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Original Article

Formulation and Evaluation of Niosomal Gel Loaded with *Asparagus racemosus* Extract for Anti-inflammatory Activity

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ABSTRACT

Background/Aim: Inflammation typically occurs when infectious microorganisms enter the body, settle in specific tissues, and/or circulate in the blood. *Asparagus racemosus* extract contains various saponins and flavonoids and plant-origin drugs have fewer side effects and toxicity. The phytoconstituents have less permeability through the skin; to enhance their permeability and effectiveness it was loaded in niosomes. Therefore, the study aimed to formulate and characterize the niosomal gel loaded with *Asparagus racemosus* extract for anti-inflammatory activity. **Materials and Methods:** The niosomes containing saponins in the extract were prepared using thin film hydration method and 2^3 full factorial design was employed to assess the influence of independent variables span 60 and cholesterol on vesicle size, PDI, zeta potential, and percentage entrapment efficiency. 10% of niosomal and conventional gels were prepared by incorporating optimized niosomes and extract containing total saponin in 1% carbopol gel. *Ex vivo* permeability studies of prepared gels were performed through goat skin using Franz diffusion cell. An anti-inflammatory study was conducted on albino rat. **Results:** The statistical analysis revealed the significant effect of independent variables on vesicle size, PDI, entrapment efficiency and zeta potential. SEM image shows vesicles are spherical in shape and uniform in size. The niosomal gel provided a significantly higher amount of steady-state flux and permeability coefficient into the skin than conventional gel. The animal model proved that niosomal gel loaded with total saponins in extract showed significant anti-inflammatory compared to the control. **Conclusion:** It was concluded that the niosomal gel had better efficacy than the conventional gel.

Keywords: *Asparagus racemosus*, Niosomes, Span 60, Cholesterol, Factorial design, Carbopol 934.

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INTRODUCTION

Inflammation is a protective response involving immune cells, immune blood vessels, and molecular mediators that is a part of the intricate biological response of body tissues to harmful stimuli such as pathogens, damaged cells, and irritants. The main criteria of anti-inflammatory are to discard the initial cause of cell injury and to discard necrotic cells and damaged tissues and initiate the repair. Plenty of drugs such as non-steroidal anti-inflammatory, and corticosteroids are used to decrease joint pain and swelling.[1] The anti-inflammatories are available in different forms such as gel ointments sterile preparation etc. Anti-inflammatories are used in combination for their differing effects. Synthetic drugs such as NSAID drugs may cause gastric haemorrhage, stomach burn, ulcers etc.[2]

Herbal extracts are considered to have fewer side effects when compared to conventional dosage forms.[3] Plant-origin drugs are safer to use because of their fewer side effects and promising therapeutic effect.[4] Novel drug delivery systems such as (niosomes,[5] liposomes,[6] ethosomes,[7] nanoparticles) loaded with herbal extract tend to decrease dose dumping, and drug degradation, and enhance the permeation of drugs through the skin.

Transdermal therapeutic systems are safe and non-interfering when compared to other routes of administration. Even though the *stratum corneum* layer of the skin is a found to be a barrier to most drug absorption, this system provides a larger surface area (1-2m^2) for drug diffusion and in order to prolong drug release, it can be incorporated into formulations like gel, patch etc. Liposomes, niosomes, ethosomes, proniosomes etc., are the other sustained release formulations. Topical route usually causes local

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irritation effect and itching whereas it also avoids the first pass effect, prolongs duration of action, provides flexibility in dosing, reduces other side effects and also ensures uniform plasma levels.[8] One of the semisolid topical drug delivery systems is gels, which are transparent and opaque preparations. Gels contain transparent gelling agents which help form three dimensional colloidal structures. They are of two types; aqueous gels that are water based and organogels that are organic solvent based. Gels have features like easily spreadable, water soluble, non-staining, less greasy and emollient. During formulation, gels require higher aesthetic value and lower energy.[9]

Niosomes are vesicular systems that, like liposomes, are used to transport lipophilic and amphiphilic drugs. These are non-ionic surfactant vesicles. They are obtained on the hydration of synthetic non-ionic surfactants that can be done with or without use of systemic circulation cholesterol or their lipids.[10]

Encapsulation of drug in the vesicular system has shown to prolong the presence of the drug in, improve penetration into target tissues and this may also reduce toxicity if selective uptake is achieved. *Asparagus racemosus*, commonly known as Shatavari, belongs to the family *Liliaceae*. It contains a major class of steroidal saponins like Shatavarin I to IV along with minor steroids and their glycosides, flavonoids such as rutin and alkaloids. Shatavari root is used to treat nervous disorders, diarrhoea, dyspepsia, tumors and inflammation.[11] The same extracts have shown to possess antiulcer, antioxidant, immunomodulatory, anti-diabetic, phytoestrogenic, anti-ageing and adaptogenic properties. Aqueous extract of *Asparagus racemosus* acts as an immunomodulator. It increases the activity of macrophages, thereby contributing to its anti-inflammatory activity.[12]

MATERIALS AND METHODS

Materials

Asparagus racemosus (Shatavri) powder was procured from Dr. Jain's Forest Herbals Pvt. Ltd., Mumbai. Cholesterol was purchased from Merck, Mumbai, India. Chloroform, span 60, disodium hydrogen phosphate, potassium dihydrogen orthophosphate, carbopol 934 and triethanolamine was obtained from Lobachemie, Mumbai, India. Ethanol was purchased from Nice chemicals, Kerala, India. All the chemicals used were of analytical grade.

Extraction of plant material (Soxhlet extraction)

Dried powder of *Asparagus racemosus* was collected from localities. Weigh approximately 150 g of powder and was kept in the thimble. Then 500ml of ethanol was added to the round bottom flask the solution was heated at 50°C for 6-7 hr to complete 5 cycles per day. The apparatus was switched off until 50 cycles were completed. The collected extract was then dried at 90°C in a water bath for concentration.[13] To determine the various phytoconstituents, the prepared extract was subjected to various chemical tests according to standard procedure.[14]

Fourier transform infrared spectroscopy (FTIR) study

FTIR spectroscopy of the extract was carried out to find the principal peak and compare with the reference standard. It was also performed to check the compatibility between constituents present in the extract, span 60 and cholesterol in the case of niosomes. The FTIR spectra of the extract and optimized niosomal formulation were recorded on an Alpha Bruker spectrometer. After obtaining the FTIR spectra for the samples, the reference peak was compared and analysed for any incompatibilities.

Determination of λ_{max} and calibration curve of *Asparagus racemosus* extract

Accurately 20 mg of extract was dissolved in ethanol and made up to 10 mL with the same solvent to get 2000µg/mL. 0.5 mL of the above stock solution was transferred into another volumetric flask. It was made up to 10 mL with the phosphate buffer pH 5.5. This solution was then scanned between 200-600 nm. The extract showed maximum absorbance at 274 and 365 nm which showed the presence of total saponins and rutin respectively. The absorbance of the 5-25 µg/mL samples was measured at the $\lambda_{max,}$ and a standard calibration curve was constructed by plotting concentration versus absorbance.

