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# ANTIPSORIATIC EFFECTS OF CLOBETASOL LOADED SOLID LIPID NANOPARTICLES ON IMIQUIMOD INDUCED PSORIASIS IN BALB/C MICE

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**Keywords:**

Solid lipid nanoparticles, Clobetasol, Imiquimod, Anti-psoriatic, ELISA

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**ABSTRACT:** The aim of the current investigation was to prepare and investigate the potential of Clobetasol (CP) loaded solid lipid nanoparticles based gel (SLN-gel) for the dermal delivery and to evaluate its anti-psoriatic efficacy by comparing with conventional ointment formulation. This study will provide an insight into the use of nanocarriers, esp. SLNs loaded with CP for the effective treatment of psoriasis. Clobetasol-loaded SLNs were formulated by the emulsification-homogenisation method and were characterized for particle size and polydispersity using DLS technique. The optimized SLNs were loaded into a gel and evaluated for drug release, spreadability. Anti-psoriatic efficacy of the SLNs gel was evaluated in imiquimod (IMQ) induced psoriatic plaque model by comparing with prepared conventional ointment formulation (0.05% w/w CP). Optimized SLNs attains a particle size of 133.3 ± 3.66 nm, polydispersity index (PDI) 0.179 ± 0.081, percentage entrapment efficiency of 78.1 ± 1.11% and Zeta potential of -36.2 ± 0.11 mV. The prepared SLNs gel was then compared with conventional ointment for drug release and efficacy. Topical application of Clobetasol-loaded SLNs gel on IMQ induced psoriatic plaque model reduced the symptoms of psoriasis, which was assessed by both Psoriasis area severity index (PASI) scoring and enzyme-linked immunosorbent assay. There was a significant reduction in disease severity and cytokines like Interleukins-17, 22, 23 and Tumor necrosis factor-a by the developed system in comparison to the negative control. To conclude, CP-loaded SLNs gel enhanced dermal delivery and was efficacious in management of psoriasis when compared to conventional ointment.

**INTRODUCTION:** Psoriasis is a chronic autoimmune skin disorder that was characterized by erythematous lesions with epidermal hyper-plasia (acanthosis) and extensive inflammation [1].

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Further, the corneocytes (dead keratinocytes) of the stratum corneum tend to retain their nuclei (parakeratosis) due to uncharacteristic terminal differentiation of the keratinocytes which manifests as red, scaly and raised patches of lesions on skin.

There is also dermal infiltration of inflammatory cells such as dendritic and T cells, which results in the activation of cytokines, including IL23, IL17, IL22, TNFα, and NFκB that promote the chronic inflammation [2]. There is burgeoning research evidence that strongly suggests that the IL1 family

of cytokines crucial roles in the pathogenesis of psoriasis. Particularly, the IL36 cytokine family (IL36 α (IL1F6), IL36 β (IL1F8) and IL36 γ (IL1F9) have been reported to augment the expressions of TH17 cytokines such as IL17A in a feedback mechanism that results in the activation of IL6, IL23 and TNFα [3, 4]. Noteworthily, diverse studies have shown the induction of IL36 cytokines in both human psoriatic skin and psoriatic-like mouse models. In this study, the novel IL36 α and its receptor, IL36R, have been demonstrated extensively to induce the expression of MAP-kinase and NFκB, which causes chronic inflammation associated with psoriasis [5].

Glucocorticoids (GCs) were highly effective drugs that were widely used in dermatology for the treatment of inflammatory diseases. However, severe adverse effects often accompany their long-term use [6, 7]. Clobetasol-17-propionate (CP) was considered as the most potent drug of choice among the currently available topical corticosteroids. It is currently approved for topical administration in different dosage forms such as cream, gel, solution, ointment, and foam [8]. Solid lipid nanoparticles (SLNs) were novel colloidal carrier systems composed of high melting point solid lipid core, coated by surfactants [9]. SLNs offer distinct advantages like negligible skin irritation, controlled release, and protection of substances as it uses physiologically tolerated, non-irritative, and non-toxic lipids [10]. SLNs were found to be safe for administration to inflamed and damaged skin. Moreover, the small size of the lipid particles ensures its close association with stratum corneum increasing the quantity of the drug penetrating into the skin.

The solid lipid matrix of SLN also permits the sustained release of drugs from the nanocarrier. SLNs reduce trans epidermal water loss and favors drug penetration through the stratum corneum and enhances dermal localization (Prow *et al.,* 2011). Transdermal drug delivery is a viable approach for consideration because of its non-invasive feature, and it circumvents the hepatic-portal circulation, which makes it an ideal delivery method for drugs that are highly susceptible to the first-pass metabolism. However, the stratum corneum is composed of lipids (ceramides, fatty acids, and cholesterol) that form a highly impermeable bilayered matrix, which makes it impossible to permeate by macromolecules and hydrophilic drugs. The aim of this study was to develop a nanogel composed of CP-loaded SLNs and evaluate its potential in the psoriatic animal model. Design of Experiments (DoE) was used in the optimization of CP-loaded SLNs. The nanogel was prepared by dispersing the CP-loaded SLNs in Carbopol 934 gel base, and its efficacy was evaluated in imiquimod-induced psoriasis in BALB/c mice based on Psoriatic Area and Severity Index (PASI) score and histopathology.

## MATERIALS AND METHODS:

**Materials:** Clobetasol-17-propionate was purchased from Yarrow Chemicals (Mumbai, India). Compritol 888 ATO was a kind gift from Gattefossé (France). Tween 80 as a surfactant, paraffin wax and Carbopol 934 were purchased from Lobachemie, Mumbai, India. Imiquimod cream (5% w/w IMQ cream) was obtained from Glenmark Pharmaceuticals (Mumbai, India). Betnovate® (betamethasone valerate ointment, 0.1% w/w) (BMV) was procured from Glaxo Smith Kline Pharmaceutical Limited (Mumbai, India). Formaldehyde was obtained from HiMedia laboratories (Thane, India). Butylhydroxytoluene (BHT) and Stearyl alcohol from Loba Chemie, Mumbai. Quantikine mouse interleukin-17 (IL-17), interleukin-22 (IL-22), interleukin-23 (IL-23), and tumor necrosis factor- α (TNF- α) ELISA kits were purchased from R&D Systems, Inc. (Minneapolis, USA). Methanol and acetone were purchased from Sigma–Aldrich (India). Distilled water was obtained from the in-house distillation system.

### Method:

**Preparation of CP-loaded SLNs:** SLNs were formulated by the emulsification-homogenisation method. The formulation procedure was divided into two portions one portion contains drug and lipid, while the second portion contains an aqueous solution of surfactant and stabilizer. The lipid mixture was melted 5 ºC above the melting point of lipid and drug was dispersed into it. The aqueous phase was heated to the same temperature as like lipid phase. When both portions attain an equilibrium, the aqueous phase was incorporated into the lipid phase and emulsified using a high-speed homogenizer (IKA® T-10 basic Ultra-Turrax®, Germany) at 10,000 rpm for 15 min.

The obtained pre-emulsion was ultra-sonicated using a probe Sonicator (Vibracell TM 700 W; Sonics, USA) at 80% amplitude for the duration of about 5 min. Cycles were repeated alternatively until it attains uniform particle size. The resulting nanoemulsion was cooled down in an ice bath to produce nanoparticle dispersion. Different formulations were prepared by varying the critical process variables [11].

**Experimental Design:** In the present work, 33 factorial designs were chosen for the optimization of CP SLN using Design-Expert software (Trial Version 11, Stat Ease Inc., and USA). 3 factors were chosen, and the responses were measured at 3 levels. Totally 30 runs were designed randomly. The design consisted of replicated center points and a set of points lying at the midpoints of each edge of the multidimensional cube. This defines the region of interest used to evaluate the main effects, interaction effects, and quadratic effects of the formulation ingredients and to optimize the formulation. The quadratic model generated by design was [18, 19, 20, 21].

$$Y = A0+A1X1+ A2X2+ A3X3+ A4X1 X2+ A5X2 X3+ A6X1 X2+ A7X12+ A8X22+ A9X32$$

In which Y is the measured response of the dependent variables associated with each factor-level combination, A0–A9 are the regression coefficients of the respective variables and their interaction terms computed from the observed experimental values of the measured response. X1–X3 are the codes for independent variables.

Independent variables selected were concentration of drug: lipid (X1), the concentration of surfactant (X2), and the high-speed homogenization (X3). Independent variables were represented by level -1, 0, and +1 corresponding to the low, middle, and high values, respectively. The measured responses Y1 = particle size (PS) and Y2 = Polydispersity index and Y3 = entrapment efficiency (%EE) with constraints were described in **Table 1**.

**TABLE 1: SELECTION OF INDEPENDENT VARIABLES AND THEIR LEVELS CHOSEN FOR EXECUTION OF $3^3$ FACTORIAL DESIGNS**

| Types of variable | Levels | | |
|---|---|---|---|
| ***Independent variable*** | **Low(-1)** | **Medium(0)** | **High(+1)** |
| $X_1$ = Drug: lipid Ratio (D:L) (%w/w) | 1:3 | 1:4 | 1:5 |
| $X_2$ = Surfactant concentration (S) (%w/w) | 2 | 3 | 4 |
| X3=HSH Time (HT) (min) | 5 | 10 | 15 |
| ***Dependent variable*** | | | |
| Y1 = Particle Size (nm) | | | |
| Y2 = Poly Dispersibility Index | | | |
| Y3 = %Entrapment Efficiency | | | |

**Data Optimization and Model Validation:** The effect of each independent CPP on CQA was analyzed for the establishment of design space with the target of ensuring the desired product quality. Hence, $3^3$ factorial designs were applied for the establishment of design space to investigate the responses of process parameters on quality attributes of CP-SLNs.

