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FORMULATION, EVALUATION, AND OPTIMIZATION OF DIACEREIN LOADED TRANSFEROSOMAL GEL FOR ARTHRITIS

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ABSTRACT

The objective of the current study was to develop and evaluate diacerein-loaded transferosomal gel to augment the permeation of diacerein into the deeper skin layer and finally to the affected area to treat arthritis. Preliminary studies included the process variable optimization (RPM) and selection of surfactant. The circumscribed central composite design was included to explore the reactions of the amount of phospholipon 90H, cholesterol, and span 60 on dependent variables like % entrapment competency, vesicle size, and release of drugs. The diacerein-loaded transferosome were assembled by reverse-phase evaporation method and evaluated for zeta potential and particle size, vesicle morphology, % entrapment competency, and in-vitro drug release study. The optimized batch of transferosomal suspension was incorporated in gel using carbopol 934P and the transferosomal gel was evaluated for various physicochemical properties, drug release, Ex-vivo permeation study, skin irritation, pharmacodynamic activity, and stability. Span 60 was selected as a surfactant for the preparation of transferosomal suspension using optimized process variables. The statistical model indicated that as the concentration of lipid increased then entrapment efficiency also improved. However, as the concentration of surfactant increased then drug release from the transferosomal gel also increased. The TEM of transferosomal suspension was elucidated sphericity. The optimized transferosomal suspension was incorporated into the gel. The in-vitro release of diacerein from optimized transferosomal gel followed Higuchi kinetic model (R^2 =0.9815). The developed formulation was stable, non-irritating, and showed a maximum amount of % swelling inhibition using carrageenan inducing hind rat paw oedema at a different time interval.

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Introduction

Inflammation is one of the natural reactions in the body during an injury or disease. It includes swelling, pain, and stiffness. Inflammation caused by arthritis results from tissue damage in joint areas where two or more bones are fixed together, such as the knee or hip. In arthritis, an area or around a joint becomes inflamed, painful, and difficult to move. They may be mild, moderate, or severe [1, 2]. One of the vesicular-based dosage forms like Transferosome has an effective therapy through the skin. Transferosome is a stress aggregate, ultra-deformable. They possess the structure of a hydrophilic and hydrophobic moiety together so accommodation of various molecules of a wide range of solubility. The primary treatment of arthritis is NSAIDs, which are more upper G.I toxic and cause a gastric or duodenal ulcer. The symptomatic slow-acting drug 'diacerein' overcomes these side effects [3, 4]. The transdermal drug delivery of diacerein overcomes the side effects of oral drug delivery such as Severe Diarrhoea, Discoloration of urine, and low oral bioavailability (35-55%). Transdermal drug delivery gives localized action without disposition in non-target organs [5-7]. The diacerein incorporated in the transferosome overcome the drawbacks of oral drug delivery. It gives a prolonged targeted drug release with better stability and reduced skin irritation. Hence, Transferosome overcomes the drawback of oral diacerein therapy. In the present investigation, we put effort to prepare and optimize the diacerein-loaded transferosomal gel for effective skin permeation using circumscribed central composite design. We evaluated anti-inflammatory activity using rat and histopathological aspects [8, 9].

Materials and Methods

Materials

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Diacerein is an API obtained from Ami life science, India while Phospholipon 90H was an offering sample obtained from Lipoid, Germany. Cholesterol was obtained from Chemdyes Corporation, India. Carbopol 934P was obtained from Astron laboratories, India. Various grades of tween and span along with organic solvents were obtained from Suvidhnath Laboratories, India. All reagents and chemicals used here were of analytical grade.

Methods

Diacerein-loaded transferosome was prepared by the reverse-phase evaporation method In which, Lipid, cholesterol, and surfactant were dissolved in Chloroform: Ethanol(2:1 v/v).The organic solvent was vaporized above the lipid transition temperature (60°C) by using a rotary flask evaporator. Diethyl ether was used to resuspend the thin film formed in a round bottom flask. The drug solution was prepared in phosphate buffer 7.4: ethanol (2:1 v/v). The lipid and drug solutions were mixed and then sonicated for 5 minutes and allowed to evaporate diethyl ether. Phosphate Buffer pH 7.4 (5ml) was poured into the suspension and allowed to hydrate for 30 minutes. The obtained suspension was refrigerated at 4°C overnight. Desired size vesicle was obtained by effective sonication process using bath sonicator at frequency 20 kh_2 for 4 min [10].