Preparation and characterization of niosomes

Design of experiment

From the literature, the factors that influenced the formation of niosomes were identified as the concentration of span 60 and cholesterol. A 3^2 factorial design was selected to analyse the influence of these factors as shown in Table 1, on responses such as vesicle size, polydispersity index (PDI), zeta potential, and entrapment efficiency by using Design Expert Software (version 11.0.3.0 64-bit, Stat-Ease, Inc. Minneapolis, MN, U.S.A). The following equation is obtained from regression analysis:

$$Yi= \beta_0+ \beta_1A+ \beta_2B+\beta_3C+\beta_4AB+ \beta_5AC +\beta_6BC+ \beta_7ABC$$

Where, β_0- $\beta_{7:}$ standardized beta coefficients for monitored experimental values of $Y_{i,}$ ABC, AB, BC and AC are the interaction term, and present the polynomial term of the independent variables. After a data analysis of the negative and positive signs of coefficient values in equations, the antagonist and agonist of independent variables can be identified. The magnitude of the beta coefficient represents the extent of the impact. Polynomial terms were included to account for the curvature effect of the independent variable. The model's suitability was determined by the statistically significant F ratio (p<0.05) and modified coefficients of determination (adjusted R^2) between 0.8 and 1.0.[15]

Table 1: Factors and their values.

Variables	Levels			Dependent variables	Goal
	-1	0	+1		
Independent variables	250	375	500	Vesicle size Y1	Minimum
Span 60	150	200	250	PDI Y2	Minimum
Cholesterol				Zeta potential Y3	Maximum
				Entrapment efficiency Y4	Maximum

Table 2: Formulation composition batches as per 2^3 full factorial design.

Formulation	Span 60 (mg)	Chloroform (ml)	Cholesterol (mg)	Extract (mg)	Phosphate buffer pH 5.5
F1	250	10	150	500	10
F2	375	10	150	500	10
F3	500	10	150	500	10
F4	250	10	200	500	10
F5	375	10	200	500	10
F6	500	10	200	500	10
F7	250	10	250	500	10
F8	375	10	250	500	10
F9	500	10	250	500	10
F10	375	10	200	500	10
F11	375	10	200	500	10

Preparation of niosomes

Niosomes were formulated using the thin film hydration method as given in Table 2. An accurate amount of surfactant that is span 60 and cholesterol was dissolved in 10ml chloroform. Then the following mixture was subjected to thin film hydration at a temperature of 50°C until the thin film was found. The film was hydrated with 10ml phosphate buffer of pH 5.5 containing extract for 3 hr with gentle shaking.[16]

Characterization of niosomes

Vesicle size, PDI and zeta potential

The vesicle size, PDI and zeta potential of prepared niosomes was used using Malvern zeta sizer which employs dynamic light scattering. Zeta potential is defined as the charge particle obtains when it is present in the medium. It helps in determining the stability of the formulation.

Entrapment efficiency

By using the centrifugation method, entrapment capabilities of niosomal formulations were assessed. At 3°C, the niosomal suspension was centrifuged for 10 min at 8000 rpm. After that, the solid mass was isolated from the supernatant, and PB was used to create the appropriate dilutions (pH 5.5). A UV-visible spectrophotometer technique was used to measure the drug concentration at 274 nm. The following equation was used to compute the drug entrapment efficiency.[16]

$$\text{Entrapment efficiency (\%)} = [(Ct - Cf)/ Ct] \times 100$$

Where Ct is the amount of total drug and Cf is the concentration of unentrapped drug

Formulation and characterization of optimized formulation

The optimized formulation was prepared as per the solution given by the software that is 355.619 mg of span 60 and 231.912 mg of cholesterol was used for the formulation. Vesicle size, size distribution and zeta potential was performed as per the factorial batches. Optimized formulation was further evaluated for the parameters like optical high-resolution microscopy, scanning electronic microscopy and *in-vitro* drug release study.

Optical high-resolution microscopy

A drop of prepared niosomes was diluted with water and placed on a glass slide and then viewed under a high-power microscope (Biovis Particle Analyser-Carl Zeiss Microscopy, NGSMIPS Advanced research centre) for the appearance of vesicles.[17]

Scanning electron microscopy

The obtained niosomes were centrifuged at 10,000 rpm for 15 min then the sediment obtained from centrifugation was subjected to freeze drying. The obtained solid residue was viewed under SEM.[18]

Preparation and characterization of niosomal gel

Preparation of gel

The extract-loaded niosomal gel (10% w/v) was prepared by adding niosomal suspension (equivalent to 1 g of total saponins in the extract) to 10 g of 1% Carbopol 934 gels. Whereas conventional gel (10% w/v) was prepared by incorporating extract equivalent to 1 g of total saponins into 10 g of 1% Carbopol 934 gel. Triethanolamine was introduced and it was stirred rapidly to achieve the gel-like consistency.[19]

Drug content

500 mg gel was added and dissolved in 100 mL phosphate buffer pH 5.5. The gel solution filled in the volumetric flask was shaken continuously with the help of a mechanical shaker for 2 hr to enhance the solubility of the drug. Then 1ml of gel solution was made up to 10 mL phosphate buffer pH 5.5 and was scanned under UV-Visual spectroscopy at λ_{max} 274 nm using phosphate buffer pH 5.5 as blank.[20]

Spreadability, Viscosity and pH measurement

The spreadability of niosomal gel was determined using two glass slides having a similar length. To one glass slide, 1 g of gel was added to the other glass slide weights were added. Time taken by the second glass slide to slip off from the first was determined.[21] The viscosity of the gel was measured using a Brookfield viscometer (LV Model D 220 NGSMIPS Advanced research centre). A small amount of gel was taken and then rotated at 10 rpm using T bar spindle number 96 and the values were noted.[21]

pH of the gel was measured using a digital pH meter.

Ex-vivo drug permeation studies

From the slaughterhouse, goat ear skin was collected; the hair which was removed from the skin and immersed in the phosphate buffer pH 5.5. By using a Franz Diffusion cell containing two compartments the *ex vivo* studies of the skin were carried. The donor compartment involves two open ends where one end of the donor compartment is covered with animal skin which was soaked previously with phosphate buffer pH 5.5. Add 500 mg of gel (equivalent to 50 mg of total saponin) on the dermal side of the skin in the donor compartment. 50 ml of pH 5.5 phosphate buffer that included a tiny magnetic bead and swirled at a fixed speed of 50 rpm was placed in the reservoir compartment. For eight hours, the investigation was conducted at 37±0.5°C. At regular intervals, 5 mL of samples were taken from the reservoir compartment, and absorbance was calculated spectrophotometrically at 274 nm. To keep the sink state constant, the reservoir compartment was consistently refilled with the same amount of new 5.5 pH phosphate buffer.[22]

Calculation of Skin Permeation Parameters

As a function of time, the cumulative amount of drug permeated by unit area was calculated. The flux was determined from the linear portion of the slope. The extract permeability coefficient (*Kp*) through goat skin was determined using the relationship established from the first law of Fick's diffusion, represented in the following equation:

$$Kp=J/C$$

Where *J* is the flux and *C* is the drug concentration in the donor compartment.[23]