The optimization was done on the basis of attaining lower particle size, PDI, and higher % EE using overlay plot (graphical) and desirability (numerical) criteria. In order to establish the reliability of the developed model, the check-point analysis was performed by taking two validation batches *viz.*, V1, and V2, whereby the magnitude of error between observed and predicted values was evaluated [17].

**Characterization of CP SLNs:** Particle size, Polydispersity index, and Zeta potential measurement Particle size, polydispersity index (PDI), and zeta potential of the SLNs were measured by dynamic light scattering technique using Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). CP SLNs colloidal dispersions were diluted 10 times with double distilled water.

Particle size and PDI measurements were performed by taking 1 ml of the diluted formulation into polystyrene cuvettes and disposable folded capillary cell for zeta potential at 25 ºC, to ensure that the light scattering intensity was within the instruments sensitivity range. Each sample was analyzed in triplicate, and the results were shown as mean ± standard deviation.

**% Entrapment Efficiency:** The entrapment efficiency (%EE) of formulated CP SLN was determined by measuring the concentration of unentrapped drugs in the lipidic dispersion by using the centrifugation method. The SLNs Samples were subjected to centrifugation at 10000 rpm for 15 min (Remi Centrifuge Pvt. Ltd., India) and the amount of Clobetasol -17-propionate in the supernatant was analyzed after suitable dilution with methanol using UV spectroscopy at 239 nm. %EE was calculated by the following equation.

% EE = (Total amount of CP-Amount of free CP)/(Total amount of CP) × 100

**Transmission Electron Microscopy:** The morphological characters of CP loaded SLNs were observed with transmission electron microscopy (TEM; Philips, Tecnai 20, Holland). A drop of the diluted sample was placed on the surface of the carbon coated copper grid and stained with a drop of 1% (w/w) aqueous solution of phosphotungstic acid (negative stain) for 30 s. Excessive staining solution was washed out by filter paper and a thin aqueous film was left on the surface. After staining, samples were dried at room temperature for 10 min to perform out investigation [12].

**Formulation of CP-SLN Topical Gel:** Direct application of SLNs is not suitable as they cannot reside on the skin for longer duration, which is crucial for skin disorders like psoriasis. In such disorders, the topical ointment/gel-based delivery is essential. CP-SLNs dispersion was converted into gel carrier system using Carbopol (CP 934) as a gelling agent. 1% w/w Carbopol 934 was dissolved in distilled water, stirred for 15 min at 1000 rpm. Subsequently, the calculated amount of freshly prepared CP-SLNs dispersion was added and mixed for 10 min, then neutralized by drops of triethanolamine until pH 5.5. Prepared gels were further allowed to stand overnight to remove entrapped air.

**Characterization of Clobetasol-loaded SLNs gel:**
**Spreadability:** Spreadability is an essential property of any topical formulation which assists in the uniform application of actives to the affected area. The spreadability of the Clobetasol-loaded SLNs gel was evaluated by parallel plate method as reported. The study was performed by taking 0.5 g of SLNs gel and placing it on the glass plate at center, another glass plate was concentrically placed above it. The gel was pressed with known weights starting from 5 to 200 g at interval of 30 s between each weight.

Spreadability of the formulation was measured by measuring the diameter of the formulation in two perpendicular directions [13]. The spreadability factor was calculated using Equation (2).

$$S_f = A/W$$

where $S_f$ ($cm^2/g$) is the spreadability factor, defined as the ratio of the maximum spread area (A) in $cm^2$ after the addition of the sequence of weights and the total weight added (W) in gm. Spreadability profile was obtained by plotting the spreading area on y-axis and weights added on x-axis.

**Preparation of Ointment:** Clobetasol ointment was prepared by simple melt dispersion. Briefly, paraffin wax and stearyl alcohol were melted under heating to form a uniform melt. To this BHT was added and mixed for some time. Finally, the drug was added and mixed thoroughly to aid uniform drug distribution. The formulation was then stirred at room temperature until it is cooled14. The obtained formulation was characterized for spreadability and drug release.

***In-vitro* Drug Release Studies:** *In-vitro* release studies of CP loaded SLNs gel, ointment and solution were carried out using modified Franz diffusion cell with a receptor volume capacity of 12.5 ml using cellulose acetate membrane (MWCO- 12,000–14,000 Da, pore size- 2.4 nm, HIMEDIA, Mumbai, India) and PBS of pH 7.4 as a dialyzing medium. The membrane was soaked in double-distilled water for about 12 h, prior to mounting the membrane (diffusion area 1.95 $cm^2$) in the Franz diffusion cell. Pre-treated membrane was placed on the modified Franz diffusion cell filled with phosphate buffer (pH7.4) in the receptor compartment. The whole assembly was placed on the magnetic stirrer at 300 rpm, and temperature was maintained at 32.0 ± 0.5 °C. The samples (CP loaded SLNs gel, ointment and solution) equivalent to 200 µl gel were kept over the membrane in donor compartment and stirred. 2 ml Samples were withdrawn from the receptor compartment at predetermined time intervals (0, 6, 8, 10, 12 and 24 h) and the same volume was refilled with diffusion medium. The samples were filtered and analyzed

using a UV spectrophotometer at 239 nm after appropriate dilutions. Percent drug release was calculated, and the graph was plotted between percent drug release against time. Release studies were performed in triplicate for each formulation.

**Anti-Psoriatic Efficacy Studies in BALB/C Mice:**

***In-vivo* Imiquimod-induced Psoriatic Plaque Model:** IMQ induced psoriatic plaque model was developed in male BALB/c mice of 8 to 11 weeks age and weight of about 15 - 20 g [15]. Animals were housed under controlled environmental conditions (temperature 23 ± 1 °C, humidity 55 ± 5% and 12 h light/dark cycles) with food and water *ad libitum*. The animals were divided into five groups and notified *i.e.*, normal (Sham), negative control, positive control, Ointment, SLN gel **Table 2**. All the animals were depilated on their dorsal side the day before starting study. Sham group was left untreated and for remaining groups, 62.5 mg of commercially available IMQ cream (5% w/w) was applied on the depilated skin and right ear for 6 consecutive days. The animals (treatment groups) were treated with respective formulations from third day of the study and continued till sixth day. Positive control group received treatment with 100 mg/d of betamethasone valerate (BMV) ointment (0.01% w/w) while other two groups received treatment with 50 mg/d of 0.05% w/w of Clobetasol loaded SLNs gel and 50 mg/d of 0.05% w/w of Clobetasol ointment. Change in body weight of the animals was noted on 0, second, fourth, and sixth day and Psoriasis area severity index (PASI) scores were assessed for all groups of animals during the treatment. All group animals were sacrificed on the seventh day by cervical dislocation method; the thickness of both ears was measured by screw gauge, and spleen weights were recorded for all the animals. Skin samples were collected, washed with buffer solution and were fixed in 10% v/v formalin solution for histo-pathological examination. Portion of skin sample was collected and stored at 80 °C for performing ELISA (to determine cytokines level).

**TABLE 2: TREATMENT PROTOCOL FOLLOWED FOR EFFICACY STUDIES IN IMIQUIMOD INDUCED PSORIASIS MODEL**

| | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| **Group** | **0** | **1** | **2** | **3** | **4** | **5** | **6** |
| Sham | -- | -- | -- | -- | -- | -- | -- |
| | -- | -- | -- | -- | -- | -- | -- |
| | -- | -- | -- | -- | -- | -- | -- |
| Negative control | -- | IMQ[a] | IMQ | IMQ | IMQ | IMQ | IMQ |
| | -- | -- | -- | -- | -- | -- | -- |
| | -- | -- | -- | -- | -- | -- | -- |
| Positive control | -- | IMQ[a] | IMQ | IMQ BMV[b] | IMQ BMV | IMQ BMV | IMQ BMV |
| | -- | -- | -- | -- | -- | -- | -- |
| | -- | -- | -- | -- | -- | -- | -- |
| CP ointment | -- | IMQ[a] | IMQ | IMQ CP ointment | IMQ CP ointment | IMQ CP ointment | IMQ CP ointment |
| | -- | -- | -- | -- | -- | -- | -- |
| | -- | -- | -- | -- | -- | -- | -- |
| CP-SLNs | -- | IMQ[a] | IMQ | IMQ CP-SLNs | IMQ CP-SLNs | IMQ CP-SLNs | IMQ CP-SLNs |
| | -- | -- | -- | -- | -- | -- | -- |
| | -- | -- | -- | -- | -- | -- | -- |

a Imiquimod. b Betamethasone valerate

**PASI Scoring:** The clinical scoring method for the Psoriasis Area and Severity Index (PASI) was used to score the severity of the inflammation-induced on the dorsal regions of the mice daily based on the extent of scaling and erythema, epidermal thickening. The scoring was recorded on the 0, 2nd, 4th, and 6th day of treatment. The scores were assigned from 0 to 4 scale based on the severity of erythema (redness), scaling, and thickening of skin *i.e.*, 0 (none), 1 (slight), 2 (moderate), 3 (severe), and 4 (very severe). These scores were plotted time points on x axis and PASI scores on y-axis.

