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Topical Delivery of Apremilast Loaded Nanostructured Lipid Carrier Based Hydrogel for Psoriasis Therapy

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Authors' contributions

This work was carried out in collaboration between both authors. The authors contributed equally to the study. Author SMS designed and conducted the study and author MKK guided the study and approved the manuscript for submission. All authors read and approved the final manuscript.

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ABSTRACT

Background: Apremilast (APR) is an orally administered selective phosphodiesterase 4 inhibitor approved to treat plaque psoriasis and psoriatic arthritis and is available as an oral tablet formulation. However, its systemic side effects limit its application. The low solubility and permeability of apremilast make it difficult to administer it through the skin. Hence an attempt is made to incorporate apremilast into a suitable nanocarrier to facilitate its topical delivery.
Aims: To formulate and characterize Apremilast loaded nanostructured lipid carriers for the management of psoriasis to reduce the systemic side effects.
Methodology: Apremilast loaded Nanostructured Lipid carriers (NLC) were prepared by melt emulsification accompanied by probe sonication. The formulation was prepared using GMS, Sefsol 218, Tween 80 and Transcutol P as Solid Lipid, Liquid lipid, Surfactant and Penetration Enhancer. The NLC was incorporated into carbapol 934 dispersion to convert it into a gel. The NLC formulation was evaluated for size, Polydispersity Index, Zeta Potential, Entrapment efficiency, Transmission Electron Microscopy. After that, the NLC gel was examined for Spreadability, Extrudabilty, Viscosity, *In vitro* drug release, *Ex vivo* permeation, Skin deposition and *In vivo* studies.

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Results: The formulated Apremilast loaded showed particle size of less than 200 nm (i.e.170.32nm) with a narrow PDI of 0.267. Entrapment efficiency revealed that 89.26±01.22% of the drug was entrapped. Transmission electron microscopy images confirmed the spherical nature of the nanocarrier. The extended-release pattern of the formulated NLC for 24h was observed in the *in vitro* release studies and followed the Higuchi model(R^2=0.9966). *Ex vivo* permeability showed a 6.14 fold increase in permeability and 74.05±0.25% deposition of apremilast loaded NLC gel compared to apremilast gel. The formulation was stable for three months without significant changes. *In vivo* skin studies showed that the prepared NLC did not have any skin irritation potential. The antipsoriatic activity demonstrated by the Apremilast loaded NLC gel in the imiquimod induced psoriasis model in mice was comparable to the standard treatment.
Conclusion: Apremilast loaded NLC demonstrated enhanced permeation, improved skin retention and extended-release compared to conventional gel. The developed formulation can be an alternative for psoriasis therapy after clinical trials in the future.

Keywords: Apremilast; nanocarrier; emulsification; topical; antipsoriatic.

1. INTRODUCTION

Psoriasis is an autoimmune skin disease characterized by recurrent flare-ups of inflammation and hyperkeratosis [1]. In the management of psoriasis, topical drug therapy is the gold standard. By minimizing systemic side effects, it allows for specific targeting of infected skin. However, patient satisfaction with the available topical treatment remains minimal [2]. Apremilast is an oral Phosphodiesterase Inhibitor (PDE4) approved by the US Food and Drug Administration in 2014 to treat moderate-to-severe plaque psoriasis. This drug could fundamentally change psoriasis therapy as it does not interfere with Immune suppression; instead, it targets the central inflammatory signalling pathways. Thus apremilast has a distinct benefit in treating psoriasis compared to other available drugs [3].

Currently, Apremilast is marketed only as an oral tablet under the brand name Otezla® in strengths of 10, 20, and 30 mg, respectively. However, side effects such as nausea, diarrhoea, upper respiratory illness, weight loss, depression, and suicidal impulses limit apremilast's efficacy in the treatment of psoriasis. Therefore it is necessary to develop an alternative topical formulation of apremilast that can overcome the limitation of the oral formulation [4].

Topical therapy as the first line of psoriasis treatment is a promising approach because it effectively delivers medications to disease sites, minimizes systemic adverse effects, and ensures high patient compliance. On the other hand, conventional topical formulations are limited in administering antipsoriatic agents due to low percutaneous penetration and targeting deeper layers of the skin [5].

Novel nano carrier-based formulations have the potential to solve the limitations of traditional formulations by reducing the dosage, dosing frequency, dose-dependent side effects, and improving effectiveness [6]. The most appropriate lipid-based nanocarriers for topical drug delivery are nanostructured lipid carriers (NLCs). These delivery systems have certain advantages like occlusiveness and complete skin adhesion due to their small size and high lipid content. Apart from these advantages, incorporating the active ingredient in lipid matrix structures improves the active ingredient's chemical stability and prevents leaching [7].

Hence an attempt was made in the current study to incorporate apremilast into a nanocarrier such as a nanostructured lipid carrier to increase its solubility and permeability, thereby improving the dermal bioavailability and antipsoriatic activity.

2. MATERIALS AND METHODS

2.1 Materials

Apremilast was received as a gift sample from Glenmark life Sciences Ltd, Ankleshwar, Gujarat**.** Sefsol 218 and Transcutol P were obtained from Gattefosse, France. Tween 80 and carbapol 934 P was purchased from LOBA Chemical Pvt. Ltd., India. Imiquad cream (5% imiquimod) of Glenmark Pvt. Ltd and Veet hair removal creams of Reckitt Benckiser India Pvt were obtained from Radha medicals Mangalore. Betagel® (Microlabs Pvt Ltd) was purchased from a local chemist shop. Ultra-pure deionized water was used

throughout the experiments. All other solvents and chemicals used were of analytical grades or higher.