Anti-inflammatory activity

Using a method to cause oedema in a rat's paw using carrageenan, anti-inflammatory activity was assessed. After receiving proper consent from the Institutional Animal Ethical Committee (IAEC) of the NGSM Institute of Pharmaceutical Sciences, Deralakatte, Mangalore, albino rats of the Wister strain (150–200g) were utilised (Approval No: NGSMIPS/IAEC/MARCH-2019/144). The animals were housed in plastic cages with plush bedding and six to a cage under the usual lighting and darkness schedules. They were given free access to food (a typical pellet diet) and tap water. Before the test, the rats were given a week to acclimate. 12 hr before the trial and throughout it, food was removed. Three animals were each made up of the three groups from which the animals were separated. The first was used as a control and received a gel base (500 mg/kg) without medication. The second group received 1% diclofenac gel (10 mg/kg) as standard, and the third group received 1% niosomal gel (500 mg/kg). All gels were applied on the dorsal surface of the paw with 50 times gentle rubbing. Before 30 min of gel application, a carrageenan solution (1% w/v in normal saline) was employed to cause inflammation. To reduce any stress-related behavioural changes, the animals were kept in isolation in observation chambers for 10 min. Paw volume was measured before injecting the carrageenan solution and after 30, 60, 120 and 180 min using of Plethysmograph.[24-26]

$$\% \text{ inhibition}=\frac{V_c - V_t}{V_c}\times 100$$

Where, Vc and Vt represents the average volume of the paw of the control and the treated animals respectively

RESULTS AND DISCUSSION

Extraction and phytochemical testing

Ethanolic extract of *Asparagus racemosus* was a yellowish-brown colour, and the percentage yield was 7.56%, respectively. The preliminary phytochemical investigation revealed that the plant extract included flavonoids and saponins and the results are shown in Table 3.

FTIR study

An FTIR spectrum of the extract was shown in Figure 1a. The peak observed at 3319.50 cm^{-1} corresponds to the hydroxyl group of the Shatavari extract. The peak at 1634 cm^{-1} is attributed to the carboxyl group. Aromatic unsaturation (C=C) peak is observed at 1416 cm^{-1} and 1016.99 cm^{-1} corresponds to C-O-C. CH_3 can be observed at 1372.90 cm^{-1} and asymmetric CH stretch at 2936.62 cm^{-1}. FTIR spectrum of optimized niosomal formulation was shown in Figure 1b, it was found that the important peaks observed in the FTIR of the extract are retained in the formulation, which indicates that there are not many considerable interactions of the extract with the components of the formulation.

Determination of λ_{max} and calibration curve of *Asparagus racemosus* extract

The U.V absorption spectra of *Asparagus racemosus* extract showed two maximum peaks at 274 and 365nm as shown in Figure 2. The 274 nm peak may be due to total saponins and 365 nm may be due to flavonoids as said rutin. The λ_{max} 274nm was selected for further investigation.[26] The calibration curve of plant extract in phosphate buffer pH 5.5 follows beers –lamberts in a given concentration range between 5-25 µg/ml with a regression coefficient of 0.9994 as given in Figure 3.

Table 3: Phytochemical testing.

Tests	Observation	Inference
Saponins	Appearance of froth	The presence of saponins confirmed
Flavonoids	Yellow colour produced in organic layer	Flavonoids present
Shinoda test	Pink colour	The presence of flavonoids confirmed

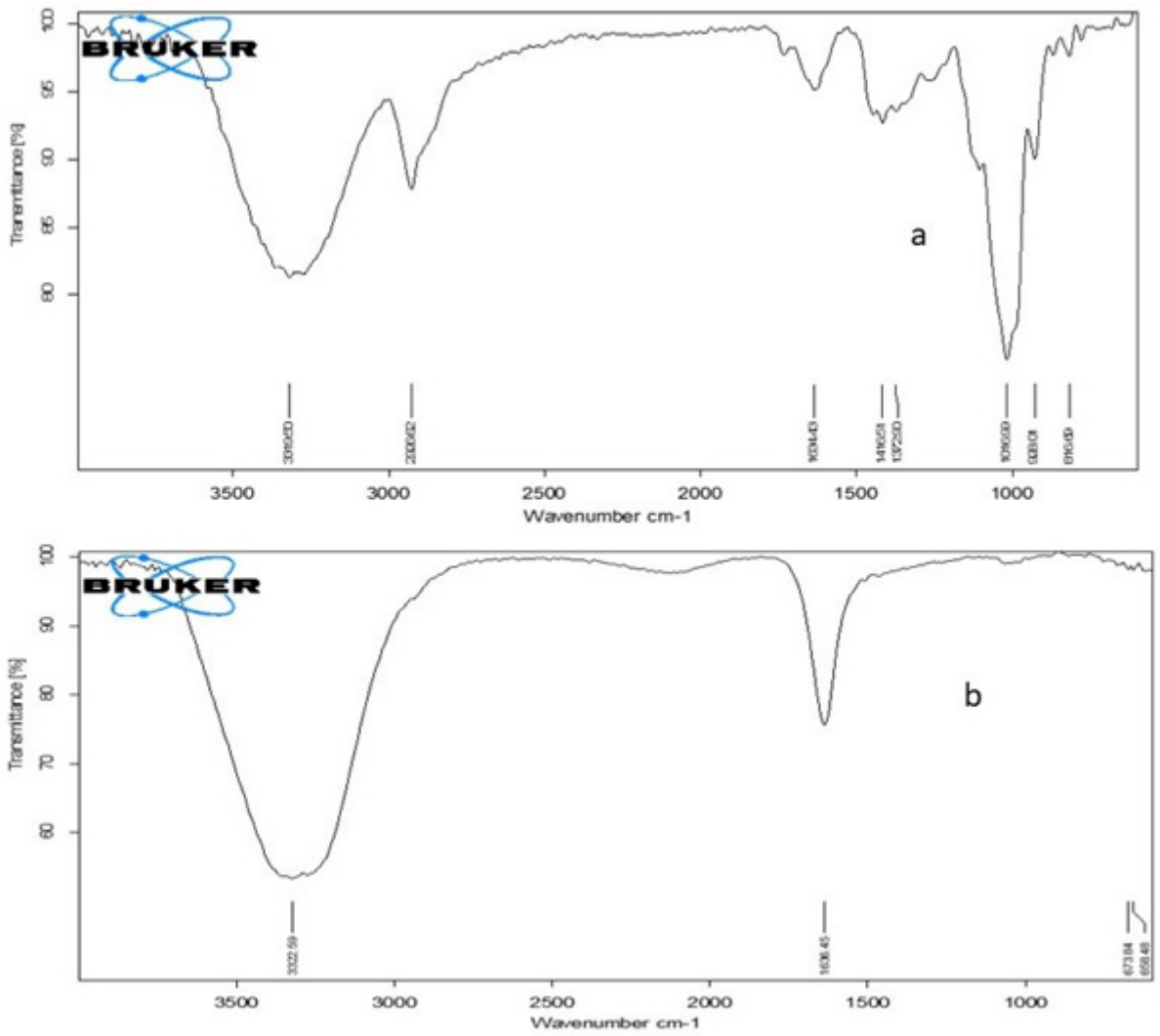

Figure 1: FTIR spectra of a) *Asparagus racemosus* root extract b) Niosomes loaded with extract.