**Histology:** Skin samples collected at the end of the study, which was fixed in formalin, were given for histopathology examination. This study was

performed to determine the pathological changes like acanthosis, inflammatory infiltrates, hyper-keratosis and parakeratosis that occurred during the treatment period. Briefly, formalin-fixed, paraffin-embedded skin sections were deparaffinized, rehydrated, and stained with hematoxylin and counterstained with eosin. The stained skin sections were mounted and viewed with the Olympus BX40 light microscope equipped with a computer-controlled digital camera (DP71, Olympus Center Valley, PA).

**Enzyme-linked Immunosorbent Assay (ELISA):** ELISA was performed to know the cytokines like IL-17, IL-22, IL-23, and TNF-levels in the treated groups. The skin samples collected at the end of the psoriatic efficacy study were stored in -80 °C until used for the ELISA. The collected tissue samples were homogenized in a double amount of an extraction buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1% TritonTM X-100) with the help of tissue homogenizer (Remi Electrokinetic, Ltd., Mumbai, India) at 4000 rpm for 5 min. The homogenates samples were centrifuged at 10,000 rpm for 15 min at 4 °C, and the supernatant was collected and stored in -80 °C until analyzed. The samples were analyzed for levels of cytokines using quantitative Mouse ELISA kits (R & D Systems, Minneapolis, MN) as per the manufacturer's protocol.

**Statistical Analysis:** Statistical analysis was performed using the trial version of GraphPad Prism version 6.01 software (GraphPad Software, San Diego, CA). The level of statistical significance was determined by analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons. The mean differences were considered significant in all experiments valued at *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

**TABLE 3: LAYOUT OF $3^3$ FULL FACTORIAL DESIGNS SHOWING THE VALUES OF DEPENDENT VARIABLES OF 30 POSSIBLE CP SLNS FORMULATIONS**

| Run | X1 | X2 | X3 | Y1 | Y2 | Y3 |
|---|---|---|---|---|---|---|
| 1 | 5 | 3 | 5 | 245.2 ± 3.21 | 0.328 ± 0.061 | 70.9 ± 3.11 |
| 2 | 4 | 3 | 15 | 173.4 ± 2.23 | 0.352 ± 0.025 | 52.6 ± 2.63 |
| 3 | 3 | 4 | 15 | 251.7 ± 4.21 | 0.432 ± 0.039 | 39.8 ± 3.12 |
| 4 | 4 | 2 | 10 | 168.3 ± 1.11 | 0.222 ± 0.042 | 68.3 ± 2.58 |
| 5 | 3 | 3 | 5 | 221.9 ± 3.55 | 0.307 ± 0.064 | 55.7 ± 2.64 |
| 6 | 4 | 3 | 10 | 140.2 ± 3.71 | 0.212 ± 0.023 | 75.3 ± 3.13 |
| 7 | 5 | 3 | 10 | 200.9 ± 5.63 | 0.284 ± 0.035 | 78.1 ± 3.45 |
| 8 | 3 | 4 | 10 | 212.2 ± 3.08 | 0.309 ± 0.045 | 52.7 ± 3.63 |
| 9 | 4 | 3 | 10 | 135.3 ± 2.05 | 0.189 ± 0.083 | 77.1 ± 3.86 |
| 10 | 4 | 4 | 10 | 225.1 ± 1.32 | 0.321 ± 0.057 | 55.8 ± 2.45 |
| 11 | 5 | 2 | 15 | 246.7 ± 2.56 | 0.445 ± 0.062 | 58.8 ± 3.12 |
| 12 | 4 | 2 | 15 | 183.9 ± 2.05 | 0.404 ± 0.039 | 48.2 ± 2.69 |
| 13 | 5 | 2 | 10 | 230.6 ± 4.12 | 0.262 ± 0.056 | 72.2 ± 3.56 |
| 14 | 3 | 4 | 5 | 280.2 ± 3.98 | 0.373 ± 0.066 | 47.2 ± 4.12 |
| 15 | 3 | 2 | 10 | 189.2 ± 4.21 | 0.247 ± 0.089 | 59.71 ± 3.23 |
| 16 | 4 | 2 | 5 | 241.1 ± 3.26 | 0.295 ± 0.056 | 57.5 ± 2.36 |
| 17 | 5 | 4 | 5 | 306.8 ± 4.25 | 0.395 ± 0.063 | 60.7 ± 3.65 |
| 18 | 3 | 3 | 10 | 175.4 ± 2.35 | 0.251 ± 0.052 | 64.1 ± 4.10 |
| 19 | 4 | 4 | 15 | 262.2 ± 3.97 | 0.457 ± 0.061 | 46.3 ± 3.25 |
| 20 | 4 | 3 | 10 | 140.5 ± 2.18 | 0.235 ± 0.073 | 74.5 ± 2.53 |
| 21 | 4 | 3 | 5 | 202.6 ± 4.91 | 0.257 ± 0.069 | 65.1 ± 3.22 |
| 22 | 4 | 4 | 5 | 286.3 ± 4.72 | 0.38 ± 0.056 | 51.2 ± 4.12 |
| 23 | 4 | 3 | 10 | 133.3 ± 3.66 | 0.179 ± 0.081 | 78.1 ± 1.11 |
| 24 | 3 | 3 | 15 | 190.2 ± 2.15 | 0.377 ± 0.071 | 44.3 ± 4.23 |
| 25 | 5 | 2 | 5 | 288.7 ± 4.13 | 0.326 ± 0.082 | 63.2 ± 3.22 |
| 26 | 5 | 4 | 10 | 240.6 ± 3.23 | 0.300 ± 0.071 | 66.6 ± 2.12 |
| 27 | 5 | 4 | 15 | 252.0 ± 2.06 | 0.440 ± 0.065 | 54.5 ± 2.81 |
| 28 | 5 | 3 | 15 | 224.0 ± 3.22 | 0.401 ± 0.059 | 60.1 ± 3.24 |
| 29 | 3 | 2 | 15 | 190.1 ± 4.89 | 0.425 ± 0.063 | 42.2 ± 3.88 |
| 30 | 3 | 2 | 5 | 261.5 ± 5.01 | 0.310 ± 0.077 | 53.1 ± 3.26 |

* All values are expressed as mean ± SD, n = 3

## RESULTS AND DISCUSSION:

**Statistical Analysis of Formulations:** Results obtained from all the formulations were analyzed using Design-Expert® Version 11.0 software **Table 3**. Response surface plots were generated using the software and new formulations with

desired responses were obtained by numerical optimization technique. The responses obtained were used to study the effect of independent variables. A quadratic model was suggested for particle size, PDI, % entrapment efficiency. The list of solutions suggested by the software was sorted in the descending order of the desirability, and the solution which fulfills the required criteria was reported. The factors which had a significant effect on the responses were identified using Analysis of variance (ANOVA). To evaluate the chosen experimental design, the optimized formulation was prepared as per the solutions obtained and subjected to evaluation parameters. The resulting experimental values were compared with predicted values.

**Effect on Particle Size:** The particle size varied for all the formulations were ranging between 133.3 nm (formulation 23) to 306.8 nm (formulation 17) at various factor-level combinations. The independent factors (variables) affecting the particle size were concentration of lipid, concentration of surfactant, and hominization speed on particle size was studied, and the responses obtained are given in **Table 3**. The suggested quadratic model with an F-value of 39.03 implies that the model is significant. The model P-value was < 0.0001 indicated that the model terms are significant. P values for, lipid to drug ratio was 0.0002, surfactant concentration was <0.0001 and that for hominization time was < 0.0001. These results indicated there was no significant effect in particle size with the change in independent variables. The regression coefficient value $R^2$ was 0.9461, adjusted $R^2$ was 0.9219 and predicted $R^2$ was 0.8652 indicating minimum variations in the experimental model. The adequate precision ratio, measuring the signal to noise ratio was 20.407 indicating an adequate signal. A ratio greater than 4 is desirable, and this model can be used to navigate the design space. The polynomial equation in terms of coded factors is given below.

Particle size: 145.83 + 14.62A + 17.61B-20.01C- 5.82AB + 1.13AC + 5.27BC + 27.69A2 + 45.91B2 + 44.46C2

The individual factor A, lipid to drug ratio had a positive effect on particle size as showed by the positive coefficient estimate value. The particle size increased with the ratio of lipid to the drug, whereas surfactant concentration showed positive effect and homogenization time showed a negative effect. The combined effect of lipid to drug ratio and surfactant concentration was negative, whereas the homogenization time with lipid to drug ratio and surfactant concentration showed a positive effect. An increase in the particle size was observed with a concomitant increase in the proportion of lipid and reduction in the surfactant concentration. Larger particle size with 5:1 lipid to drug ratio could be attributed to the increased viscosity of the system due to higher lipid concentration. Another possible explanation to this may be a film of loosely arranged surfactant molecules at the interface of the two layers in Nano dispersion due to the lower concentration of surfactant in comparison to the lipid. On the other hand, a lower lipid with higher surfactant resulted in fine Nano dispersion with smaller sized particles. The size of SLN decreased with an increase in homogenization time. This could be due to the sheer energy induced by homogenization. The effect of lipid: drug ratio, sonication time, and surfactant concentration on the particle size is presented in the form of response surface plots in **Fig. 1A**.

