ORIGINAL ARTICLE

Investigating Skin Permeation Behavior, Skin Irritation and Anti-Inflammatory Activity of Diclofenac Diethylamine Novel Formulation Prepared using Shea Butter as Absorption Base with Nerolidol as Permeability Enhancer

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Abstract

Background: The present investigation was to study the drug permeation of diclofenac diethylamine (DDEA) cream prepared using shea butter as the absorption base and nerolidol as a permeability enhancer and also to evaluate skin irritation and anti-inflammatory study of prepared cream on Wistar rats in comparison with marketed formulation. **Materials and Methods:** The cream prepared using shea butter and nerolidol was assessed for permeation study through rat skin. The three formulations mainly F0 (without nerolidol), F5 (containing 0.5% nerolidol), and the marketed formulation were assessed for % drug permeation through rat skin along with an estimation of flux and permeability coefficient. A skin irritation study of a placebo, F0, and F5 formulation was carried out on Wistar rats. Anti-inflammatory study of transdermal cream formulation of control, placebo, F0, F5, and marketed formulation was evaluated for anti-inflammatory study on Wistar rat using carrageenan-induced rat paw edema method. **Results:** The F5 formulation showed enhanced drug permeation as compared to the F0 and marketed formulation. The flux value F5 formulation was found to be 0.3942 ± 0.009 g/cm^2/min as compared to F0 and marketed formulation which were found to be 0.2789 ± 0.013 g/cm^2/min and 0.2730 ± 0.0110 g m/cm^2/min, respectively. The permeability coefficient for the F5 formulation was found to be 2.370×10^{-5} m/s as compared to F0 and marketed formulations which were found to be 1.680×10^{-5} and 1.640×10^{-5} m/s, respectively. The F0 and F5 formulations were found to be non-irritant and no edema was observed on its application. F5 formulation showed significant inhibition of edema in rat paw volume induced by carrageenan as compared to control, placebo, Std, and F0 batches. **Conclusion:** The study demonstrates that the F5 formulation prepared using shea butter as an absorption base and containing 0.5% w/v of nerolidol as a permeability enhancer showed a better permeation of DDEA through rat skin, non-irritant in nature and significant anti-inflammatory activity as compared to F0 and marketed formulation.

Key words: Diclofenac diethylamine, nerolidol, permeability coefficient, shea butter and absorption base

INTRODUCTION

The bioavailability of active pharmaceutical ingredients (APIs) is primarily achieved through innovation in drug delivery methods. Oral delivery systems are the most preferred method due to their advantages such as a range of dosage forms, painlessness, ease of administration, self-administration, convenience, patient compliance, and high safety. However,

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there are drawbacks to oral delivery methods, including first-pass metabolism and poor drug stability in the gastrointestinal tract. Transdermal drug delivery systems, on the other hand, use the skin to administer medication, bypassing first-pass metabolism, being less invasive, simple to apply and administer, and not requiring specialized staff.[1-3] Shea butter, a natural fat made from the seeds of the shea tree (*Butyrospermum parkii* Kotschy) which belongs to the *Sapotaceae* family is used in pharmaceutical ointments for its humectant, emollient, and anti-inflammatory properties.[4] Therefore, shea butter possesses the exceptional ability to be utilized as an excipient as well as an active ingredient.[5] Non-steroidal anti-inflammatory medicines are commonly used to reduce pain and inflammation by blocking the cyclooxygenase (COX-2) enzyme. Diclofenac diethylamine (DDEA) is a viable option for creating transdermal dosage forms due to its effectiveness in traversing membrane barriers, accumulating in the neutral intracellular region where COX-2 enzymes are found.[2,6] Therefore, shea butter was used as a carrier base in this present study to formulate diclofenac cream for transdermal application.[7] Terpenes' solubility in stratum corneum (SC) lipids is determined by their lipophilic characteristics, as evidenced by their high LogP. However, terpene molecules have the ability to facilitate the penetration of both hydrophilic and lipophilic APIs due to the inclusion of both polar and non-polar groups.[8,12] Natural terpenes with high-enhancing impact and minimal skin irritation are increasingly being used as permeation enhancers in pharmaceutical and cosmetic formulations.[11] Nerolidol showed the highest enhancing activity for hydrocortisone penetration among the terpene series. Several additional publications agree with this view. Among the series of terpene enhancers that were studied, Cornwell and Barry found that nerolidol was the most effective in facilitating the penetration of 5-fluorouracil through the skin.[9,13] This was ascribed to nerolidol amphiphilic nature, which was suitable for upsetting the SC lipid packaging.[10]

