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FORMULATION, *IN VITRO* AND *IN VIVO* EVALUATION OF ADAPALENE NIOSOMAL GEL

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MATERIALS AND METHODS

Materials

Adapalene was gifted from Cipla Pharmaceuticals, Ahmedabad, India. Benzoyl Peroxide was gifted from Sun Pharmaceuticals, Hosur, India. Remaining all ingredients was purchased from Himedia laboratories, Hyderabad.

PREPARATION OF NIOSOMAL GEL FORMULATIONS

Preparation of Adapalene niosomal gel

Carbopol 940 gel bases are prepared by homogenizing 1 % (w/w) carbopol dispersion in sufficient water using a magnetic stirrer for 30 minutes and leaving it to equilibrate for 24 hours. After that, pH is adjusted to 4.5 – 6.5 with Triethanolamine (Saleem M.A, et al.,, 2010). The Adapalene loaded niosomes is added to the prepared plain gel base during the stirring process and the step is completed as mentioned for carbopol plain gel bases.

EVALUATION OF NIOSOMAL GEL FORMULATIONS

The prepared Adapalene niosomal gel was subjected for various *in vitro* evaluation tests such as drug content analysis, pH measurements, rheological studies, *in vitro* drug release and stability studies.

***In vitro* permeation study**

Permeation study of prepared antiacne niosomal gel

In vitro skin permeation studies were performed using vertical Franz diffusion cells with an effective diffusion area of 2.54 cm^2. The study was conducted using wistar rat skin. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The donor compartment was filled with weighed amount 200 mg of antiacne Niosomal gel containing 0.1% Adapalene and 0.600% benzoyl peroxide. A 25 ml aliquot of 1:1 (ethanol / methanol: saline) v/v was used as receptor medium to maintain a sink condition. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 600 rpm. At appropriate time interval 3 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution up to 24 h. The samples were analyzed by UV spectrophotometer at 234 nm for benzoyl peroxide and 237 nm for Adapalene. The flux of was calculated for each component from niosomal gel formulation using wistar rat skin.

***In vitro* skin retention study**

The skin was cleaned with cotton dipped in saline solution and blotted with tissue paper to remove any adhering formulation. Subsequently, the skin sample was homogenized with 20 ml of chloroform: methanol/ethanol mixture (2:1, v/v), for the extraction of homogenate suspension thus obtained was filtered using filter paper and quantified for the drugs content using UV spectrophotometer at respective absorption maxima for benzoyl peroxide and Adapalene.

In vivo study

The rabbit ear model was used to study comedo formation in order to assess the comedogenicity of cosmetics, toiletries and dermatological preparation and to evaluate the potential of anti-acne drugs. This comedo induction takes place after about 2 weeks of repeated topical application of a chemical comedogen such as 50% oleic acid. One rabbit was treated as control which receives no treatment, remaining two rabbit receives treatment of 50% oleic acid and dimethyl sulphoxide up to 28 days on the ventral aspect of the pinnas once a day. Rabbits were treated for 3, 7 and 28 days and skin biopsies performed from the treated pinnas at the end of treatment.

Total Score:

The neck skin was shaved carefully. The niosomes prepared with optimized parameters, were incorporated into carbopol gel base. Simultaneously plain drug was also incorporated into gel base as control.

Both the formulations of same strength (0.05%) were applied on shaved rabbit skin for the determination of irritation characteristics. The applied area was covered by cotton and bandage. The observations were carried out at regular intervals of 12, 24, 48 hours for various symptoms such as scaling, lesions and erythema by the in charge pharmacologist.

The symptoms, lesions and erythema were graded as

3 = severe
2 = moderate
1 = mild and
0 = absent

And scaling as

1 = present
0 = absent

RESULTS AND DISCUSSIONS

Drug Content analysis

Adapalene niosomal gel (100 mg) is dissolved in 50 ml of phosphate buffer (pH 7.4) that has been accurately weighed. These solutions are transferred quantitatively to a volumetric flask and diluted appropriately with the same buffer solution. The resulting solutions are then filtered through membrane filters (0.45 mm pore size) prior to spectrophotometric analysis at 237 nm for adapalene.