2.2 Preparation of Apremilast Loaded Nanostructured Lipid Carriers

Nanostructured lipid carriers of Apremilast was formulated using the melt emulsification-probe sonication method. Previously, the NLC was optimized in terms of particle size, polydispersity index and entrapment efficiency (data not shown). The procedure for the optimized batch is briefly discussed here. Weighed quantities of Sefsol 218 and Glyceryl monostearate were taken in 6:4ratios to prepare the lipid phase. Tween 80 (surfactant) and Transcutol (co-surfactant) were dissolved in Milli-Q water to make the aqueous phase. Both of these phases were held at 75°C. Apremilast (0.1%) was introduced to the molten lipid mixture when stirring constantly, and then the aqueous phase was added drop by drop to the lipid phase under steady stirring at 2000 rpm for 30 minutes until a homogeneous emulsion was created. The resulting primary emulsion was ultrasonicated for 15 minutes at 60% amplitude under hot condition. After cooling the solution to room temperature, the NLC dispersion was obtained [8].

2.3 Characterisation of Apremilast Loaded Nanostructured Lipid Carriers

2.3.1 Determination of particle size, polydispersity Index and zeta potential

The mean particle size, polydispersity index, and zeta potential of the formulated NLC were determined using Zetasizer (Malvern, Worcs, UK). During the study, the cell temperature was maintained at 25°C, and the scattering angle was 90°. Before the analysis, samples were diluted with double distilled water for better accuracy. The measurements were taken three times overall, and the average was considered.

2.3.2 Entrapment efficiency

The NLC dispersion was subjected to controlled centrifugation at 2000 rpm for thirty minutes for entrapment efficiency determination. The NLC dispersion was decanted without disturbing the sedimented drug pellets obtained after centrifugation. The entrapped drug was determined using the NLC dispersion, and the free drug was estimated using the drug pellets. The analysis was carried out by using a UV visible spectrophotometer after dilution with methanol [9].

2.3.3 Transmission electron microscopy (TEM)

The surface morphology of the prepared NLC was examined by TEM. The prepared NLC was diluted with ultra-pure water, and a drop of the dispersion was placed on a carbon-coated copper grid and kept for 1 min. Excess dispersion was removed by adsorbing on the filter paper. The grid was then air-dried, and images were taken by TEM.

2.4 Incorporation of the NLC Dispersion into the Hydrogel

Carbopol 934 P was chosen as a gelling agent because of its excellent adhesive properties, elegant appearance, and ease of removal from the skin. Initially, carbopol 934 P was dispersed in purified water under magnetic stirring to obtain three different hydrogels concentrations: 0.5%, 1%, and 1.5%. Propylene glycol(10%) and Glycerol (30%) was then added to the carbopol dispersion. The optimised NLC was then added to the aqueous dispersion, followed by dropwise addition of triethanolamine until the gel was formed.

2.5 Characterisation of Apremilast loaded NLC based hydrogel

2.5.1 Visual appearance, spreadability, extrudability, and measurement of pH

The prepared gels were examined for their clarity, colour and homogeneity by visual inspection. The gels' spreadability was measured by positioning 0.5 g of the gel within a 1 cm diameter circle pre-marked on a glass plate, which was then filled with a second glass plate. For 5 minutes, a weight of 500 g was allowed to rest on the upper glass plate. The diameter of the gel increased as a result of the spreading, which was noted. For determining the extrudability, a collapsible tube holding 20g of the gel was pressed by adding a steady load of 1kg at the crimped end. When the cap was removed, the gel was pushed out of the tube until the pressure was released. The pH of the gel was determined by using a pen pH meter [10].

2.5.2 Determination of viscosity and Percentage drug content

The optimised NLC gel's viscosity measurement was carried out by Brookfields viscometer using the T 90 Spindle, which was dipped in the gel preparation. Viscosities were determined at different shear rates to understand the flow behaviour of the formulation. For estimating the drug content, a specific quantity (100 mg) of the gel was diluted with100 ml of methanol. This gel solution was then placed on a mechanical shaker for two hours to solubilize the drug. The solution was then filtered, and the amount of the drug was determined using a UV spectrophotometer (UV-1601, Shimadzu) at 230 nm [11].

2.5.3 *In vitro* drug release studies

Phosphate buffer 6.8: Methanol(8:2) was used as the diffusion medium for drug release studies. The cumulative release of apremilast from the NLC gel and conventional gel was carried out using Franz diffusion cell over 24h at 37±0.5°C with a stirring speed of 50rpm. The NLC and conventional gels formulations containing 1mg of apremilast were kept on the dialysis membrane(MW 12-14kDa, Merck). The Dialysis membrane was previously activated and kept in the donor compartment. Samples were taken from the receptor compartment and refilled with the diffusion medium at predetermined time intervals to maintain the sink conditions. The amount of apremilast in the withdrawn samples was assessed by UV spectroscopy at 230nm. All the measurements were carried out in triplicate [12].

2.5.4 Kinetic analysis of the release data

To understand the release kinetics of the formulations, the *in vitro* release data was fitted into different kinetic models such as zero order, first order, Higuchi diffusion, and Korsmeyer-Peppas. The model with the highest correlation coefficient (r2) was chosen as the best fit.

