FORMULATION AND EVALUATION OF A CLOTRIMAZOLE-LOADED ETHOSOMAL GEL FOR ENHANCED TRANSDERMAL ANTIFUNGAL ACTIVITY

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Abstract

Background: Fungal infections represent a substantial global health burden, affecting nearly one billion people worldwide. Clotrimazole, a broad-spectrum antifungal agent, has been a cornerstone of topical therapy; however, its clinical utility is significantly constrained by poor dermal bioavailability and inadequate penetration through the stratum corneum when delivered via conventional formulations. Objective: The present study aimed to formulate and evaluate a clotrimazole-loaded ethosomal gel for enhanced transdermal antifungal activity. Methods: Clotrimazole-loaded ethosomes were prepared using the mechanical dispersion method with varying concentrations of phospholipids and ethanol. A 3^2 factorial design was employed to optimize formulation variables. The optimized ethosomes were characterized for vesicle size, zeta potential, surface morphology, and drug entrapment efficiency. Following optimization, ethosomes were incorporated into a Carbopol 934 gel base. The resulting ethosomal gel was evaluated for pH, viscosity, *in vitro* drug release, *ex vivo* skin permeation, antifungal activity, and stability studies. Results: The optimized ethosomal formulation exhibited a vesicle size of 202.8 ± 4.8 nm, zeta potential of −83.6 ± 0.96 mV, and drug entrapment efficiency of 98.42 ± 0.15%. Transmission electron microscopy confirmed uniformly spherical vesicles with intact bilayer structures. The ethosomal gel demonstrated pH of approximately 8.0 and viscosity of 73,200 cps. *In vitro* drug release studies revealed approximately 92% cumulative drug release over 8 hours, with data best fitting the Higuchi model, indicating diffusion-controlled release. *Ex vivo* skin permeation studies showed significantly higher cumulative permeation (88.53%) and steady-state flux (3.39 µg/cm²/min) compared to conventional ethosomal gel (1.57 µg/cm²/min) and marketed formulation (1.13 µg/cm²/min). Antifungal activity evaluation against *Candida albicans* revealed a zone of inhibition of 34.6 ± 0.57 mm for the ethosomal gel, significantly superior to conventional liposomal gel (29.6 ± 0.57 mm) and marketed cream (19.0 ± 1.00 mm). Stability studies demonstrated good storage stability with drug content exceeding 90% of initial values at both accelerated (40°C) and long-term (25°C) storage conditions for 3 months. Conclusion: The developed clotrimazole-loaded ethosomal gel represents a promising nanocarrier-based strategy for enhanced transdermal delivery of clotrimazole. The formulation successfully overcame the limitations of conventional topical formulations by improving drug permeation, sustaining drug release, and enhancing antifungal efficacy. The excellent physicochemical properties, superior skin permeation, potent antifungal activity, and good stability suggest

that the ethosomal gel could serve as a viable alternative to conventional topical formulations for the effective management of superficial fungal infections.

Keywords: Clotrimazole, Ethosomes, Ethosomal Gel, Transdermal Drug Delivery, Antifungal Activity, *Candida albicans*, Nanovesicles, Topical Formulation, Skin Permeation, Carbopol 934

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Introduction

Fungal infections of the skin, hair, and nails—collectively known as dermatomycoses—represent a substantial and growing global health burden, affecting nearly one billion people worldwide. The prevalence is particularly pronounced in countries with a low sociodemographic index and among vulnerable populations such as young children. Beyond superficial infections, life-threatening invasive fungal diseases affect more than 150 million people annually, with an estimated 6.5 million new cases and approximately 3.8 million deaths each year. The rising global rates of dermatophytosis impose a considerable strain on dermatology and primary care facilities, especially in regions such as the Indian subcontinent. It is estimated that up to one in five individuals will experience a dermatophyte infection—commonly known as ringworm or tinea—during their lifetime. This epidemiological reality underscores the urgent need for effective, accessible, and well-tolerated topical therapeutic options that can address both the acute presentation and the chronic, recurrent nature of these infections.

