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EVALUATION OF TAZAROTENE LOADED TOPICAL LIPOSOMAL GEL FOR MANAGEMENT OF ACNE

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ABSTRACT

The aim was to design a liposomal delivery system for topical administration of tazarotene capable of providing controlled and localized release of the encapsulated drug in order to minimize adverse effects associated with its topical use. Liposomal drug delivery strategies can play a pivotal role in improving the topical delivery by enhancing their dermal localization with a concomitant reduction in their side effects. Tazarotene liposomal gels were formulated and evaluated comparatively with commercial gel with respect to antibacterial activity and *in - vivo* anti acne activity. The *in-vitro* and *in-vivo* effect of prepared formulation was confirmed by *in-vitro* antibacterial activity against *Propionibacterium acnes* (ATCC) and *in-vivo* anti acne activity in wistar rats. An attempt was made evaluate the topical liposomal tazarotene gel formulation for the treatment of acne in patients. Liposomal gel showed greater percentage of inhibition of acne infection against *Propionibacterium acnes*. The prepared liposomal gel formulations exhibit superior stability thereby increasing its potential application in transdermal delivery systems. The developed novel drug delivery system demonstrated anti acne activity *Propionibacterium acnes*. The findings of this investigation therefore underscore potential utility of commercialization of liposomal gel in the treatment of acne.

Keywords: Tazarotene, Liposome, Acne, Antibacterial, Gel, Topical

INTRODUCTION

Acne is a disease that involves the oil glands of the skin. It is not dangerous, but can leave skin scars. Your skin has pores (tiny holes) which connect to oil glands located under the skin. The glands are connected to the pores via follicles - small canals. Sebum, an oily liquid, is produced by these glands. The sebum carries dead skin cells through the follicles to the surface of your skin. A small hair grows through the follicle out of the skin. Pimples grow when these follicles get blocked. In humans, when pimples appear they tend to do soon the patient's face, back, chest shoulders and neck. Acne develops when follicles get blocked and infected. Simply put - skin cells, sebum and hair can clump together into a plug, this plug gets infected with bacteria, resulting in a swelling. A pimple starts to develop when the plug begins to break down [1-4].

Tazarotene is a third generation retinoid which is effective for topical treatment of acne vulgaris and has cosmetic benefits for photoaging [5]. However, there are adverse effects accompanied with its use where

local cutaneous irritation including burning, itching, erythema, peeling or dryness occurs in approximately one-quarter of patients using tazarotene [6,7]. In comparative trials 0.1% tazarotene gel demonstrated the highest irritation score followed by tazarotene cream compared to different concentration of tretinoin cream [8]. In a recent study, tazarotene foam 0.1 % was found to be an alternative to tazarotene gel with less systemic exposure [9]. Therefore, the development of new effective topical drug delivery system intended to modulate tazarotene release rate, enhance its localization in the skin and reduce its percutanous absorption might minimize its adverse effects and could be of particular usefulness.

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of phospholipids with or without some additives [10]. Among a variety of drug lipid carriers, liposomes seem to have the best potential as localizers of topically applied drugs [11]. It was reported that conventional liposomes only enhanced skin deposition, with mostly reduction effect on percutaneous permeation or systemic absorption of drugs with better drug localization at site of action[12]. The aim of this work was to incorporate tazarotene in suitable liposomal formulation may modify its diffusion parameters in the skin and hence reduce its systemic absorption and consequently its adverse effects. The study was aimed to design a liposomal delivery system for topical administration of tazarotene capable of providing controlled and localized release of the encapsulated drug in order to minimize adverse effects associated with its topical use.

MATERIALS AND METHODS

Tazarotene and Hydroquinone was gift sample from Bioplus life science, Banglore, India. Soya lecithin was purchased from Hi Media Ltd. Mumbai. Cholesterol was purchased from Thomas baker, Ethanol Methanol, Chloroform, Propylene glycol, was purchased from S. D. Fine Chem. Ltd., Mumbai. Ethanol was procured from Qualigens fine chemicals, Mumbai. All other chemicals were of analytical grade and double distilled water used throughout the experiment.

Method of preparation for Liposomes

Liposomes were prepared by lipid film hydration method using rotary vaccum evaporator. Drug Tazarotene (constant), SPC: Cholestrol: rotation speed ratio was altered and vesicle size and drug entrapment efficiency were studied. Briefly, a chloroform: methanol (1:2) mixture of different ration of drug Tazarotene (Constant) SPC: CHOL evaporator under vaccum at $40^0 \pm 0.5^0$ C to form a lipid film on the wall of a round bottom flask. The resulting lipid film was then hydrated with phosphate buffer for 2 hours at $37^0 \pm 0.5^0$ C. The preparation was sonicated at 4^0C in 3 cycles of the 5 minutes and rest of 5

minutes between each cycle by using probe sonicator. The formulation was homogenized at 15,000 psi pressure in 3 cycles using high-pressure homogenizer to get liposome [13].

