.43	-32.90	7.3	7.89	-30.16
PVA	D-114	200	200	30	1.5:0.5	1.5:0.5	240	221.2	227.55	-37.27	74.2	75.43	-38.01	6.3	5.76	-24.24
PLX-188	D-116	100	150	30	0.5:1.5	1.5:0.5	180	241.2	242.48	-44.22	84.2	82.81	-36.54	9.8	9.97	-25.66
PLX-188	D-116	200	100	20	1.5:0.5	1.0:1.0	120	214.6	218.78	-39.08	84.2	82.81	-33.51	11.2	9.97	-22.86
PLX-188	D-116	150	200	10	1.0:1.0	0.5:1.5	240	152.3	150.88	-43.79	85.6	82.81	-36.54	10.3	9.97	-17.13
PLX-188	D-114	200	150	20	0.5:1.5	0.5:1.5	240	190.2	185.47	-38.55	83.8	82.81	-38.01	7.6	7.84	-23.09
PVA	D-114	150	150	20	1.0:1.0	1.0:1.0	180	198.9	200.40	-43.54	75.6	75.43	-32.90	6.3	5.76	-16.01
PVA	D-116	200	200	10	0.5:1.5	1.0:1.0	180	167.5	165.82	-43.04	76.2	75.43	-41.07	7.4	7.89	-17.56

The mean size of all the formulations was ranging from 213.3 ± 2.8 nm to 221.9 ±3.9 nm The PDI was ranging from 0.114 to 0.197, indicating the narrow size distribution. The SLN formulations exhibited negative surface charge with the inclusion of acitretin which clearly suggested the orientation of acitretin in the lipid matrix. The zeta potential values of SLN formulations were found to be in between -18.9 ± 4.89 mV to -20.7 ± 5.48 mV. Total entrapment efficiency of the nanoparticles formulations was determined and found to be ranging from 85.12 ± 0.37 % to 86.08 ± 0.18 %. The percent drug loading of the formulations was found to be in the range from 10.98 ± 2.12 % to 11.20 ± 1.72 (Table 2).

Table 2: The mean particle size, PDI, zeta potential, entrapment efficiency and % drug loading of optimized formulations

Batch	MPS ± SD (nm)	PDI	ZP ± SD (mV)	% EE ± SD	% DL ± SD
1	213.3 ±2.8	0.118	-20.7 ± 5.48	85.42 ± 0.82	10.98 ± 1.72
2	219.9 ± 2.2	0.114	-19.6 ± 7.79	85.12 ± 0.37	11.12 ± 2.12
3	221.9 ± 3.9	0.197	-18.9 ± 4.89	86.08 ± 0.18	11.20 ± 0.94

n = 3 (p < 0.05)

Drug release study

Dissolution rates of Acitretin pure drug and solid lipid nanoformulations were evaluated. All formulations showed an increase in dissolution over pure drug, in vitro drug release of optimized SLN formulation (F2) was found to be 95.63±1.52%, whereas pure drug release was 30.12 after 60 min. The in vitro drug release pattern of drug from the optimized batches recorded. The in vitro drug release pattern of drug from the optimized batches is as shown in Figure 1 and Table 3.
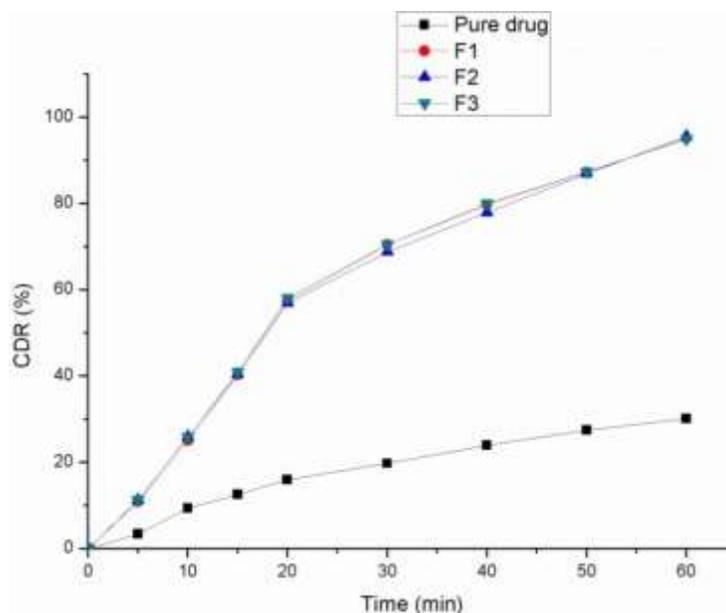

Figure 1: *In- vitro* release of Acitretin from solid lipid nanoparticles

Table 3: Dissolution profile of Acitretin solid lipid nanoparticles (Optimized batches)