Process Variables Optimization

In the preparation of trial batches, the process was optimized using process variables such as RPM, and the effect of this was checked on the properties of a thin film [11, 12].

Screening of Various Surfactants

The various surfactants such as Span 20, Span 40, Span 60, and Tween 80 have been used as shown in **Table 1** earlier in the preparation of transferosome. Here the same concentrations of different surfactants were taken for the preparation of the thin film. 4 trial batches were prepared using 30 mg of each surfactant to select a suitable surfactant for the preparation of a thin film and vesicle size was determined [13, 14].

Table 1. Screening process of various surfactants

Surfactant (mg)					
Span 20	**Span 40**	**Span 60**	**Tween 80**	**Phospholipon 90 H (mg)**	**Cholesterol (mg)**
30	-	-	-	100	15
-	30		-	100	15
-	-	30	-	100	15
-	-	-	30	100	15

Optimization Studies using Central Composite Design

Transferosome was fabricated via Central Composite design. Amount of Phospholipon 90H (X1), amount of Cholesterol (X2), and amount of Surfactant (X3) were identified as independent variables and their effect on dependent variables % Drug entrapment (Y1), Vesicle size (Y2), In-Vitro drug release (Y3) was evaluated as shown in **Table 2**. The different levels of independant variables were as per shown in **Table 3** and drug concentration was kept constant. The response was investigated by the following polynomial and interactive denominate:

$$Y= b0 + b1X1 + b2X2 + b3X3 + b11X12 + b22X22 + b33X32 + b12X1X2 + b13X1X3 + b23X2X3 \quad (1)$$

Here Y denotes the dependent variable, b0 represents the mean response of the twenty runs; b1, b2, and b3 are the estimated coefficients for the factors X1, X2, and X3 respectively. X1, X2, and X3 are the main effect whereas X1X2, X1X3, and X2X3 as interactive notations, demonstrates how the feedback changes when two factors were simultaneously changed [15, 16].

Table 2. Central composite design variables

Central Composite Design Variables		
Independent variables	**Variable levels**	
	Low Level (-1)	High level (+1)
Amount of lipid (mg) (X1)	100	160
Amount of Cholesterol (mg) (X2)	8	28
Amount of Surfactant (mg) (X3)	20	60

Table 3. Central composite design variables

Batch No	**Drug (mg)**	**Independent Variable Levels in actual unit (mg)**		
		X1	X2	X3
F1	10	100	8	20

F2	10	160	8	20
F3	10	100	28	20
F4	10	160	28	20
F5	10	100	8	60
F6	10	160	8	60
F7	10	100	28	60
F8	10	160	28	60
F9	10	79.5462	18	40
F10	10	180.454	18	40
F11	10	130	1.18207	40
F12	10	130	34.8179	40
F13	10	130	18	6.36414
F14	10	130	18	73.6359
F15-F20	10	130	18	40

Validation of Experimental Model

For the model to be approved, and check the reliability of the equations where the effects of the independent variables on % Entrapment Efficiency, Vesicle Size, In-Vitro Drug Release (Q8) were examined, the additional checkpoint batches were evaluated. The % relative error between the predicted values and the observed values was calculated.

Optimization by Numerical Method

The fundamental goal of product development was to explore the desired level of variables that make sure that a finished product got the desired or targeted characteristics. Thus the optimization was carried out by employing the desirability function. Criteria were set for various dependent factors and out of the possible formulation, the optimized formulation was selected which had a value of D (Desirability function) closest to 1.

Evaluation Parameter of Transferosome

Particle size and Zeta Potential

Zeta sizer was used to measure the mean particle/globule size and Zeta potential (ZP) of the transferosome. The mean particle/globule size was an important parameter that governs the degree of permeation through the skin. The stability of the colloidal system in terms of globule size was evaluated based on Zeta Potential values and was established based on an electrophoretic light scattering technique. For each formulation, three replicate analyses were performed, and data were presented as mean± S.D [17, 18].