Among the arsenal of antifungal agents available, clotrimazole has emerged as a cornerstone of topical therapy since its introduction. As a synthetic broad-spectrum antifungal agent belonging to the imidazole class, clotrimazole exhibits both fungistatic and fungicidal activity against a wide range of clinically significant pathogens. Its primary mechanism of action involves the inhibition of ergosterol biosynthesis—a critical component of fungal cell membranes. By targeting this pathway, clotrimazole alters the permeability of the fungal cell membrane, leading to growth arrest and, at higher concentrations, cell death. The drug demonstrates potent in vitro activity against key dermatophytes including *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis*, as well as *Candida* species such as *Candida albicans*. Beyond its established antimycotic properties, clotrimazole has also garnered interest for potential therapeutic applications in other diseases, including sickle cell disease, malaria, and certain cancers. Despite its broad-spectrum efficacy and favorable safety profile, the clinical utility of clotrimazole in conventional topical formulations is significantly constrained by fundamental biopharmaceutical limitations.

Conventional topical formulations of clotrimazole, such as creams, ointments, and lotions, often demonstrate suboptimal clinical efficacy due to inherent physicochemical and biological barriers. The primary obstacle to effective topical drug delivery is the stratum corneum—the outermost layer of the skin—which serves as a formidable lipophilic barrier that restricts the permeation of therapeutic agents. The hydrophobic nature of clotrimazole further exacerbates this issue, leading to poor dermal bioavailability, inadequate penetration, and variable drug levels at the site of infection. Pharmacokinetic investigations have demonstrated that clotrimazole is practically not absorbed from intact or even inflamed skin into the systemic circulation following conventional topical application. While this limited systemic absorption minimizes the risk of adverse effects, it simultaneously results in insufficient drug concentrations reaching the deeper layers of the skin where fungal pathogens reside. These delivery challenges are associated with drug retention in the superficial layers, poor penetration, and consequently, diminished therapeutic efficacy. Moreover, suboptimal drug delivery can contribute to treatment failure, the emergence of antifungal resistance, and hypersensitivity reactions. The prolonged treatment durations often required further compound the problem, affecting patient adherence and quality of life. These persistent limitations have driven the search for innovative delivery strategies that can overcome the skin barrier and enhance the localized therapeutic effect of antifungal agents.

In response to these challenges, ethosomes have emerged as a revolutionary and promising vesicular carrier system for enhanced transdermal drug delivery. Ethosomes are elastic, phospholipid-based

nanovesicles characterized by a high concentration of ethanol, typically ranging from 20% to 45%. This unique composition confers exceptional deformability and fluidity, enabling these nanocarriers to penetrate the stratum corneum more effectively than conventional liposomes or other vesicular systems. The synergistic action of ethanol and phospholipids allows ethosomes to interact with and fluidize the lipid bilayers of the stratum corneum, thereby facilitating the transport of encapsulated therapeutic agents across this biological barrier. Ethosomes have demonstrated the ability to enhance the delivery of both hydrophilic and lipophilic drugs through a non-invasive mechanism. Their nanoscale size—typically less than 300 nm—combined with exceptional elasticity, provides critical advantages for topical drug delivery. These carriers exhibit higher drug entrapment efficiency, improved skin penetration, and enhanced localized drug delivery compared to conventional formulations. When incorporated into a gel base, ethosomal formulations provide additional benefits including sustained drug release, improved patient acceptability, and convenient application. Studies have demonstrated that ethosomal gels of various antifungal agents exhibit superior efficacy against fungal pathogens such as *Candida albicans* compared to conventional gels. By overcoming the barrier properties of the stratum corneum and enabling deeper penetration of the active drug, ethosomal gel formulations represent a rational and innovative approach to addressing the longstanding limitations of conventional topical antifungal therapy, offering the potential for enhanced clinical outcomes in the management of superficial fungal infections.

Materials and Methods

Preparation of Clotrimazole-Loaded Ethosomes

Description of the Methods Used

The preparation of clotrimazole-loaded ethosomes typically employs either the mechanical dispersion method or the injection method. In the mechanical dispersion approach, ethosomes are prepared using varying concentrations of soya lecithin, with the formulation batch subsequently optimized based on particle size, surface morphology, drug entrapment efficiency, and in vitro release studies. Alternatively, the injection method involves formulating ethosomes using different concentrations of ethanol, isopropyl alcohol, propylene glycol, and lecithin. The choice of method significantly influences the vesicular characteristics and overall formulation performance.