Table 1: Drug content in niosomal gel

S. NO	Formulation	Drug content %
1	F2	91.11
2	F6	94.21

3	F8	96.99

***pH* measurements**

Using a pH meter, the pH of each niosomal gel is determined. Before each use, this is calibrated with buffered solutions at pH 4, 7, and 10. One gram of gel is diluted with one hundred milliliters of distilled water and stored for two hours. The pH is determined in triplicate, with an average value of 6.8 being recorded.

Table 2: pH Values of niosomal gel

S.No.	F2	F6	F8
1	6.46	6.77	6.82
2	6.77	6.68	6.96
3	6.98	6.79	6.78
Average	**6.73**	**6.74**	**6.85**

Rheological Studies

Using a viscometer (Brookfield+ II LV viscometer), the viscosities (in cps) of the polymers were measured. The spindle (TF 59) was rotated at speeds between 0.5 and 100 rpm. Before taking measurements, samples of the gels were to settle for 30 minutes at the assay temperature (28 /1oC). It was determined that the viscosity of F8 gel was 23,500 cps in 0.5 rpm. In 0.5 rpm, the viscosity of F2 gel was determined to be 18500 cps.

Table 3: Rheological studies of niosomal gel

S.No.	RPM	Viscosity in CPS		
		F8	F6	F2
1	0.1	23500	19500	18500
2	0.5	7000	5500	4700
3	1.0	4500	2500	1650

4	5.0	2400	1620	1200
5	10.0	950	650	550
6	20.0	640	350	200
7	50.0	310	200	118
8	100.0	180	95	70

Transmission electron microscopy

Figures display the results of a transmission electron microscopy (TEM) examination of niosomal gel prepared from FG2 and FG3 formulations. The majority of vesicles were easily identified, spherical and discrete with sharp boundaries, and contained a substantial amount of aqueous space within.

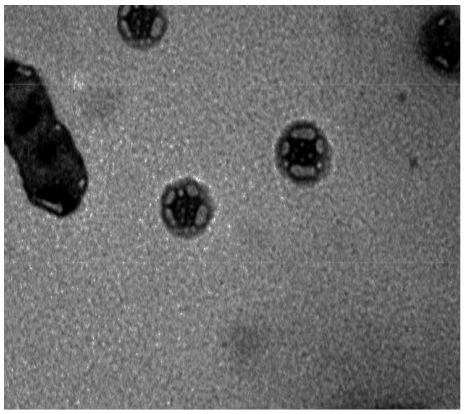

Figure 1: TEM image of niosomal gel F8

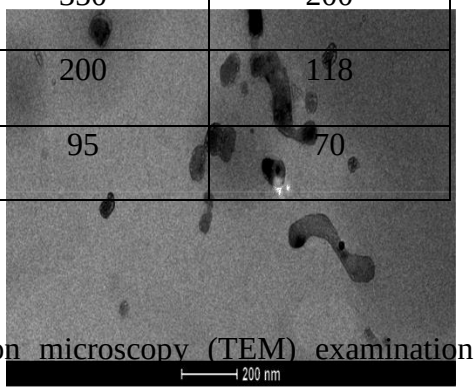

Figure 2: TEM image of niosomal gel F2

***In-vitro* release studies**

The dialysis bag method was used to conduct *in vitro* release investigations for niosomal gel. At 12 hours, the cumulative percentage of drug release from formulations containing (F8) ordinary Adapalene gel was 99.27%. At 12 hours, the cumulative percentage of drug release from a formulation containing (F2) span 60 niosomal gel was 68.93%. At 12 hours, the cumulative

percentage of drug release from a formulation containing (F6) niosomal gel was 88.91%. The results showed that F8 was more effective than F6 and F2 than F6.