Time (min)	% CDR			
	Pure drug	F1	F2	F3
0	0	0±0	0±0	0±0
5	3.34	10.8±1.28	11.19±2.62	11.31±1.72
10	9.36	25.05±1.52	25.94±1.12	25.87±0.76
15	12.56	40.30±1.25	40.41±1.34	40.92±2.01
20	15.93	57.40±1.19	56.92±0.96	58.12±1.32
30	19.72	70.50±1.27	68.83±1.56	70.46±1.65
40	23.98	79.76±1.28	77.93±2.21	80.02±0.64
50	27.43	87.27±1.58	86.93±1.35	87.42±1.89
60	30.12	95.12±1.52	95.63±0.97	94.91±0.62

Evaluation of SLN based gel of Acitretin

The Acitretin content of the gel formulation was found to 99.86 ± 0.012% w/w of the theoretical value (0.05% w/w). The results indicate that the processes employed to prepare solid dispersions in this study were capable of producing formulation with uniform drug content. The diameter of gel formulation was 6.9 ± 0.021 cm and that of marketed gel was found to be 5.7 ± 0.06 cm, indicating better spreadability of SLN based gel formulation.

The pH of the gel was found to be 7.1 ± 0.06 indicating that it could result in less irritation to the skin. The viscosity of gel formulation at 5 rpm was found to be 6.1 x 103± 0.4 x 103 cp.

The release rate (flux) of Acitretin across the membrane and excised skin differs significantly (Table 4), which indicates about the barrier properties of skin. Interaction between skin and nano formulation component may justify these differences. Association and fusion of lipid based nanoparticles to the skin surface resulted in higher flux due to the direct transfer of drug from the vesicles.

Table 4: Flux of Acitretin from SLN based gel formulation

Formulation	Flux ($\mu g \; cm^{-2} \; h^{-1}$)	
	Egg membrane	Rat skin
F2	49.231 ± 2.68	182.754 ± 3.126
Marketed formulation	38.326 ± 3.14	122.345 ± 4.786
Control	29.115 ± 2.37	49.265 ± 5.12

The flux value for SLN based gel formulation (182.754 ± 3.126µg cm−2 h−1) was found to be higher than that for marketed gel (122.345 ± 4.786µg cm−2 h−1). The higher flux values of SLN based gel suggest that it might be able to enter the skin easily as compared with marketed gel with an advantage of low inter facial tension of the emulsifier film that ensures an excellent contact to the skin.

The accumulative amount of Acitretin in skin from SLN based gel formulation and marketed gel were 38.26 ± 0.24 mg and 28.12 ± 0.16 mg respectively. This result supported our hypothesis made in skin permeation studies on rat skin. The more Acitretin permeates the less Acitretin is retained in the skin and might lead to systemic adverse side effects. This was one of the reasons to employ lipid based formulations for topical delivery of Acitretin as its epidermal localization is highly desirable for enhancing the treatment of skin diseases such as psoriasis.