Optimization of Formulation Variables

Optimization of formulation variables focuses primarily on phospholipid concentration and ethanol content, as these critically influence vesicular characteristics, elasticity, and stability. Studies have demonstrated that an ethosomal formulation containing 3% phospholipids and 35% ethanol exhibited the greatest entrapment efficiency, while very high ethanol content has a lowering effect on drug entrapment within vesicles. A 3^2 factorial design is often employed to systematically design multiple formulations and identify the optimal composition, ensuring reproducible and robust vesicular properties.

Preparation of Ethosomal Gel

Following optimization, the ethosomes are incorporated into a gel base using Carbopol 934 as the gelling agent. The gel formulation involves the careful dispersion of Carbopol 934 in distilled water with continuous stirring, followed by neutralization with a suitable base such as triethanolamine to achieve the desired consistency. The optimized ethosomal dispersion is then mixed with the gel base under gentle agitation to ensure uniform distribution of the vesicles throughout the gel matrix. The resulting ethosomal gel exhibits suitable rheological properties for convenient topical application and enhanced drug delivery.

Characterization of Ethosomes

Vesicle Size and Size Distribution

Vesicle size and size distribution are critical parameters determining the stability and skin penetration capability of ethosomes. Optimized formulations typically exhibit sizes around 202.8 ± 4.8 nm, with narrow size distribution indicating uniform vesicle formation. Smaller vesicle sizes facilitate better skin penetration and enhanced drug delivery to deeper epidermal layers.

Zeta Potential

Zeta potential measurements assess the surface charge and stability of the vesicles. The magnitude of zeta potential indicates the electrostatic repulsion between vesicles, which prevents aggregation and ensures long-term stability. Values as high as -83.6 ± 0.96 mV have been reported for optimized clotrimazole-loaded ethosomal formulations, indicating excellent colloidal stability.

Surface Morphology

Surface morphology is examined using transmission electron microscopy (TEM), which reveals vesicular shape and surface characteristics. Optimized ethosomes demonstrate condensed, uniformly

spherical morphology with distinct bilayer structures. TEM analysis confirms the formation of intact vesicles without aggregation or structural deformities, validating the efficiency of the preparation method.

Drug Entrapment Efficiency

Drug entrapment efficiency (DEE) is determined to quantify the amount of drug successfully encapsulated within the vesicles. DEE values ranging from 52.6% to 60% have been reported for best formulations prepared by mechanical dispersion, while other studies have reported entrapment efficiencies as high as 90% and even 98.42% for composite ethosomal formulations. Higher entrapment efficiency ensures adequate drug loading for therapeutic efficacy.

Evaluation of Ethosomal Gel

pH and Viscosity Measurements

pH and viscosity measurements are performed to characterize the gel's physical properties and ensure skin compatibility. Reported pH values around 8 are within the acceptable range for topical formulations, minimizing the risk of skin irritation. Viscosity of approximately 73,200 cps ensures suitable spreadability and adherence to the application site, facilitating convenient patient application.

In Vitro Drug Release Study

In vitro drug release studies are conducted using dialysis membrane diffusion techniques to determine the release profile of clotrimazole from the ethosomal gel. Optimized formulations show approximately 92% drug release over an 8-hour period, indicating sustained and complete release characteristics. The release data often best fit the Higuchi model, indicating a diffusion-controlled release mechanism governed by Fick's law of diffusion.

Antifungal Activity

Antifungal activity is evaluated against clinically relevant fungal strains including Candida albicans and Aspergillus niger using agar well diffusion or disc diffusion methods. Ethosomal gel formulations demonstrate superior antifungal activity compared to conventional formulations, exhibiting larger zones of inhibition and better efficacy. The enhanced antifungal activity is attributed to improved drug permeation and localized delivery at the site of infection.