2.5.5 *Ex vivo* permeation studies

The permeation studies were conducted to determine the amount of apremilast permeated from the formulations. Before the study, pig ears were cleaned, and the dorsal part of pig ear skin was shaved using an electric razor, and subcutaneous fat was removed using a scalpel. The skin was hydrated for 24 h in phosphate buffer (pH 7.4): Methanol (8:2). The excised pork skin was sliced into a suitable size and clamped between the donor and receptor compartments of the Franz diffusion cell with the stratum corneum facing the donor compartment. The experiments were carried out in the same way as in *in-vitro* release studies(section 2.4.3) except for the change that porcine ear skin was used as the membrane instead of the dialysis membrane.

The cumulative amount of drug that permeated through the skin per unit area over time was plotted. The steady-state flux (Jss) was calculated using the slope of the linear portion of the plotted curve, and the permeability coefficient (Kp) was calculated using the equation below;

.Kp=Jss/Co

Jss is the drug flux at steady-state, and Co is the initial drug concentration in the donor cell [13].

2.5.6 Drug deposition studies

The skin mounted on Franz diffusion cells' receptor compartment was carefully removed after the permeation study. The amount of drug that remained over the skin was estimated by washing the skin surface three times with 20 percent Methanolic PBS and measuring the drug's absorbance at 230 nm. A cotton swab was used to clean away any remaining solvent on the skin gently. After that, the skin was sliced into small parts followed by the addition of methanol and sonicated for 20 minutes at 25°C. Finally, the resulting mixture was centrifuged, and the absorbance was measured triplicate using a UV spectrophotometer at 230 nm [14].

2.6 Tability Studies

Apremilast-loaded NLC was subjected to stability studies for 90 days at three different temperature conditions in accordance with ICH guidelines. Sampling was done at day 0, day 30, day 60 and day 90, respectively and evaluated for parameters like pH, Drug content, Viscosity spreadability and phase separation [15].

2.7 *In Vivo* Studies

2.7.1 Skin irritation studies

The skin irritation potential of the developed nanoformulations was assessed by conducting skin irritation studies on Wistar rats. The rats were divided into four groups, namely group 1(control, Untreated), group 2 (Plain gel treated) & Group 3 (NLC gel treated). The gels, i.e. Plain

gels, & NLC gels, were applied to the rats' shaved dorsal area in group 2 and Group 3. The excess formulation was carefully removed after 24 hours, and the treated area was visually examined for erythema (redness) and edema (swelling). Animals were then sacrificed by cervical dislocation, and the skin was isolated and fixed in 10% formalin. After that, the skin samples were dehydrated in ethanol, waxed in xylene, and embedded in paraffin wax to make blocks. For histopathological examinations, the blocks were sliced into sections and stained with hematoxylin and eosin. [16].

2.7.2 In vivo anti psoriatic activity

The antipsoriatic activity of the Apremilast loaded nanostructured lipid carriers gel was assessed by using the imiquimod induced plaque psoriatic model on swiss albino mice previously established in our lab. The animals were divided into five groups (six animals per group). Two days before experimentation, the mice's dorsal skin was depilated (Veet, Reckitt Benckiser India Pvt. Ltd.). Imiquimod (5%) cream was applied on the mice's shaved backs at a dose of 62.5 mg/d for six days in the morning to induce psoriasis in all the groups except normal control. The animals were treated with formulations in the evening from the third day until the sixth day. PASI (Psoriasis Area Severity Index)scoring was given to each mice to score the severity of the inflammation, skin thickening, and scaling, induced on the dorsal region of mice groups. Scoring was done on a scale of 0–4 scale depending on the severity of thickness, erythema and scaling, i.e. 0, none; 1, slight; 2, moderate; 3, marked; 4, Severe. The scoring was performed on the 0^{th}, 2^{nd}, 4^{th} and 6^{th} day of the study period. On the seventh day, all group mice were sacrificed by cervical dislocation under sodium pentobarbital anaesthesia. For histopathological evaluation, skin samples were obtained, washed in buffer solution, and fixed in a 10% v/v formalin solution [17].

3. RESULTS AND DISCUSSION

Apremilast loaded NLC gels were successfully prepared by using the melt emulsification-probe sonication method. The composition of the prepared NLC is given in Table 1. The formulated NLC gels were further characterised for *in vitro* and *in vivo* investigations.

Table 1. Composition of the apremilast loaded NLC

Components	% in formulation(w/w)
Apremilast	0.1
Sefsol 218	2.52
Glyceryl Monosterate (GMS)	1.68
Tween 80	2.4
Transcutol P	4.8
Deionised water	88

3.1 Characterisation of Apremilast Loaded NLC

3.1.1 Determination of particle size, polydispersity index and zeta potential

The particle size and polydispersity index of the prepared NLC shown in Table 2. The particles were found to be in the nano range, and the narrow PDI Indicates uniform size distribution. Zeta potential plays a vital role in determining NLCs stability as it suggests the attraction or repulsion between particles. The Zeta potential of the prepared formulations is displayed in Table 2. The negative charge is due to the presence of free fatty acids of the liquid and solid lipids used in the formulation of NLCs.

3.1.2 Entrapment efficiency

The entrapment efficiency of the optimised NLC was 89.26±1.22%, which suggests minimum loss of drug in the formulation. The high entrapment efficiency of the NLC can be attributed to the matrix structure of the NLC. The solid lipid matrix of the NLC encloses tiny oil droplets(liquid lipid) with significantly high drug solubility. These liquid lipids significantly affect the entrapment efficiency by producing imperfections in the highly ordered crystal matrix and allowing a substantial amount of the drug to be entrapped successfully [18].