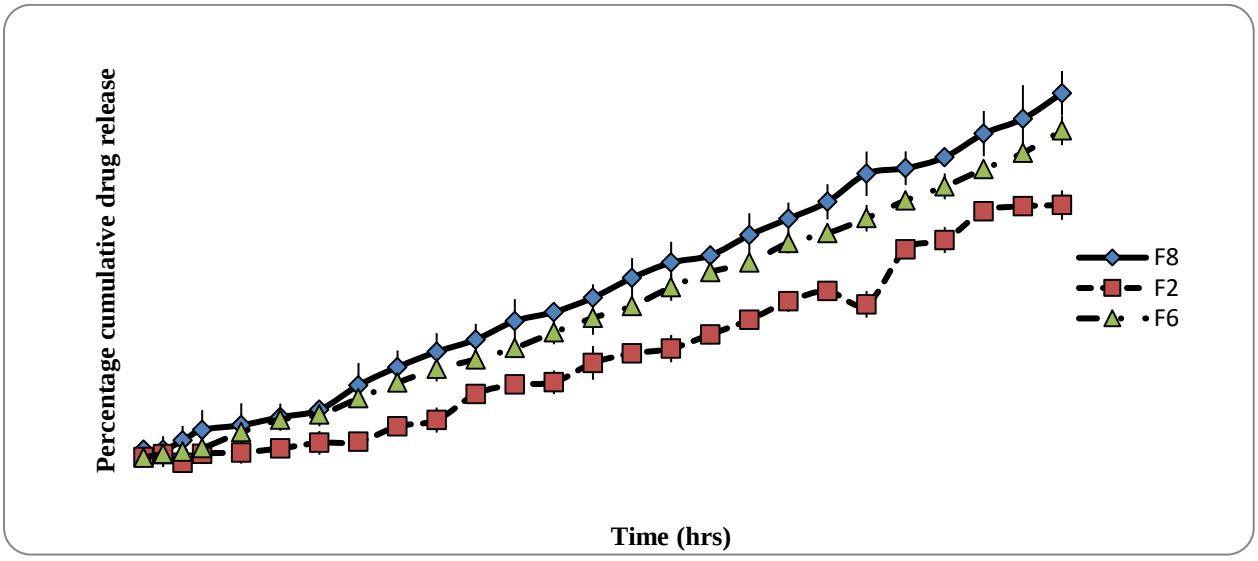

Figure 3: Cumulative % drug release of niosomal gel

Kinetics of drug release

A linear regression analysis was performed to determine the optimal order of drug release. All formulations are governed by zero order kinetics. The calculation of the Higuchi correlation coefficient verifies that the drug's release was proportional to the square root of time, indicating that the release of Adapalene from niosomal gel was controlled by diffusion. According to the Higuchi diffusion-controlled model, the formulations F8 (0.997), F2 (0.994), and F6 (0.995) were developed.

Table 4: Release kinetics of niosomal gel

Formulation code	**R^2 Values of mathematical models of dissolution studies**				
	Zero order	**First order**	**Higuchi model**	**Peppas model**	
				R^2	N
F2	0.980	0.849	0.994	0.985	0.920

F6	0.981	0.871	0.995	0.964	0.728
F8	0.986	0.875	0.997	0.952	0.781

***In vitro* permeation study:**

During experiments lasting 24 hours, the mean quantity of Adapalene and Benzoyl peroxide permeated per unit of surface area was determined. Permeation profiles of niosomal gel (cumulative quantities of Adapalene and Benzoyl peroxide permeated versus time) are depicted in Figure 51. The quantity of Adapalene that permeated from niosomal gel in 24 hours was 6.25± 0.14 g/cm^2 and the amount of Benzoyl peroxide that permeated was 5.04± 0.014 g/cm^2.