Vesicle Morphology

A transmission electron microscopy image was taken to study the surface morphology and characteristics of diacerein-loaded transferosome [19].

% Entrapment Efficiency

Unentraped drug was separated from entrapped drugs in transferosome to measure the %EE. Using the centrifugal method. The sample of transferosomal dispersion was placed at -20°C in Eppendorf tubes and froze. They were then expelled from the cold storage and let to thaw at room temperature, then centrifuged at 14000 rpm for 50 min at 4°C. Transferosomal pellets were re-suspended in phosphate buffer pH 7.4 and then centrifuge again. This process was rerun to confirm that the % EE values would be accurate. The supernatant containing free drugs was quantified using the UV method using phosphate buffer pH 7.4 [20, 21].

In Vitro Drug Release Study

The dialysis diffusion bag made of a cellophane membrane was used to predict the in-vitrorelease of diacerein-loaded transfersomes. The receptor medium was 40 ml phosphate buffer pH 7.4 was taken which was retained at 37±0.5 ^{0}C and constantly stirred at 100 rpm in a thermostatically controlled water bath shaker. The transfersome pellets were loaded in a dialysis bag which was immersed in the donor compartment. 4 ml of samples were withdrawn from the receptor compartment at regular intervals of 1 hour up to 8 hours and the same is replaced with an equal quantity of fresh medium. Triplicate experiments were conducted for each study and the sink condition was maintained throughout the experiment. All samples were quantified at a wavelength of 257.5 nm against phosphate buffer pH 7.4 and the amount of drug release in 8 hours (Q8) was calculated [22].

Preparation of Transferosomal Gel

The transferosomal suspension containing an equivalent amount of drug was centrifuged at 14000 RPM using a centrifuge. After centrifugation residues were collected and disseminated in 10 ml of Phosphate buffer pH 7.4 and added to the above dispersion is prepared carbopol gel [23, 24].

Evaluation Parameter of Gel

Spreadability of the Gel

The spreading efficiency of the prepared diacerein-loaded transferosomal gel was measured by keeping the 0.5 g of content on a glass plate which is marked with a circle of 1 cm diameter and then putting a second glass plate. 3g of weight was rested on the upper glass plate for 3 min. The gel spread thus increasing the diameter [25].

Ex-Vivo skin Diffusion Study

Evaluation of penetration power of the prepared transferosomal gel was carried out by using human cadaver skin through 6 stages of Franz static diffusion cell assembly. The human cadaver skin acted as a semi-permeable membrane and was placed between the donor compartment where 2 gm of the drug-loaded formulation was placed and the receptor compartment was filled with 25 ml Phosphate buffer Ph under constant stirring. The sink condition was maintained by replacing the withdrawn sample with a freshly prepared medium. The temperature remained at 37±5ºC. The samples were analyzed using a UV spectrophotometer at 257.5 nm. After 24 hours, the skin was cut into small pieces and extracted with dimethylacetamide. The extract was diluted with methanol and analyzed in a UV spectrophotometer at 257.5 nm [26, 27].

Skin Irritation

The pathophysiological condition of the prepared formulation on the skin was evaluated by measuring skin sensitivity. For that skin irritation study using four groups of rats having 3 nos in each group was prepared. (Positive control, Negative control, Diacerein simple gel, Diacerein transferosomal gel) was identified for each group. Hair was depleted from the backside of rats with the help of depilatories and the area was marked on both sides. Gel (500mg/rat) was applied once a day for 14days.skin sensitivity parameters like redness, edema, and skin rashes were observed in each group. The skin irritation effect of the gel was graded as a 0-no reaction; 0.5-slight, patchy erythema; 1-moderate but patchy erythema; 2-moderate erythema, and 3-severe erythema with or without edema [28, 29].

Histopathology

To diagnose the anatomical and physiological changes in the skin, the excised rat skin was fixed in 10% formalin solution. Sections were prepared and stained with hematoxylin and eosin. Any symptom of inflammation linked with tissue damage was observed [30, 31].