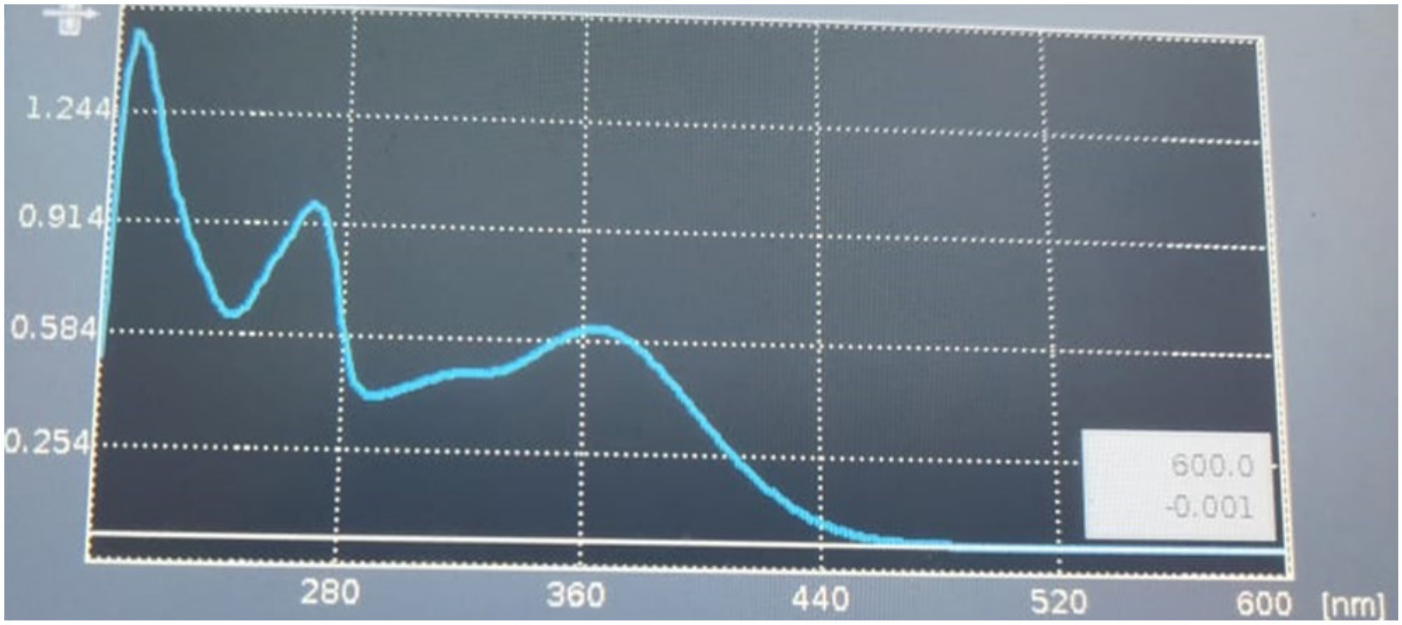

Figure 2: UV spectra of *Asparagus racemosus* root extract in phosphate buffer pH 5.5.

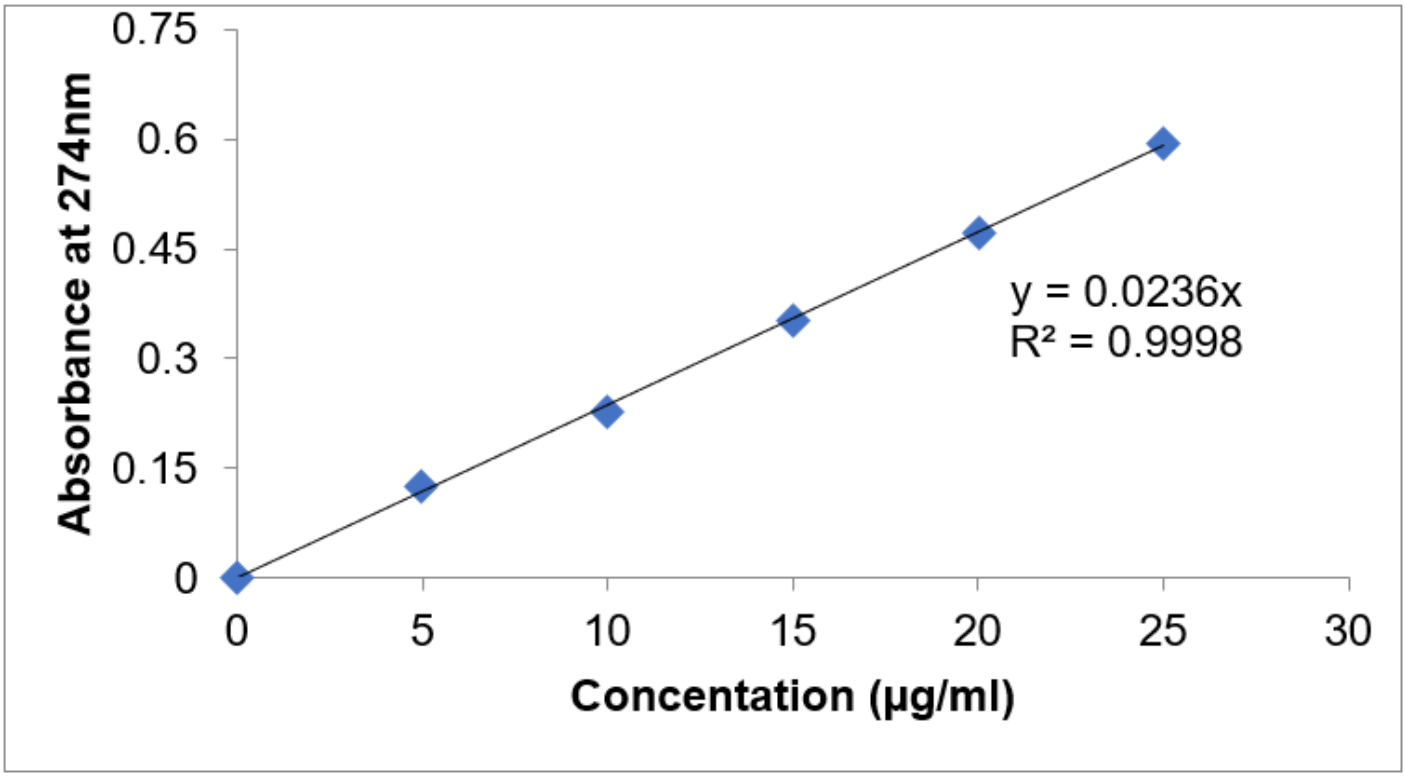

Figure 3: Calibration curve of *Asparagus racemosus* extract in phosphate buffer of pH.

Formulation and characterization of niosomes

Statistical analysis of experimental design

The extract containing total saponin loaded with niosomes was successfully formulated employing 3^2 factorial designs to comprehend how cholesterol and the nisomal component span 60 affect its characteristics.