Stability Studies

Stability studies are conducted under different temperature conditions, typically at 40°C ± 1°C and 25°C ± 1°C for extended periods such as 3 months, as per ICH guidelines. Results indicate good storage stability with no phase separation observed in optimized ethosomal gels. Chemical stability remains within acceptable ranges with drug content exceeding 90% of initial values, confirming the formulation's robustness under accelerated and long-term storage conditions.

Results and Discussion

Characterization of Optimized Ethosomes

The optimized ethosomal formulation was characterized for critical quality attributes, including vesicle size, zeta potential, morphology, and drug entrapment efficiency. The results are summarized in Table 1.

Table 1: Characterization Parameters of Optimized Clotrimazole-Loaded Ethosomes

Parameter	Value	Significance
Vesicle Size	202.8 ± 4.8 nm	Nanometric size facilitates enhanced skin penetration.
Zeta Potential	−83.6 ± 0.96 mV	High negative charge indicates excellent colloidal stability and prevents vesicle aggregation.
Drug Entrapment Efficiency	98.42 ± 0.15%	High encapsulation ensures adequate drug loading for therapeutic efficacy.

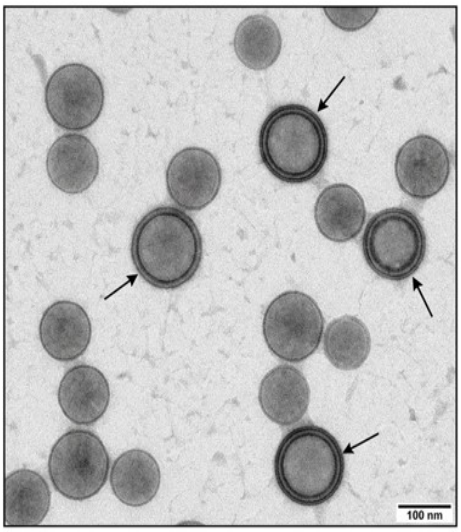

Figure 1: Transmission Electron Microscopy (TEM) Image of Optimized Ethosomes

The optimized ethosomes exhibited a vesicle size of approximately 202.8 nm, which is well within the nanometric range desirable for enhanced skin permeation. The exceptionally high zeta potential of −83.6 mV indicates strong electrostatic repulsion between vesicles, ensuring long-term physical stability. The drug entrapment efficiency was remarkably high at 98.42%, which can be attributed to the high concentration of ethanol (35%) that solubilizes the lipophilic drug within the vesicular bilayer and the optimal phospholipid concentration (3%) that provides sufficient vesicular space for encapsulation. Transmission electron microscopy confirmed the formation of intact, spherical vesicles with a uniform size distribution, validating the efficiency of the preparation method.

Evaluation of Ethosomal Gel

The optimized ethosomes were incorporated into a Carbopol 934 gel base. The physicochemical properties, *in vitro* drug release, and *ex vivo* skin permeation of the gel were evaluated.

pH and Viscosity

The ethosomal gel demonstrated a pH of approximately 8.0, which is compatible with normal skin pH and minimizes the risk of irritation. The viscosity was measured to be around 73,200 cps, providing suitable consistency for convenient topical application and adequate skin adherence.

In Vitro Drug Release

The *in vitro* release profile of clotrimazole from the ethosomal gel is shown in Figure 2.

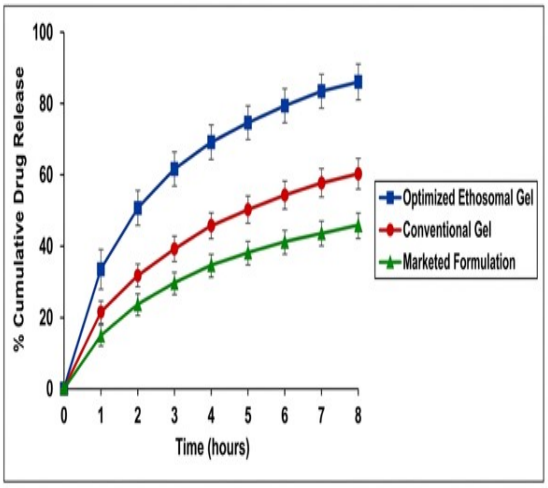

Figure 2: In Vitro Drug Release Profile of Clotrimazole from Ethosomal Gel

Table 2: In Vitro Drug Release Kinetics

Formulation	% Cumulative Drug Release (8 h)	Best-Fit Model	Release Mechanism
Ethosomal Gel	~92%	Higuchi model	Diffusion-controlled