Pharmacodynamic Activity

Carrageenan-induced rat paw edema technique was used for evaluation of the anti-inflammatory activity of diacerein-loaded transferosomal gels using white male albino rats. Animals were divided into three groups (Control, Diacerein Simple Gel, Diacerein Transferosomal Gel). A certain amount of gel was applied topically to the right hind paw of the rats by gentle rubbing for 15 seconds. The size of edema was expressed as the change in paw thickness in cm after carrageenan injection and the obtained results were recorded [32, 33]. The % of Swelling Inhibition was calculated using the following formula:

$$\% = Swelling\ inhibition = 1 - \frac{V\ treated}{V\ control} \times 100 \quad (2)$$

Residual Solvent Levels in the Optimized Formulation of Transferosome[43]

In the preparation of transferosome Chloroform, Ethanol and Diethyl ether were used as solvents in the reverse-phase evaporation method. The flask was kept overnight after film formation so the solvent may get evaporated during the process and only traces remain in the transferosomal suspension. Chloroform is a Class II solvent. Ethanol and Diethyl ether is class III solvents so the residual level of these three solvents was determined in transferosomal suspension by Gas chromatography using Thermo scientific, DSQ 2 [34].

Stability Study[44]

As per ICH guidelines, Short term accelerated stability studies were carried out. The prepared transferosomal gel was subjected to stability studies in the aluminium collapsible tube at two different temperatures Refrigerated (5°C ±3°C) and at Accelerated Condition (25°C ± 2°C & 60% ± 5% RH) and evaluated for drug release, viscosity, pH appearance, drug content, after a period of 30 days [27].

Results and Discussion

Process Variable Optimization

The process variable RPM in rotary vacuum evaporation was varied from 70,80 to 90 rpm to check the film formation and temperature was maintained at 60 ˚C. From the above trials, a smooth and uniform films was found with 90 rpm, it was concluded that the optimum RPM for film formation was 90 rpm at the temperature of 60˚C.

Screening of Surfactant

During finalization of suitable surfactants, span 20,40,60 and 80 grade were tried in film making process where, Span 60was selected for further preparation of screening batches as it produced desired vesicle size and it has a low HLB value as compared to other excipients, so it was selected for further studies.

Evaluation of Central Composite Design Batches

Table 4. Result of central composite design batches

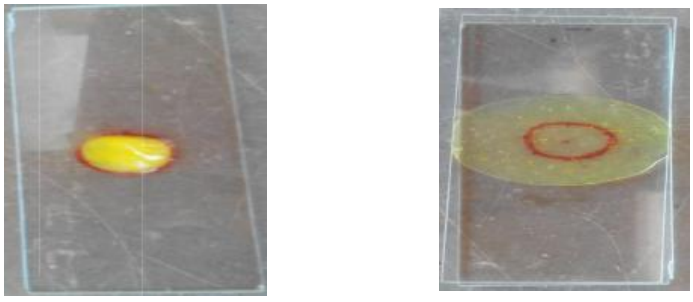
Sr. No.	Batches	%EntrapmentEfficiency	Vesicle Size (nm)	%Drug Release (Q8)
1	F1	41.09 ± 0.52	144.9	70.22 ± 0.16
2	F2	48.12 ± 1.39	402.1	76.36 ± 0.39
3	F3	39.05 ± 0.29	167.7	65.24 ± 0.15
4	F4	52.28 ± 0.59	399.5	71.69 ± 0.21
5	F5	36.93 ± 0.35	113.3	85.34 ± 0.32
6	F6	43.68 ± 1.25	323.6	90.2 ± 0.23
7	F7	37.02 ± 0.96	198.1	78.63 ± 0.18
8	F8	49.25 ± 0.28	323.9	83.21 ± 0.59
9	F9	34.39 ± 1.68	192.4	73.53 ± 0.11
10	F10	53.03 ± 0.55	503.3	78.23 ± 0.01
11	F11	41.18 ± 0.29	191.6	87.33 ± 0.32
12	F12	43.20 ± 0.58	237.4	71.73 ± 0.02
13	F13	49.38 ± 1.39	206.8	66.79 ± 0.03
14	F14	39.06 ± 0.32	184.7	90.47 ± 0.02
15	F15-F20	50.98 ± 1.21	179.2	88.33 ± 0.16

Transmission Electron Microscopy of Optimized Batch

The TEM study of the optimized batch of transferosomal suspension elucidated its spherical shape

Spreadability Study

The transferosomal gel had spreadability 0.92 gm.cm/sec. which indicated good spreadability



Before After

Figure 1. Spreadability of Gel formulation

In Vitro Drug Release Study

The prediction of in vivo behavior and how effectively the formulation is permeated thru skin was evaluated by in vitro release study. The diacerein-loaded transferosomal suspensions were subjected to in vitro drug release studies. The drug release data obtained for all formulations was tabulated in the above table.

