Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [7] June 2023 : 08-13

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

In-Vivo Evaluation of Ethosomal Gel of Betamethasone Valerate for the Treatment of Psoriasis

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ABSTRACT

Psoriasis is a common, chronic, non-contagious, auto-immune disease that primarily affects the skin. It is characterized by excessive growth and abnormal differentiation of keratinocytes. It is located on the elbows, knees, scalp, arms, nails, legs, hands, and feet. The limitation of ointment, creams is that they do not efficiently undergo to the skin and biologics are available as injectables and have low patient compliance and also cause irritation at the site of action. The main reason of selecting ethosomes is that it is the promising vehicle for topical drug delivery. Due to enhanced permeation it can easily penetrates to the epidermal layer of skin.Tazarotene Cream (0.1%) and Ethosomal gel of Betamethasone valerate (0.1%) were used for the in-vivo study.The optimized ethosomes formulation was selected to be incorporated in the gel system based on the good stability results and the optimized in-vitro study. The specified amount of carbopol 934 powders is to be slowly added to ultrapure water and kept at 100 °C for 20 min. Triethanolamine were added to it drop wise. Appropriate amount of 1.5% w/w formulation containing drug were incorporated into gel base. Water q.s. were added with other formulation ingredients should be achieved. The ethosomal gel formulation on relative epidermal thickness (%), orthokeratosis (%) and drug antipsoriatic activity (%) in the mouse tail test showed 27.82±1.47% orthokeratosis. The scaly areas presented parakeratosis. Tazarotene formulation (standard) showed 67.58±2.05% orthokeratosis, while Ethosomal gel of Betamethasone valerate showed an almost 66.24±1.15% orthokeratotic differentiation, respectively. These findings present the potency of ethosomal gel-based approach to provoke normal epidermal differentiation in psoriasis.

Keywords: *Psoriasis, Keratinocytes, ethosomal gel, Betamethasone valerate, orthokeratosis etc.*

Received 14.02.2023 Revised 27.04.2023 Accepted 21.05.2023

INTRODUCTION

Psoriasis is a common, chronic, non-contagious, auto-immune disease that primarily affects the skin. It is characterized by excessive growth and abnormal differentiation of keratinocytes. It is located on the elbows, knees, scalp, arms, nails, legs, hands, and feet [1]. The most common symptoms of psoriasis include red, raised, inflamed patches of skin, whitish-silver scales or plaques on the red patches, dry skin that may crack and bleed soreness around patches itching, burning sensations around patches, thick, pitted nails, painful and swollen joints [2,3]. Psoriasis is a skin disease which is till now treated by ointment, creams, biologics more commonly but these formulations have certain limitations with them. The limitation of ointment, creams is that they does not efficiently undergoes to the skin and biologics are available as injectables and have low patient compliance and also cause irritation at the site of action.

The main reason of selecting ethosomes is that it is the promising vehicle for topical drug delivery.Due to enhanced permeation it can easily penetrates to the epidermal layer of skin.They have the capabilities to encapsulate large and diverse groups of drug. Ethosomes are the novel lipid carriers composed of ethanol, phospholipids and water.These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are the slight modification of well established drug carrier liposome [4].The size range of ethosomes may vary from tens of nanometers to microns. They are reported to improve the skin delivery of various drugs. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization. They permeate through the skin layers sooner and possess significantly higher transdermal flux. The present investigation was to design the ethosomes containing Betamethasone valerate using different concentration of ethanol and phospholipid (Soya Lecithin). It is steroid ester which is used to help relieve redness, itching, swelling or other discomfort caused by skin conditions. It is a BCS class II drug (low solubility and high permeability) [5].

MATERIAL AND METHODS

Materials

Tazarotene Cream (0.1%) and Ethosomal gel of Betamethasone valerate (0.1%) were used for the in-vivo study.

Methods

Acute Dermal Toxicity Study

The acute dermal toxicity test (LD50) of Ethosomal transdermal gel was determined according to the OECD (Organization for Economic Corporation and Development) guidelines no. 402 on Swiss albino mice. Healthy young adult albino mice (approx. 20-25g) were used. Animals were acclimatized to the laboratory conditions for 5 days prior to the test. Animals were divided in to 3 groups, each group consisting of 3 animals (n=3). About 24 hours prior the test, fur was removed from the 10% of the body surface area from dorsal area of the back of the test animals by using hair remover cream avoiding any abrasion on skin. Gradually increasing dose (topically) of Ethosomal gel was applied to all three groups (n=3). The treated animals of all groups were examined for 14 days for any change in fur, eyes, sleep pattern, central nervous system activity, behavior pattern, toxic reactions and time of death occurring during the dermal toxicity studies. During overall toxicity study, animals were placed at optimum environmental conditions (25-30ºC temperature, 30-70% humidity) with 12:12 hours light and dark cycle with regular supply of drinking water and conventional laboratory diet. The suitable dose of the formulation was determined for anti-psoriatic activity.