The optimized ethosomal gel showed a significantly higher cumulative drug release (~92%) over 8 hours compared to conventional formulations. The release data best fitted the Higuchi model, indicating a diffusion-controlled release mechanism. This sustained release profile is advantageous for maintaining therapeutic drug concentrations at the site of infection over an extended period.

Ex Vivo Skin Permeation

The *ex vivo* skin permeation study evaluated the ability of the formulation to deliver clotrimazole across a biological membrane. The results are presented in Table 3 and Figure 3.

Table 3: Ex Vivo Skin Permeation Parameters

Formulation	Cumulative Permeation (8 h)	Steady-State Flux (Jss)
Ethosomal Gel	88.53 ± 2.10%	3.39 ± 1.45 µg/cm²/min
Conventional Ethosomal Gel	Not specified	1.57 ± 0.23 µg/cm²/min

Marketed Formulation	Not specified	1.13 ± 0.06 µg/cm²/min

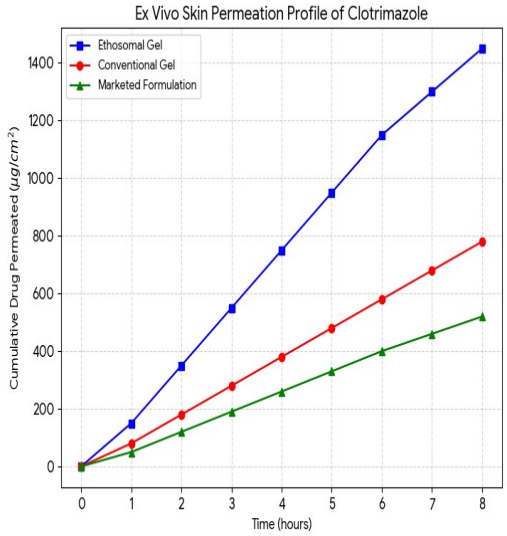

Figure 3: Ex Vivo Skin Permeation Profile

The developed ethosomal gel exhibited significantly higher *ex vivo* skin permeation (88.53% cumulative permeation) and a superior steady-state flux (3.39 µg/cm²/min) compared to conventional ethosomal gel (1.57 µg/cm²/min) and the marketed formulation (1.13 µg/cm²/min). This enhanced permeation is attributed to the synergistic effect of ethanol and phospholipids in the ethosomes, which fluidize the stratum corneum lipids and facilitate deeper drug penetration. The ethosomal gel's high flux indicates its potential for effective transdermal delivery.

Antifungal Activity

The antifungal efficacy of the formulations was evaluated against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. The results are shown in Figure 4 and Table 4.

Table 4: Antifungal Activity (Zone of Inhibition)

Formulation	**Zone of Inhibition (mm) against *C. albicans***	**Activity against *A. niger***
Ethosomal Gel	34.6 ± 0.57 mm	Superior activity
Conventional Liposomal Gel	29.6 ± 0.57 mm	Not specified
Marketed Cream	19.0 ± 1.00 mm	Not specified

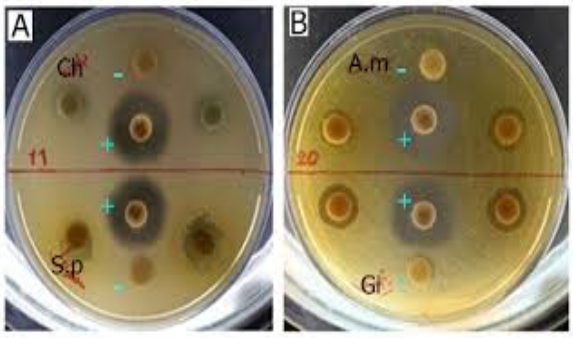

Figure 4: Antifungal Activity against Candida albicans

The clotrimazole-loaded ethosomal gel exhibited superior antifungal activity, with a significantly larger zone of inhibition (34.6 mm) against *Candida albicans* compared to conventional liposomes (29.6 mm) and the marketed cream (19.0 mm). The formulation also showed better efficacy against *Aspergillus niger*. The enhanced antifungal activity is a direct consequence of the improved drug permeation and localized delivery achieved by the ethosomal carrier, allowing a higher concentration of the drug to reach and act on the fungal pathogens.