**Effect on PDI:** The suggested quadratic model with F-value of 44.89 implies that the model is significant. The model P value was < 0.0001 indicated that the model terms are significant. P values for, lipid to drug ratio was 0.1083, surfactant concentration was <0.0001 and that for hominization time was < 0.0001 the results indicated there was no significant difference in PDI with the change in hominization time. The regression coefficient value $R^2$ was 0.9528, adjusted $R^2$ was 0.9316 and predicted $R^2$ was 0.9059 indicating minimum variations in the experimental model. The adequate precision ratio, measuring the signal to noise ratio was 20.160 indicating an adequate signal. A ratio greater than 4 is desirable and this model can be used to navigate the design space. The polynomial equation in terms of coded factors is given below.

PDI: 0.2131+0.008A+0.026B+0.042C-0.002AB-0.0006AC-0.013BC+0.026A2+0.048B2+0.1089C2

As per the polynomial equation, the effect of lipid to drug ratio, surfactant concentration and homogenization time was shown a positive effect.

All the interaction effects showed negative coefficient estimate value indicating the indirect relationship with the PDI. It was observed that when 4% w/w tween 80 was used, PDI was increased **Table 3**. This was because; during homogenization process alkyl chain of the surfactant molecule covers the surface of lipid particle via hydrophobic interaction to form a stable lipid matrix. Once this stable matrix is formed, excess surfactant may lead to accumulation of surfactant particles on the surface of stable lipid matrix causing increase in PDI as was observed in the form of response surface plots in **Fig. 1B**.

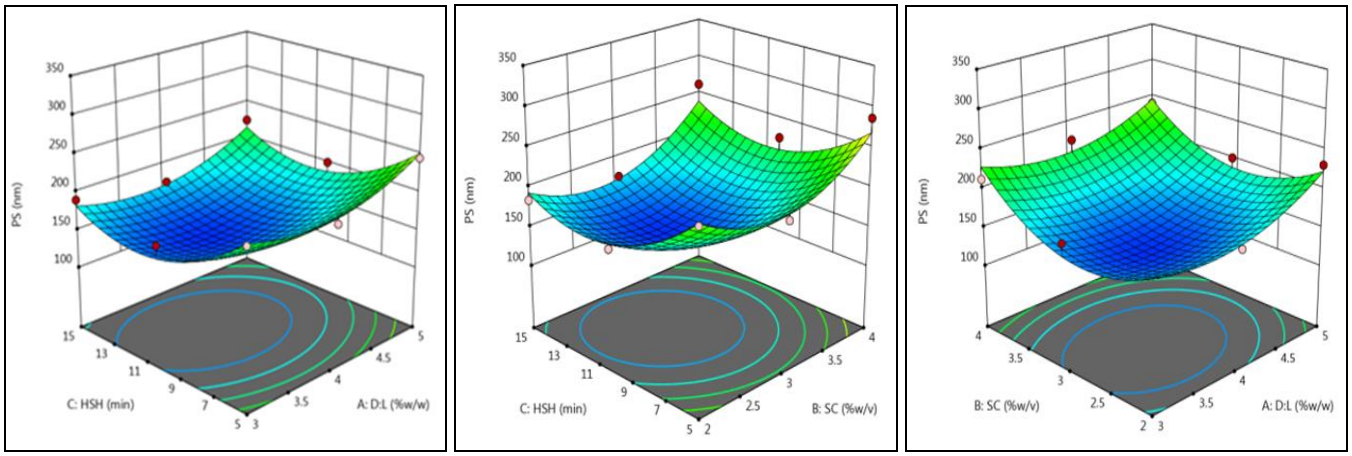


**FIG. 1A: 3D RESPONSE SURFACE PLOT FOR EFFECT OF LIPID: DRUG RATIO, HOMOGENISATION TIME AND SURFACTANT CONCENTRATION ON PARTICLE SIZE**

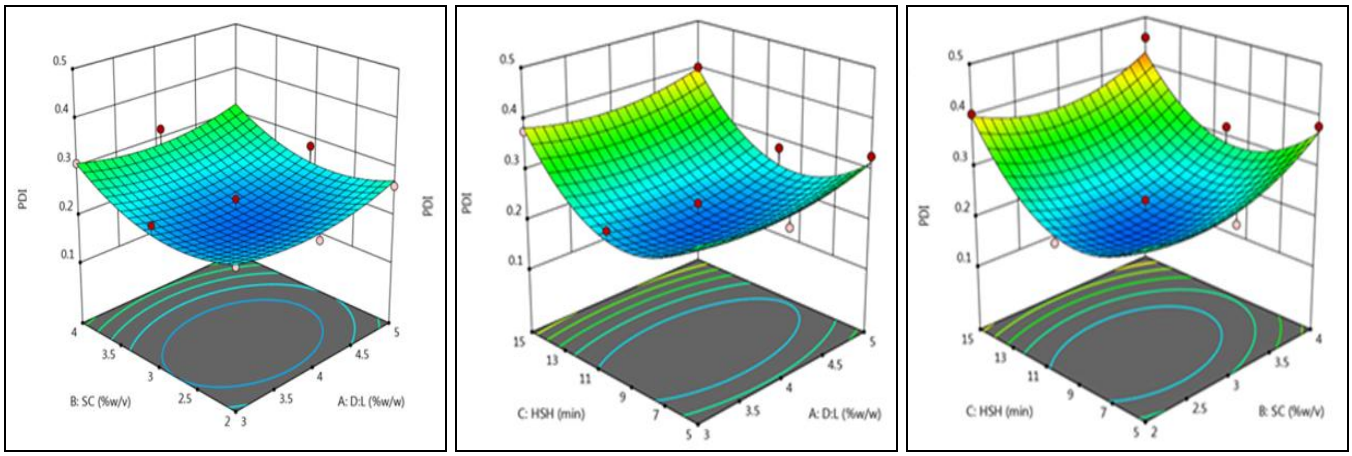


**FIG. 1B: 3D RESPONSE SURFACE PLOT FOR EFFECT OF LIPID: DRUG RATIO, HOMOGENISATION TIME AND SURFACTANT CONCENTRATION ON POLYDISPERSITY INDEX**

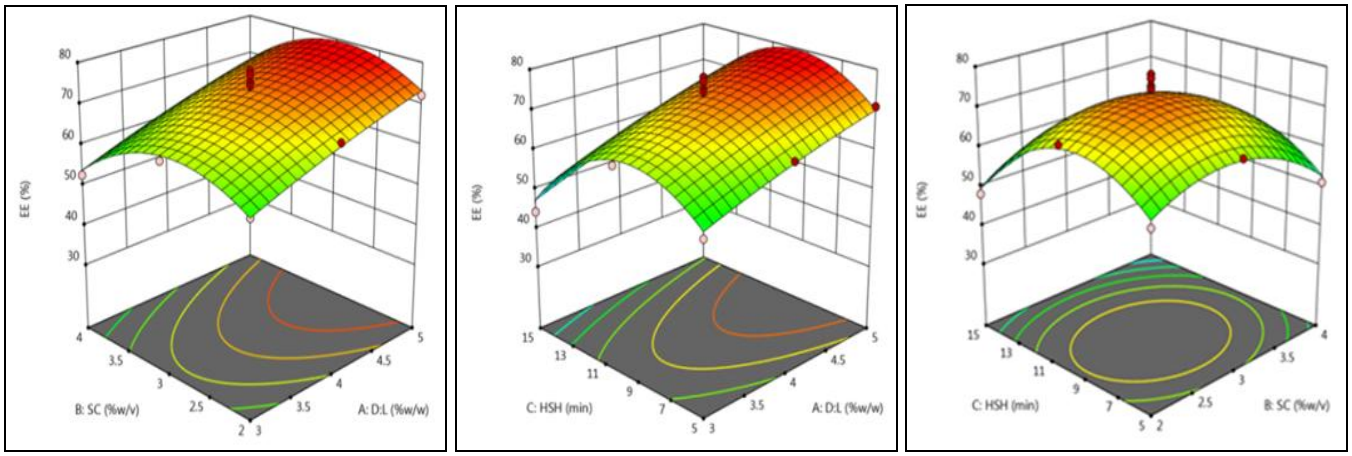


**FIG. 1C: 3D RESPONSE SURFACE PLOT FOR EFFECT OF LIPID: DRUG RATIO, HOMOGENISATION TIME AND SURFACTANT CONCENTRATION ON ENTRAPMENT EFFICIENCY**

**Effect on % EE:** The suggested quadratic model with an F-value of 45.47 implies that the model is significant. The model P-value was < 0.0001 indicated that the model terms are significant. P values for, lipid to drug ratio was 0.0001, surfactant concentration was 0.0008 and that for hominization time was < 0.0001 the results indicated there was no significant difference in %EE with the change in hominization time. The regression coefficient value $R^2$ was 0.9534, adjusted $R^2$ was 0.9324, and predicted $R^2$ was 0.8971 indicating minimum variations in the experimental model. The adequate precision ratio, measuring the signal to noise ratio was 25.75 indicating an adequate signal. A ratio greater than 4 is desirable, and this model can be used to navigate the design space. The polynomial equation in terms of coded factors, is given below.