In the present study, transdermal cream of DDEA (1.16%w/w) was prepared with and without permeability enhancer (nerolidol) using shea butter as an absorption base.[14] Franz diffusion cells were used to quantify DDEA permeation from cream formulations as compared to commercial formulations. The goal of the present study was to evaluate that the prepared cream formulations are non-irritant to the skin by performing a skin irritation study and have significant anti-inflammatory action in comparison with commercial formulation by performing carrageenan-induced rat paw edema study using shea butter as absorption base and nerolidol as permeability enhancer.

MATERIALS AND METHODS

DDEA was given a complimentary sample by Magnus Biotech Pvt. Ltd, Karnal. Tert-butylhydroquinone (TBHQ) was given a complimentary sample by Aarnee International Pvt. Ltd, Ahmedabad. Span 80 (MONEMUL-80Hi) was given a complimentary sample by Mohini Organic Pvt. Ltd, Mumbai. Shea butter was purchased from Mangalam Agro CitSpray Aroma Sciences, Nagpur. Butylparaben, propylparaben, butylated hydroxytoluene (BHT), and Methanol-AR grade were procured from Modern Science, Nashik.

Formula design

Creams were prepared using the trituration method. The drug, excipient, and cream base were taken in different amounts as per the formula. The formula with ingredients is shown in [Table 1].

Formulation of DDEA anti-inflammatory cream

The amounts of drug and other materials were measured according to [Table 1], and the formulation was made in the way shown below.

a. All glassware was cleaned and dried in a hot-air oven
b. Given quantities of all ingredients and DDEA drug were weighed
c. Formulation of transdermal cream of DDEA was prepared using previously cleaned and dried mortar and pestle
d. Beaker A: Accurate quantity of shea butter was weighed and allowed to melt using a water bath. To it, BHT and TBHQ were added and mixed well. DDEA drug was added to the melt and was mixed well
e. Beaker B: Accurate quantity of glycerol, butylparaben, propylparaben, and water was taken in a beaker. Add one measured drop (0.1 g) of Span 80 in the mixture. The nerolidol was measured accurately and added to it according to the given batch mixture
f. Beaker C: Shea butter mixture containing the drug was mixed in the aqueous mixture by dropwise addition with continuous stirring on the ice bath
g. The cream so prepared was then stored in a light resistance container.

Table 1: Composition of diclofenac diethylamine transdermal cream

Ingredients	F0 (I, II, III)	F5 (0.5%) (I, II, III)
Diclofenac diethylamine	0.116 g	0.116 g
Shea butter (purified)	7.232 g	7.182 g
Nerolidol	-	0.055 µL (equivalent to 0.05 g)
Span 80	0.1 g	0.1 g
Glycerol	2 g	2 g
BHT	0.01 g	0.01 g
TBHQ	0.002g	0.002 g
Butyl paraben	0.01 g	0.01 g
Propyl paraben	0.03 g	0.03 g
Water	0.5 g	0.5 g

BHT: Butylated hydroxytoluene, TBHQ: Tert-butylhydroquinone

In situ permeation study

For the penetration studies, Wistar rat skin was used, which was obtained from control group rats that were sacrificed as part of routine pharmacological experiments. The rat skin was washed with cold water to remove the dirt. The hair was removed with hair removal cream. The rat skin was then soaked in hot water at a temperature of 60°C for 70 s. The rat skin membrane was isolated with the help of a blade and forceps. For permeation investigations, Franz diffusion cells with a surface area of 3.14 cm^2 were employed. Phosphate-buffered saline with a pH of 7.4 was used to fill the receptor compartment. Using an external continuous water circulator, the temperature was kept at 37 ± 0.5°C to replicate the physiological condition during the experiment. To avoid any boundary layer effects, a tiny magnetic bead was used to continuously swirl the receiver medium. The donor and receptor compartments were separated by the rat skin membrane. 1 g of cream was placed on the membrane surface. 2 mL samples were collected at 15, 30, 60, 120, 180, 240, and 360 min and replaced with fresh receptor solution. Spectrophotometric analysis of the collected sample was performed at 276 nm.

Skin irritation test and anti-inflammatory studies

The Animal Ethical Committee examined and approved the animal protocol, which was assigned protocol approval number KBH/IAEC/2023/12/12.

