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Original Article

Novel Deformable Vesicle for the Transdermal Delivery of Terbinafine Hydrochloride-Formulation and Cytotoxic Evaluation

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ABSTRACT

Aim/Background: According to estimates, about a billion people in the world have fungal infections of the skin, nails, and hair. Skin disorders are a significant contributor to disability, deformity, and misery among people. In the present study, a versatile vesicular delivery of terbinafine hydrochloride through the dermal route in a biocompatible platform consisting of glycerine, phospholipids, and cholesterol is proposed for the treatment of mycoses. **Materials and Methods:** The glycerosomes of terbinafine hydrochloride were formulated by thin film hydration technique using central composite design to explore the composition effects on the formation of nanosized stable glycerosomes and drug loading. The optimized formulation was evaluated for deformability, antifungal activity, compatibility by FTIR, surface morphology, *in vitro* and *ex vivo* drug diffusion, skin irritation, histopathology, and cytotoxicity studies on HaCaT cell lines. **Results:** Numerical optimization of the design revealed that the particle size and entrapment efficiency of the drug were remarkably affected by the composition. Surface imaging by TEM revealed the formation of well-defined vesicles with no aggregation. The elasticity of the vesicles was established by determining their deformability index. The *in vitro* and *ex vivo* drug release study revealed better permeation of the drug compared to the marketed formulation. The histopathology and cytotoxicity study conceded the suitability of the glycerosomal carrier system for the transdermal delivery of terbinafine hydrochloride. **Conclusion:** Hence it can be concluded that the glycerosomes of terbinafine hydrochloride possess a potential formulation approach for treating dermal infection.

Keywords: Glycerosomes, Terbinafine hydrochloride, Transdermal delivery, Skin permeation, Cytotoxicity.

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INTRODUCTION

Mycoses are fungal infections of the skin that exert superficial, subcutaneous, or even systemic diseases. Fungal infection of the skin is prevalent worldwide. Initially, the infection is confounded at the skin surface, later it penetrates the deeper layer of the skin due to desquamation and results in subcutaneous mycosis. According to the WHO, skin disorders are a significant contributor to disability, deformity, and misery.[1]

Topical delivery of drugs is perhaps the best way to treat the skin condition to ensure direct access to the skin, reduce systemic toxicity, and avoid systemic metabolism of the drugs.[1]

Terbinafine Hydrochloride is an anti-fungal and a BCS II drug and the drug is mainly used in the treatment of dermatophyte infections of nails, jock itch, ringworm, athlete's foot, and pityriasis versicolor. It acts by inhibition of squalene epoxidase thereby inhibiting ergosterol synthesis and thus hindering cell wall synthesis of fungi.[2]

It is available in the market in the form of tablets and conventional topical products. The drug has low oral bioavailability due to its high first-pass effect and urinary elimination. Conventional topical products are associated with skin irritations and also exert a slow onset of action. It requires repeated administration and increases the risk of local toxic reactions. In the progressive state of infections like subcutaneous mycoses, conventional therapy remains unresolved and shows a lack of tolerability of the antifungal agents, Hence, a novel delivery of terbinafine hydrochloride is envisaged in the present study to ameliorate the local side effects and enhance efficacy. A specially designed carrier with increased permeation and penetration through the stratum corneum to localize the drug in the skin, as well as deeper tissues, can be beneficial to treating dermal fungal infection.[3]

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Glycerosomes are novel carrier systems for drugs for dermal and transdermal delivery. They are vesicles of phospholipids, cholesterol, and glycerol in differing quantities, where glycerol is present at high concentrations varying from 10-30% v/v. Glycerol is a triglyceride and non-toxic in nature. The incorporation of glycerol in the vesicular system increases the fluidity, deformability, and stability of the vesicles. The vesicular delivery of glycerosomes provides a better bio-platform for skin penetration compared to liposomes and Niosomes.[4] Moreover, glycerin as an excipient possesses antifungal, healing, and restorative properties.

Glycerosomes, the versatile vesicular delivery, are capable of enclosing both hydrophilic and hydrophobic drugs. The presence of phospholipids aids in the formation of a bilayered structure, cholesterol enhances the packing efficiency of bilayers and improves the rigidity of the bilayers, and glycerol increases the fluidity and stability of the vesicles. The composition enables the glycerosomes for easy deformation to a lesser diameter and quick penetration through the intercellular pathway to the subcutaneous tissue. The driving force for this elastic transport is created by water retention due to the presence of a high concentration of glycerol.[5]

The present research work explores the possibility of delivering terbinafine hydrochloride in a biocompatible bio platform of glycerosomes to achieve better penetration and an alternative to the conventional dosage form.

MATERIALS AND METHODS

Materials

Terbinafine hydrochloride was gifted by Viatris, Hyderabad, India. Phospholipon® 90G (SPC) was purchased from Central Drug House (P) Ltd, Cholesterol CH) from Loba Chemie, Glycerin, Chloroform, Ethanol, Methanol, Hydrochloric acid, and other reagents were procured from SD Fine Chemicals, Bengaluru, India.

