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Research Article

Evaluation of Antifungal Properties of Fluconazole-Loaded Ethosomal Gel

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Abstract:

Ethosomal gels are advanced drug delivery systems that enhance the penetration of active pharmaceutical ingredients through the skin and mucosal layers. Fluconazole, a broad-spectrum antifungal agent, is commonly used to treat fungal infections. The present study aims to formulate an ethosomal gel containing Fluconazole and evaluate its antimicrobial activity against various fungal and bacterial strains. The study involves the preparation of ethosomes using various ingredients followed by incorporation into a gel base. The antimicrobial activity is determined using in-vitro techniques such as the agar well diffusion method. The results indicate enhanced antifungal activity of the ethosomal formulation compared to conventional Fluconazole gels, demonstrating its potential as an improved topical therapy.

Keywords: Fluconazole, Ethosomal Gel, Antimicrobial Activity, Fungal Infections, Drug Delivery System.

Introduction

Transdermal drug delivery systems (TDDS) offer numerous advantages, including non-invasiveness, controlled drug release, and avoidance of first-pass metabolism. However, the major challenge in transdermal delivery is overcoming the stratum corneum, the outermost layer of the skin, which acts as a protective barrier, limiting drug absorption. To address this issue, novel drug carriers like ethosomes have been developed. Ethosomes are lipid-based vesicular systems composed of phospholipids, ethanol, and water, which enhance drug permeation through the skin. The presence of ethanol fluidizes the stratum corneum, allowing deeper penetration of the drug into the systemic circulation. Ethosomal gels combine the benefits of ethosomes and gels, offering better stability, controlled drug release, and improved patient compliance. Due to their versatile applications, ethosomal gels have gained significant attention in pharmaceutical and cosmeceutical fields. [1,2]

Ethosomal gel is an advanced drug delivery system that enhances the penetration of antifungal agents through the skin and deeper layers. Ethosomes, composed of phospholipids, ethanol, and water, improve drug permeability by disrupting the skin's lipid barrier, allowing efficient drug delivery to fungal infection sites [3,4]. This system is particularly beneficial for treating superficial and deep-seated fungal infections such as dermatophytosis, candidiasis, and onychomycosis. The presence of ethanol enhances membrane fluidity, facilitating drug diffusion into fungal cells, leading to better therapeutic outcomes. Compared to conventional creams and ointments, ethosomal gels

exhibit prolonged drug retention, improved bioavailability, and reduced systemic side effects. Their non-greasy, patient-friendly formulation further increases treatment adherence, making them a promising alternative in antifungal therapy. [5]

Antifungal Activity

Pathogenic fungus used: The pathogenic fungi Aspergillus niger and Candida albicans were acquired from the Microbial Culture Collection at the National Centre for Cell Science in Pune, Maharashtra, India.

Media preparation: Composition of nutrient agar media is given below:

Table 1: composition of nutrient agar media.

Potatoes extract	200gms
Dextrose	20 gms
Agar	15 gms
Distilled Water	to make 1000ml
pH (at 25°C)	5.6±0.2

The agar was dissolved in distilled water and heated in a conical flask of adequate volume. The dry components were added to a conical flask along with the necessary amount of distilled water and boiled until they were fully dissolved. [6]

Sterilization culture media: The flask containing the medium was sealed with a cotton stopper and subjected to sterilization in an autoclave at a pressure of 15 pounds per square inch and a temperature of 121 degrees Celsius for duration of 15 minutes.

Preparation of plates: Following sterilization, the liquefied agar in the flask was promptly dispensed (20 ml each plate) into sterile Petri dishes on a flat surface. The poured plates were allowed to harden and incubated at a temperature of 37°C overnight to assess the sterility of the plates. Prior to usage, the plates were subjected to a drying process at a temperature of 50±0.5°C for duration of 30 minutes.

Revival of the microbial cultures: The investigation utilized lyophilized microbial cultures. Using aseptic procedures, the lyophilized cultures are introduced into sterile nutrition broth and potato dextrose broth for fungal growth. They are then incubated at a temperature of 37±0.5 °C for a period of 24 hours. Following the incubation period, the presence of growth can be detected by the appearance of turbidity. The broth cultures were subsequently inoculated onto nutritional agar and potato dextrose agar plates using a bacterial loop. The plates were then incubated at a temperature of 37±0.5 °C for 24 hours to obtain a pure culture. The pure cultures were preserved as stocks for future research purposes.

Antimicrobial Sensitivity: The antimicrobial susceptibility test is conducted on the fungus utilized in the current investigation using the antifungal gel formulation. In this experiment, 6 mm diameter Whatman filter paper discs were soaked in a solution containing 100 mg/ml of the stock, and then dried under sterile circumstances. Using the spread plate approach, specific bacteria are added to a nutrient agar plate, while specific fungus is added to a potato dextrose agar plate. The plates are then kept undisturbed for duration of 5 minutes. The drug-soaked filter paper discs were positioned in the middle of culture plates that had already been inoculated, and then incubated at a temperature of 37±0.5 °C for a period of 24 hours. Following incubation, the plates were examined to assess the susceptibility of the extracts to the test bacteria at a certain dose by measuring the size of the inhibitory zone. [7,8]

Antibiogram Studies:

Broth cultures of the pure culture isolates of microorganisms Aspergillus Niger and Candida albicans, which are susceptible to the 100 mg/ml concentration of gel formulation used in the current study, were prepared by transferring a small amount of culture into sterile nutrient broth. The cultures were then incubated at a

temperature of 37±0.5℃for a duration of 48 hours. A loopful of the broths was inoculated onto sterile nutritional agar and potato dextrose agar plates using a sterile cotton swab to cultivate a dense and spread-out bacterial culture.