Entrapment efficiency (%): 73.28+7.02A- 2.69B- 4.32C+ 0.242AB+ 0.691AC+ 0.508BC- 0.911A2- 8.56B2-13.00C2

The results for drug entrapment in solid lipid nanoparticle formulations were shown in **Table 4**. The term A, lipid to drug ratio showed positive coefficient estimate value indicating the direct relationship with the entrapment efficiency, whereas surfactant concentration and homo-genization time showed a negative effect. The interaction effect of lipid to drug ratio with surfactant concentration and homogenization time showed a positive effect. Formulation with lipid: drug ratio of 4:1 showed greater drug entrapment, *i.e.*, 78.1%. A higher %EE could be due to the presence of higher amount of lipid which provides additional space for a drug molecule to embed in, thereby decreasing total surface area **Table 3**. This can lead to reduction in the diffusion rate of the solute molecule as the viscosity of the lipidic phase is higher and thus showed higher % EE s compared to others. %EE was found to increase with the increasing amount of lipid to drug molar ratio. Thus, % EE was found to be mainly dependent on the drug: lipid ratio of the formulation. **Fig. 1C** represents the response surface plots depicting the effect of various factors on % EE.

**Data Optimization and Model Validation:** In Design Expert Software, graphical optimization was done by superimposing the critical response contours on a contour plot. This gave the overlay plot **Fig. 2** comprising of two regions *viz.*, yellow region describing an area of design space with feasible response values and grey region describing an area where response values did not fit the quality product criteria. On 13 the basis of overlay plot and desirability criteria, the optimized batch was selected. It had three CPP *viz.*, X1-D: L of 1: 4, X2- 3 % w/w of tween 80, X3- HT of 10 min, and fix levels were 1% w/w Pol-188 and HSH RPM 10000. The predicted value for size, PDI and %EE was respectively 145.831 nm, 0.213 and 73.27 as shown in overlay plot, while the result for observed value was shown in **Table 4**. Composition of validation formulation observed versus predicted values of all responses and their magnitude of error. The $R^2$ value of >0.9 for predicted versus observed value were found to be linear for all CQA responses indicating good correlation between them. Magnitude of error is useful to establish the reliability of generated equations and to express the relevant domain of model.

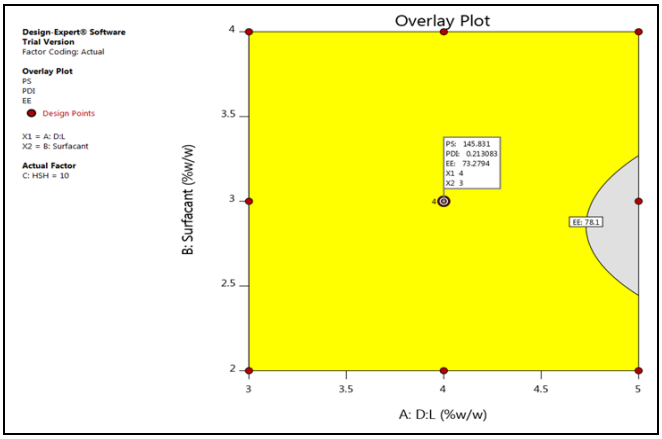


**FIG. 2: OVERLAY PLOT SHOWING A LOCATION OF OPTIMIZED CP SLNS IN DESIGN SPACE**

**TABLE 4: PREDICTED AND OBSERVED RESPONSES FOR THE OPTIMIZED FORMULATION**

| Factor 1 Lipid: Drug Ratio | Factor 2 Surfactant Concentration | Factor 3 HSH | Responses | Observed | Predicted | Relative error (%) |
|---|---|---|---|---|---|---|
| 3 | 2 | 10 | Ps(nm) | 143.8 ± 3.026 | 145.17 ± 2.36 | 1.37 |
| | | | PDI | 0.209 ± 0.15 | 0.213 ± 0.23 | 0.004 |
| | | | %EE | 71.35 ± 2.45 | 73.27 ± 5.36 | 1.92 |
| 3.64 | 2.71 | 14.03 | Ps(nm) | 153.81 ± 10.1 | 155.02 ± 9.21 | 1.21 |
| | | | PDI | 0.318 ± 1.35 | 0.320 ± 0.12 | 0.002 |
| | | | %EE | 58.53 ± 3.53 | 60.21 ± 2.31 | 1.68 |

* All values are expressed as mean ± SD, n=3

**Selection of Preparation Method:** Emulsification-homogenization method was selected to formulate CP loaded SLNs. The particulate characters were tabulated in **Table 5**.

As per the results Particle size, polydispersity index and zeta potential were found to be 133.3 ± 3.66 nm, 0.179 ± 0.081, 78.1 ± 1.11% and -36.2 ± 0.11 mV respectively.

**TABLE 5: OPTIMIZATION OF FORMULATION PARAMETERS FOR SOLID LIPID NANOPARTICLES**

| Optimization Parameters | | | | Particulate Characters | | |
|---|---|---|---|---|---|---|
| S. no. | Surfactant Concentration (%w/v) | Drug to lipid ratio (w/w) | Homogenization time | Particle size (nm) | PDI | %EE |
| 1 | 2 | 1:3 | 5 | 261.5 ± 5.01 | 0.310 ± 0.077 | 53.1 ± 3.26 |
| | 3 | | 10 | 175.4 ± 2.35 | 0.251 ± 0.052 | 64.1 ± 4.1 |
| | 4 | | 15 | 251.7 ± 4.21 | 0.432 ± 0.039 | 39.8 ± 3.12 |
| 2 | 2 | 1:4 | 5 | 241.1 ± 3.26 | 0.295 ± 0.056 | 57.5 ± 2.36 |
| | 3 | | 10 | 133.3 ± 3.66 | 0.179 ± 0.081 | 78.1 ± 1.11 |
| | 4 | | 15 | 262.2 ± 3.97 | 0.457 ± 0.061 | 46.3 ± 3.25 |
| 3 | 2 | 1:5 | 5 | 288.7 ± 4.13 | 0.326 ± 0.08 | 63.2 ± 3.22 |
| | 3 | | 10 | 209.6 ± 5.63 | 0.284 ± 0.035 | 78.1 ± 3.45 |
| | 4 | | 15 | 252.0 ± 2.06 | 0.440 ± 0.065 | 54.5 ± 2.81 |

Values are expressed as mean ± SD (n = 3)

**Optimization of Surfactant Concentration:** Clobetasol-loaded SLNs were optimized for different surfactant concentrations from 2 to 4% w/v. The lowest particle size, polydisperse index and % entrapment efficiency was obtained with Tween 80 at 2%, 3%, and 4% w/v by keeping homogenization speed at 10,000 rpm for 10 min with solid lipid to drug ratio constant (4:1) as shown in **Table 5**. Prepared SLNs with three concentrations of Tween 80 at 2 and 4% w/v concentration, higher particle size, PDI, and lower % entrapment efficiency was observed and the lower particle size, PDI, and higher % entrapment efficiency were shown Tween 80 at 3% w/v. Based on the particle size and PDI observations, formulation with Tween 80 (3% w/v) was selected for further studies to keep the surfactant concentration minimum.

**Optimization of Solid Lipid and Drug Ratio:** Clobetasol-loaded SLNs were optimized for the ratio of solid lipid to the drug. Batches for optimization were prepared in three ratios 3:1, 4:1 and 5:1. The ratio having lowest particle size, PDI and higher % entrapment efficiency was selected for further studies with Tween 80 at 3% w/v concentration and 10 min homogenization as constant. The lowest particle size, PDI and higher % entrapment efficiency for the batches were with ratio 4:1 *i.e.* 133.3 ± 3.66 nm, 0.179 ± 0.081 and 78.1 ± 1.11% respectively.

**Optimization of Homogenization Time:** Effect of homogenization time on particle size, PDI, and % entrapment efficiency was studied at homogenization speed of 10,000 rpm. The homogenization time was varied from 5 to 15 min. From the study, it was observed that the particle size reduces with an increase in homogenization time for 10 min. After this, there was an increase in particle size, PDI, and % entrapment efficiency. It was observed that %EE was less in 15 min than at 10 min. This was owing to the removal of surfactant particles from the lipid surface, thereby causing lipid disruption and escape of entrapped drug into an aqueous phase.

**Morphology of SLNs:** Size and shape of the optimized batch of nanoparticles were evaluated by TEM. TEM images of the SLNs confirmed the oval and nearly spherical shape of nanoparticles with narrow size distribution. They further confirmed the non-aggregation of nanoparticles in **Fig. 3**. The diameters of the nanoparticles observed in the micrographs were in good agreement with data obtained from the Malvern particle size analyzer.

**% Entrapment Efficiency:** The interaction effect of lipid to drug ratio with surfactant concentration and homogenization time showed a positive effect. Formulation with lipid: drug ratio of 4:1 showed greater drug entrapment, *i.e.*, 78.1%. A higher %EE could be due to the presence of a higher amount of lipid, which provides additional space for a drug molecule to embed in, thereby increasing the total surface area. This can lead to a reduction in the diffusion rate of the solute molecule as the viscosity of the lipidic phase is higher and thus

showed higher %EE s compared to others. % EE was found to increase with the increasing amount of lipid to drug molar ratio. Thus, % EE was found to be mainly dependent on the drug: lipid ratio of the formulation.