Below **Figure 2** showed the plot of % Cumulative drug release in 8 hours as a function of time for all formulations. the formulation provided no hindrance to drug release. Drugs were released slowly and steadily over the period.

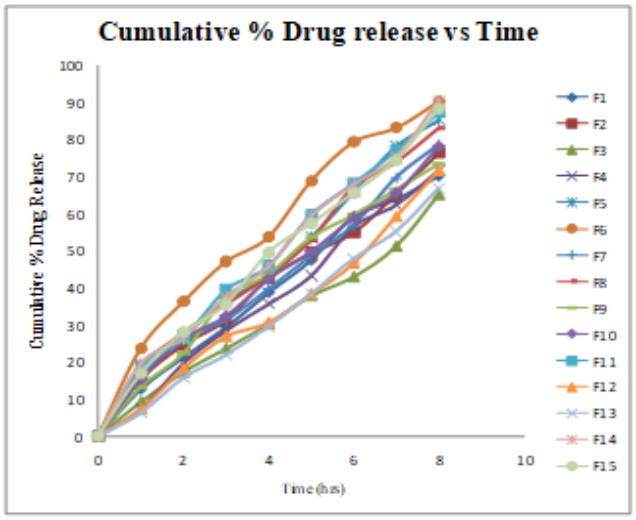


Figure 2. Cumulative % drug release of batches

Response Surface Plot Analysis

Effect of Formulation on % Entrapment Efficiency

The response plot showed that with an increased amount of lipid and Cholesterol the %Entrapment Efficiency increased while with the increased amount of surfactant the %Entrapment Efficiency decreased. The coefficient amount of lipid, cholesterol, and surfactant indicated these trends.

$$\%\text{Entrapment Efficiency} = +50.32 + 5.20X1 + 0.91X2 - 2.33X3 + 1.38X1X2 - 0.12X1X3 + 0.63X2X3 - 2.33X12 - 2.86X22 - 2.16X32 \quad (3)$$

Effect of Formulation on Vesicle Size

The response plot showed that with an increased amount of lipid and Cholesterol the Vesicle Size increased while with the increased amount of surfactant the Vesicle Size decreased. The coefficient amount of lipid, cholesterol, and surfactant indicated these trends.

$$\text{Vesicle Size} = +176.66 + 98.70\ X1 + 13.35\ X2 - 14.09\ X3 - 13.74\ X1X2 - 19.11X1X3 + 8.11\ X2X3 + 60.89X12 + 13.74X22 + 7.12X32 \quad (4)$$

Effect of Formulation on % Drug Release(Q8)

The response plot showed that with an increased amount of lipid and Surfactant the drug release increased while with the increased amount of Cholesterol the drug release decreased. The coefficient amount of lipid, cholesterol, and surfactant indicated these trends.

$$\text{Drug Release (Q8)} = +88.94 + 2.19\ X1 - 3.63\ X2 + 6.86\ X3 + 3.750E\text{-}003X1X2 - 0.39X1X3 - 0.51\ X2X3 - 4.57\ X12 - 3.27\ X22 - 3.59\ X32 \quad (5)$$

Statistical Analysis Using ANNOVA Study

The result of the experiments was analyzed using ANNOVA study and all results were significant with $P \leq 0.05$.