The gel formulation's antifungal activity was determined using the paper disc diffusion method, following the conventional procedure. Three concentrations, namely 25, 50, and 100 mg/ml, were utilized in the gel formulation for antibiogram experiments. The key aspect of this process involves the placement of filter paper discs containing antibiotics onto the agar surface just after inoculating it with the tested organism. Undiluted broth cultures that have been left overnight should never be employed as an inoculum.

It is not advisable to directly apply discs to plates that have been seeded with clinical material on a regular basis due to issues with controlling the inoculum and the presence of mixed cultures. The plates were placed in an incubator at a temperature of 37±0.5 ℃ for duration of 48 hours. After this time, the plates were inspected for distinct areas of inhibition surrounding the discs that were infused with a specific concentration of the medication. [9]

Results and Discussion

Antifungal activity of Ethosomal Gel

Microbial cultures: For the studies of antimicrobial effect of antifungal gel formulation, there was one, MCC microbial strains procured from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India. The lyophilized cultures of microbial strain upon culturing in nutrient and potato dextrose broth for 48 hours at $37^{o}\pm0.5^{o}$ C in an incubator resulted into turbid suspension of activated live microbial cell ready to be used for microbiological study. From the broth of respective revived cultures of micro organism loop full of inoculum is taken and streaked on to the nutrient and potato dextrose agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on to the surface of the agar culture that are successfully stored in refrigerated conditions at 4^{o}C as stock culture to be used for further experimentation.

Antifungal studies: The lawn cultures were prepare with the pathogenic microorganism used under present study and sensitivity of microorganism towards the anti fungal gel formulation studied at the concentration of 100 mg/ml using disc diffusion method.

Antifungal activity: *Aspergillus niger* / *Candida albicans* were inhibited by the standard antifungal used in present work i.e., Fluconazole, at all the concentration (20, 50 and 100 mg/ml) used in the study for comparison. The results of anti-fungal activity are shown in Table 2-3.

In present work, ethosomal and marketed gels showed antifungal activity against *Aspergillus niger and Candida albicans* with maximum zone of inhibition lying in the range of 17 to 29 mm (Figure 1).

Ethosomal gel showed greater percentage of inhibition of fungal infection against *Aspergillus niger* and *Candida albicans*- On comparison of formulated gels with marketed gel of Fluconazole, ethosomal gel showed greater percentage of inhibition of fungal infection against *Aspergillus niger* and *Candida albicans.*

Conclusion

The study successfully formulated and evaluated an ethosomal gel containing Fluconazole with enhanced antimicrobial activity. The results indicate that ethosomal drug delivery systems can significantly improve the therapeutic efficacy of topical antifungal agents. Further clinical investigations are recommended to validate these findings for commercial applications.

Table 2: Antifungal activity of different gel formulations against Aspergillus niger

Sample	Zone of Inhibition (mm)		
	20mg/ml	50 mg/ml	100mg/ml
Marketed Fluconazole Gel	17±0.22	21±0.12	25±0.11
Ethosomal Gel	16±0.15	24±0.23	29±0.25

Table 3: Antifungal activity of different gel formulations against *Candida albicans*

Sample	Zone of inhibition (mm)		
	20mg/ml	50 mg/ml	100mg/ml
Marketed Fluconazole Gel	15±0.20	20±0.15	25±0.13
Ethosomal Gel	17±0.11	24±0.21	29±0.20

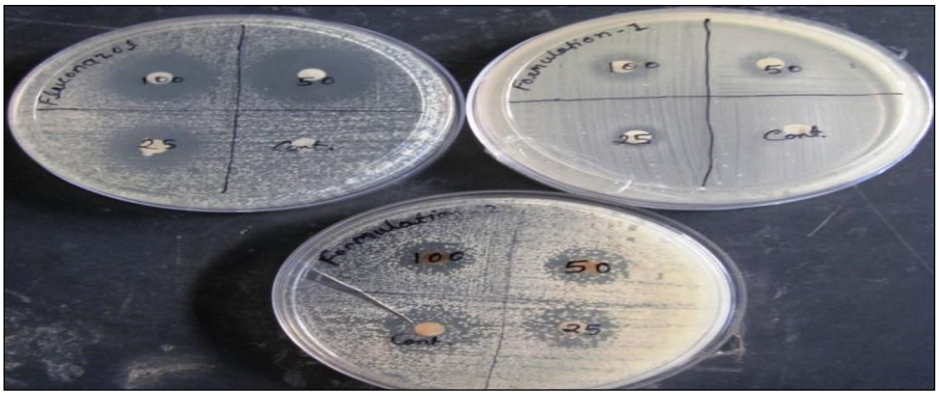

Figure 1: Photograph showing antifungal activity

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