Vesicle size

Vesicle size plays a important role in the permeation of niosomes through the skin as per literature. The impact of span 60 and cholesterol on vesicle size was shown in Table 4 and Figure 4. It was found that as the concentration of surfactant was increased, vesicle sizes were increased may be higher contraction led to the aggregation of vesicles. As shown in Table 5, the model created

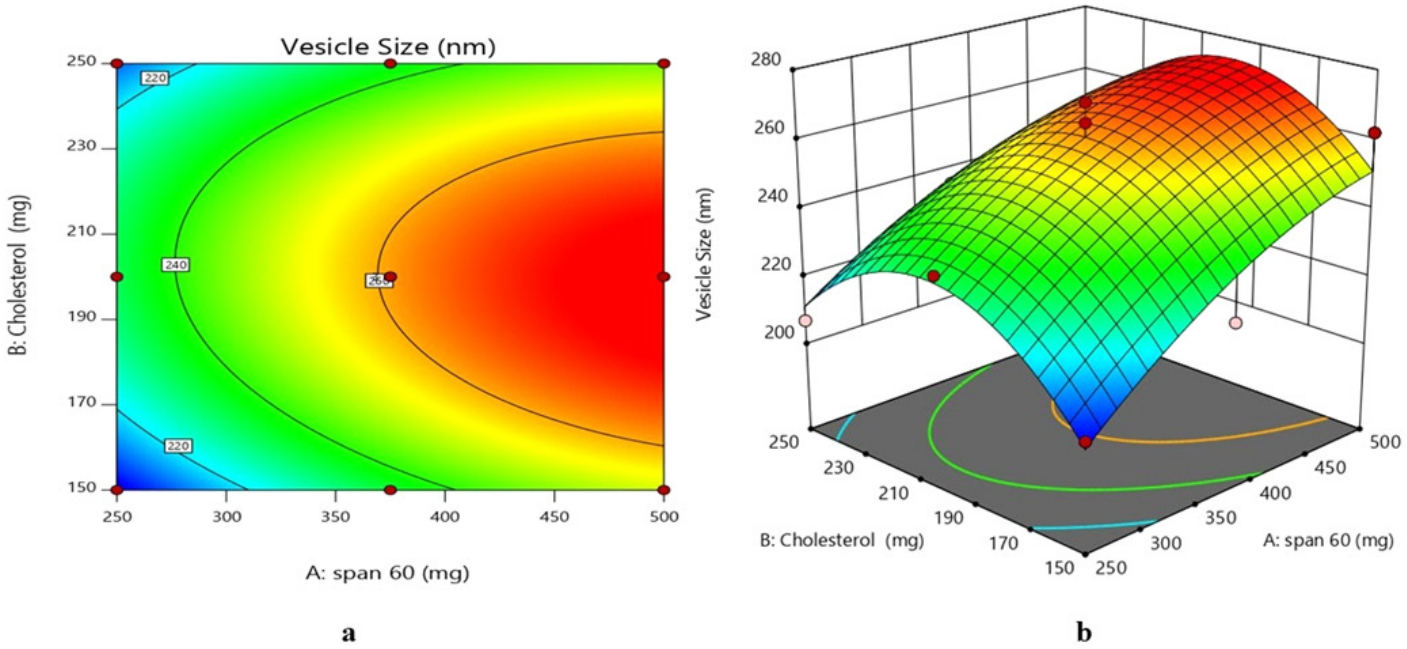

Figure 4: Responses surface curve representing a) Contour and b) 3D effect of span 60 and cholesterol on vesicle size.

Table 4: Results of responses of niosomes as per full 3^2 factorial.

Form. No.	Vesicle size nm	PDI	Zeta potential mV	Entrapment Efficiency %
F1	204.8±2.30	0.36±0.020	-30.8±2.3	45± 3.3
F2	221.4±1.59	0.39±0.028	-29.8±2.6	60± 2.4
F3	262.2±2.60	0.485±0.015	-25.9±1.8	48± 4.1
F4	234.6±2.35	0.372±0.019	-24.6±1.5	56± 2.7
F5	270.8±3.95	0.395±0.025	-29.3±2.10	65± 3.1
F6	258.4 ±2.55	0.42±0.023	-28.6±1.65	56±3
F7	206.9±4.78	0.386±0.016	-26.1±2.35	50± 4.1
F8	235.5±2.78	0.41±0.024	-28.5±2.10	55± 2.7
F9	250±3.45	0.398±0.022	-31.5±1.98	34± 3.6
F10	260.8±3.90	0.398±0.013	-28.4±2.30	63± 3.2
F11	265.9±5.86	0.3870.019	-29.6±2.24	62± 2.3

for the vesicle size has a *p*-value of less than 0.05 and a F value of 6.83 and model was significant. The value of 8.75 indicates a non-significant lack of fit, indicating that the model can be used to determine and calculate the particle size. The adjusted R^2 value (0.5541) and anticipated R^2 value (0.3565) differed by less than 0.2, showing fair agreement between the two.

The analysis's findings led to the following quadratic equation

$$\text{Vesicle Size} = +260.95 + 20.65A^{*} + 0.6667B - 3.58AB - 7.78A^{2*} - 25.63B^{2*}$$

Where A is the concentration of span 60 and B is the cholesterol concentration, the co-efficient in this equation represents the stranded beta co-efficient and the asterisk sign indicates the significance of the variables. With high adjusted R^2 values of 0.5541, the derived regression model was proven to be statistically significant ($p<0.05$).

PDI

PDI show the homogeneity of vesicle size. The lower value of PDI (<0.5) shows formulation is more homogeneous in nature. Results show that as the cholesterol concentration was increased PDI value decreased as shown in Table 4 and Figure 5. The Polynomial model implied to be significance with a model having *F*-value of 14.97. The *p*-value of 0.1092 indicates that the model is a non-significant lack of fit, and was significant to calculate the

Table 5: Summary of regression analysis of niosomes.

Factor	Vesicle size (Adjusted R^2=0.9174)		PDI (Adjusted R^2=0.8074)		Zeta potential (Adjusted R^2=0.5096)		Entrapment efficiency (Adjusted R^2=0.9030)	
	Estimated Beta Co-Efficient	*P* value	Estimated Beta Co-Efficient	*P* value	Estimated Beta Co-Efficient	*P* value	Estimated Beta Co-Efficient	*P* value
Intercept	260.95	0.0275	0.4001	0.0020	-28.46	0.0472	64.58	0.0027
A-Span 60	20.65	0.0082	0.0308	0.0011	-0.7500	0.2588	-2.17	0.1228
A-cholesterol	0.6667	0.8965	-0.0068	0.2799	0.0667	0.9161	-2.33	0.1023
AB	-3.58	0.5752	-0.0282	0.0055	-2.58	0.0108	-4.75	0.0210
A^2	-7.78	0.3469	-	-	-	-	-10.45	0.0021
B^2	-25.63	0.0189	-	-	-	-	-8.95	0.0042
Lack of Fit	-	0.1043	-	0.1092	-	0.1200	-	0.1660