In-Vivo Anti-Psoriatic Activity-Mouse Tail Model

The mouse tail model is widely accepted as a testing method for measurement of anti-psoriatic activity of drugs. Principle of this model is that topical application of a mouse-tail with anti-psoriatic drugs enhances orthokeratotic cell differentiation in the epidermal scales. This characteristic was used for evaluation of drug efficacy in animal model. The anti-psoriatic activity was executed according to mouse tail model as described in Vogel 2002, with slight modification. Total 24 animals were used in the present research work (Table 1). Animals were divided into four groups of six each (n=6). The first group was the negative control which was left untreated and the second group was the positive control group treated with TNF-α. The marketed ointment (Tazarotene- 0.1% w/w) was applied to third group. The fourth group was treated with the 0.1% of optimized Ethosomal transdermal gelof Betamethasone valerate.

Table 1: Study Plan

Group Name (n=6)	Treatment	Dose	Route	Dose & Frequency
Vehicle Control	Normal Saline	1-1.5 ml	Topical	14 days
Negative control	TNF- α	0.1 ml	SC	Once
Standard group	Tazarotene cream	0.1%	Topical	14 days
Test group	Ethosomal gel (Betamethasone valerate)	0.1%	Topical	14 days

Preparation of Betamethasone valerate Ethosomal Gel

The optimized ethosomes formulation was selected to be incorporated in the gel system based on the good stability results and the optimized in-vitro study. The specified amount of carbopol 934 powders is to be slowly added to ultrapure water and kept at 100 °C for 20 min. Triethanolamine were added to it drop wise. Appropriate amount of 1.5% w/w formulation containing drug were incorporated into gel base. Water q.s. were added with other formulation ingredients should be achieved. Gel containing free drug were prepared by similar method using 1.5% Carbopol. 1.5% carbopol gel base was prepared by dispersing 1.5 gm carbopol 934 in 90 ml hot distilled water in which 10 ml glycerol was previously added. Accurately weighed quantity of methyl paraben and propyl paraben was added into it. The mixture was stirred until thickening occurred and then neutralized by the drop wise addition of 50 % w/w triethanolamine to achieve a transparent gel.

Table 2: Composition of Gel base

Ingredients	Concentration
Carbopol 934	1.5 %
Glycerol	5 %
Methyl paraben	0.02 %
Propyl paraben	0.01 %
Distilled water	Upto 100 %

In this gel base ethosomal formulation was slowly added with gentle stirring. Finally the ethosomal gel was mixed using a mechanical stirrer for 5 minutes.

Evaluation of Betamethasone valerate ethosomal Gel

The Betamethasone valerate ethosomal gel was checked for appearance, pH, viscosity, in-vitro release study, spreadability, drug content uniformity, homogenicity etc. The results of evaluation parameters were depicted in table 3.

Table 3: Evaluation Parameters for Optimized Betamethasone valerate ethosomal gel formulation

S. No.	Parameter	Inference
1.	Appearance	White appearance, consistent, no grittiness, no phase separation
2.	pH	5.6 ± 0.03
3.	Viscosity	4980 ± 0.45 cP
4.	Spreadability	37.88/4 ± 0.05

In-vitro Release study of optimized Betamethasone valerate Ethosomal Gel Formulation

The in-vitro release results are depicted in table 4 and Figure 1.