Preparation of Gels

Preparation of carbopol gel base

0.5 g Carbopol 934 was weighed and dispersed in water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerin was added to for gel consistency. Preservatives also added to it. Similarly, 1 and 2% carbopol gels were prepared [14, 15].

Preparation of liposomal gels

1g of liposome formulation was dissolved in 10ml of ethanol and centrifuged at 6000 rpm for 20 minutes to remove the unentrapped drug. The supernatant was decanted and sediment was incorporated into the gel vehicle [16].

The incorporation of the tazarotene loaded liposomes (equivalent to 0.1%) and direct incorporation of hydroquinone (4%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes. The optimized formulation was incorporated into three different gel concentration 0.5% (LF1), 1 %(LF2) and 2 %(LF3) w/w.

The viscosity and Spreadability values indicate that the formulations are efficient to hold up the liposomal formulation. Moreover, the LF 2 formulation has an optimum viscosity which can be spread evenly over the skin and will maintain contact for longer period of time with the skin and thereby leading to maximum therapeutic effect.

Also the pH of the formulations indicates to be in the range of 7.0 to 7.2 and hence they are suitable for the application to the skin and will not lead to any irritation and adverse effects.

The release of the drug from liposomal gel was found to follow the order: LF2>LF3>LF1.

In drug release study it was observed that the maximum drug release rate was shown in case of LF2 liposomal gel formulation. On the basis of results LF2 was selected as optimized gel formulation based in the drug release and other attributes viz viscosity and Spreadability which was found to be optimum for LF2.

Antibacterial activity

Pathogenic bacteria

The pathogenic bacteria used in the current study *Propionibacterium acnes* was obtained from Microbial type culture collection, Institute of microbial technology, Chandigarh, Punjab, India.

Media preparation: Composition of nutrient agar media

Agar - 1.5 gms.

Beef extract - 0.3 gms.

Peptone - 0.5 gms.

Sodium chloride - 0.55 gms.

Distilled water - to make 100 ml.

PH - 7

This agar was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients were transferred to a conical flask containing required quantity of distilled water and were heated to dissolve them completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121 ^{o}C) for 15 minutes.

Preparation of plates

After sterilization, the molten agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{0}C overnight to check the sterility of plates. The plates were dried at 50^o±0.5 ^{o} C for 30 minutes before use.

Revival of the bacterial cultures

The bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques, the lyophilized cultures are inoculated in sterile nutrient broth for bacteria than incubated for 24 hours at 37^o±0.5 ^{o}C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient agar plates with loop full of bacteria and further incubated for next 24 hours at 37^o±0.5 ^{o}C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antibacterial sensitivity

The antibacterial sensitivity test is employed on to the *Propionibacterium acnes* used under present study with liposomal formulation compared with marketed formulation. For this experiment 6 mm diameter wells, stock of equivalent to 100 mg/ml of liposomal gel applied on it. A nutrient agar plate is seeded with particular bacteria with the help of spread plate technique prior and left for 5 minutes then incubated for 24 hours at 37 ºC. After incubation, plates were observed to see the sensitivity of extracts towards test bacteriums at particular concentration in the form zone of inhibition.

Antibacterial study

Broth cultures of the pure culture isolates of microorganisms *Propionibacterium acnes* which are sensitive towards the 100 mg/ml concentration of gel formulation used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37º±0.5 ºC for 48 hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antibacterial activity of gel formulation using standard procedure. There were 3 concentration used which are 25, 50 and 100 µg/ml for gel formulation for antibiogram studies. Undiluted overnight broth cultures should never be used as an inoculum. Routine direct application of discs to plates seeded with clinical material is not recommended because of problems with inoculam control and mixed cultures. The plates were incubated at 37º±0.5 ºC for 48 hours and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug [17].

In - vivo anti acne activity

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Chemicals

Clindamycin (Cipla, India) was procured from the authorized distributor of the company.

Anti-acne activity

Groups of 5 adult male Wistar rats weighing 150–200 g are shaved in the interscapular area. Twenty-four hours later, standard (Clindamycin) & test preparations (Formulation-I & II) were applied locally to the shaved area. The treatment was continued times per 3 times per week. Pieces of skin from the interscapular region are excised and processed for evaluation by electron microscopy. The measurement the thickness of the smooth endoplasmic reticulum vesicles is measured [17].