Table 2. Particle size, PDI and Zeta potential of the prepared apremilast loaded NLC

Formulation	Particle size	Polydispersity Index	Zeta Potential
Apremilast Loaded NLC	170.32±2.14 nm	0.267±0.023,	-18.34±1.32 mV

3.1.3 Transmission electron microscopy (TEM)

TEM images of the apremilast loaded NLC showed uniform size distribution of the colloidal particles having nano range and coarsely spherical shape. As seen from Fig. 1 there are no signs of particle aggregation or particle adherence.

3.2 Characterisation of Apremilast Loaded NLC Gel

Gel bases containing different concentrations of carbopol 934 P(0.5, 1 and 1.5%) were prepared in distilled water to which apremilast loaded NLC was added. The prepared NLC gels were evaluated for their physicochemical properties such as spreadability, extrudability, consistency and pH drug content. The results are as shown in Table 3. All the prepared gels were visually appealing and firm. The gel was homogenous and free from coarse particles or aggregates, thus acceptable. Based on good flowability, excellent spreadability, Maximum drug content and extrudability and near to neutral pH, carbopol 934 P gel prepared with a concentration of 1%w/w was the most suitable for incorporating into the optimised NLC formulation and was selected for further studies.

3.3 Rheological Properties of Apremilast Loaded-NLC Gel Formulation

As the gels are meant to be topically applied to psoriatic skin, understanding the rheological flow pattern is very important. The optimised apremilast loaded NLC gel and Plain apremilast gels viscosities were determined at different shear rates (rpm). The NLC gel's viscosity decreased as the shear rate increased from 0.5 to 100 rpm, suggesting that the formulation was exhibiting shear-thinning behaviour. [19]. From the rheogram obtained Fig. 2 pseudoplastic flow behaviour of the NLC gel was evident.

3.4 *In-vitro* Drug Release Studies

For drug release studies, methanol: phosphate buffer saline pH 7.4 (2:8) was selected as the receptor media. The cumulative percentage drug release from the drug-loaded gel and NLC loaded gel was studied over 24 hours [20]. The ideal topical formulation should show controlled release for a more extended period to avoid frequent application for better patient compliance. From the *in vitro* release study, apremilast gel showed the faster release of the drug, i.e.100% in 12h, whereas apremilast loaded NLC gel demonstrated a sustained release profile(93.03% for 24h). This could be attributed to the apremilast's hydrophobic tendency, which hinders its rapid release from the system and retards the release confining more into the lipidic system. As seen from Fig. 3, Apremilast loaded NLC gel demonstrated an initial burst effect followed by the sustained release for an extended period. This may be attributed to unencapsulated drug diffusion for the first two hours, followed by NLC surface diffusion, and finally core diffusion. Further prolongation in drug release was observed, which can be attributed to the probable retention of the drug inside the microchannel structures of the carbopol gel system [21].