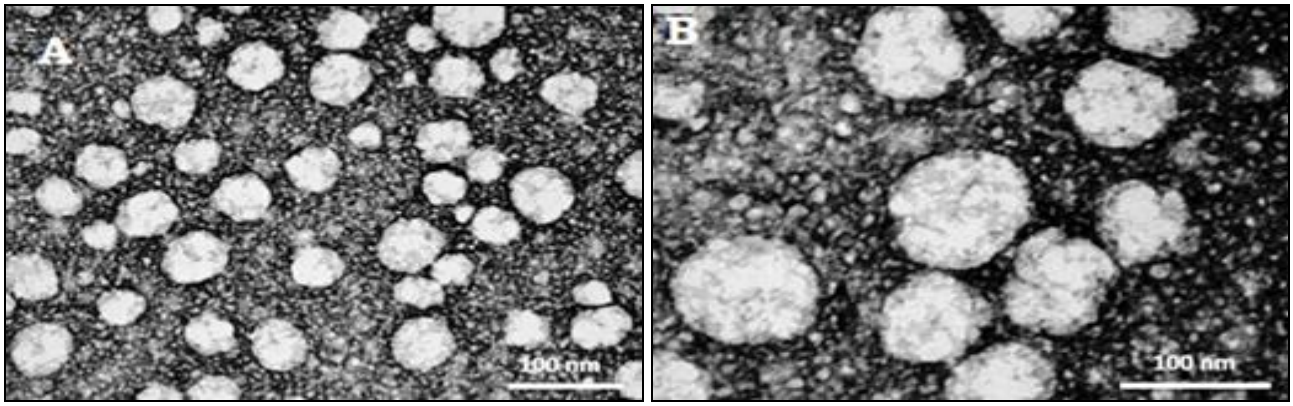


**FIG. 3: CP LOADED SLNS (OPTIMIZED FORMULATION) VISUALIZED BY TRANSMISSION ELECTRON MICROSCOPY. (A) OVERALL VIEW SHOWING PARTICLE POLYDISPERSITY (B) MAGNIFIED VIEW SHOWING PARTICLE INTERNAL STRUCTURE**

**Spreadability:** The CP ointment and CP-SLNs were evaluated for spreadability, and comparison of spreadability of the ointment and gel with increasing weights was shown in **Fig. 4**. The initial spreading area for CP ointment was 1.7 ± 0.32 $cm^2$ whereas CP-SLNs Gel showed a spreading area of 6.8 ± 0.23 $cm^2$. After the addition of 200 g weights to the formulations, the spreading area was 4.46 ± 0.35 $cm^2$ for CP ointment, and it was 21.9 ± 0.15 cm2 for CP-SLNs-Gel. A significant difference was observed between the spreadability of ointment and SLNs gels. The spreading area of the CP-loaded SLNs gel was higher as compared to the ointment, which means gel can be applied to the skin with less force of application.

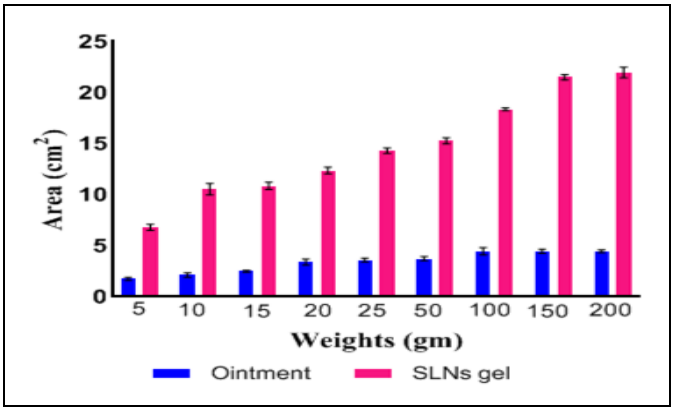


**FIG. 4: COMPARISON OF SPREADABILITY PROFILE OF CP OINTMENT AND CP-SLNS GEL.** Values are expressed as Mean ± S.D. (n = 3)

***In-vitro* Release Study:** The cumulative percentage of drug release of CP from CP solution, CP ointment, and CP loaded SLNs gel was investigated *in-vitro* over a period of 24 h. Each sample was analyzed in triplicate, and release curves have been shown in **Fig. 5**. The drug release from CP solution was faster, with 93.9 ± 3.84% release of CP within 7 h. Whereas drug release from the ointment and SLNs gel was comparatively slow, it showed 33.9 ± 4.29 and 67.3 ± 3.36%, respectively, in 24 h. In SLNs gel, the drug is protected from oxidation as it is encapsulated along with the presence of anti-oxidants, and hence such trend was not observed in the release profile of SLNs gel. For topical application, the formulations were expected to remain on the skin for 24 h; for this reason, the in vitro release was studied for 24 h. To understand the best fit model and possible mechanisms governing the drug release in CP-ointment and CP-SLNs formulation.

Various kinetics models were fitted including zero-order ($r^2$ = 0.906), first-order ($r^2$ = 0.994), Higuchi ($r^2$ = 0.978) and Korsmeyer–Peppas models ($r^2$ = 0.980) for SLNs gel. Among that, the Peppas model was found to be the best fit model for SLNs gels. For CP-ointment, regression values were zero-order ($r^2$ = 0.901), first-order ($r^2$ = 0.990), Higuchi ($r^2$ = 0.971) and Korsmeyer–Peppas models ($r^2$ = 0.975) respectively. Further analysis of the diffusion exponent (n) in CP-SLNs gel by Korsmeyer –Peppas equation revealed that values of n were greater than 0.5 in the formulation. Hence, it follows non Fickian diffusion kinetics, which is a combination of both diffusion and erosion controlled rate release.

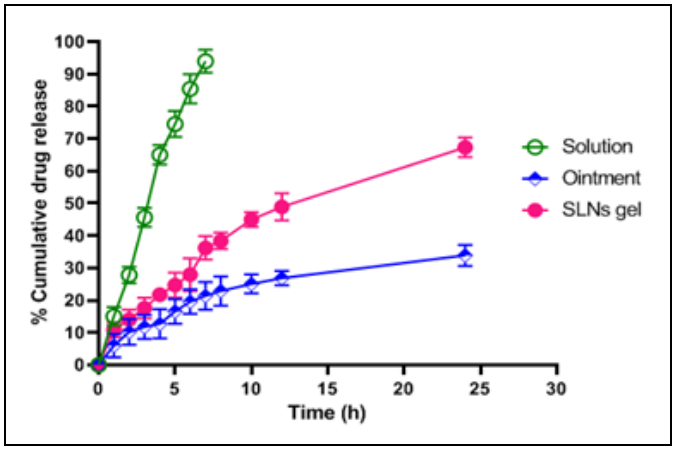


**FIG. 5: *IN-VITRO* DRUG RELEASE PROFILE FROM CP-SLNS GEL, OINTMENT AND SOLUTION IN PHOSPHATE BUFFER (PH 7.4).** Values are expressed as Mean ± S.D. (n = 3)

**Anti-psoriatic Efficacy in BALB/C Mice:**

**IMQ Induced Psoriasis Plaque Model:** A murine model of human psoriasis was developed in this study to investigate the efficacy of topical CP-SLNs carriers in psoriasis. IMQ-induced psoriasis-like inflammation acts as a model for the analysis of pathogenic mechanisms in psoriasis.

The topical application of IMQ on mouse skin leads to the rapid proliferation of dendritic cells and keratinocytes to increase cytokine production. These effects in the mouse skin closely resemble human plaque-type psoriasis with respect to erythema, skin thickening, scaling, epidermal alteration (acanthosis, parakeratosis) **Fig. 6**. From the 3rd day after the IMQ application, the back skin of IMQ-treated mice began to display psoriatic features, *i.e.*, erythema, thickening, and scales. Phenotypical changes of mouse skin after the sixth day of treatment with respect to formulation are shown in **Fig. 6**. Observations were made in comparison with the negative control, positive control, clobetasol ointment, clobetasol-loaded SLNs gel, and efficacy of these groups was compared with the sham group. Ear thickness was measured, and the difference between the right and left ear thickness was interpreted. The negative control showed a significant difference between the right ear and left ear thickness among the treatment groups; no difference was observed in other treatment groups shown in **Fig. 7E**.