Group I- Normal

Group II - Clindamycin served as reference standard

Group III- Formulation I (Marketed formulation of Tazarotene cream)

Group IV- Formulation II (Liposomal gel (LF2) containing Tazarotene and hydroquinone)

Statistical analysis

The values were expressed as mean ± SEM. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$, $P<0.01$, and $P<0.001$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Topical formulations containing tazarotene loaded liposomes embedded into Carbopol gel containing hydroquinone have been prepared. Visual inspection showed that Tazarotene liposomal gels were milky white homogenous gels. The present research work could be concluded as successful production of liposomes using lecithin and cholesterol. Tazarotene liposomal gels were formulated and evaluated comparatively with commercial gel with respect to antibacterial activity and in - vivo anti acne activity. The in-vitro and in-vivo effect of prepared formulation was confirmed by in-vitro antibacterial activity against *Propionibacterium acnes* (ATCC) and *in-vivo* anti acne activity in wistar rats.

The results of anti - bacterial evaluation showed that the topical formulation showed anti-bacterial activity against acne organism. Anti acne gel formulations except control also showed inhibitory effect on *Propionibacterium acnes* . Zone of inhibition of formulation was higher than marketed formulations.

The tazarotene loaded topical liposomal gel showed significant (P<0.001) anti-acne activity as compared to control.

Table 1: Antibacterial activity of different gel formulations against *Propioni bacterium acnes*

Sample	Zone of Inhibition (mm)		
	25µg/ml	50 µg/ml	100µg/ml
Marketed Gel (Tazarotene cream)	15±0.22	20±0.12	22±0.11
Liposomal Gel (Tazarotene loaded liposomal gel LF2)	18±0.12	24±0.10	26±0.20

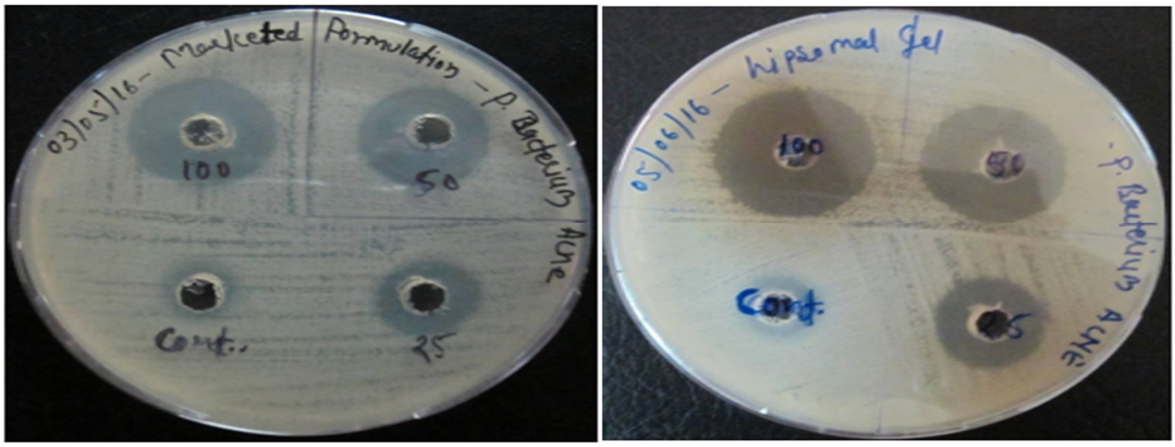


Fig. 1: **Photograph showing antibacterial activity**

Liposomal gel (LF2) showed greater percentage of inhibition of microbial infection against *Propionibacterium acnes*- On comparison of formulated gels with marketed gel of Tazarotene, Liposomal gel showed greater percentage of inhibition of bacterial infection against *Propionibacterium acnes*. This may be due to the fact that the liposomal gel released the drug in more efficient manner.