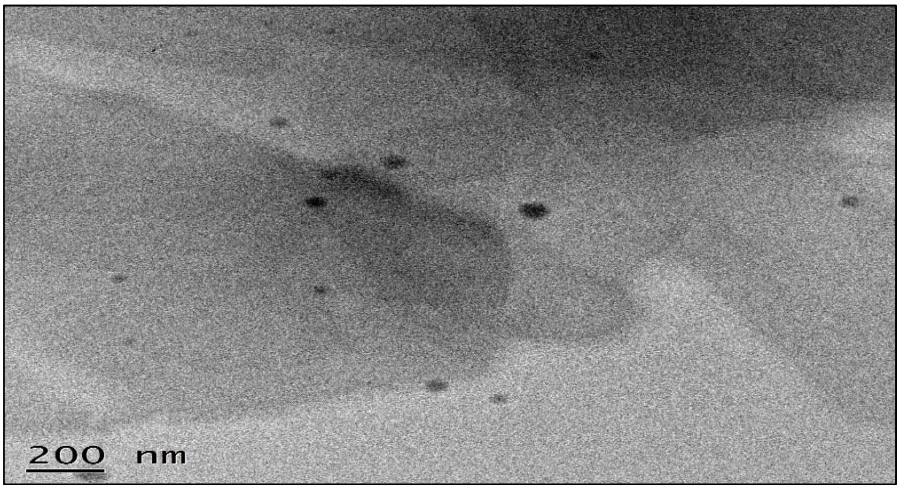

Fig. 1. TEM image of the optimised apremilast loaded NLC

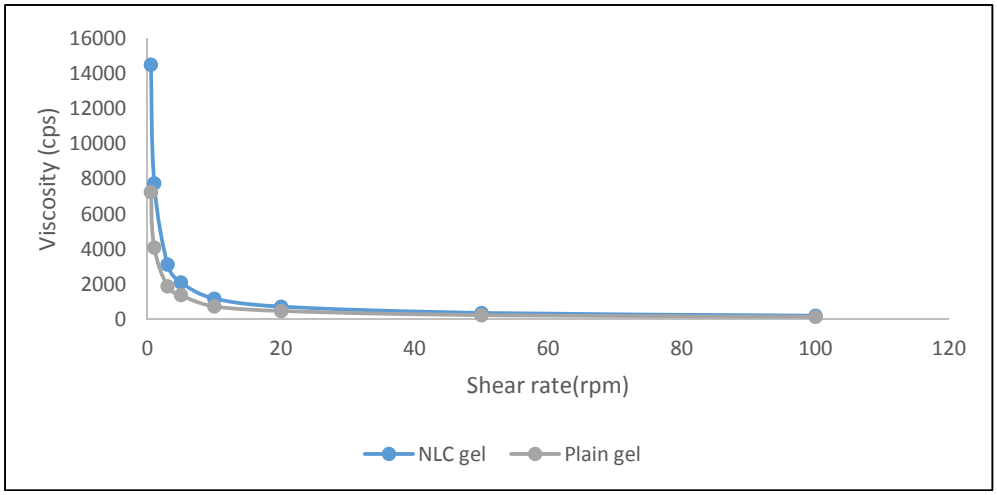

Fig. 2. Viscosities of the NLC gel and plain gel under different shear rates

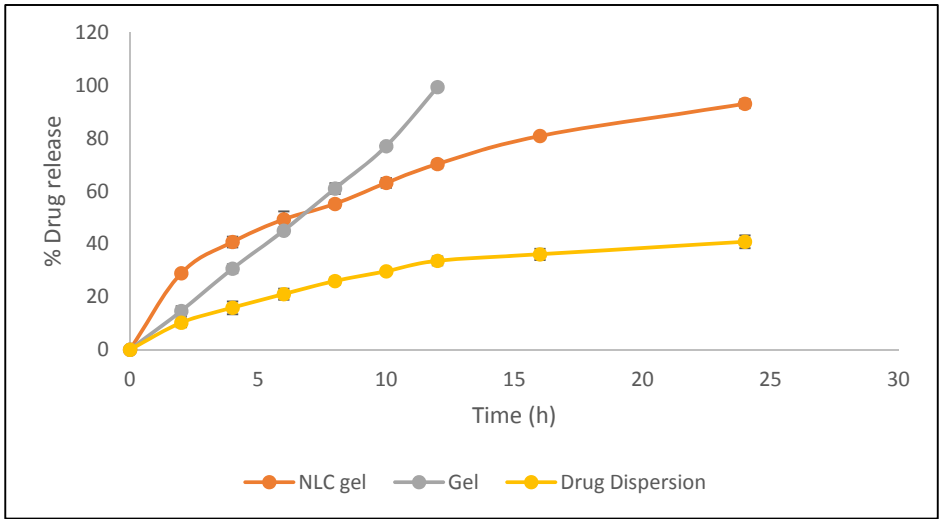

Fig. 3. *In vitro* drug release of apremilast from Apremilast dispersion, Apremilast Gel and NLCgel through cellophane membrane using Phosphate buffer 7.4:Methanol (8:2) as the diffusion medium

It was observed that *in vitro* release of APM gels followed zero-order release kinetics (r^2 =0.9848) whereas NLC (r^2=0.9946)and NLC gel (r^2=0.9966) followed Higuchi kinetics which explains that the drug incorporated into the solid-liquid lipid matrix was released in a sustained manner via the diffusion-controlled and swelling controlled mechanism.

3.5 *Ex vivo* Permeation Studies

The *ex vivo* permeation profiles Fig. 4 revealed that 202 µg/cm^2 apremilast permeated through the porcine skin from the NLC gel compared to 33 µg/cm^2 apremilast permeated from the aqueous dispersion of apremilast. This enhanced permeation showed by NLC can be attributed to the composition of the NLC. NLC consists of a mixture of lipids and surfactants, and the latter enhances the lipid bilayers disruption and denaturation of keratin in the stratum corneum, thus allowing drug-loaded NLCs to permeate passively across the skin via the intercellular route. Since Apremilast loaded NLC contains a higher amount of liquid than solid lipid (6 parts of Sefsol 218 and 4 parts of oil), multiply type NLC may be assumed. Apremilast could be dissolved in these oil compartments due to its higher

solubility in sefsol 218 than GMS. Also, the release of apremilast from these compartments with further erosion of lipid particles, overall high permeation of apremilast was noted. The higher dynamic viscosity of the NLC loaded gel and slow diffusion from the carbopol matrix of the NLC Gel seemed to retard the drug release further, similar to that obtained in the *in vitro* release study [22].

Additionally, apremilast loaded NLC gels and apremilast loaded gels had a flux value of 21.43 µg/cm^2/h, whereas the flux of apremilast loaded gel & aqueous apremilast dispersion was found to be 2.22 µg/cm^2/h &1.45 µg/cm^2/h. NLC gel displayed 13.75 fold increase in the permeation coefficient compared to the aqueous dispersion of apremilast. The lower flux and permeation coefficient of apremilast in the aqueous dispersion is due to poor solubilisation and partition of apremilast in the skin layers. The low permeation of apremilast from the normal gel might be due to the low solubilisation of the gel in the hydrophilic matrix and insufficient partitioning of the drug between the matrix and skin layers. It was observed that there was a difference in the order of drug release in the *in vitro* and *ex vivo* studies. This is mainly due to different characteristics of the cellophane membrane and the animal skin.