Experimental design

Glycerosomes are bilayered vesicles of phospholipids for dermal and transdermal delivery of drugs. The bilayer fluidity of glycerosomes is obtained using different phospholipids and a high concentration of glycerol. These versatile vesicular carriers permit their transdermal penetration through the pores and the flexibility of the transport is facilitated by their nano vesicular size and their ability for deformation. The vesicular size and entrapment efficiency of the drug play a critical role in the formulation of glycerosomes. The preliminary preformulation study revealed that the vesicular size and entrapment efficiency were dependent on the composition - the proportion of Phosphatidylcholine (SPC) and Cholesterol (CH), and the percentage of glycerol in water. To assess the impact of the composition on the glycerosomal formulation a Central Composite Design (CCD) was created using Design Expert V11 software. Table 1 depicts the list of factors studied and responses evaluated. The experimentation was carried out for thirteen trials which consisted of 5 center points and 4-star points.

Preparation of terbinafine hydrochloride glycerosomes

The glycerosomes were formulated using thin film lipid hydration. During this process, the drug (50 mg), the calculated quantity of phospholipid, and cholesterol were dissolved thoroughly in chloroform in a round bottom flask. The rota evaporator was set at 40°C, 100 rpm, and a vacuum was applied.[5] Formation of thin lipid film occurred which was hydrated with a glycerol-water mixture and the process was carried out at 30°C for 1 h at 100 rpm. The vesicular dispersion was loaded into a dialysis tube made from 12-14 kDa MV cut off and dialyzed against water at room temperature for two hours to remove the unentrapped drug. The purified samples were sonicated on a bath ultra-sonicator at 25°C for 20 min, and taken for further studies. Thirteen formulations were prepared as per the design.

Vesicle size

Dynamic Light Scattering (DLS) principle was used to evaluate all thirteen experimental trials for vesicle size through a computerized system (HORIBA scientific). To prevent aggregation, the samples were diluted with Millipore water prior to performing the analysis and were evaluated in triplicate at 25°C.[5]

Entrapment Efficiency (EE)

All thirteen experimental trials were evaluated for % EE. During the purification process, the dialysate was analyzed for free drug and was diluted suitably with 0.1N HCl and analyzed by U.V spectrophotometry at 283 nm to estimate the free drug.[6] The total drug was estimated by disrupting the vesicles of glycerosomes with an equal volume of methanol, vortexed for 5 min, filtered through a 0.45 µm syringe filter, diluted suitably with 0.1N HCl, and analyzed by U.V spectrophotometry at 283 nm. The experiments were conducted in triplicate. %EE of terbinafine HCl glycerosomes was calculated using the formula.[7]

Table 1: List of Factors and Responses for Central Composite Design.

Independent variables	Levels		Dependent variables	Target
	Low	High		
SPC: CH	10:1	30:1	% Entrapment efficiency	Maximize
% Glycerol in water	10	30	Vesicle size	Minimize

$$\% \text{ EE} = \frac{(\text{Total drug} - \text{Unentrapped drug}) \text{ X } 100}{\text{Total drug}}$$

Characterization of the Optimized Glycerosomes (TRFOP)

Fourier Transformation Infrared spectroscopy (FTIR) study

FTIR was used to identify the compatibility of terbinafine hydrochloride with the physical mixture of the composition of the optimized formulation. Pure drug, blank, and optimized formulation physical mixture were taken for the study. The study was performed using the ATR model Nicolet iS50 (Thermo Fisher Scientific, USA).[8]

Surface morphology study

The surface characteristics of TRFOP were analyzed through TEM (Jeol, 100 CX-TEM, Tokyo, Japan) at 80 kV. A drop of the glycerosomes was applied on the paraffin sheet and a carbon-coated grid was positioned on the sample. The grid was then placed on a drop of phosphotungstate (1%) for a few seconds. The excess solution was absorbed with filter paper, and was air-dried, and examined for analysis of shape and aggregation.[9]

Zeta potential

The electrophoresis principle is used in the measurement of zeta potential using nanoPartica SZ-100V2 (HORIBA scientific). TRFOP was subjected to electrophoresis and the velocity of the formulated particles was analyzed. The results gave information on the charge and stability of the optimized sample.[10,11]

Degree of deformability

Extrusion method was used to determine the elasticity of the glycerosomes. The glycerosomes were forwarded through a polycarbonate membrane (pore diameter, 0.2 microns), (Merck, India) through a stainless-steel filter holder (25 mm diameter) at an applied pressure of 2.5 bar.[12] Vesicle size was noted before and after passing through the membrane by DLS technique.