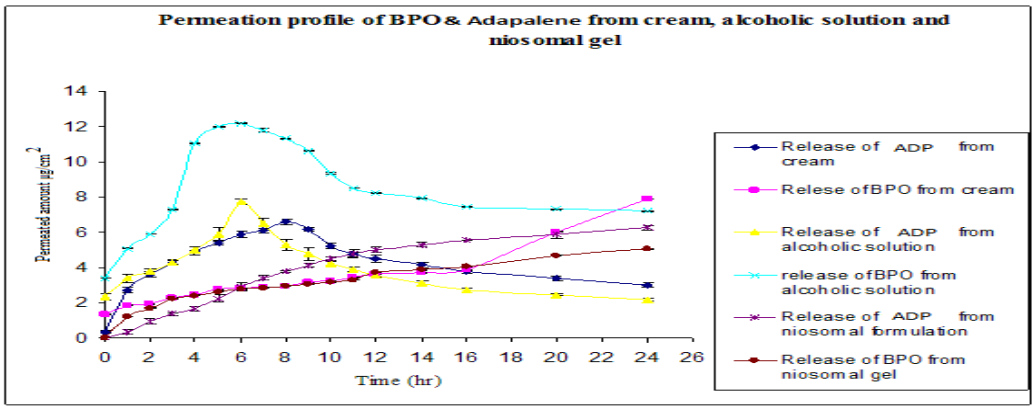

Figure 4: Permeation profile of BPO and Adapalene from niosomal gel

***In vitro* skin retention study:**

The drug content retained in the layers of skin from cream were 3.36 µg from F2 Formulation, 12.28 µg from F4 Formulation, 16.27 µg from F5 Formulation, 60.11 µg from F6 Formulation, 69.37 µg from F7 Formulation, and 148.12 µg from F8 Formulation from niosomal gel.

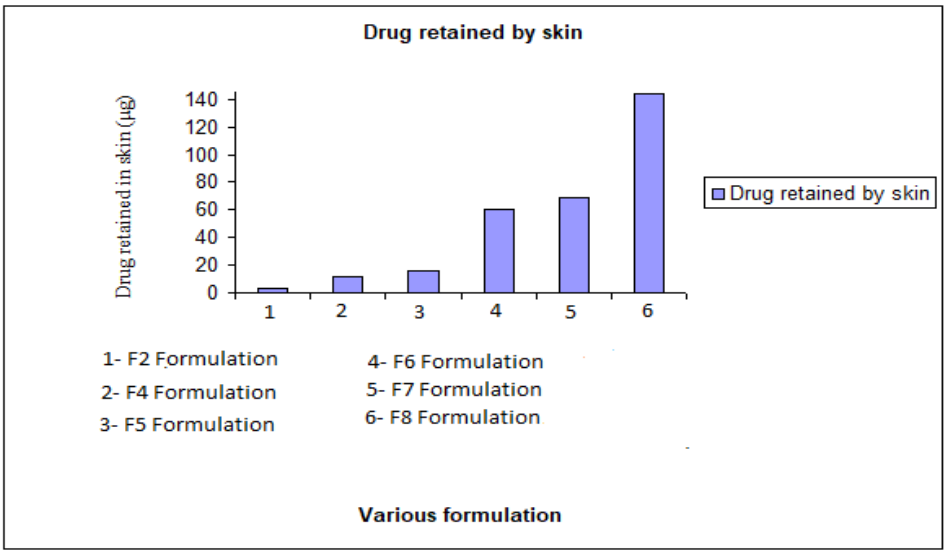

Figure 5: Comparison of *in vitro* retention study of niosomal gel

In vivo studies

Histological investigations revealed that the prepared niosomal formulation was effective for the treatment of acne. A comparison of untreated and treated pinna samples revealed a significant increase in sebaceous gland volume and the presence of multiple comedones. The acne-induced pinna treated with prepared niosomal gel for 14 days showed a significant reduction in sebaceous gland volume and no follicle dilatation, according to histological reports.

Histological investigations demonstrated that the prepared niosomal formulation was effective for the treatment of acne. Comparing the control sample to the treated pinna revealed a significant increase in the volume of the sebaceous gland and the presence of multiple comedones in the treated pinna. In addition, histological reports revealed a significant reduction in the size of the sebaceous gland and the absence of follicular dilatation in acne-affected pinnas treated with the prepared niosomal gel for up to fourteen day.