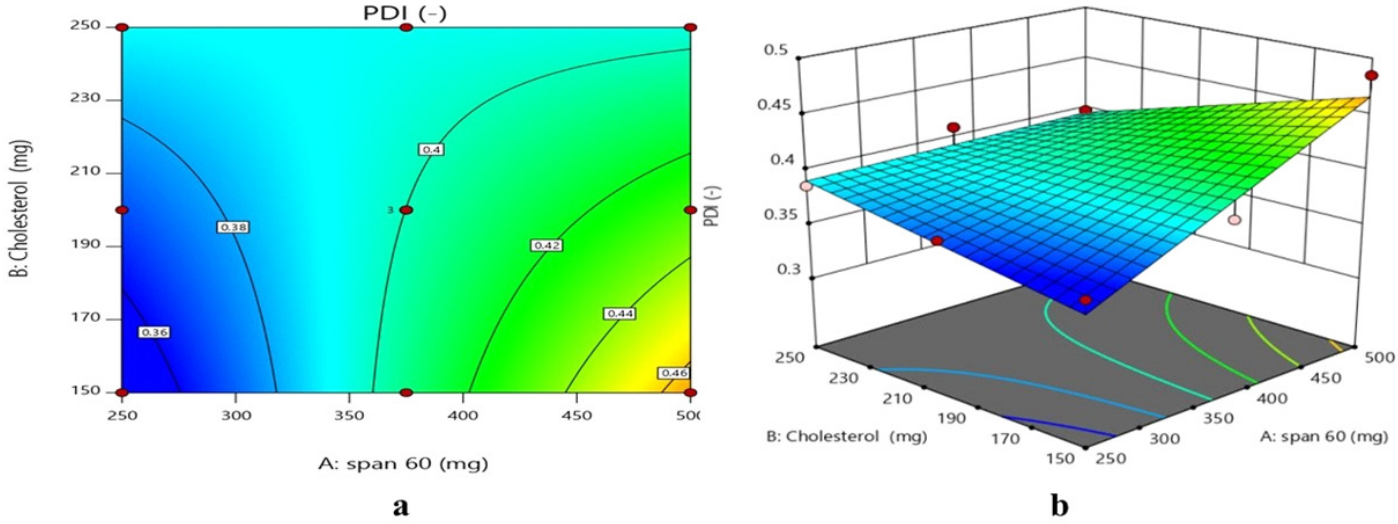

Figure 5: Responses surface curve representing a) Contour and b) 3D effect of span 60 and cholesterol on PDI.

size of the particle. The equations for the response i.e., PDI based upon the 2FI (interactive effect) show the equation as;

$$PDI=0.4001 + 0.0308 (A^*) -0.0068 (B) -0.0283 (AB^*)$$

Where A is the concentration of span and B is the concentration of cholesterol the co-efficient in this equation represents standardized beta co-efficient and the asterisk sign indicates the significance of the variables The obtained regression model was proven to be statistically significant (p<0.05) with high adjusted R^2 values of 0.8074 as shown in Table 5.

Zeta potential

Zeta potential can affect the stability of the vesicles. During storage, the aggregation of vesicles is stopped by the electrostatic repulsive force produced by charged particles. The cholesterol at all SPC concentrations prevents or at least delays the formation of electrostatic repulsions cause vesicle clump to develop as shown in Table 4 and Figure 6. A p-value of <0.05 and a F Value of 4.46 in the model developed for particle size indicated that the model was significant. Since the value of 7.62 shows that the lack of fit is not statistically significant, the model can be used to determine the particle size. The polynomial equations for the response i.e., Zeta potential based upon the 2FI (interactive effect) are

$$Zeta\ potential= -28.46 - 0.750\ A^* +0.0667) - 2.58\ AB^*$$

Table 5 shows that the generated regression model had high adjusted R^2 values of 0.5096 and was statistically significant (p<0.05).

Entrapment efficiency

As the concentration of span, 60 increases at all concentrations of cholesterol, the percentage efficiency of the entrapment was marginally decreased as shown in Table 4 and Figure 7. It may be chances of vesicle aggregation rising at higher concentrations of span 60 which often reduces the potential to form a stable film surface. As a consequence, drug leaching happens, and thus the efficiency of entrapment reduces. As can be seen in Table 5, the model developed for the particle size had a significant p-value of

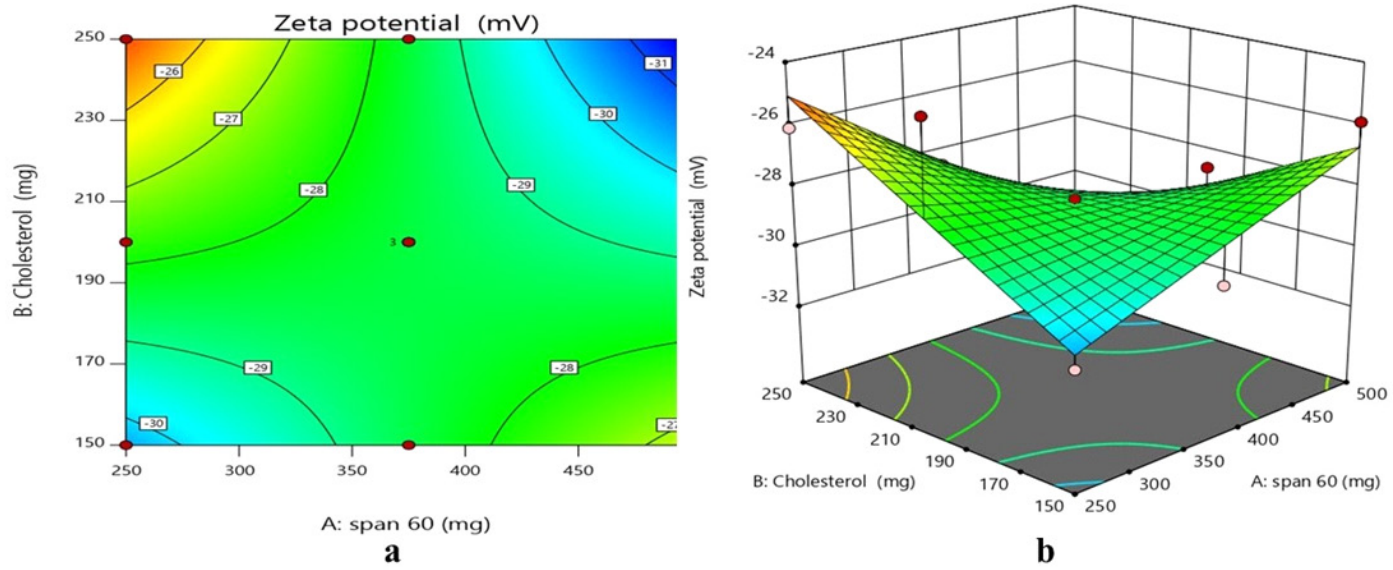

Figure 6: Responses surface curve representing a) Contour and b) 3D effect of span60 and cholesterol on zeta potential.