Antifungal study

The antimicrobial study was performed using broth dilution technique on the various strains of Candida (*Candida albicans* ATCC90028, *Candida pappsilosis* ATCC 22019, and *Candida tropicalis* ATCC750) using CLSI (Clinical Laboratory Standards Institute) method in RPMI 1640 medium and incubated overnight at room temperature (30°C) at a constant shaking. The microbes were procured from the Microbiology Department, Maharani College of Arts and Science, Bangalore. A series of dilutions were made for the TRFOP in the dilution range of 25-200 µg/mL and 1 mL of the formulation was added to the media (9 mL) containing 1×10^3 to 2×10^3 colony-forming units per mL. The tubes were incubated for 24 h at 30°C. The minimum inhibitory concentration of the TRFOP was determined.[13]

In vitro drug diffusion study

A modified Franz diffusion cell was used to carry out the *in vitro* release study. The dialysis membrane (MWCO 14000, Hi-Media) was soaked in phosphate buffer pH 5.5 overnight and washed thoroughly with distilled water. The receiver compartment was filled with phosphate buffer pH 5.5 which was used as the release medium. The dialysis membrane was then placed tightly between the donor compartment and the receiver compartment. The formulation TRFOP (1 mg equivalent of terbinafine hydrochloride) was spread evenly on the membrane. The apparatus was set on a magnetic stirrer (2 MLH REMI) maintained at 32°C and at 100 rpm. A specific volume (1 mL) of the medium was collected at predetermined time intervals and reloaded with freshly prepared buffer and the samples were analyzed spectrophotometrically at 283 nm. The results were the mean of three trials.[14,15]

Ex vivo drug diffusion study

The *ex vivo* study was performed on a modified Franz diffusion cell for the formulation TRFOP and a marketed formulation (1% Terbinafine hydrochloride cream available in India). Permission for animal usage was obtained from the Institutional Animal Ethics Committee (IAEC) vides the reference number KCP/IAEC-407/2021-2022. A full-thickness rat's abdominal skin was excised from the animal house. The hair was removed carefully using an electric clipper. The subcutaneous fatty layer was removed, and the skin was washed thoroughly with saline water. The skin was sandwiched between the donor and receptor compartments. The receptor compartment was filled with phosphate buffer solution pH 5.5. The formulation TRFOP (1 mg equivalent of terbinafine hydrochloride) was spread evenly on the skin facing the donor compartment and constantly stirred on a magnetic stirrer maintained at 32°C.[16] Samples were withdrawn at regular time intervals and replaced with fresh buffer and analyzed spectrophotometrically at 283 nm. Flux and permeation constant is calculated using the equation.[17]

$$\text{Jss} = \frac{dQ/dT}{A}$$

$$P = \frac{dQ/dT}{ACd}$$

Where,

A- Diffusion area of the patch (4.512 cm^2).

dQ/dT- slope of the linear region of the *ex vivo* diffusion curve.

Cd- donor concentration (µg/mL).

Skin irritation study

Eighteen albino Wistar rats were taken for this study and were distributed in three groups. The animals in group I served as control. Group II and III animals were treated with a placebo and TRFOP respectively. After the depletion of the hairs from the back of the animals, the areas were marked. The blank glycerosomal formulation and TRFOP were applied once daily for 7 consecutive days and observed for any sensitive reactions.[18]

Histopathology studies

Histopathology test was conducted with the skin of Wister albino rats. The skin was treated with TRFOP for 24 h and the untreated skin served as a control. The skin was initially treated with saline water containing formalin (10%v/v). The skin tissues were stained with hematoxylin and eosin and were observed under a microscope.[19]

Cytotoxicity study

MTT assay was carried out with HaCaT cell line to estimate the cell viability with the administration of TRFOP. The cell line was cultivated in DMEM media fortified with 10 %v/v fetal bovine serum at 37°C in a 5% carbon dioxide rich environment. A concentration of 0.05% Trypsin/EDTA was added for the detachment of the cells before centrifugation.[20]

For the cytotoxicity test, the TRFOP dispersion (20-80 µg/mL) was added in DMEM and cultured with HaCaT cells at 37°C for 24 h, 36 h, and 72 h. After the specified period, the media was treated with 10% MTT, and incubated for 3 h at 37°C and 5% carbon dioxide rich environment. To this DMSO was added to assist the formation of purple formazan, and estimated at 570 nm spectrophotometrically to quantify the data related to growth inhibition (%) and from there % viability was calculated.[21]

RESULTS

Design optimization for the preparation of glycerosomes

The experimental batches of 13 trials showed a variation in % entrapment efficiency from 46.54% to 97.89%. The vesicle size (nm) also showed a variation in the range of 100.6 nm to 719.8 nm as listed in Table 2. The model and parameter sensitivity analysis are represented in Table 3. The contour and response surface graphs for both responses are shown in Figure 1. The curvature in the response surface graphs showed the factors and the responses were in quadratic relation in a logarithm scale. The relation is expressed by the following equations

$$\text{Log}_{10}\text{ (Particle Size)} = +2.86 - 0.1239A - 0.0240B + 0.0306AB - 0.0051A^2 - 0.2080B^2$$

$$\text{Log}_{10}\text{ (Entrapment Efficiency)} = +1.94 - 0.0273A + 0.0510B + 0.0137AB + 0.0142A^2 - 0.0894B$$

Where A represents SPC: CH and B represents %glycerol in water.