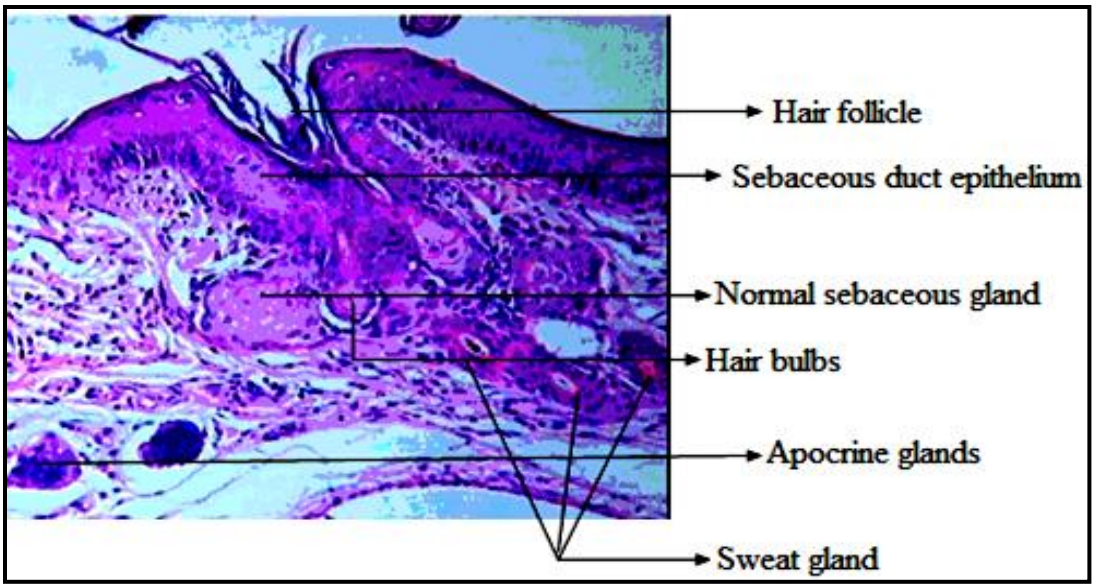

Figure 6: Light micrograph at 10X of normal rabbit ear pinna (control) at 0 day

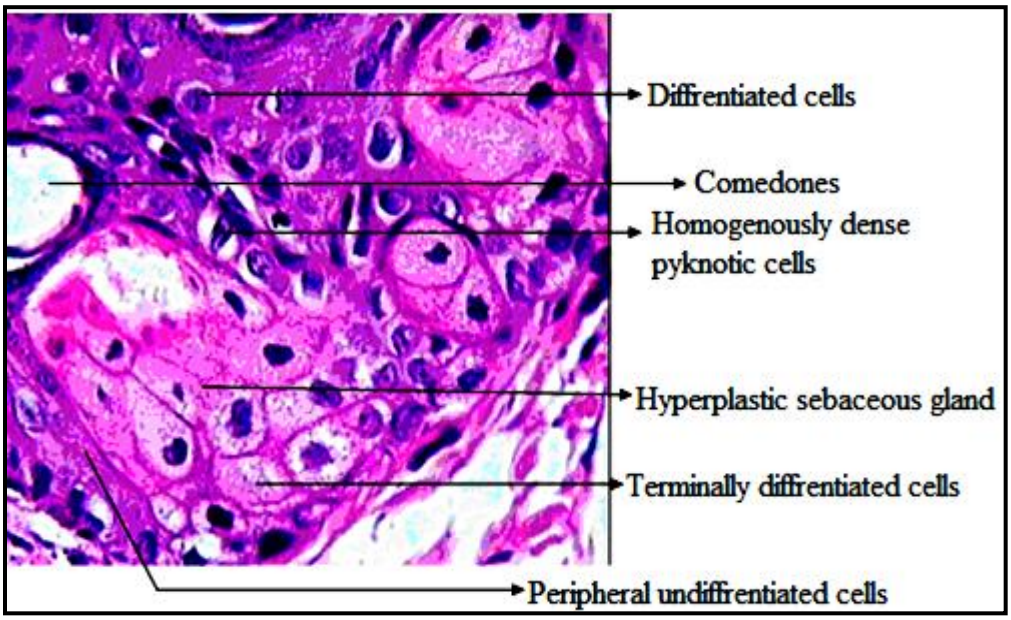

Figure7: Light micrograph of gel treated rabbit ear pinna at 10X (treated pinna) at 7th day