Table 2: Effect of clindamycin (standard), formulation I & II on acne

Sr. No	Group	Mean Thickness ± SEM(excised skin)
1	Normal	1.18 ± 0.09
2	Clindamycin	0.30 ± 0.09***
3	Formulation I	0.65 ± 0.06 **
4	Formulation II	0.45 ± 0.06***

*Values expressed as mean ± SEM *P<0.05, **P<0.01, *** P<0.001 as compared to normal*

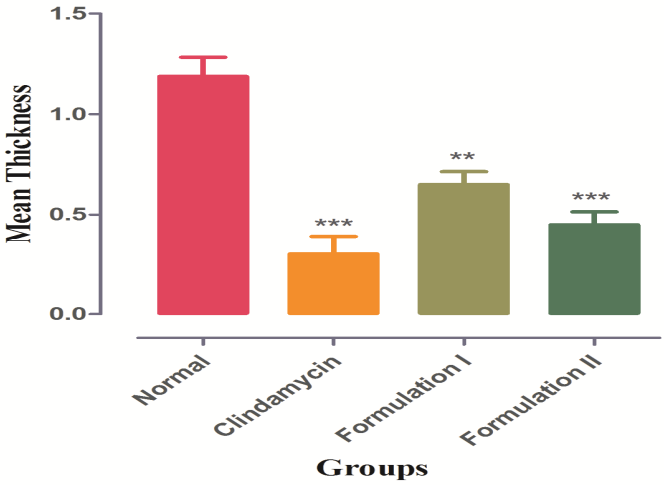
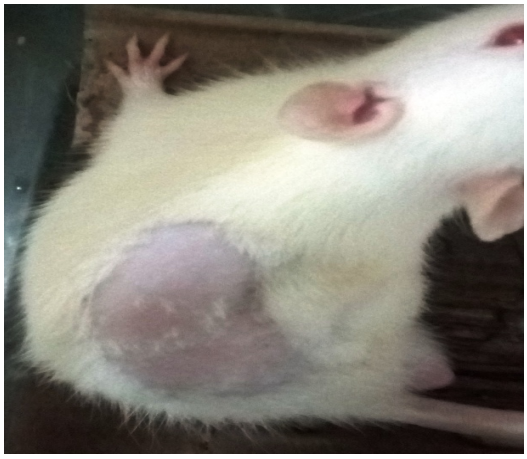
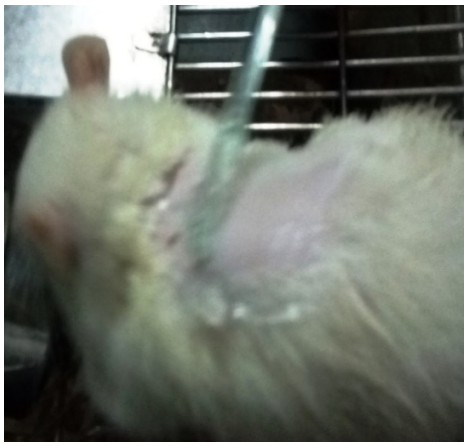


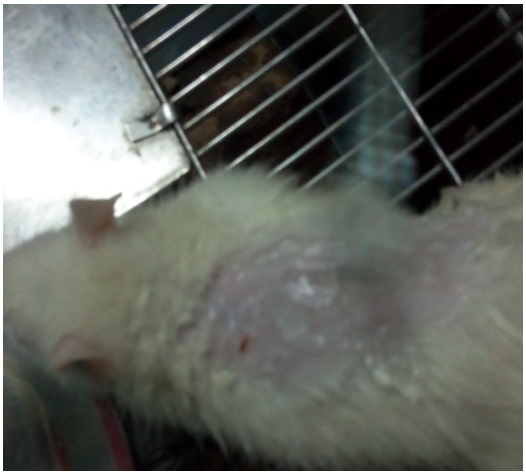
Fig. 2: Effect of clindamycin (standard), formulation I & II on acne



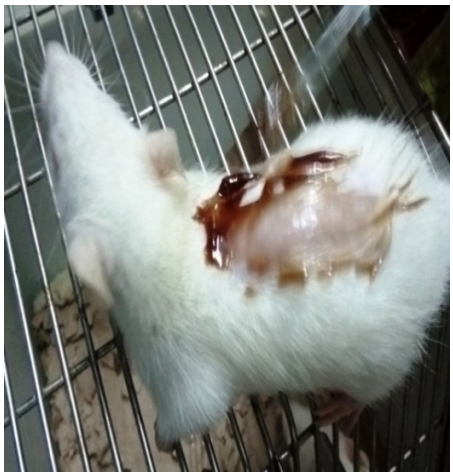
Acne Induction



Standard Drug



Formulation I



Formulation II

Fig. 3: Photo Plates of *In - Vivo* anti acne activity

CONCLUSION

In present in-vivo anti acne activity Clindamycin was selected as a standard drug and showed the effect of Clindamycin, Formulation- I (marketed Tazarotene gel) and formulation II (liposomal gel LF2) on acne and mean thickness compared to the normal. Results showed in table 2 and figure 2-3.

It was observed that Formulation-I (marketed Tazarotene gel) and formulation-II (liposomal gel LF2) showed a significant reduction in the acne without necrosis as compared with the standard Clindamycin.