Table 4: In-vitro Release Study of Optimized Betamethasone valerate Ethosomal Gel Formulation

S. No	Time (hrs)	pH 7.4 PBS
1	0	0
2	0.5	31.23
3	1	45.67
4	2	57.26
5	4	67.98
6	6	74.73
7	8	80.12
8	10	85.87
9	12	88.92

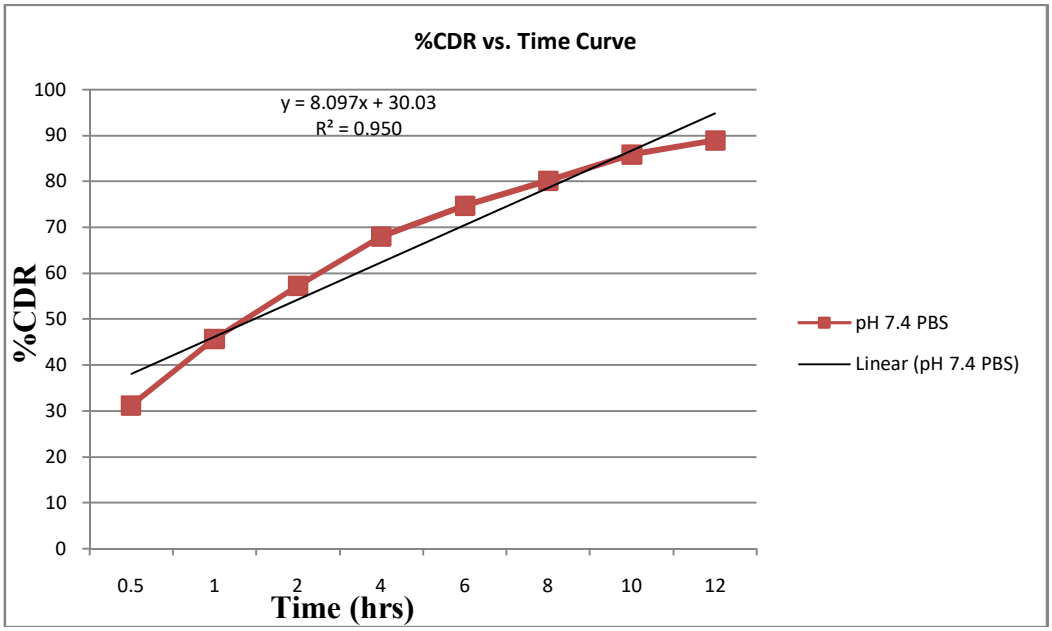

Figure 1: In-vitro Release Study of Optimized Betamethasone valerate Ethosomes Formulation

Experimental Design

Hairs were removed from the 10% of the body surface area from dorsal area of the back portion of all the test animals by using hair remover cream. Psoriasis was induced by a single subcutaneous injection of 0.1 ml of tumor necrosis factor on dorsal area of the back portion of all the test animals. After 4 days of administration of tumor necrosis factor, there was the presence of granular layer in the shaved area of skin which after a period of time was transformed in psoriatic lesion. After the induction of psoriasis, animals were treated with respective dose of standard (Tazarotene- 0.1% w/w) and test formulation (0.1% of optimized Ethosomal transdermal gel) once daily, for 14 days to evaluate the therapeutic effect. During this period, animals were visualized daily to record the symptomatic effect and the photographs of

every animal were taken from each group. Two hours after the last treatment the animals were sacrificed using deep ether anesthesia by cervical dislocation, and the sections of skin were cut from each group and stored in 10 % formalin in saline. Longitudinal sections of about 5 µm thickness were prepared by microtomy and were stained with hematoxylin-eosin dye for histological examination.

Evaluation Parameters for Anti-psoriatic Activity

Measurement of Percent Ortho-keratosis (OK)

An antipsoriatic drug that targets the epidermis is a compound that restores skin homeostasis by suppressing keratinocyte, hyper proliferation, abnormal differentiation, or both. The granular layer is greatly reduced or almost absent in epidermis of psoriatic lesions. This parakeratosis condition is one of the most important hall marks of psoriasis. Granular layer formation around the epidermis is known as orthokeratosis condition. The main principle behind the mousetail test is conversion of parakeratosis to orthokeratosis. Percent orthokeratosis in those parts which normally have a parakeratotic differentiation is quantified measuring the length of the continuous granular layer (A) and the length of the scale (B) and expressed as a percentage of total number of scales region per section.

$$\textbf{\% Orthokeratosis} = \frac{\text{Length of continuous granular layer (A)} \times 100}{\text{Length of Scale (B)}}$$

Measurement of Epidermal Thickness (ET)

It was obtained by measuring the distance between the dermo-epidermal borderline and the beginning of the horny layer. Five measurements per animal were made in every 10 scales and the mean of the different animals was calculated. The change in epidermal thickness of standard and formulation treated group were then calculated.

$$\textbf{Epidermal Thickness (\%)} = \frac{\text{ET of treated group-ET of Control group} \times 100}{\text{100 - ET of Control group}}$$

ET = Epidermal thickness

Measurement of Drug Activity

Drug activity is calculated by the percentage increase of orthokeratotic regions.

$$\textbf{\% Drug Activity} = \frac{\text{Mean OK of treated group-mean OK of control group} \times 100}{\text{100-Mean OK of the control group}}$$

OK = Orthokeratosis

Statistical Analysis

Data obtained in the present study was presented as mean±standard error. In the mouse tail test for statistical comparisons, probabilities were obtained by the Tukey's multiple range tests. Statistical calculations were performed using Graph Pad Prism software. Values with $p<0.05$ was considered significant [6-9].