Model optimization was carried out in a numerical way to achieve maximum entrapment of the drug in the vesicles and minimum vesicle size for effective dermal penetration. The optimization yielded a desirability of 0.845 and suggested a formulation condition of SPC: CH at coded value (-0.75) and % glycerol in water (+1) with a prediction of particle size 299.01 nm and %EE 86.64. The optimized formulations were prepared as per the predicted coded ratio and the optimized product exhibited entrapment efficiency of 90.54% with a particle size of 309.6 nm and the % bias was found to be <5%.

FTIR study

FTIR spectroscopy of pure Terbinafine Hydrochloride showed characteristic peaks at 2967 cm^{-1} (C-H stretch), 2562 cm^{-1} (aliphatic C-H stretch), 2224 cm^{-1} (aromatic C-C stretch), 1516 cm^{-1} (C-C stretch), and 1172 cm^{-1} (C-N band). The FTIR overlay spectra are shown in Figure 2.

Surface morphological study

The nano-sized glycerosomal vesicles as observed from the TEM study are shown in Figure 3.

Degree of deformability

The vesicle size of the glycerosomes was found to be 309.6±3.49 nm and 294±2.21 nm before and after extrusion respectively. The deformability index of the glycerosomes was calculated and found to be 0.951.

Antifungal study

The minimum inhibitory concentration of the formulation against the fungi was found to be 100 µg/mL, 125 µg/mL, and 100 µg/mL for the strains *Candida albicans* ATCC90028, *Candida pappsilosis* ATCC 22019, and *Candida tropicalis* ATCC750 respectively.

Comparative drug diffusion studies

The drug diffusion study of the glycerosomal formulation showed enhancement in the diffusion of the drug compared to the marketed formulation through the dialysis membrane and the animal skin. The *in vitro* and *ex vivo* graphs are almost close as shown in Figure 4. It was observed that the permeation parameters were improved by 3 folds as presented in Table 4.

Skin irritation study

The skin irritation test was done for the eighteen albinos Wistar rats such that the first group received no application of the formulation, the second group applied with blank glycerosomal formulation, and the third group with TRFOP.

Table 2: Observations of the CCD Experimental Design.

Experimental runs	Actual values		Dependent factors	
	SPC:CH	% Glycerol in water	% EE±SD	Particle size(nm)±SD
1	1.41421	0	97.23±0.16	719.8±6.41
2	-1.41421	0	86.83±0.58	436±6.90
3	1	-1	56.68±0.003	623.4±7.56
4	1	1	72.41±0.005	547.3±7.98
5	0	0	97.89±0.003	583.6±5.83
6	0	0	90.14±0.001	583.6±6.67
7	0	1.41421	69.89±0.001	265.2±4.21
8	0	0	80.87±0.002	583.6±7.34
9	0	0	93.78±0.001	583.6±5.59
10	-1	1	94.72±0.001	315±7.09
11	0	0	78.7±0.001	583.6±6.65
12	0	-1.41421	46.54±0.01	248.1±4.48
13	-1	-1	84.12±0.001	100.6±5.29

Table 3: Model and Parameter Sensitivity Analysis.

Responses	Model and parameters	Sum of squares	d_f	Mean square	F-value	*p*-value	Significance
Particle size	Model	0.4354	5	0.0871	19.78	0.0005	Significant
	A-SPC:CH	0.1227	1	0.1227	27.88	0.0011	Significant
	B-% glycerol in water	0.0046	1	0.0046	1.04	0.3412	
	AB	0.0038	1	0.0038	0.8522	0.3867	
	A^2	0.0002	1	0.0002	0.0411	0.8451	
	B^2	0.3008	1	0.3008	68.33	<0.0001	Significant
Entrapment efficiency	Model	0.0878	5	0.0176	5.02	0.0284	Significant
	A-SPC:CH	0.0060	1	0.0060	1.71	0.2324	
	B-% glycerol in water	0.0208	1	0.0208	5.94	0.0449	Significant
	AB	0.0008	1	0.0008	0.2150	0.6570	
	A^2	0.0014	1	0.0014	0.4016	0.5464	
	B^2	0.0555	1	0.0555	15.89	0.0053	Significant

Histopathology studies

The histopathology of normal skin for the control group of animals and treated skin is presented in Figure 5.

Cytotoxicity study

The *in vitro* MTT assay of the glycerosomal terbinafine hydrochloride on HaCaT cells revealed the absence of the cytotoxic effect of the TRFOP formulation over a concentration range of 20-80 µg/mL. The cells were viable (>95% viability) over 24 h in the wide concentration range of the formulation (Figure 5C). The highest concentration of TFROP (80 µg/mL) on the cell sustainability, for 72 h revealed 94% cell viability (Figure 5D).

DISCUSSION

Statistical interpretation of CCD model

CCD is a booming technique of surface response methodology that assesses the degree to which the individual variables in an experiment have an impact on the outcome. The effect of composition on the prime attributes of glycerosomes was investigated.