Various antibiotics like tetracycline, Clindamycin, and erythromycin etc and other drugs like benzoylperoxide are used for acne treatment. The various drawbacks of synthetic drugs are different side effects and resistant developed towards these drugs. Formulation therapy is required to overcome the above draw backs and treat the acne. The Formulation-I showed a reduction in the acne as compared to the clindamycin formulation but the marked reduction in the acne was found in case of Formulation II. This shows that most effective reduction in the acne could be have had by the developed formulation which is due to the fact that the developed formulation consisted on liposomes loaded with tazorotene. The liposomes penetrated to deeper layer of the skin thereby providing enhanced reduction in the bacterial population of the bacterium located deeper within the acne. Also since the formulation released the drug in a controlled fashion owing to controlled release nature of the delivery system which ensured the gradual delivery of drug for a longer period of time and hence the therapeutic concentration of drug was maintained at the infection site for longer period of time. All the features resulted in efficient delivery there by leading to marked reduction in the infection. Also since the gel contains hydroquinone which also aids in the reduction of the acne and protects the skin from any untoward effects and therefore the combination therapy as is evident result in efficient drug delivery leading to enhanced treatment for acne.

Tazarotene would offer the efficacy in the treatment of acne and offer patient compliance.

REFERENCES

1. Gollnick H, Cunliffe W, Berson D, Dreno BA, Leyden J, Shalita A and Finlay TD. Management of acne: A report from a global alliance to improve outcomes in acne. J Am Acad Dermatol. 2003; 49: S1-S37.
2. Krautheim A and Gollnick H. Transdermal penetration of topical drugs used in the treatment of acne. Clin Pharmacokinet. 2003; 42: 1287-1304.
3. Date AA, Naik B and Nagarsenkar MS. Novel drug delivery systems: Potential in improving topical delivery of antiacne agents. Skin Pharmacol Physiol. 2006; 1: 2-16.

4. Schδfer T, Nienhaus A, Vieluf D, Berger J and Ring J. Epidemiology of acne in the general population: The risk of smoking. Br J Dermatol. 2001; 145:100-104.
5. Antoniou C, Kosmadaki MG, Stratigos AJ and Katsmaba AD. Photoaging: Prevention and topical treatments. Am J Clin Dermatol. 2010; 11 :95-102.
6. Guenther LC. Topical tazarotene therapy for psoriasis, acne vulgaris, and photoaging. Skin Therapy Letter. 2002; 7: 1-4.
7. Chiricozzi A and Chimenti S. Effective topical agents and emerging perspective in the treatment of psoriasis. Expert Rev Dermatol. 2012;7(3): 283-293.
8. Thielitz A and Gollnick H. Topical retinoids in acne vulgaris. Am J Clin Dermtol 2008; 9(6): 369-381.
9. Jaratt M, Werner CP and Saenz AB. Tazarotene foam versus tazarotene gel: A randomized relative bioavailability study in acne vulgaris. Clin Drug Invest 2013; 33(4): 283-289.
10. El-Sayed MA, Abdallah OY, Naggar VF and Khalafallah, NM. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int J Pharm. 2007; 332 (1-2): 1-16.
11. El-Maghraby GM, Williams AC and Barry BW. Liposomes and skin: From drug delivery to model membranes. Eur J Pharm Sci 2008; 34 (4-5): 203-222.
12. El-Maghraby GM, Williams AC and Barry BW. Can drug bearing liposomes penetrate intact skin? J Pharm Pharmacol. 2006; 58 (4): 415-429.
13. Mansoori MA, Jawade S, Agrawal S and Khan MI. Formulation development of ketoprofen liposomal gel. J Pharmaceu Cosmetol. 2012; 2:22-29.
14. Chung BK and Chung S. Proliposomes as prolonged intranasal drug delivery systems. STP Pharma Sci. 2002; 12 (1):33-38
15. Katare OP, Vyas SP and Dixit VK. Proliposomes of indomethacin for oral administration. J Biochim Biophys Acta. 1985; 817 (1): 67–74.
16. Patel VB, Misra AN and Marfatia YS. Preparation and comparative clinical evaluation of liposomal gel of benzoyl peroxide for acne. Drug Dev Ind Pharm. 2001; 27 (8): 864-870.
17. Daud FS, Wankhede S, Joshi M and Pande G. Development of herbal anti acne gel and its evaluation against acne causing bacteria *Propionibacterium acne* and *Staphylococcus epidermidi*. Int J Res Ayurveda Pharm. 2013; 4(5): 781-786.