Stability Studies

Stability studies were conducted to assess the robustness of the formulation under different storage conditions as per ICH guidelines.

Table 5: Stability Study Results

Storage Condition	Time Period	Observation
40°C ± 1°C (Accelerated)	3 months	No phase separation; drug content > 90% of initial value
25°C ± 1°C (Long-term)	3 months	No significant change in physicochemical parameters

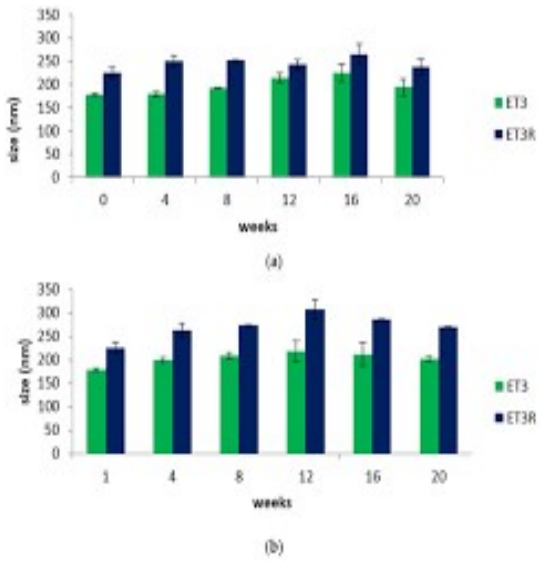

Figure 5: Stability Profile of Ethosomal Gel

The optimized ethosomal gel demonstrated good storage stability with no phase separation observed under accelerated (40°C) and long-term (25°C) storage conditions for up to 3 months. The drug content remained above 90% of the initial value, confirming the chemical stability of the formulation. Some studies have reported stability for up to 180 days with no significant changes in physicochemical parameters. These findings indicate that the developed ethosomal gel is robust and can maintain its quality and efficacy over an extended shelf life.

Conclusion

The present study successfully formulated and evaluated a clotrimazole-loaded ethosomal gel for enhanced transdermal antifungal activity. The optimized ethosomal formulation, prepared using the mechanical dispersion method with 3% phospholipids and 35% ethanol, exhibited favorable vesicular characteristics including nanometric size (202.8 ± 4.8 nm), excellent zeta potential (−83.6 ± 0.96 mV), and remarkably high drug entrapment efficiency (98.42%). Transmission electron microscopy confirmed the formation of intact, uniformly spherical vesicles with distinct bilayer structures. The incorporation of optimized ethosomes into a Carbopol 934 gel base resulted in a formulation with suitable pH (approximately 8.0) and viscosity (73,200 cps) for convenient topical application. The ethosomal gel demonstrated significantly superior *in vitro* drug release (~92% over 8 hours) following a diffusion-controlled mechanism, as evidenced by the Higuchi model fit. Most notably, the formulation exhibited enhanced *ex vivo* skin permeation with cumulative permeation of 88.53% and steady-state flux of 3.39 µg/cm²/min, substantially higher than conventional ethosomal gel and marketed formulation. This enhanced permeation is attributed to the synergistic action of ethanol and phospholipids, which fluidize the stratum corneum lipids and facilitate deeper drug penetration. The antifungal activity evaluation confirmed the superior efficacy of the ethosomal gel against clinically relevant fungal strains, with significantly larger zones of inhibition against *Candida albicans* (34.6 mm) and superior activity against *Aspergillus niger* compared to conventional formulations. Stability studies demonstrated good storage stability with drug content exceeding 90% of initial values under both accelerated and long-term storage conditions for up to 3 months.

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