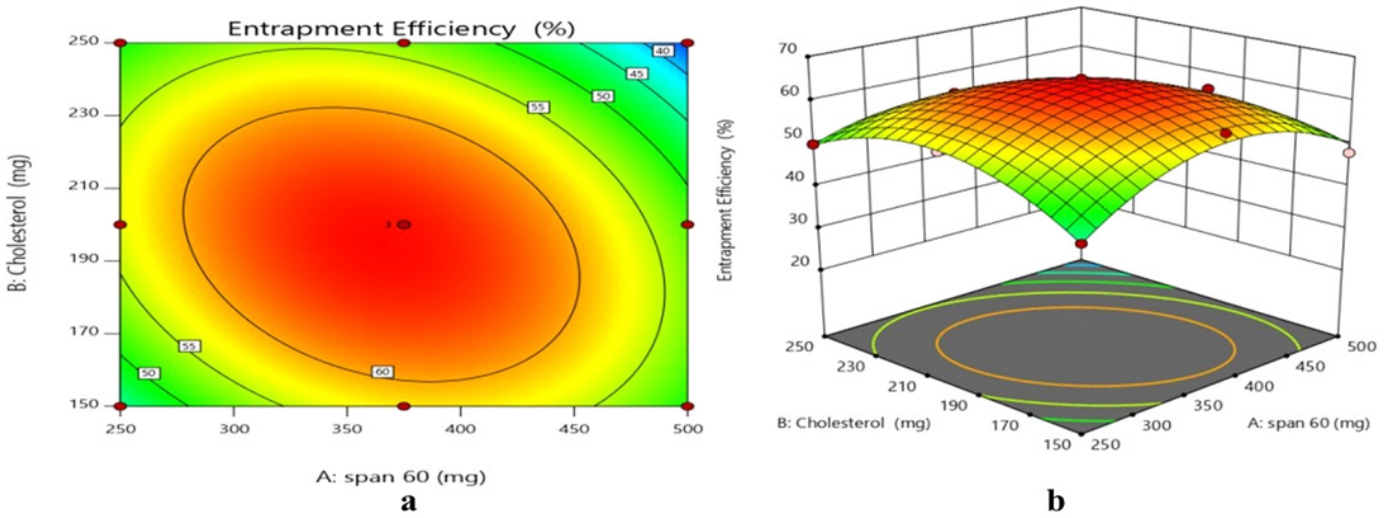

Figure 7: Responses surface curve representing a) Contour and b) 3D effect of span60 and cholesterol on entrapment efficiency.

<0.05 and a F value of 19.61. The difference between the adjusted (R^2 0.9030) and the predicted (R^2 0.7357) model R-squared value was less than 0.2 showing fair agreement between the two. The polynomial equation which was obtained from the results of the analysis as shown below:

$$\text{Entrapment Efficiency} = 64.58 - 2.17\,(A) - 2.33\,(B) - 4.75\,(AB^*) - 10.45(A^{2*}) - 8.95\,(B^{2*})$$

Where A is the concentration of span and B is the concentration of cholesterol the co-efficient in this equation represents standardized eta co-efficient and the asterisk sign indicates the significance of variables The obtained model for regression was found to be statistically significant (p<0.05) having a high adjusted R^2 values of 0.9030.

Optimization of niosomes loaded with extract

Niosomes were optimized based on constraints such as minimum vesicle size and PDI, maximum zeta potential and entrapment efficiency. Based on a desirability value greater than 0.8, the optimal formula was chosen. The formulation which was desirable and selected was prepared according to the solution given by the software containing 355.619 mg of span 60 and 231.912 mg of cholesterol. The vesicle size, PDI, zeta potential, and entrapment efficiency values given by the software were 245.90 nm, 0.394, -28.05 mV, and 60.8% respectively, whereas the experimental value was found to be 248.8 nm, 0.3734, -28.4 mV, and 62.3% as shown in Figure 8. The observed values were found to be within ± 5% error of the predicted value which is acceptable.

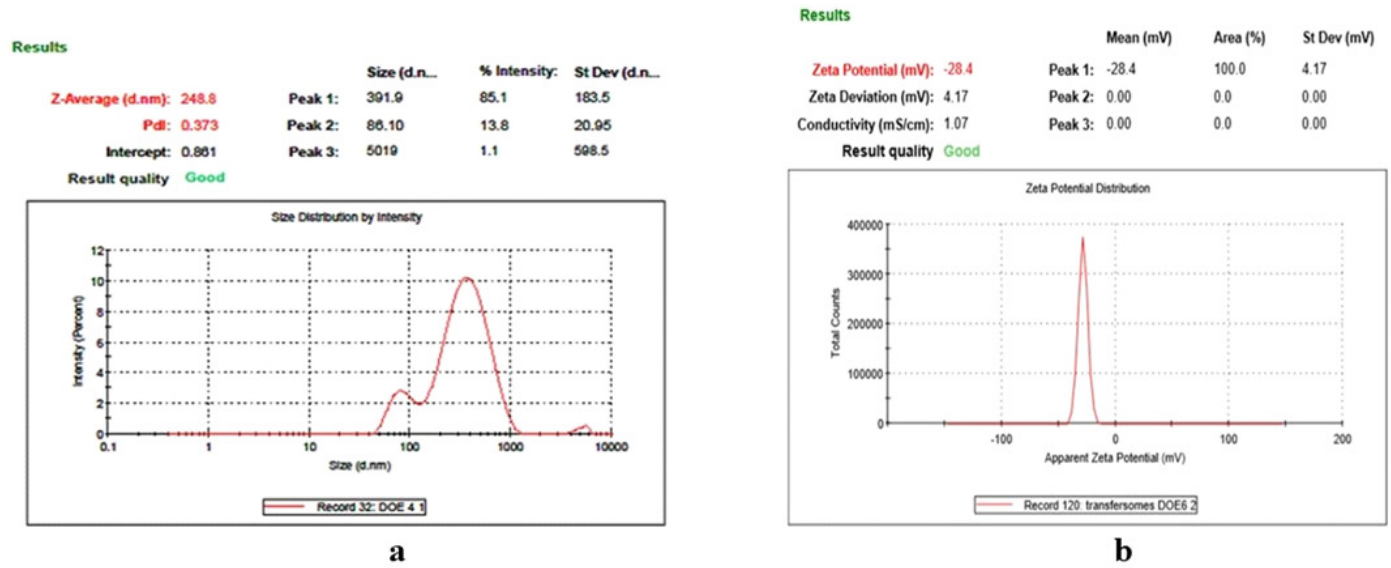

Figure 8: Optimized niosomal formulation a) Vesicle size b) Zeta potential.

Table 6: Result of pH, spreadability, drug content and viscosity of gels.

Form. code	Measurement of pH	Spreadability (g/cm²)	% Drug content	Viscosity (cps)
NGL	6.4	15.26	85.61±1.63	4968.4 ± 12.61
CGL	6.2	14.94	87.56±1.34	4297.7 ± 10.92

Table 7: The permeated amount of extract at 480 min, flux, and permeability coefficient.

Form. Code	Permeated amount at 480 min (µg/cm²)	Flux (µg/cm²).min	Permeability constant (K_p) × 10^{-3} (cm/hr)
Conventional gel	2800 ±6.23	5.283	0.5283
Niosomal gel	4200±7.95	8.119	0.8119

Table 8: Effect of gels on carrageenan-induced rat paw edema.

Sl. No	Treatment Group	Mean Volume of Edema Induced By Carrageenan (Ml)			
		30 min	60 min	120 min	180 min
1	Control	0.36 ± 0.05	0.47± 0.09	0.52± 0.06	0.57± 0.10
2	1% Diclofenac Gel	0.25 ± 0.02	0.20 ± 0.03	0.18 ± 0.04	0.16 ± 0.03
3	Niosomal Gel (1%)	0.31± 0.06	0.27± 0.04	021± 0.06	0.20 ± 0.05

(Mean±SD, *n*=3)

Scanning electron microscopy

The vesicle shape and the surface morphology of the formulations was determined by Scanning electron microscopy (SEM) as shown in Figure 9. The vesicles observed were spherical and uniform. The surface of the vesicles was found to be smooth and also the SEM analysis also supported that the vesicle size was less than 500 nm.

Formulation and characterization of Niosomal gel

The niosomal (NGL) and conventional (CGL) gels that were created were uniform, off-white in colour, smooth, and gritty-free. The findings of measuring the pH, spreadability, medication content, and viscosity of both gel formulations are displayed in Table 6.