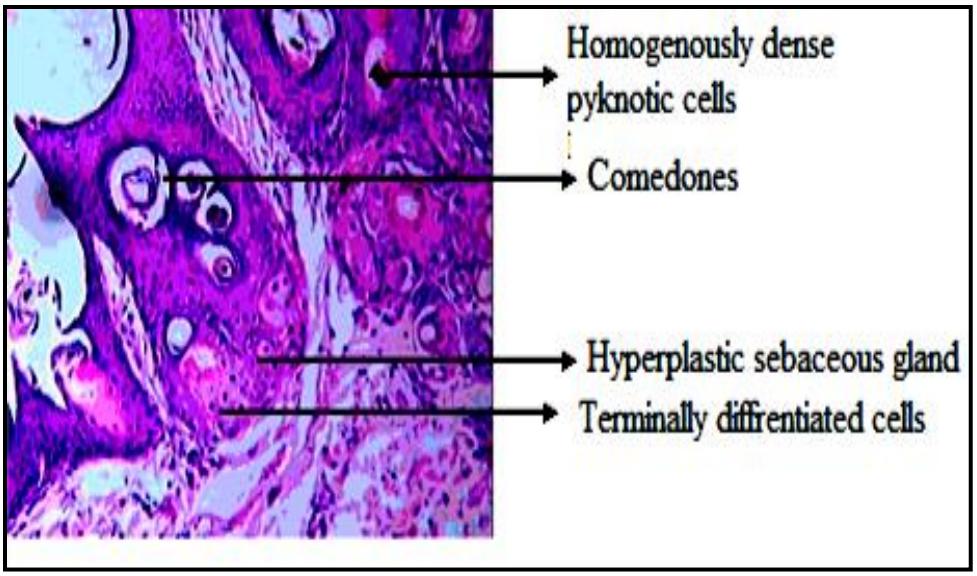

Figure 8: Light micrograph of gel treated rabbit ear pinna at 10 X (treated pinna) at 28th day

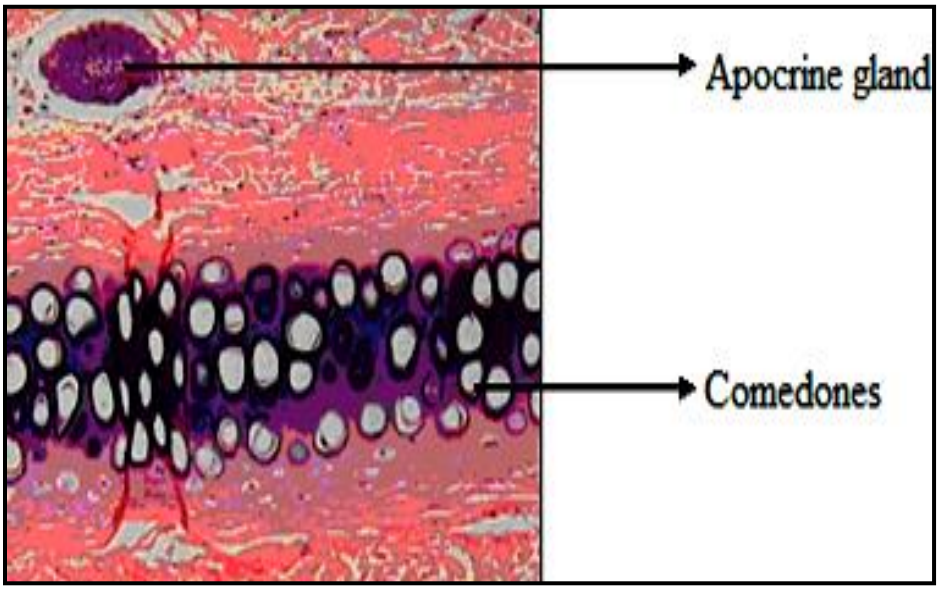

Figure 9: Light micrograph of treated rabbit ear pinna further treated with antiacne niosomal gel for 14 days