3.6 Skin Deposition Studies

As seen in Fig. 5, the highest drug deposition among different formulations found in NLC gel (74.05±0.25%). This is due to nanosized particles that enhance the solubilisation of apremilast and the sustained release of apremilast from the polymeric gel matrix. The NLCs tend to directly penetrate the junctions of corneocyte clusters and furrows owing to their small particle size, potentially favouring drug retention for many hours. Furthermore, the high affinity of NLC lipids for lipophilic skin layers and other tissues led to apremilast skin accumulation after topical application of NLC gel. The increase in the deposition of NLC gel can also be attributed to the improved adhesiveness of the hydrogel with the skin. Thus NLC based gels are suitable for topical administration in treating disease like psoriasis, where the local effect is desired [23].

3.7 Stability Studies

The stability of the NLC Gel was examined in terms of pH, Drug content, viscosity, spread-ability, signs of fungal growth and visual appearance (Phase separation). The results obtained from the studies Table 4 indicated no significant changes in pH, Drug content, Spreadability and Viscosity after subjecting the tested formulations to stability studies at 4°C & 25°C as per ICH guidelines. Whereas at 45°C, there were slight changes concerning spreadability and viscosity. These results indicate that the prepared NLC can be best stored at refrigerated or room temperature conditions.

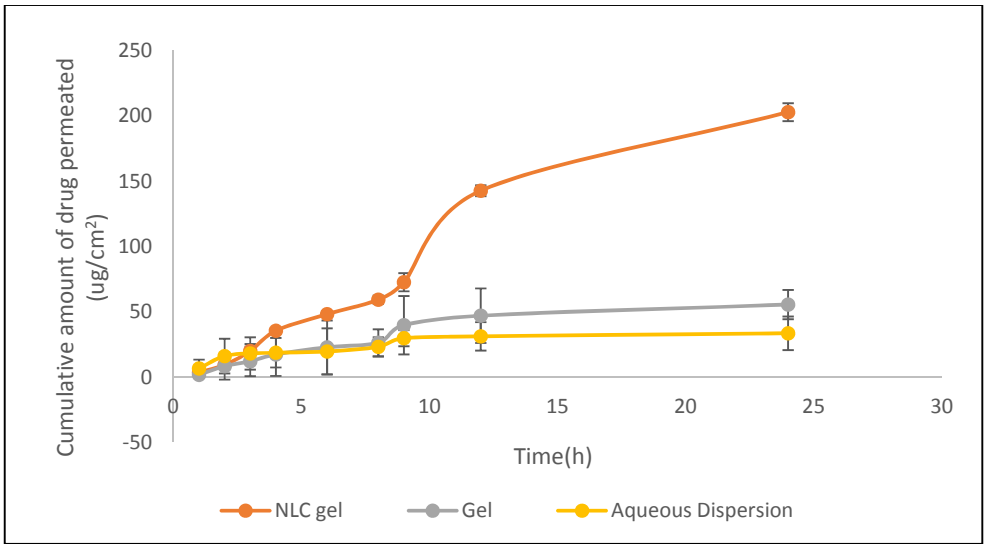

Fig. 4. *Ex vivo* Permeability studies of apremilast from different formulations through pork skin using Phosphate buffer 7.4: Methanol(8:2) as the diffusion medium

Table 3. Characterisation of the optimised APM loaded NLC gels

Concentration(%)	Spreadability	Extrudability	pH	Drug Content	Consistency
0.5	12.35 cm	87.21±0.32%	5.9±0.13	97.84±0.28%	Excellent flowability
1	11.23cm	90.36±0.45%	6.2±0.20	99.23±0.65%	Good flowability
1.5	8.11cm	88.47±0.72%	5.7±0.34	98.73±0.58%	Poor flowability

All data are represented as Mean±Standard deviation

Table 4. Stability studies of the optimised NLC gel under different temperature conditions for 90 days

S.No	Time	Storage Condition	pH	Drug content (%)	Viscosity at 5 rpm (cps)	Spreadability (cm)	Phase Separation
1	Day 0	4±2°C	6.2±0.20	99.78±0.23%	1068±44	11.6±0.34	No
		25±2°C	6.2±0.36	99.84±0.63%	1075±82	11.4±0.26	
		45±2°C	6.2±0.32	99.21±0.36%	1181±26	11.3±0.26	
2	Day 30	4±2°C	6.1±0.45	99.70±0.29%	1065±23	11.3±0.85	No
		25±2°C	6.2±1.02	99.24±0.17%	1057±39	11±0.21	
		45±2°C	6.1±0.12	99.19±0.31%	1272±45	9.8±0.33	
3	Day 60	4±2°C	6.2±0.31	99.57±0.39%	1051±40	11.1±0.15	No
		25±2°C	6.2±0.20	99.81±0.37%	1068±18	10.6±0.74	
		45±2°C	6.2±0.76	99.21±0.98%	1146±29	9.4±0.84	
4	Day 90	4±2°C	6.2±0.54	99.51±0.55%	1064±26	10.6±0.76	No
		25±2°C	6.2±0.15	99.54±0.69%	1036±21	10.0±0.39	
		45±2°C	6.2±0.87	99.14±0.17%	936±38	8.1±0.22	