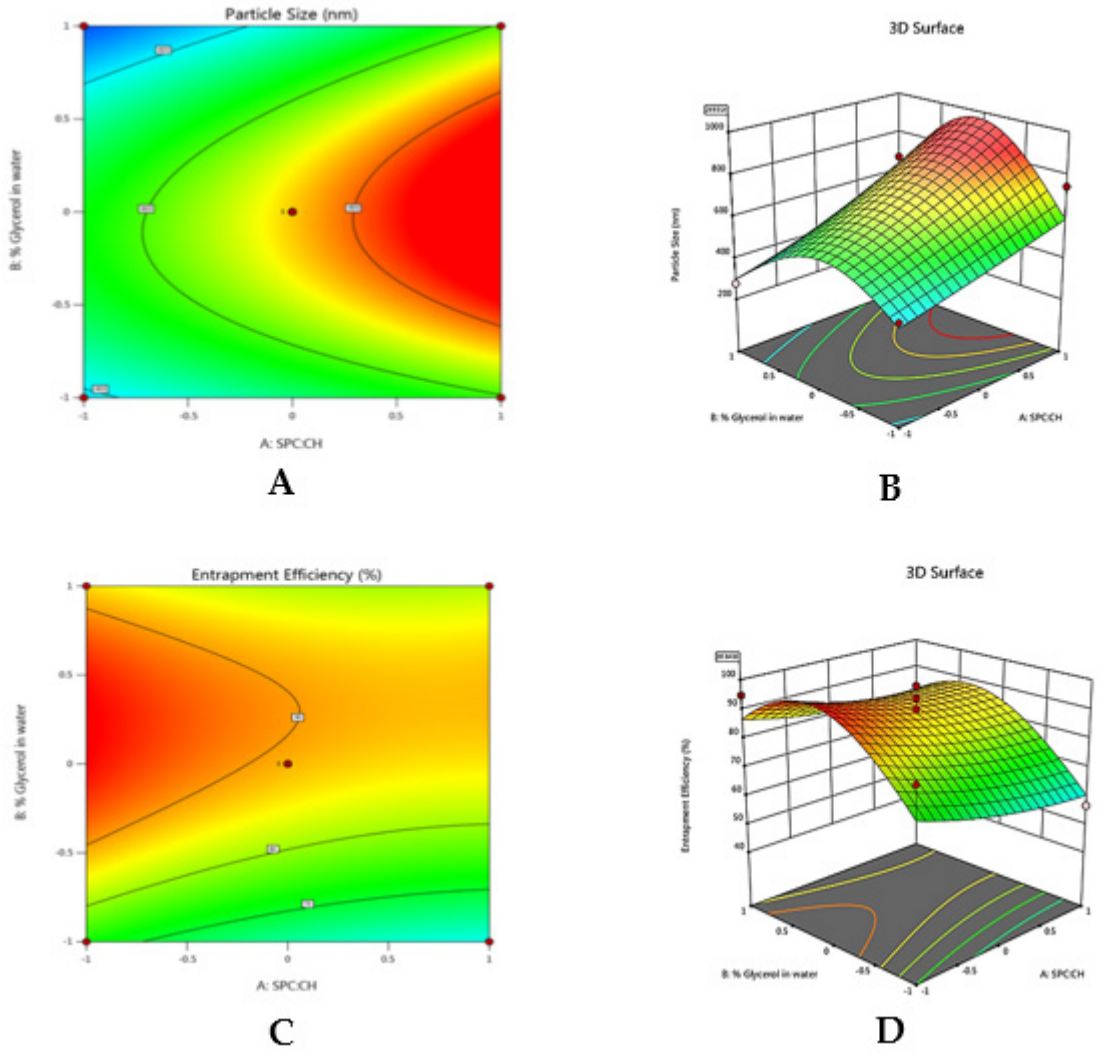

Figure 1: 2D Contour Plot of Particle Size(A) and Entrapment Efficiency(C) and 3D Response Surface Graph of Particle Size(B) and Entrapment Efficiency(D) illustrating the effect of factors in the formulation of glycerosomes.

The experimental model was evaluated at a significance level of $p<0.05$ and log transformation was employed to determine the model's significance. The sensitivity analysis proved the efficacy of the model in estimating the factor's contribution to the responses. The composition of lipids has a profound effect on the particle size, drug entrapment efficiency was remarkably affected by the % glycerol in water and was evidenced by the curvature in the response surface diagram. The optimized product was found to be within the predicted range of the design. Therefore, the employed model for the preparation of nanosized glycerosomes was validated. The high drug loading of the terbinafine hydrochloride might be due to its amphiphilic nature and encapsulation in the aqueous and bilayers of lipids. The blank optimized formulation showed a zeta potential close to zero (-0.6 mv) while the optimized formulation showed a slightly high negative zeta potential. The formulations were stored at 4°C for further studies for 90 days and revealed that the aggregation of the TRFOP was less compared to the blank; it might be due to their high negative zeta potential which extends the stability of the optimized formulation of terbinafine hydrochloride.