For topical formulations, pH evaluation is a crucial factor because if it differs from the pH of normal skin, it could irritate the skin. The pH of the niosomal and conventional gel was observed to be 6.4 and 6.2 respectively shown in Table 6, which are closer to the skin pH. The mechanical and physical characteristics of the preparation, such as its spreadability, consistency, and hardness, which in turn are connected to how easily the product can be removed from the container, are all influenced by viscosity, an

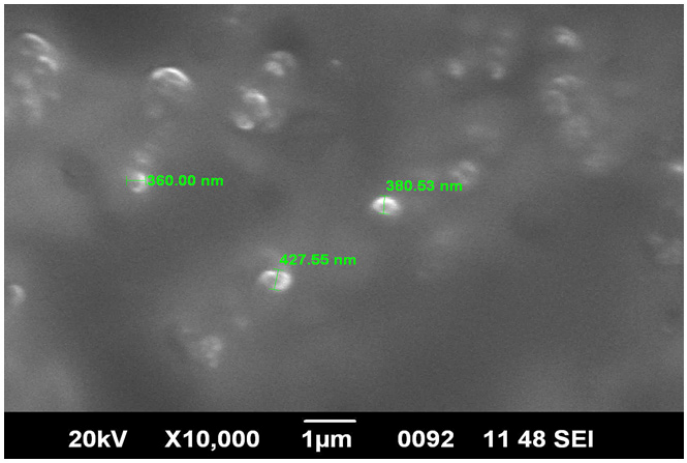

Figure 9: SEM image of optimized niosomal formulation.

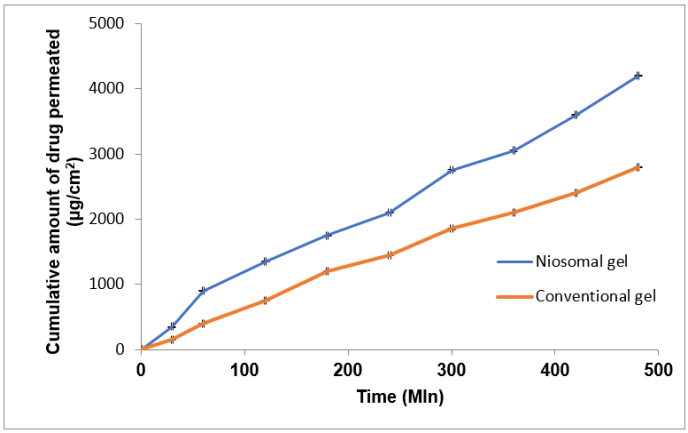

Figure 10: Comparative e*x vivo* drug permeability study of niosomal gels with conventional gel.

important rheological parameter. Both the gels showed required viscosity in order to ease the application of the gel. The drug content of the niosomal gel and conventional gel was found to be 85.61±1.63% and 87.56±1.34% respectively as shown in Table 6. It showed that the drug was uniformly distributed throughout the gel. Drug content uniformity is essential for semi-solid preparation to confirm the homogeneity of the dispersed drug throughout the formulation.

Ex-vivo drug permeation studies

An *ex vivo* permeation study was conducted to determine the amount of drug released through the goat skin. The permeation profile of total saponins present in an extract from niosomal and conventional gel formulation is shown in Figure 10. The total quantity of saponins delivered from niosomal gel was 4200 µg; this was far more than the amount supplied by conventional gel which was substantially higher, which was 2800 µg ($p< 0.0001$). The inclusion of surfactant, which aids in the solubilization of the lipid in the stratum corneum and permits high vesicle penetration, may have contributed to the improved permeation of the niosomal gel extract. Parameter of permeability, as shown in Table 7, in the case of niosomal gel the steady-state flux was

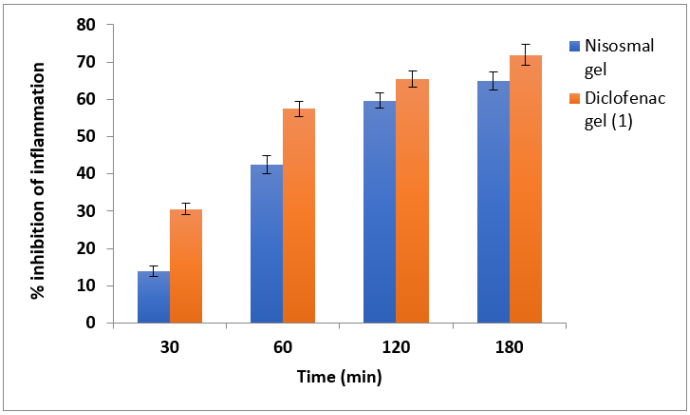

Figure 11: % inhibition of inflammation by niosomal gel (test) and diclofenac gel (Standard) on carrageenan-induced rat paw edema (*n*=3).

found to be greater than the conventional gel. The steady-state flux and permeability coefficient of niosomal gel was 8.119 µg/cm^2. min and 0.8119 cm/min respectively whereas conventional gel is shown to be 5.283 µg/cm^2.min and 0.5283 cm/min respectively after 480 min (8 hr). It was discovered that steady-state flow and permeability coefficient are directly related. Results indicated that the flux and permeability coefficient of niosomal gel was 1.55-fold higher than conventional gel.

Anti-inflammatory activity

The niosomal gel of *Asparagus racemosus* significantly reduced the inflammation brought on by the carrageenan-induced paw edema model. After 30 min of induction of edema niosomal gel caused a significant anti-inflammatory effect comparable to control group at the respective time. The extract eventually achieved a maximum inhibitory effect as time went on. 1% Diclofenac gel was used as a standard drug and it produced a significant reduction of paw edema compared to that of the control group as shown in Table 8 and Figure 11. As previously discovered in research, the niosomal gel present in *Asparagus racemosus* extract displayed an anti-inflammatory activity that may have resulted from the inhibition of the production of inflammatory mediators such as histamine, serotonin, cytokines, and prostaglandins.[27]

CONCLUSION

From the results, it concluded that the 3^2 factorial design was capable of obtaining an optimized niosomal formula, with small vesicle size and high EE. The gel-containing niosomes performed through goat skin showed increased effectiveness in penetrating the drug through the skin. The animal model proved that the niosomal gel loaded with an extract containing total saponin showed a significant anti-inflammatory effect.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SEM: Scanning electron microscopy; **µg:** Microgram; **FT-IR:** Fourier transform infrared spectroscopy; **PDI:** Polydispersity index.

SUMMARY

Asparagus racemosus extract contains various types of saponins and flavonoids. The phytoconstituents of the extract have less permeability through the skin; so to enhance their permeability and effectiveness it was loaded in nisomes. The main objectives of the study were to design, formulate and characterize the niosomal gel loaded with *Asparagus racemosus* extract for anti-inflammatory activity. The niosomes containing total saponins in the extract were prepared by using 3^2 factorial designs using the thin film hydration method. The span 60 and cholesterol were selected as the independent variable. Vesicle size, PDI, zeta potential, and percentage entrapment efficiency were considered for responses. The prepared gel was evaluated for *ex vivo* studies on goat skin using a modified version Franz diffusion cell. When compared to conventional gel, the niosomal gel provided significantly more steady-state flux as well as permeability coefficient into the skin. The anti-inflammatory study proved that niosomal gel loaded with total saponins in extract showed significant anti-inflammatory compared to the control.

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