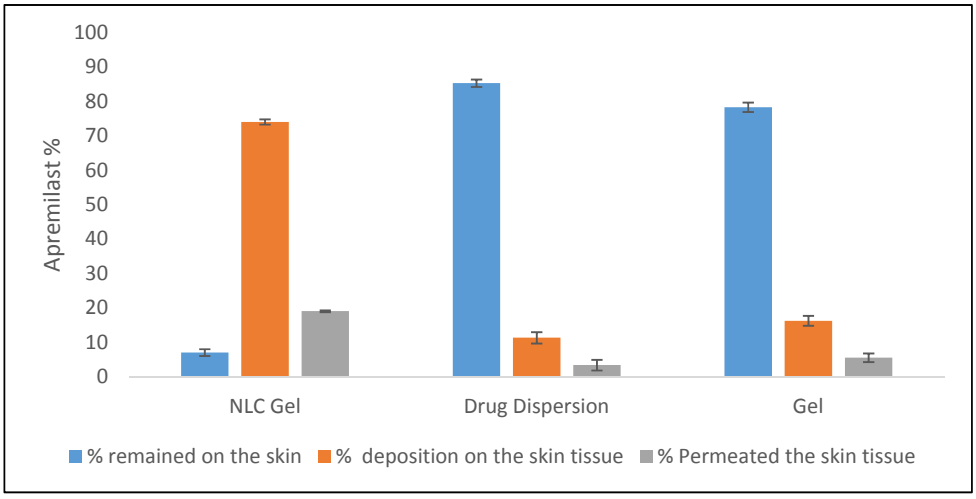

Fig. 5. *Ex vivo* Permeability studies of apremilast from different formulations through pork skin using Phosphate buffer 7.4:Methanol(8:2) as the diffusion medium

3.8 *In vivo* Studies

3.8.1 Skin irritation studies

There were no visual signs of localised allergic reactions on the rat skin 24h after the topical application of the formulations. The study observed that there were no erythema or edema or any other signs of skin irritation. H & E images showed of the control group showed normal skin architecture with well-defined epidermis and dermis. The groups treated with the gel formulations did not show any characteristic features of inflammation like fibrosis, edema or inflammatory cells in the dermis. Thus the developed formulation does not have any skin irritation potential and is tolerable.

3.8.2 Antipsoriatic activity studies

3.8.2.1 Psoriatic area severity index scoring

The result for the scoring of the severity of psoriasisl like skin inflammation is depicted in Fig. 7. Imiquimod treated groups revealed high PASI scores based on increased thickness, scaling and erythema due to daily dosing of imiquimod. Apremilast gel showed a significant decrease ($p < 0.01$) in PASI scores compared to that of the Imiquimod treated group. However, NLC Gel showed a higher reduction in PASI scores ($p < 0.0001$) at the same dose of the drug. The extent of improvement in PASI scores by the NLC gel was comparable to that of the standard treatment group(Betagel). This can be attributed to the sustained release and increased drug retention in skin achieved by the Apremilast loaded NLC gel for a longer duration.PASI scoring is considered as one of the direct methods employed in evaluating improvements in psoriasis condition. The decrease in PASI scores achieved by the Apremilast loaded NLC Gel indicates their efficacy in topical psoriasis therapy.

3.8.2.2 Histopathological examination

The effect of the prepared apremilast nanoformulations was examined using Imiquimod induces psoriasis in a mouse model. Daily application of imiquimod on mice dorsal skin induces dendritic cells and keratinocytes proliferation and thus increases cytokine production leading to human plaque psoriasis-like symptoms like erythema, thickening of the skin, scaling and epidermal alteration (acanthosis, parakeratosis). As seen in Fig. 8, the Healthy mice control group had normal tissue architecture characterised by regular epidermis up to 1 to 2 cell layers and dermis. Very Small amounts of keratin were seen in the stratum corneum, and no inflammatory cells were seen in the normal control group.

The occurrence of conditions similar to that of Human Plaque psoriasis was confirmed by the H & E examination in the disease control group. Compared to normal control, the epidermis showed increased layers of cells consisting of 5 to 9 layers (Epidermal hyperplasia) in the disease control group. The rete pegs were bulbous and rounded. There was slightly more keratin in the stratum corneum as compared with

the control group. Lymphocytic inflammatory infiltrates and many dilated capillaries were seen in the dermis. Some area of the dermis showed an abundance of neutrophils. The sections also displayed marked acanthosis and inflammation. In the case of the Apremilast gel group, Hyperkeratosis, epidermal hyperplasia and acanthosis were seen in the skin samples similar to the disease control group indicating that treatment was not effective. Animals treated with NLC showed a marked decrease in acanthosis, epidermal hyperplasia and inflammatory cells, and no inflammatory infiltrates, neutrophils and dilated capillaries were seen. This can be attributed to improved permeation and controlled release of the drug from the NLC. The Groups receiving Betagel® showed similar observations as that of the NLC treated group though some areas showed slightly more keratin in the stratum corneum than normal control [24].