FTIR study

The FTIR study revealed that the peaks of the pure drugs were preserved in the optimized formulation TRFOP. There is neither appearance nor disappearance of any peak, a slight reduction and shift in peak intensities was observed for a few peaks which might be due to the formation of the H-bond. Hence, it can be confirmed that the excipients used are compatible with the drug.

Surface morphology study

TEM images revealed nano-sized glycerosomal vesicles with no aggregation. The vesicles were found to be in uniform size and there were no disruptions of structure which confirms the stability and integrity of the vesicles during the sonication procedure.

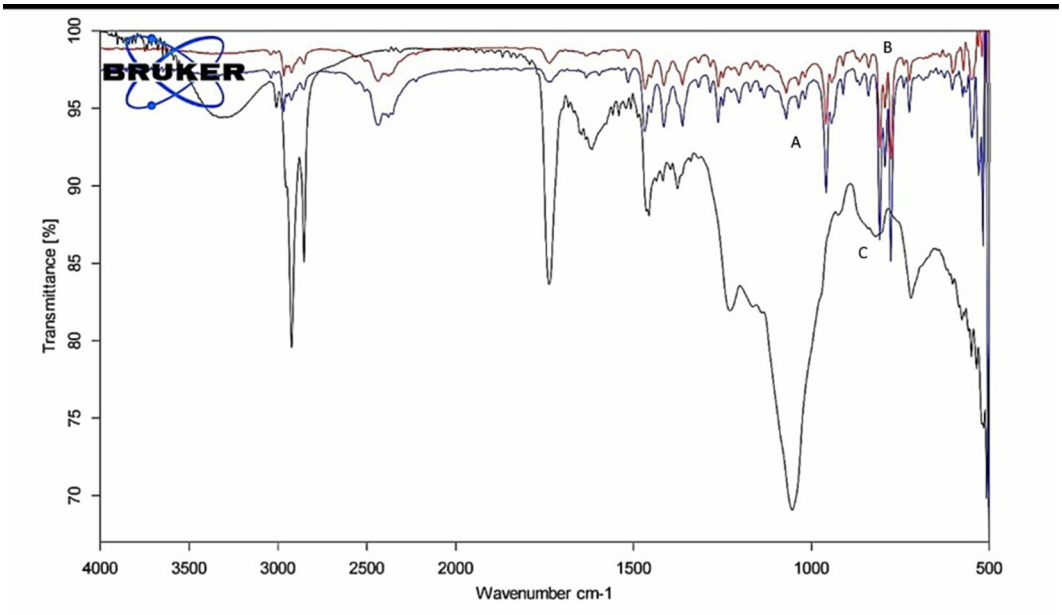

Figure 2: FTIR study of the pure drug (A), blank (B), and optimized formulation (C).

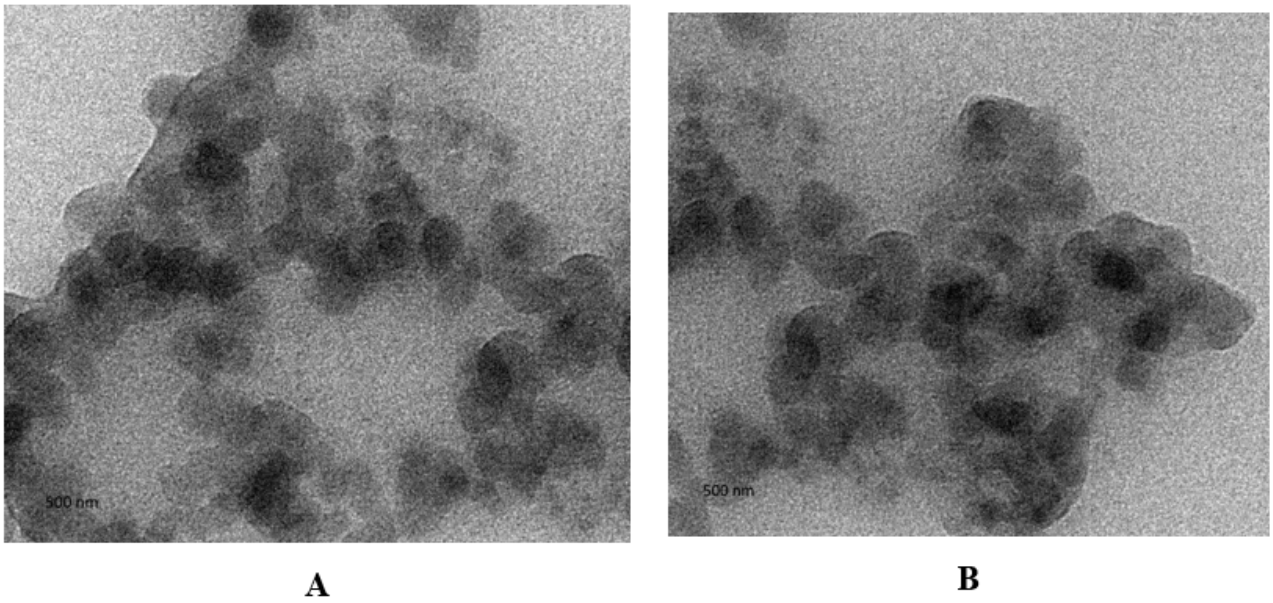

Figure 3: TEM Images of TRFOP.