Skin irritation study

Approximately 24 h before the test, animals were divided into three groups Group 1 (placebo cream), Group 2 (F0), and Group 3 (F5) each group consisted of $n = 6$ animals. Close clipping was used to remove the animal's hair from the dorsal portion of the trunk. Group 2 (F0) and Group 3 (F5) each group of animals were applied creams directly to the shaved dorsal skin of weanling rats. Applications were twice daily for 15 consecutive days. During the study, rats in all three groups were fed on normal food chow pellets and tap water ad libitum. After 15 days of application, the degree of irritation was assessed in terms of dermal reactions (erythema), edema, and responses scored as per the score given in [Tables 2 and 3].

Anti- inflammatory study

The "Carrageenan-induced hind paw edema method" was used to assess the anti-inflammatory activity. Rats (male, Wistar, 200–250 g) were given 0.1 mL of 1% carrageenan (w/v) in saline to induce inflammation. The rats' left hind paw's plantar area received this injection.

Five animal groups with carrageenan-induced paw edema were studied to assess the formulations' topical anti-inflammatory activity: control, positive control, F0, optimized F5 batch, and placebo groups. After 1/2 h, the edematous paw was topically treated with positive control, F0, optimized batch, and placebo.

The initial set of rats served as an untreated control group. A plethysmometer will be used to measure the increase in paw thickness before (time 0) and 0.5, 1, 2, 3, 4, 5, and 6 h after the administration of carrageenan. The percentage increase in paw thickness from time 0 was calculated. The treatment groups of DDEA formulations for the anti-inflammatory study are shown in [Table 4] and the experimental protocol for carrageenan-induced rat paw edema is shown in [Table 5].

Table 2: According to the grades, dermal reactions were graded and recorded

S. No	Erythema and eschar formation grade	Grade
1	No erythema	0
2	Very slight erythema (barely perceptible)	1
3	Well defined erythema	2
4	Moderate-to-severe erythema	3
5	Severe erythema to eschar formation preventing grading of erythema	4

Table 3: Based on the grades, edema formation was graded and recorded

S. No	Edema formation	Grade
1	No edema	0
2	Very slight edema (barely perceptible)	1
3	Slight edema (edges of the area well raised)	2
4	Moderate edema (raised approximately 1 mm)	3
5	Severe edema (raised more than 1 mm and extending 4 beyond the area of exposure)	4

At 1, 24, 48, 72, 7, and 15 days, the skin reaction at the application location was subjectively evaluated and scored once every day

RESULTS AND DISCUSSION

In situ permeability study

The results are shown in [Tables 6 and 7].

The results showed that F5 has 23.70% permeated as compared to F0 and the marketed formulation (omnigel) which is 13.90% and 13.42%, respectively. Furthermore, the permeability coefficient for F5 formulation was found to be 2.370×10^{-5} m/s as compared to F0 and marketed formulation which were found to be 1.680×10^{-5} and 1.640×10^{-5} m/s, respectively. This indicates that the F5 formulation containing

Table 4: Treatment groups of DDEA formulations for anti-inflammatory study

Group no.	Treatment group	No. of animals	Parameters to be evaluated
I	Control (untreated)	5	Paw thickness: Paw thickness values were calculated from the difference between the left and the right paw volumes
II	Placebo (without drug)	5	
III	STD	5	
IV	F0 (without penetration enhancer)	5	
V	F5	5	

DDEA: Diclofenac diethylamine

Table 5: Experimental protocol for carrageenan-induced rat paw edema

Groups (*n*=5)	Treatment	Evaluation parameter
I Control	Carrageenan (1%) 0.1 mL subplanter	Edema volume (mL) 6.0 h after carrageenan administration
II Placebo	Carrageenan (1%) 0.1 mL subplanter+placebo cream	
III STD	Carrageenan (1%) 0.1 mL subplanter+Std cream	
IV Fo	Carrageenan (1%) 0.1 mL subplanter+Fo cream	
V F5	Carrageenan (1%) 0.1 mL subplanter+F5 cream	

Table 6: Percent permeation of DDEA through rat skin by F0, F5, and marketed formulations