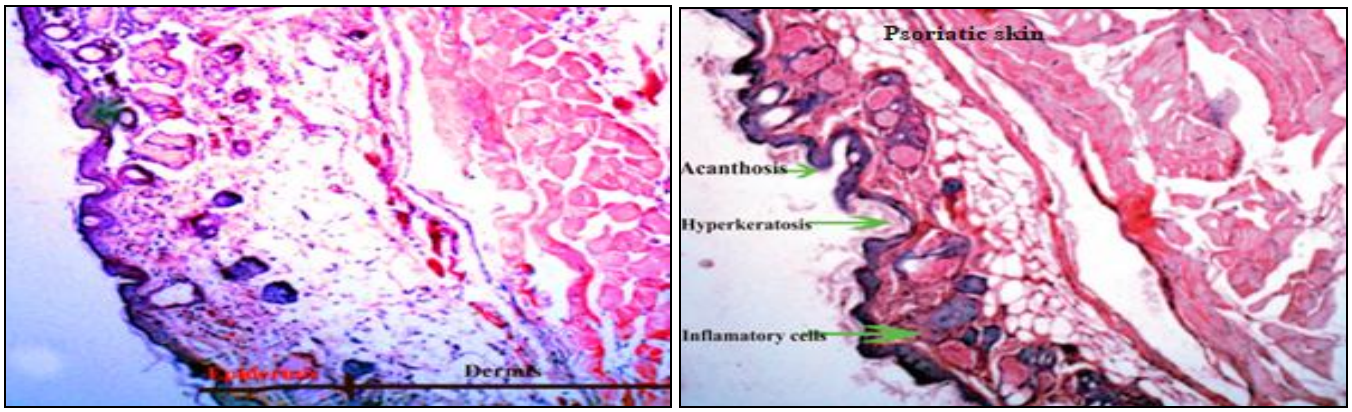


**FIG. 6: HISTOPATHOLOGICAL IMAGES OF NORMAL MICE SKIN AND IMIQUIMOD INDUCED PSORIATIC MICE SKIN**

**Body Weight and PASI Scoring:** Body weight of all the groups was measured during the treatment period of the sixth day. A slight increase in body weights was observed in positive, ointment, CP-SLNs and negative control showed slight decrease in body weights was observed except Sham group showed a constant increase in body weight from 0th day to 6$^{th}$ day **Fig. 7A**. To evaluate the efficacy of the formulation PASI scoring is one of the measurements. It is done on the scale of 0 - 4 on 0, 2, 4, 6 day and plotted **Fig. 7B, C, D**. Erythema represents the degree of vasodilation due to release of cytokines (IL-1 and TNF-a) and other compounds like NO, phospholipase A2 metabolite, histamine in the dermis from dendritic cells, keratinocytes, mast cells, *etc.* An increase in skin thickness refers to the proliferation of keratinocytes due to pro-inflammatory cytokines (IL-20 and IL-22). Scaling reflects the poor differentiation of keratinocytes. Negative control exhibits erythema, scaling, and thickness with a score of 2-3 (severe) on the sixth day of IMQ treatment, which indicates the inflammatory responses were developed as compared to sham control group16.In comparison to a negative control group, positive control group, ointment and CP-SLNs gel showed a reduction in with significant difference in p-value < 0.05. Significant reduction in skin thickness and scaling

was observed in the positive control, ointment, and CP-SLNs gel group in comparison with negative control. Spleen enlargement is an important marker of the immunological disorder. IMQ induces splenomegaly by increasing the number of Th17 cells. Spleen weight to body ratio (SWBR) for all study groups was calculated on the seventh day of the experiment. An increase in SWBR was observed in the negative control group, which indicates the induction of psoriasis after the application of IMQ. Other treatment groups resulted in the reduction of SBWR with no significant difference between **Fig. 7F**.

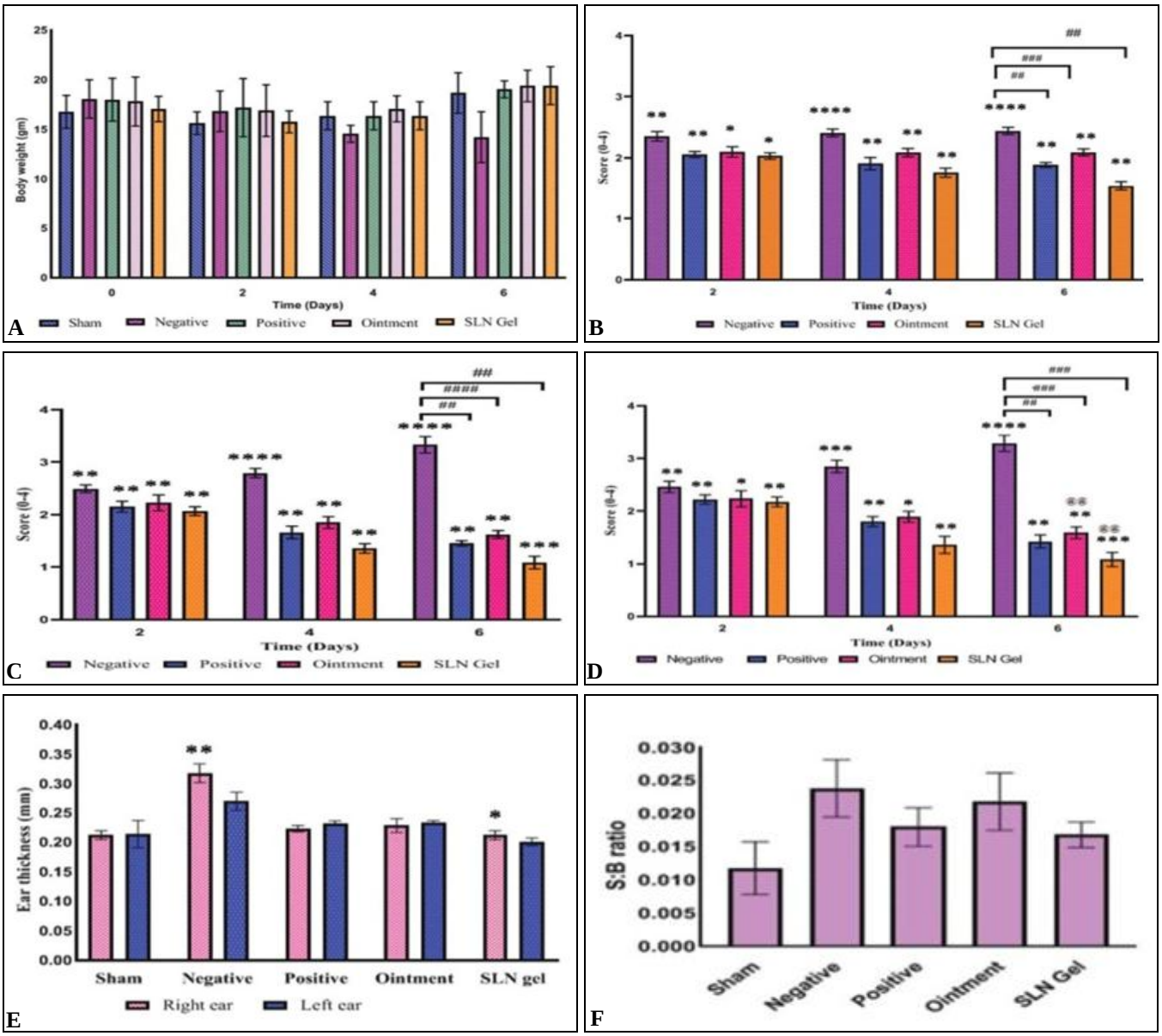


**FIG. 7: BODY WEIGHT ASSESSMENT AND PASI SCORING. A) CHANGES IN BODY WEIGHT OF DIFFERENT GROUPS DURING SIX DAY TREATMENT WITH RESPECTIVE FORMULATIONS, B) PASI SCORING FOR ERYTHEMA, C) PASI SCORING FOR SCALING AND D) PASI SCORING FOR SKIN THICKNESS, E) MEASUREMENT OF EAR THICKNESS OF MICE FROM DIFFERENT GROUPS AFTER 6 DAYS OF TREATMENT WITH RESPECTIVE FORMULATIONS, F) SPLEEN WEIGHT: BODY WEIGHT RATIOS OF MICE FROM DIFFERENT GROUPS AFTER 6 DAYS OF TREATMENT WITH RESPECTIVE FORMULATIONS. DATA REPRESENT MEAN ± SD (N = 5 ANIMALS). RESULTS ARE PRESENTED AS GROUP MEANS ± SD (N = 5) FOR DIFFERENT GROUPS; SCORING WAS PERFORMED ON DAYS 0, 2, 4 AND 6 USING THE ERYTHEMA, SCALES AND SKIN THICKNESS ELEMENTS OF THE PASI TO ASSIGN A SCORE OF 0 –4 TO EACH ANIMAL AND THEREBY ASSESS THE EFFECTS OF DAILY TREATMENT WITH RESPECTIVE FORMULATIONS. *P < 0.05 (VS SHAM), **P < 0.01 (VS SHAM), ***P < 0.001 (VS SHAM), ****P <0.0001 (VS SHAM), #P < 0.05 (VS NEGATIVE CONTROL), ##P < 0.01 (VS NEGATIVE CONTROL), ####P < 0.0001 (VS NEGATIVE CONTROL), @P < 0.05 (VS POSITIVE CONTROL). ***P < 0.001 (VERSUS SHAM), ###P < 0.001 (VS NEGATIVE CONTROL) AND (C), **P < 0.01 (VERSUS RIGHT EAR), *P < 0.05 (VS RIGHT EAR)**

**Histology:** Histopathology images of skin samples were collected from mice, and its representation of each group was shown in **Fig. 8**. Sham represents histology of normal skin, while negative control exhibited hyperkeratosis (thickening of stratum corneum) and parakeratosis (retention of nuclei in stratum corneum), acanthosis (proliferation of the epidermis) and discreet chronic inflammatory infiltrate in the dermis. The positive control group showed a reduction in the thickness of epidermis in comparison to the negative control group. Clobetasol-loaded SLNs gel and Clobetasol ointment treated group showed very similar histopathological results to that of the normal group where epidermis was normalized and less infiltrated were observed. The histopathological results are in agreement with phenotypic images taken from each group of animals.