Table 4: Permeation Parameters.

Permeation parameters	*Ex vivo* studies	
	MKTD	TRFOP
Drug flux(mcg/cm²/h)	10.73	29.11
Permeation constant(cm/h)	0.01	0.03

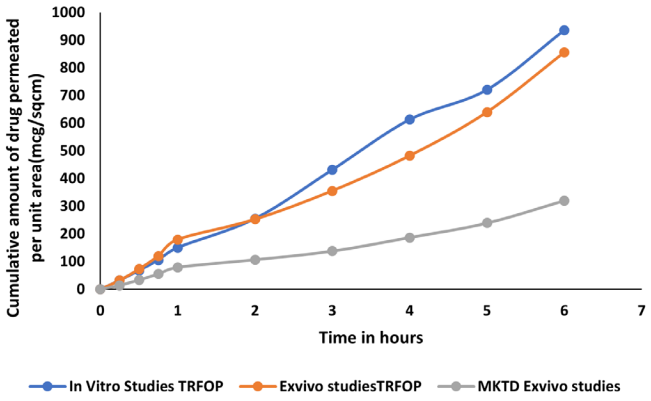

Figure 4: Comparative diffusion Studies of Terbinafine hydrochloride from the formulation (TRFOP) and Marketed product (MKTD).

Degree of deformability

The formulation TRFOP was passed through the polycarbonate membrane of pore size smaller than the vesicular size of the formulation and was subjected to size analysis by DLS techniques. There was a very slight change in the vesicular size. The elastic characteristic of the formulation and retainment of its size was evidenced by the deformability index. Hence, it can be interpreted that the vesicular system has the efficacy to pass through the different barriers of the skin without vesicular leakage.

Antifungal activity

The antifungal study disseminated that the optimized formulation (TRFOP) was found to be potent at the lowest concentration against various strains of fungi.