Time	F0 mean% permeated±standard deviation	F5 mean% permeated±standard deviation	Marketed formulation mean% permeated±standard deviation
0	0	0	0
15	0.181±0.10	0.335±0.13	0.137±0.07
30	0.778±0.25	1.861±0.39	0.667±0.31
60	1.529±0.67	4.690±1.07	1.463±0.47
120	5.950±0.27	4.889±0.96	5.972±0.29
180	7.343±0.75	8.801±1.18	7.475±0.73
240	13.099±1.04	12.935±1.76	13.090±0.93
360	13.908±0.86	23.700±1.75	13.421±0.63

Values represented as mean±SD, *n*=3, Where, *n*=Number of replicates

Table 7: Observation of flux and permeability coefficient of F0, F5, and marketed formulation of DDEA

S. No.	Batches	Flux (Wb) g/cm²/min	Permeability coefficient (m/s)
1	F0	0.2789±0.013	1.680×10^{-5}
2	F5	0.3942±0.009	2.370×10^{-5}
3	Marketed Formulation	0.2730±0.0110	1.640×10^{-5}

DDEA: Diclofenac diethylamine. Values represented as mean±SD, *n*=3, Where, *n*=Number of replicates

(0.5% nerolidol) has higher permeation as compared to the F0 and marketed formulation.

Skin irritation study

No dermal irritation and no edema were observed in any groups. The treated skin of all rats in all the groups appeared normal throughout the observation period. Results are displayed in [Table 8 and Figure 1].

Table 8: Effect of application of placebo and DDEA test cream (F0 and F5) on the skin of Wistar rats

Groups	Erythema and eschar formation grade	Grade	Edema formation	Grade
Placebo	No erythema	0	No edema	0
F0	No erythema	0	No erythema	0
F5	No erythema	0	No edema	0

DDEA: Diclofenac diethylamine

Anti-inflammatory Study

The anti-inflammatory study's results are displayed in [Table 9].

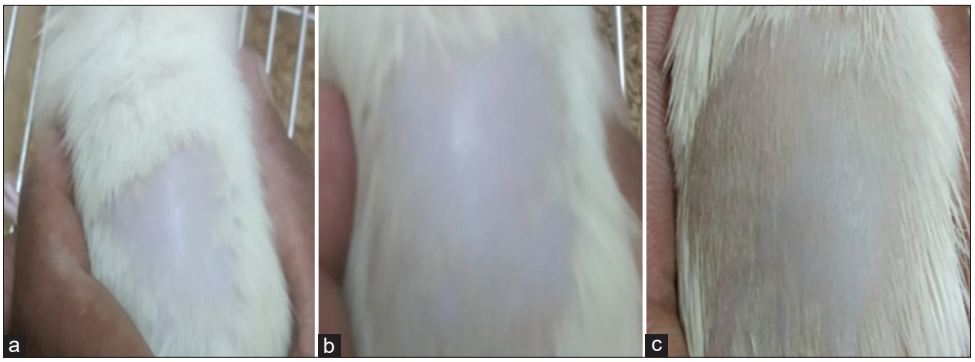

Figure 1: (a) Placebo-treated rat skin (b) skin treated with F0 (c) skin treated with F5, after 15 days of treatment.

Table 9: Comparative anti-inflammatory Effect of Control, Placebo, F5, F0 and Standard formulation of DDEA on carrageenan induced rat paw edema

Groups	Edema Volume in mL (Mean percent inhibition)						
	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Control	0.35±0.00	0.43±0.00	0.54±0.00	0.58±0.01	0.63±0.00	0.82±0.00	0.93±0.0
Placebo	0.34±0.00	0.42±0.00	0.54±0.00	0.55±0.01	0.62±0.00	0.89±0.00	0.88±0.0
F5	0.27±0.00**	0.23±0.00**	0.16±0.00**	0.15±0.00**	0.05±0.00**	0.08±0.00**	0.06±0.0**
F0	0.32±0.00	0.34±0.00	0.33±0.00*	0.31±0.00**	0.26±0.00**	0.24±0.00**	0.22±0.0**
STD	0.31±0.00	0.34±0.00	0.32±0.00**	0.37±0.00**	0.26±0.00**	0.21±0.00**	0.23±0.00**

DDEA: Diclofenac diethylamine. Results expressed in mean±SEM (n=6) ANOVA followed by Dunnett's test *P<0.05, **P<0.01 when compared to control group. From above results it can be concluded that F5 showed significant inhibition of edema in paw volume induced by carrageenan. As compared to Control, Placebo, Std and F0 batch

CONCLUSION

In situ, drug permeation study carried out on F0, F5, and marketed formulations on rat skin reveals that F5 formulation has significantly penetrated the DDEA through rat skin as compared to F0 and marketed formulation (OMNIGEL). Furthermore, the flux and permeability coefficient of the F5 formulation was significantly higher as compared to the F0 and marketed formulation. The skin irritation study carried out on placebo, F0, and F5 formulations revealed that the prepared formulation is non-irritant to the skin and safe for topical application. The study on anti-inflammatory effects using carrageenan.