Validation of the Experimental Model

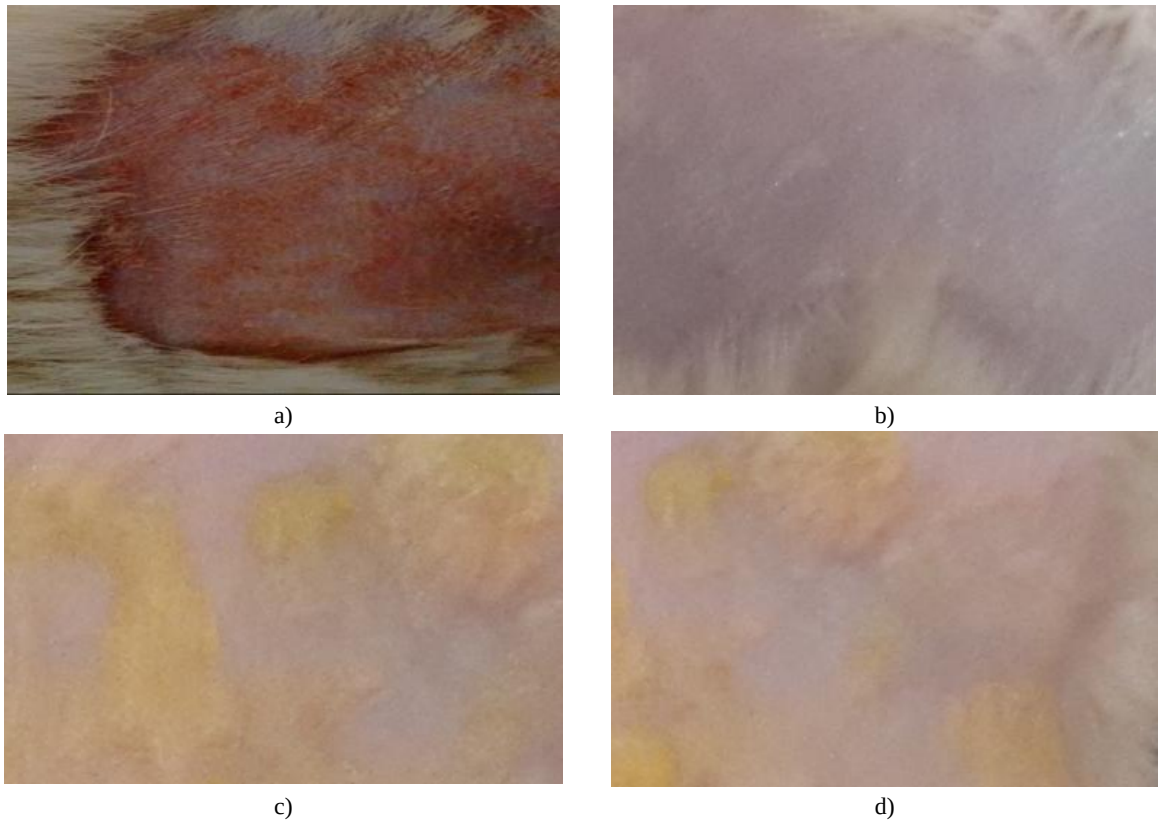
A checkpoint batch was designed in accordance with the desirability function and to access the validity of the prediction. From the below table, it was concluded that the % Bias was within limits and satisfactory. Thus, as the observed response was similar to the predicted response. It can be concluded that the experimental model was a validated one.

Ex-Vivo Permeation and Skin Retention Study

The studies showed that the higher drug permeates through the skin. The pathological condition and target receptors of the skin were preferable to the topical application of gel to improve the effectiveness of therapy. The % Drug in receptor medium was found around 56.23%, from where 18.87% was remained as unabsorbed, around 22.9% of drug retained in to the skin and % drug loss was found to be around 2% [35, 36].

Skin irritation study

From the below **Figure 3** It can be concluded that the positive control (10% formalin solution) was more irritant than the negative control (saline solution), Diacerein simple gel, and Diacerein transferosomal gel. There was no irritation observed in diacerein simple gel and transferosomal gel [25, 27, 37].