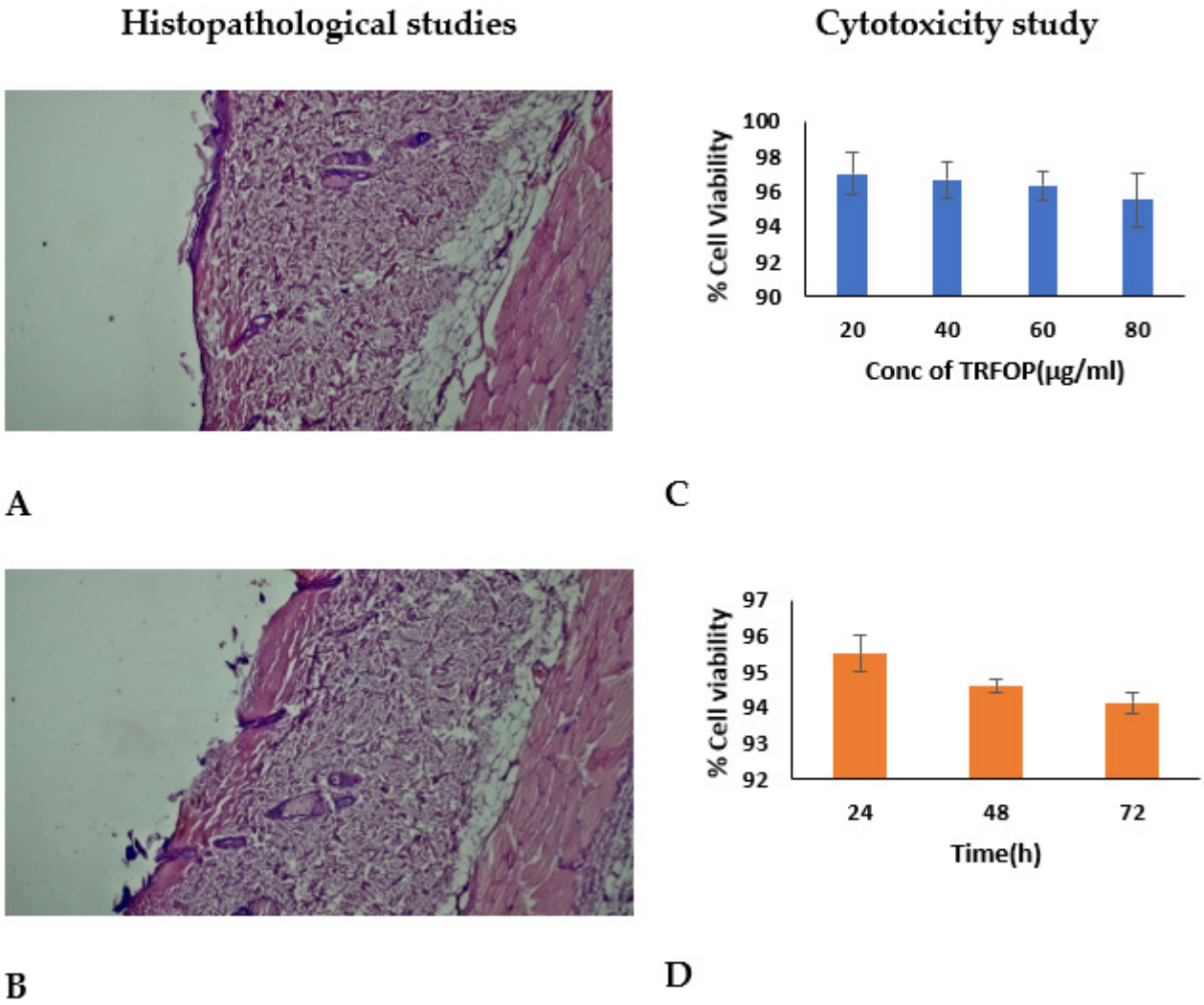

Figure 5: Histopathology (control group (A), and treated group (B)) and cytotoxicity studies of TRFOP (at different concentrations (C) and at the highest concentration (80 µg/mL) for long hours (D)).

Drug diffusion studies

The comparative diffusion study revealed that higher diffusion of terbinafine hydrochloride from TRFOP, and indicated higher permeation of the drug, compared to the marketed cream. The diffusion data were compared for drug flux and permeation rate where a 3 times improvement in permeation was observed.

Skin irritation study

The skin irritation study disclosed that animals in the test groups showed no hypersensitivity reactions on the skin. Hence the suitability of TRFOP for dermal application was established.

Histopathology study

Mechanism of penetration, enhanced penetrability, and interaction of glycerosomes with skin were observed from the histopathology studies. The normal skin for the control group of animals showed an intact epidermis with the lamellar bilayers of lipids. The treated skin showed some degree of thickening, while the control group did not show any noticeable thickening. Infiltration of leukocytes into the deeper layer of skin was an indication of growth factor stimulation.[22] The penetration of the deformable vesicles through the skin was notably visible in the histopathological study through the disarrangement of the intercellular lipid bilayer.

Cytotoxicity study

The nontoxic nature of the formulation was established through a cytotoxicity study in HaCaT cell lines. The effect of the highest concentration of TFROP (80 µg/mL) on the cell sustainability, showed 94% viability after 72 h. This proved the nontoxic nature of the formulation and ruled out the possibility of time-dependent toxicity.

Hence the overall results and observations supported the desired outcome of the proposed research objectives.

CONCLUSION

In the present research, a central composite design was employed to optimize the composition of glycerosomal formulations of terbinafine hydrochloride. The optimization of the design yielded nanosized (309 nm) stable glycerosomes with a high drug entrapment. The prepared glycerosomes showed remarkably enhanced percutaneous delivery of the drug and no sensitization on the skin of the animals. Histopathology and cytotoxicity study revealed the good transdermal permeability and biocompatibility of the glycerosomal terbinafine. The exposure of the live cells with the formulation for long hours established safety and biocompatibility. Deeper penetration of drug through this nanocarrier can be beneficial for the prevention of subcutaneous mycosis, easy healing, and restoration of skin function. Hence it has been established as an efficient carrier for the transdermal delivery of terbinafine hydrochloride. Therefore, it can be concluded that glycerosomes of terbinafine hydrochloride demonstrated to be a potential formulation approach for treating dermal infection and can be a better alternative to commercially available products.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CH: Cholesterol; **CCD:** Central Composite Design; **DLS:** Dynamic Light Scattering; **DMEM:** Dulbecco's Modified Eagle Medium; **DMSO:** Dimethyl sulfoxide; **EE:** Entrapment efficiency; **FTIR:** Fourier Transformation Infrared Spectroscopy; **HaCaT:** High sensitivity of human epidermal keratinocytes; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **TEM:** Transmission Electron Microscope; **TRFOP:** Optimized glycerosomes of terbinafine hydrochloride.

SUMMARY

Glycerosomes of terbinafine hydrochloride to enhance dermal penetration for the treatment of dermal mycoses were proposed in the study. A central composite design was employed to obtain a nanosized vesicular system, The glycerosomes exhibited good deformability, antifungal activity, and enhanced permeation of the drug. Skin irritation, histopathological studies of skin, and cytotoxicity studies on HaCaT cell lines further proved the applicability of the proposed delivery of the drug. The overall observations concluded that loading of terbinafine hydrochloride in a deformable vesicular system can be a promising way to improve its efficacy over dermal mycoses.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The institutional ethical clearance was obtained for animal usage vide the approval number KCP/IAEC/PCOL/PCEU/91/2022. The research does not involve any human volunteers.

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