Results

In-vivo Anti-psoriatic Activity

The mouse tail test is employed widely for psoriatic investigations because it is simpler model, smooth to carry out and displays good reproducibility, fine sensitivity and high correlation with the activity of oral or topical antipsoriatic therapeutics at present clinically. Additionally, this model facilitates the quantitative and histometric investigations of fabricated formulation response on epidermal differentiation.

a) Mouse Tail Model

Ethosomal gel was screened for its possible anti-psoriatic activity using mouse tail model. Ethosomal formulation (0.1% w/w) and standard drug (0.1% w/w) were applied on the induced psoriatic lesions. Ethosomal gel has increased the orthokeratotic regions by 66.24±1.15%, in comparison to control group. The standard drug showed an increase in the orthokeratotic regions by 67.58±2.05%. Ethosomal gel has decreased the epidermal thickness (42.75±2.16% while standard drug decreases the epidermal thickness 43.64±1.56%. Percent drug activity of Ethosomal gel (0.1%) was found to be 55.56±1.38% which showed a significant antipsoriatic effect in comparison to standard drug which has shown the drug activity of about58.31±1.27%. The results were represented in table 5.

Table 5: Anti-psoriatic Potential of Ethosomal Gel using Mouse Tail Model

S.No.	Formulation	Relative epidermal thickness (%)	% Ortho-keratosis	Drug activity (%)
1.	Vehicle Control	100.00±0.00	27.82±1.47	0.00±0.00
2.	Negative control	100.00±0.00	0.00±0.00	0.00±0.00
3.	Tazarotene- 0.1% w/w	43.64±1.56%*	67.58±2.05%*	58.31±1.27%
4.	Ethosomal gel-0.1% (Betamethasone valerate)	42.75±2.16%*	66.24±1.15%*	55.56±1.38%

The results were presented in mean±S.D and results were significant in comparison to control group at $p<0.05$ (*)

Histological Examination of Mouse Skin

Ethosomal gel of Betamethasone valerate also showed considerable change in epidermal thickness compared to control group's animals. Granular layer of the epidermis is more reduced in psoriatic lesions. Parakeratotic condition is seen in the skin which is one of the hallmarks of psoriasis. Formation of granular layer in the region of the epidermis is known as orthokeratosis state. The key theory following the mouse tail test is alteration of parakeratosis condition to orthokeratosis. Drugs which show their mechanism of action with multiple mechanisms in the treatment of psoriasis are more significant than other drugs performing by one solitary mechanism.

Conclusion

In the present work, Figure 2, illustrates the histological appearance of haematoxylin and eosin-stained sections of mouse tail skin on treatment with various samples. From control group (Group I), distinct pieces of evidence associated with parakeratosis, a higher density of nucleated keratinocytes and thinning of the granular layer were noticed. Fourteen days of treatment therapy with exhibited considerable histological changes in sections of tail skin with respect to marketed product. Throughout this time, the orthokeratotic stratum corneum provinces spread longitudinally, in the previous parakeratotic condition. The influence of topical application of Ethosomal gel formulation on relative epidermal thickness (%), orthokeratosis (%) and drug antipsoriatic activity (%) in the mouse tail test was displayed in table 2. The control group showed 27.82±1.47% orthokeratosis, while the scaly areas presented parakeratosis. Tazarotene formulation (standard) showed 67.58±2.05%orthokeratosis, while Ethosomal gel of Betamethasone valerate showed an almost 66.24±1.15%orthokeratotic differentiation, respectively. These findings present the potency of ethosomal gel-based approach to provoke normal epidermal differentiation in psoriasis.