Induced rat paw edema methods were carried out on control, placebo, F5, F0, and Std groups. The study showed that F5 showed significant inhibition of edema in paw volume induced by carrageenan. As compared to the control, placebo, Std, and F0 batches. Thus from the study, it was concluded that the F5 formulation prepared using shea butter as an absorption base and containing 0.5%w/v of nerolidol as a permeability enhancer showed a better permeation of DDEA through rat skin, non-irritant in nature, and significant anti-inflammatory activity as compared to F0 and marketed formulation.

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ETHICS APPROVAL

The Animal Ethical Committee examined and approved the animal protocol, which was assigned protocol approval number KBH/IAEC/2023/12/12.

REFERENCES

1. Waghule T, Patil S, Vamshi K, Rapalli V, Girdhar S, Saha RN, *et al*. Improved skin-permeated diclofenac-loaded lyotropic liquid crystal nanoparticles: QbD-driven industrial feasible process and assessment of skin deposition. Liquid Crystal 2021;48:1-19.
2. Hagen M, Baker M. Skin penetration and tissue permeation after topical administration of diclofenac. Curr Med Res Opin 2017;33:1623-34.
3. Ramado D, Mccrudden TC, Courtenay AJ, Donnelly RF. Enhancement strategies for transdermal drug delivery systems: Current trends and applications. Drug Deliv Transl Res 2022;12:758-91.
4. Femi-Oyewo MN, Ajala TO, Mabadeje A. The evaluation of shea butter from *Butyrospermum parkii* as

a vehicle in sulphur ointment formulations. West Afr J Pharm 2013;24:58-65.
5. Oyagbemi AA, Oridupa OO. Formulation and *in vivo* anti-inflammatory properties of diclofenac multiple emulsions prepared using *Vitellaria paradoxa* fat (Shea Butter). Afr J Med Med Sci 2019;48:307-17.
6. Shah SN, Rabbani ME, Shahzad Y, Badshah A, Meidan VM, Murtaza G. Developing an efficacious diclofenac diethylamine transdermal formulation. J Food Drug Anal 2012;20:464-70.
7. Oyedele AO. Delivery of metronidazole from purified Nigerian shea butter in comparison to standard and modified ointment bases. Ife J Sci 2012;14:253-7.
8. Ju L, Wenlan W, Yanping Z, Dongju Z, Yawei C, Shan C, *et al*. Use of Shea Butter as Transdermal Osmosis Promoter; 2012 Available from: https://patents.google.com/patent/CN102671212A/en
9. El-Kattan AF, Asbill CS, Michniak BB. The effect of terpene enhancer lipophilicity on the percutaneous permeation of hydrocortisone formulated in HPMC gel systems. Int J Pharm 2000;198:179-89.
10. Escribano E, Calpena AC, Queralt J, Obach R, Doménech J. Assessment of diclofenac permeation with different formulations: Anti-inflammatory study of a selected formula. Eur J Pharm Sci 2003;19:203-10.
11. Schafer N, Balwierz R, Biernat P, Ochędzan-Siodłak W, Lipok J. Natural ingredients of transdermal drug delivery systems as permeation enhancers of active substances through the stratum corneum. Mol Pharm 2023;20:3278-97.
12. Chen J, Jiang QD, Chai YP, Zhang H, Peng P, Yang XX. Natural terpenes as penetration enhancers for transdermal drug delivery. Molecules 2016;21:1709.
13. Cornell PA, Barry BW. Sesquiterpene components of volatile oils as skin penetration enhancer for hydrophilic permeant 5-Fluorourasil. J Pharm Pharmacol 1994;46:261-9.
14. Karmarkar RR, Tiwari RS, Aher RS, Lokhande TN, Pillewan PS, Bachhav YS. Formulation and evaluation of transdermal cream of curcumin prepared using novel absorption bases and permeability enhancer. Afr J Bio Sci 2024;6:443-71.

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