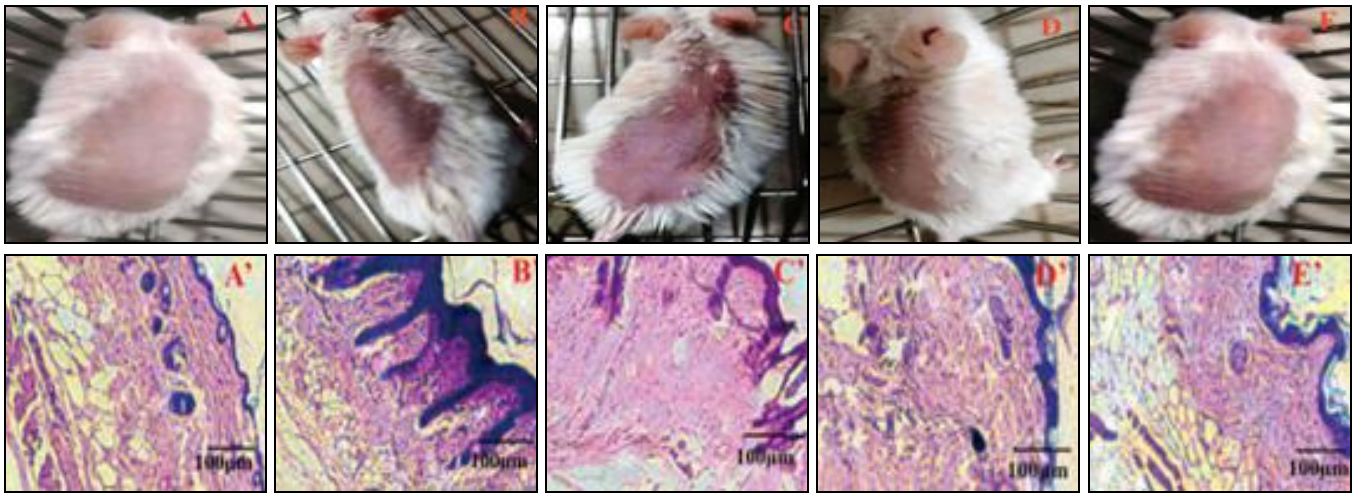


**FIG. 8: PHENOTYPIC AND HISTOPATHOLOGICAL FEATURES OF SKIN DURING EFFICACY STUDIES ON IMQ INDUCED PSORIATIC PLAQUE MODEL. (A AND A') NORMAL GROUP (ARROWHEAD AND DOUBLE-HEADED ARROW INDICATE EPIDERMIS AND DERMIS RESPECTIVELY), (B AND B') NEGATIVE CONTROL GROUP, (C AND C') POSITIVE CONTROL GROUP, (D AND D') OINTMENT GROUP AND (E AND E') SLNS GEL GROUP. EACH IMAGE IS REPRESENTATIVE OF RESPECTIVE GROUP (N = 5)**

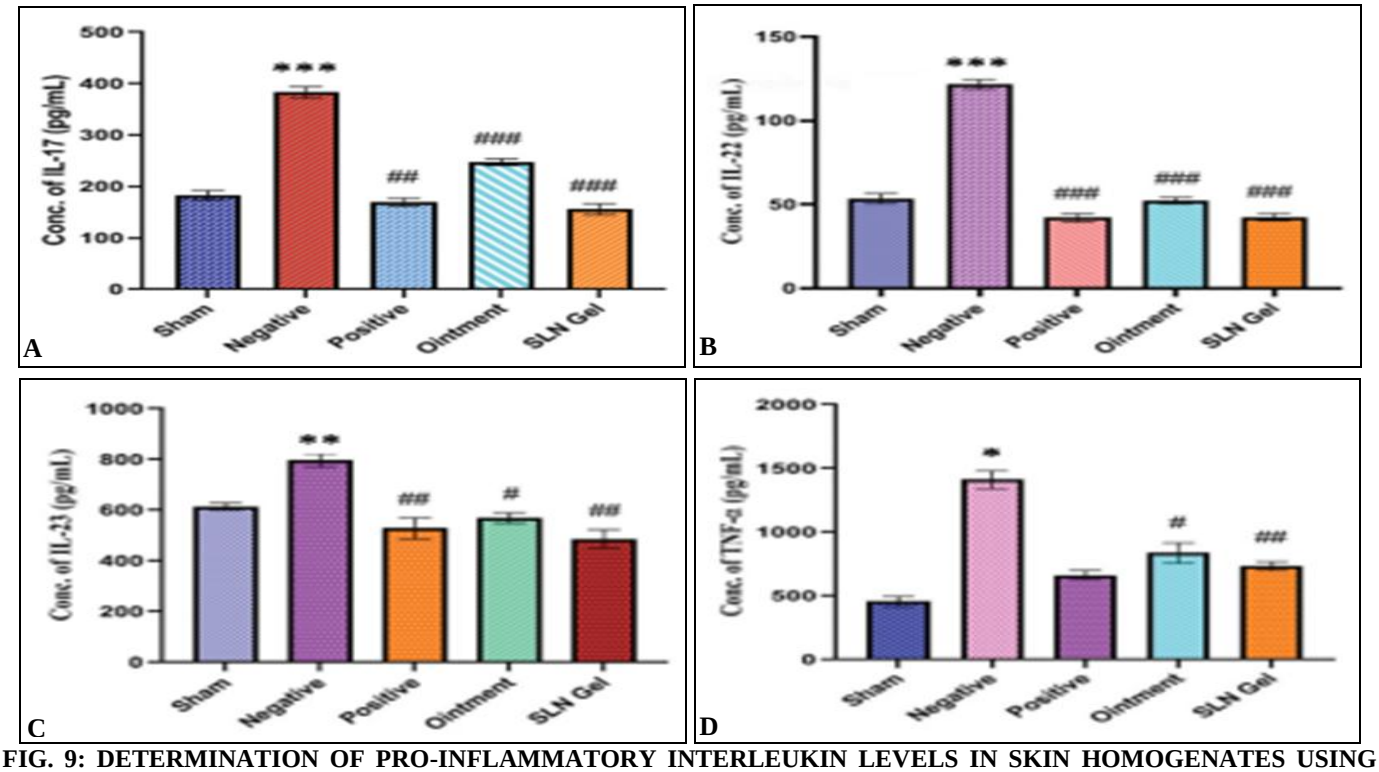


**FIG. 9: DETERMINATION OF PRO-INFLAMMATORY INTERLEUKIN LEVELS IN SKIN HOMOGENATES USING ELISA. RESULTS ARE PRESENTED AS GROUP MEANS ± SD (N = 5) FOR (A) IL-17, (B) IL-22, (C) IL-23 AND (D) TNF-A LEVELS. ***P < 0.001 (VERSUS SHAM), *P < 0.05 (VERSUS SHAM), ###P < 0.001 (VS NEGATIVE CONTROL), #P < 0.05 (VS NEGATIVE CONTROL)**

**Enzyme-linked Immunosorbent Assay:** Psoriasis is a Th1/Th17 mediated inflammatory process associated with overexpression of Th1 and Th17-associated cytokines (IL-17, IL-22, IL-23, TNF- α) leading to inflammation and keratinocyte hyperproliferation. IMQ induced psoriasis model mimics biochemical parameters characteristic of human psoriatic lesions. Topical application of the IMQ cream was reported to increase the levels of cytokines such as IL-17, IL-22, IL-23, and TNF- α in the treated skin tissues [16].

In this present study, CP-loaded SLNs gel showed 2.4 folds reduction in IL-17 level, 2.9 folds reduction in IL-22 level and 1.8 folds reduction in IL-23 in compared with the negative control group, positive control showed 1.9 folds, 2.5 folds, and 1.5 folds reduction in IL-17, IL-22 levels, and IL-23 respectively and CP-ointment showed 1.5 folds 2.3 folds and 1.2 folds in IL-17, IL-22, and IL-23 levels respectively compared with the negative control. CP-loaded SLNs gel, positive control, and CP-ointment resulted in 2.0 folds, 1.6 folds, and 1.4 folds reduction of TNF- α level as compared with negative control **Fig. 9**. Treatment with the CP-SLNs gel decreased the cytokines levels, which confirmed the efficacy of formulation in treating psoriasis.

**CONCLUSION:** CP loaded SLNs were prepared using Compritol 888 ATO as lipid matrix, poloxamer-188 as stabilizer employing emulsi-fication-homogenisation technique. The important parameters like a drug: lipid ratio, surfactant concentration, and homogenization time were optimized by 33 full factorial design to obtain a minimal PS, Narrow PDI, and highest % EE. Thus, desirable goals could be achieved by systematic formulation approach the shortest possible time with a reduced number of experiments. Solid lipid nanocarriers of clobetasol were developed with the objective of enhancing the skin penetration of Clobetasol and thereby improving the efficacy of topical treatment psoriasis. To prepare CP-loaded SLNs and the obtained Particle size, polydispersity index,% entrapment efficiency, and zeta potential obtained from this method were 133.3 ± 3.66 nm, 0.179 ± 0.081, 78.1 ± 1.11% and -36.2 ± 0.11 mV respectively. *In-vitro* release profile suggested the sustained release of CP for a prolonged time, which would be beneficial in avoiding frequent administration of CP. CP-SLNs gel system provided ease of application, deeper penetration, and slow release of the drug. The topical application of the SLN gel showed a reduction in psoriatic symptoms in the IMQ induced psoriatic plaque model. Efficacy of SLN gel and ointment were compared to the results like PASI scoring, histopathological study, and ELISA showed that CP-SLNs gel has the potential to treat psoriasis. To conclude, SLNs gel was found to be more effective than ointment in treating psoriasis.

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**AUTHORS CONTRIBUTIONS:** All the authors have contributed equally.

**CONFLICTS OF INTEREST:** The authors confirm no conflict of interest for this manuscript.

**ETHICAL APPROVAL:** All experimental procedures were performed in accordance with the ethical guidelines for the study and was approved by the Institutional Animal Ethical Committee (IAEC no. 16/KTPC/IAEC/2018:1/03/2018), Krishna Teja Pharmacy College, Chadalawada Nagar, Tirupati, Andhra Pradesh, India.

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