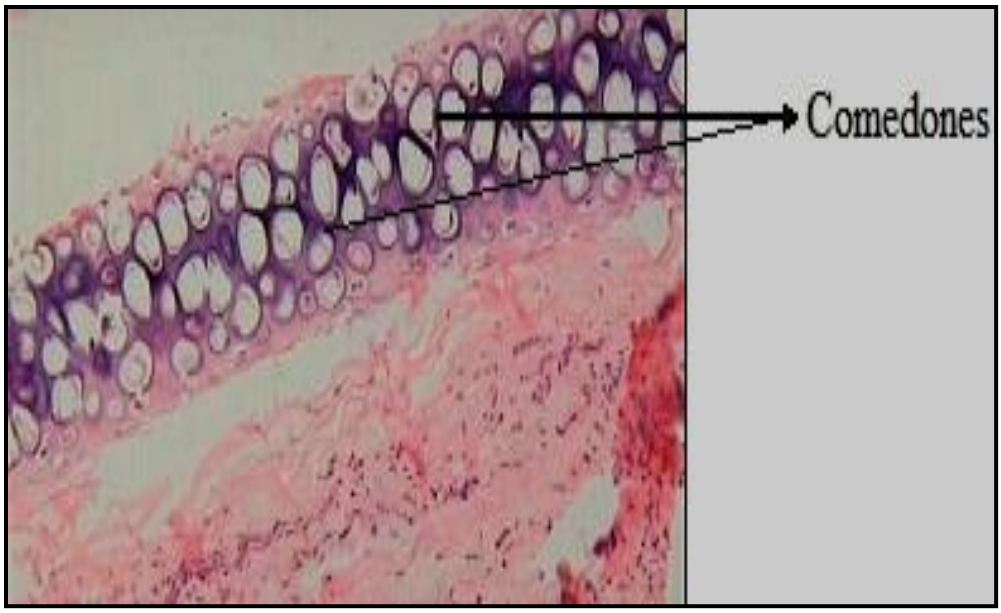

Figure 10: Light micrograph of treated rabbit ear pinna further treated with antiacne suspension for 14 days at 10X magnification

Total Score:

In terms of increased skin permeation and retention, the results indicate that niosomal formulations are preferable to conventional drug formulations. Consequently, it was deemed beneficial to examine the impact of Adapalene niosomal formulations. The rodents treated with plain gel exhibit more signs of irritation. The erythema signs are continually increasing at regular intervals (they were initially marked as 1, but 48 hours later they were marked as 3). Scaling was initially absent with ordinary gel, but appeared after 12 hours on the applied skin portion. As the duration of therapy increased, so did the severity of the lesions.

Table 5: Observations and calculations of i*n-vivo* studies

	Plain drug gel			**Niosomal gel**		
Symptoms	**A**	**B**	**C**	**A**	**B**	**C**
Scaling	0	1	1	0	0	0
Lesions	1	2	2	1	0	1
Erythema	1	2	3	0	1	1
Total score	2	5	6	1	1	2

Where,

A = observation after 12 hours, B = observation after 24 hours

C = observation after 48 hours

The rabbits treated with niosomal gel are exhibiting significantly less irritation. Initially, there were no erythema symptoms, but after 48 hours, there were minor erythema symptoms. With niosomal gel, the scaling was wholly absent initially and even after 48 hours. The lesions were initially and even after 48 hours completely absent. Consequently, based on the above comparisons, niosomal gel is more efficacious than regular gel.