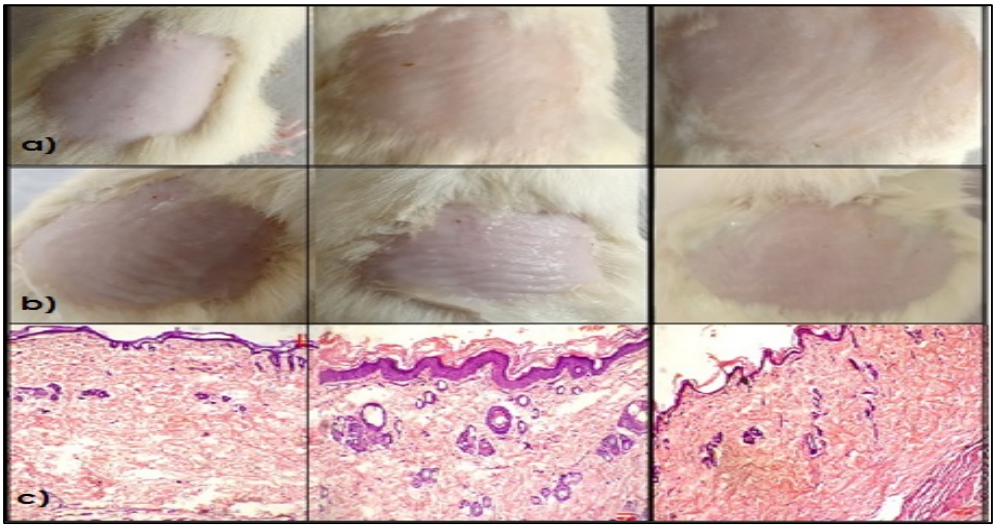

Fig. 6. Skin Irritation study of different apremilast formulation a) Before treatment b) 24 h after Application c) H&E images of dorsal skin 24h after treatment

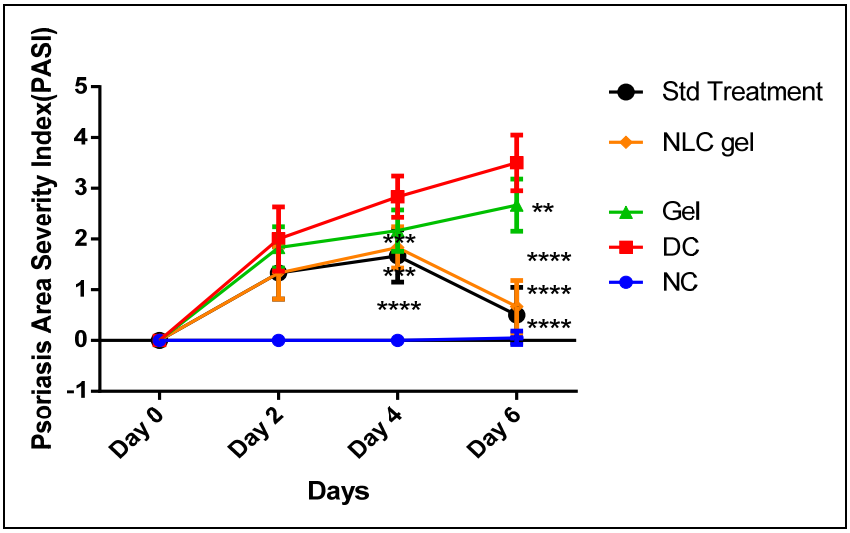

Fig. 7. PASI scores of different groups on day 0, day2, day 4 and day 6. Data are represented as Mean ±SD. One way ANOVA followed by Tukey's multiple comparisons test. *P<0.05 considered statistically significant. **p<0.01, ***p<0.001 and ****p,0.0001 is compared with the disease control group*

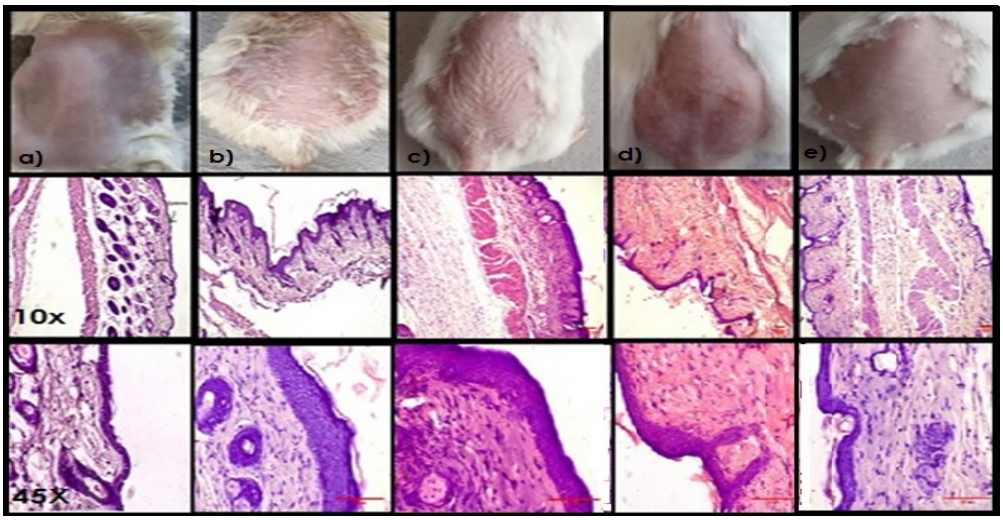

Fig. 8. *In vivo* antipsoriatic activity of different groups a) Normal Control b) Disease Control c) Apremilast Gel d) Standard treatment e) Apremilast loaded NLC gel

4. CONCLUSION

In the current study, apremilast was successfully incorporated into a nanostructured lipid carrier. Apremilast loaded nanostructured lipid carrier demonstrated superior permeability and skin retention compared to conventional dosage forms demonstrating its potential to overcome challenges posed by the stratum corneum. The antipsoriatic activity of the topical nanoformulations of Apremilast was successfully tested using the Imiquimod plaque psoriasis model and produced similar efficacy as compared to standard treatment. Thus the developed nanoformulation could be an effective alternative for oral apremilast therapy where there is less patient compliance due to GIT related and other prominent side effects.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved Institutional Animal ethics committee (IAEC), NGSMIPS, Mangalore (Approval no NGSMIPS/IAEC/NOV-2019/164) and studies were conducted according to the CPCSEA guidelines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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