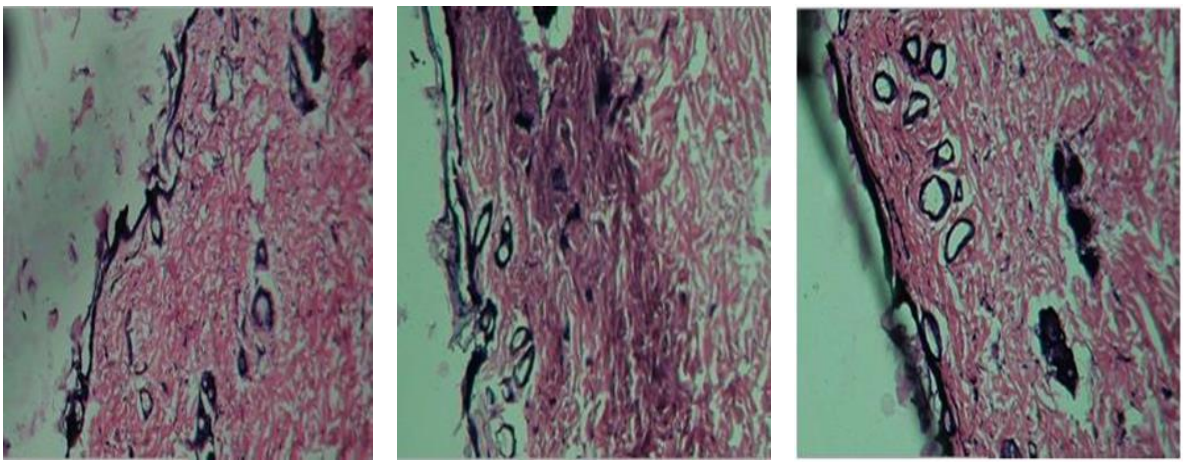


a) b) c) d)

Figure 3. Images of skin irritation studies showed (b) Normal skin before any treatment and after treatment with (a) 10 % Formalin solution (c) Diacerein simple gel (d) Diacerein transferosomal gel

Histopathology Studies

As shown in the **Figure 4**, No noticeable variations in skin morphology or irritation were realized after the application of Diacerein simple gel and Diacerein transferosomal gel. They did not show any histological changes in the skin. No inflammation in the dermal layers was found in the skins applied with Diacerein simple gel and Diacerein transferosomal gel. Hyperplasia or migration of inflammatory cells was not observed. However, some damage and inflammation were noticed in the skin treated with formalin solution indicating the growth of nuclei. Thus, the developed formulation was safe for topical use [31, 38].



a) b) c)

Figure 4. Histopathology of rat skin after treatment with (a) 0.8% formalin solution (b) Diacerein simple gel (c) Diacerein transferosomal gel

Pharmacokinetic Activity

The % swelling inhibition at different time interval from 0.5 hours to 24 hours was carried out, It was Concluded that the % Swelling Inhibition at 24 hours in Transferosomal Gel was more than the Simple Gel. The % Swelling Inhibition in transferosomal gel was 70.34% and in the simple gel was 48.83% [39, 40].

Residual Solvent Levels in the Optimized Formulation of Transferosome

9.62 ppm of chloroform,1713.29 ppm of ethanol, and 709.81 diethyl ether were detected in drug-loaded transferosomal suspension while 60 ppm is the permissible limit for chloroform and 5000 ppm is the permissible limit for ethanol as well as diethyl ether has approved by ICH. So, the Prepared Transferosomal formulation was found to be safe to use as a topical application [41, 42].

Stability Studies

The similarity factor (f2) was noted at 51.31 and 51.20 for refrigerated and accelerated conditions respectively. Results of stability studies depicted no remarkable alteration in drug content, viscosity, in vitro drug release, appearance, and pH when stored at room temperature and under accelerated conditions for a period of 30 days [43, 44].

Conclusion

Diacerein-loaded transferosome was successfully formulated using phospholipon 90H, cholesterol, and span 60. Circumscribed Central composite design was selected to enhance the transferosomal formulation and identify the effect of independent variables such as Phospholipon 90H (X1), Cholesterol (X2), and Surfactant (X3) on the dependent variables such as %entrapment efficiency (Y1), vesicle size (Y2) and % drug release in 8 hours (Y3). The design-generated response surface plot indicated that the build-up in the lipid concentration led to an increase in entrapment efficiency and a drop in surfactant concentration. However, a boost in surfactant concentration led to increasing drug release. A morphological study of transferosomal suspension was done by TEM which elucidated the spherical shape and size of the vesicle. Thus, it can be concluded that the developed diacerein-loaded transferosomal gel has a better potential to treat arthritis. The developed formulation showed sustained drug release, increased skin permeation, and efficient anti-inflammatory activity.

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Conflict of interest: None

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Ethics statement: None

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