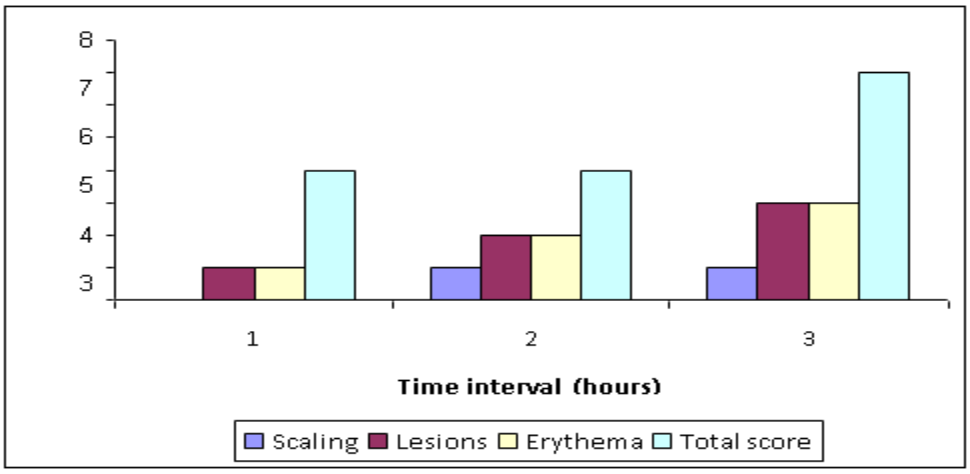

Figure 11: Graphical presentation of in-vivo studies of plain drug gel

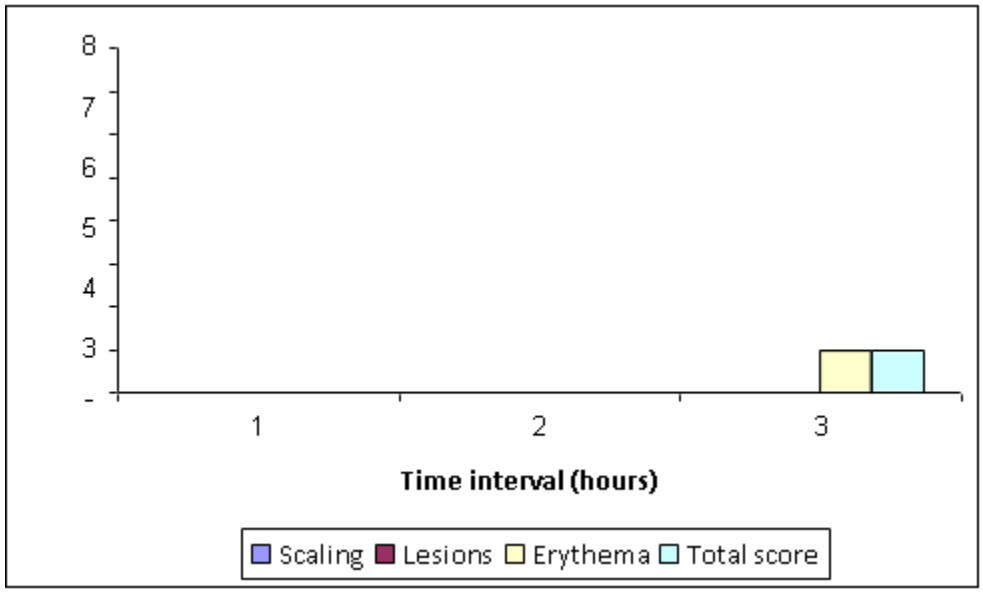

Figure 12: Graphical presentation of in-vivo studies of niosomal gel

Stability studies of niosomal gel

There was no change in the niosomal formulation containing loaded BPO and Adapalene. On the basis of stability study we can concluded that niosomal gel formulation was stable.

Table 6: stability studies of niosomal gel

Formulation	Condition	Percent drug retained (Time in weeks)					
		Initial	**1**	**2**	**3**	**4**	**6**
Niosomal gel	**2 –8 ˚C**	100.27	100.69	98.82	97.55	96.19	93.43
	25˚C /60 %RH	100.27	98.15	97.74	96.84	95.27	90.76
	40˚C /75 %RH	100.11	92.25	84.16	81.51	72.47	61.63

Conclusion

When applied to the epidermis for the treatment of acne, adapalene produces erythema. The thin film hydration technique employing a rotary flask evaporator demonstrates superior efficiency and vesicle-forming properties. At the same time interval, the ordinary gel formulation of 0.05% adapalene showed greater release than the niosomal gel formulation. The niosomal gel exhibited properties of sustained release. The niosomal gel applied to the rat's skin exhibited no erythema, whereas the conventional drug gel did. This research concludes that the novel formulation of niosomal gel is significantly superior to conventional gel for topical use.

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