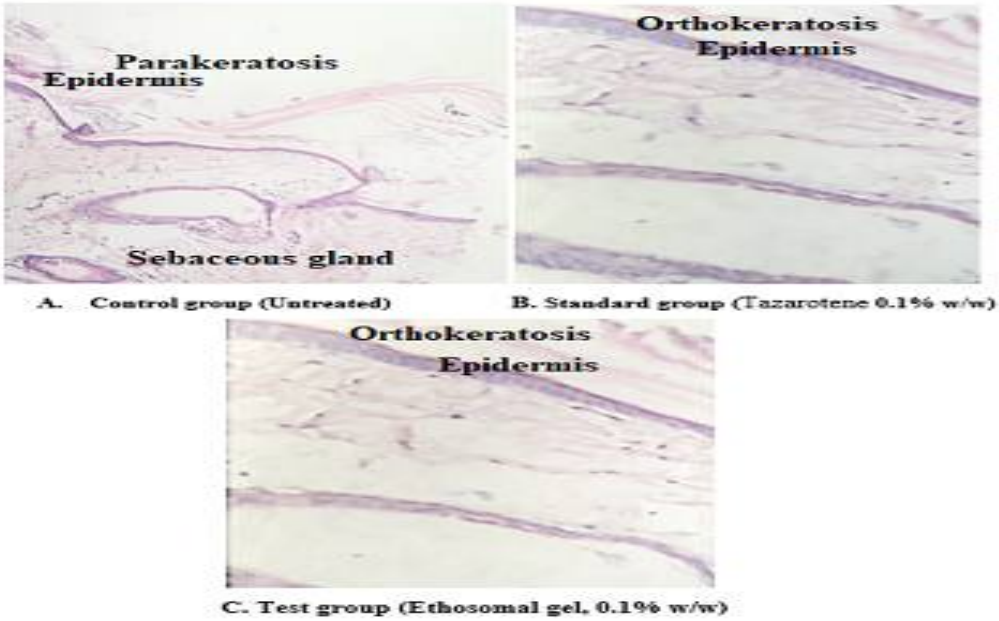

Figure 2: Histopathological evaluation of mice tail skin after various treatments. A. Control; B. Standard drug formulation; C. Ethosomal gel of Betamethasone valerate.

REFERENCES

1. Raychaudhuri SK, Maverakis E, Raychaudhuri SP. (2014). Diagnosis and classification of psoriasis. Autoimmunity reviews. 2014 May 31; 13(4):490-510.
2. Syed ZU, Khachemoune A. (2011). Inverse psoriasis: case presentation and review. American Journal of Clinical Dermatology. 12:143–146.
3. Boehncke, WH, Schön, MP. (2015). Psoriasis. Lancet . 5:983-94.
4. M. Mezei, V. Gulasekharam, (1980). Liposomes - a selective drug delivery system for the topical route of administration I. Lotion dosage form, Life Sci. 26; 1473–1477, https://doi.org/10.1016/0024-3205(80)90268-4.
5. Yade Metri Permata, Muchlisyam Bachri, Julia Reveny, Fitri Mardiyanti Sibuea, (2019). Formulation and Quantitative Analysis of Betamethasone Valerate and Neomycin Sulfate Cream by High Performance Liquid Chromatography and Spectrophotometry, Open Access Maced J Med Sci.; 7(22):3841-3846.
6. Patsariya S.K., Middha Anil., (2014). "Antipsoriatic activity of dithranol transdermal proniosomes gel on swiss albino mice", Int. J. Res.Dev.Pharm. L. Sci., 3(6), pp. 1287-1294.
7. Poonam Negi, Ishita Sharma, Chetna Hemrajani, Charul Rathore, Alpna Bisht, Kaisar Raza and O. P. Katare. (2019). Thymoquinone-loaded lipid vesicles: a promising nanomedicine for psoriasis. Negi et al. BMC Complementary and Alternative Medicine 19:334.
8. Kumar, S., Singh, K. K., & Rao, R. (2019). Enhanced anti-psoriatic efficacy and regulation of oxidative stress of a novel topical babchi oil (Psoralea corylifolia) cyclodextrin-based nanogel in a mouse tail model. Journal of Microencapsulation, 1–28. doi:10.1080/02652048.2019.1612475.
9. Sunil Kumar, Babu Lal, Jangir Rekha Rao,(2022). A new perspective for psoriasis: Dithranol nanosponge loaded hydrogels. Applied Surface Science Advances,(12), 100347.

CITATION OF THIS ARTICLE

Mayuri J, Neha J, Vinay P, Upendra N. *In-Vivo* Evaluation of Ethosomal Gel of Betamethasone Valerate for the Treatment of Psoriasis. Bull. Env. Pharmacol. Life Sci., Vol 12[6] June 2023: 08-13.