The rat skin is a multilayered organ with many histological layers. The histology of excised rat skin in pure drug and treated with optimized Acitretin SLN gel formulation, Acitretin SLN gel formulation and marketed gel is shown in Figure 2 (A-D).
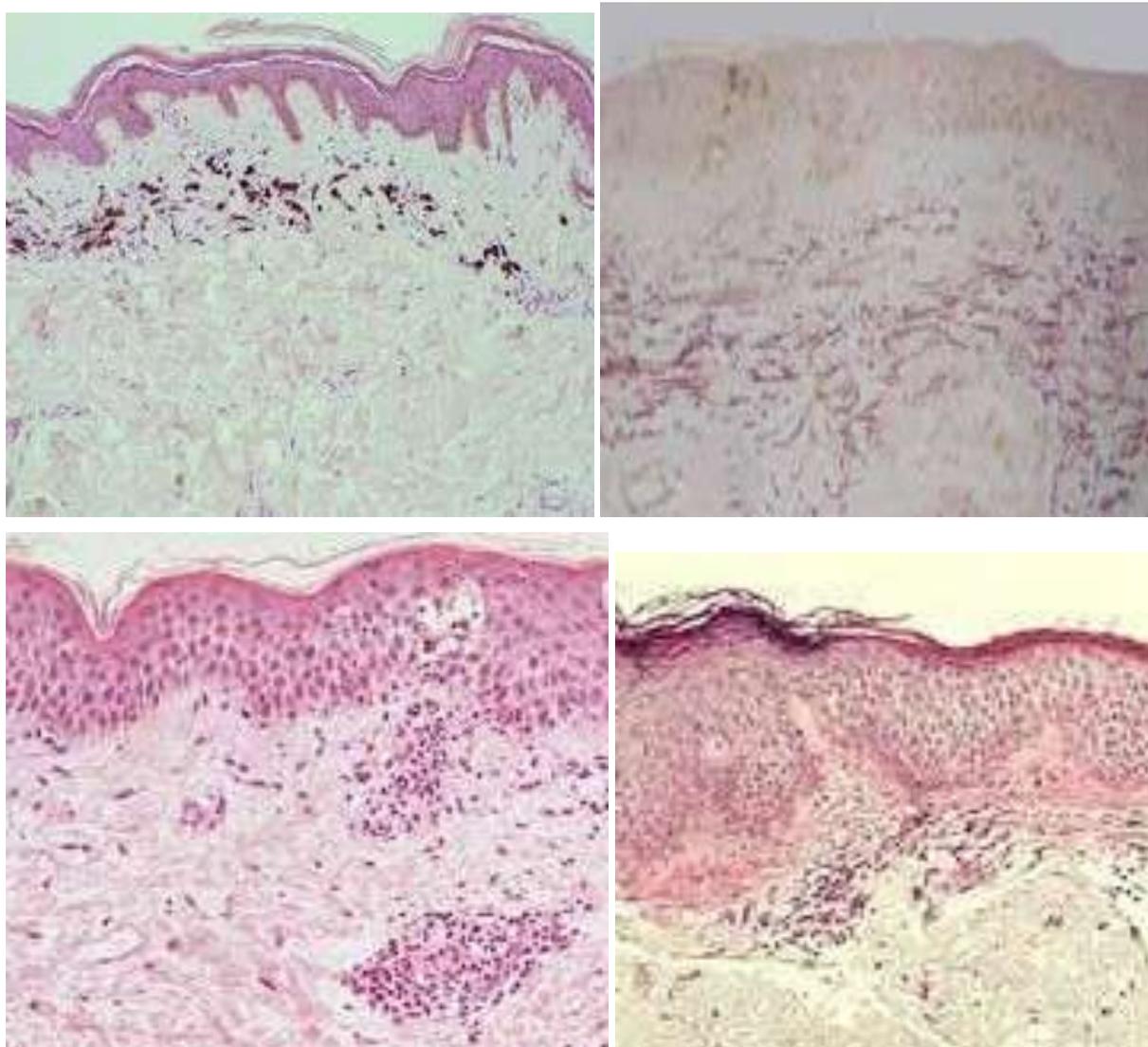
Figure 2: Light microscopic photographs of in vitro histological study. (A) Pure drug. (B)The rat skin treated with optimized SLN formulation. (C) The rat skin treated with SLN based gel formulation. (D)The rat skin treated with marketed gel.

The microscopic observations indicate that the optimized SLN formulation, SLN based gel formulation and marketed gel has no significant effect on the microscopic structure of the skin. The surface epithelium lining and the granular cellular structure of the skin were totally intact. No major changes in the ultrastructure of skin morphology could be seen and the epithelial cells appeared mostly unchanged.

One of the major disadvantages associated with the Acitretin therapy is skin irritation (erythema), which strongly limits its utility and acceptability by the patients. Ideally, the delivery system of Acitretin should be able to diminish or abolish these erythematic episodes. However, most of the currently marketed conventional dosage forms such as creams, lotion sand gels are not able to reduce the irritation caused by topical application of Acitretin. It was hypothesized that encapsulation of Acitretin in the lipid structure would reduce the skin irritation (Table 5).

Table 5: Mean Erythemal scores for various Acitretin formulations obtained at the end of 24 , 48 and 72 h

Formulations	Erythmeal score (n = 3)		
	24 h	48 h	72 h
Control (Group 1)	0	0	0
Marketed gel (Group 2)	1	2	2
SLN based gel without Acitretin (Group 3)	0	0	0
SLN based gel containing Acitretin (Group 4)	0	0	0

Draize patch test is a reliable method and the results obtained from this study can be linked to that obtained in humans. The skin-irritation studies indicated that SLN based gel containing Acitretin did not show any sign of skin irritation as compared to moderate erythema shown by marketed gel formulation (Tazret® gel) after 72 h of application (Table 5). Thus, SLN based gel formulation demonstrated advantage over marketed formulation in improving the skin tolerability of Acitretin indicating their potential in improving patient acceptance and topical delivery of Acitretin.

CONCLUSION

The present research work could be concluded as successful production of SLN's may be a more promising approach for the topical delivery of Acitretin. It is assumed that SLN based gel due to its appropriate physicochemical properties, high skin permeation ability, higher accumulative uptake in skin, and improved skin tolerability may be more suitable as a novel regime for topical

administration of Acitretin. This topically oriented SLN based gel formulation could be useful in providing site-specific dermal treatment of psoriasis with minimal drug systemic availability, and high skin tolerability hence, future clinical studies on psoriatic patients are recommended.

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