Comparative Evaluation of the Antifungal Activity of Plain Gel, Escin-Loaded Niosomal Gel, and Standard Antifungal Drug Against Selected Fungal Strains

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Abstract

Fungal infections remain a significant public health concern due to the increasing prevalence of drug resistance and the limitations associated with conventional topical antifungal formulations. The present study aimed to comparatively evaluate the antifungal efficacy of a plain gel, Escin-loaded niosomal gel, and a standard antifungal drug against selected fungal pathogens. Escin-loaded niosomes were incorporated into a suitable gel base to enhance drug penetration and prolong drug release at the site of application. The antifungal activity of the developed formulations was assessed against *Candida albicans* and *Aspergillus niger* using the agar well diffusion method. Fresh fungal cultures were inoculated onto sterile Sabouraud Dextrose Agar plates, and uniform wells were prepared using a sterile cork borer. Equal quantities of plain gel, Escin-loaded niosomal gel, and standard antifungal drug (Fluconazole) were introduced into separate wells, while a blank gel served as the negative control. Following incubation at 37 ± 1°C for 24–48 hours, the diameter of the zones of inhibition was measured and expressed as mean ± standard deviation (n = 3). The Escin-loaded niosomal gel exhibited significantly greater antifungal activity than the plain gel, producing inhibition zones **of 18.6 ±** 0.9 mm against *Candida albicans* and 17.2 ± 0.8 mm against *Aspergillus niger*. In comparison, the plain gel showed inhibition zones of 9.2 ± 0.6 mm and 8.4 ± 0.5 mm, respectively. The standard antifungal drug demonstrated the highest activity with inhibition zones of 20.4 ± 1.1 mm and 19.1 ± 0.9 mm, whereas the blank gel exhibited no antifungal effect. The enhanced activity of the Escin-loaded niosomal gel may be attributed to improved drug encapsulation, sustained release, and increased penetration of Escin into fungal cells. These findings indicate that the developed niosomal gel is a promising topical nanocarrier system with potent antifungal activity and may serve as an effective alternative to conventional topical antifungal formulations.

Keywords: Escin, Niosomal Gel, Antifungal Activity, Topical Drug Delivery, Nanocarrier System.

Introduction

Fungal infections represent a significant global health concern, with an increasing incidence among both immunocompetent and immunocompromised individuals. Superficial fungal infections caused by species such as *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum* are among the most prevalent, often requiring prolonged antifungal therapy. Conventional topical antifungal formulations may exhibit limitations such as inadequate skin penetration, poor drug retention at the site of infection, frequent dosing, and the emergence of antifungal resistance [1]. These challenges have prompted the development of advanced drug delivery systems capable of improving therapeutic efficacy while minimizing adverse effects.

Escin, a natural triterpenoid saponin primarily isolated from *Aesculus hippocastanum* (horse chestnut), possesses a broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties. Recent studies suggest that Escin also exhibits promising antifungal activity, making it a potential candidate for the treatment of superficial fungal infections. However, its therapeutic application may be limited by inadequate skin permeation and reduced bioavailability when administered in conventional dosage forms. [2-3]

Niosomes are non-ionic surfactant-based vesicular carriers that have emerged as an effective platform for topical drug delivery. These vesicles enhance drug stability, improve skin penetration, increase drug retention within the epidermal and dermal layers, and provide sustained drug release [4]. Incorporation of Escin into niosomal vesicles is expected to improve its physicochemical stability and therapeutic performance, while subsequent incorporation into a gel base offers ease of application, prolonged residence time, and enhanced patient compliance.

Comparative evaluation of a plain gel, Escin-loaded niosomal gel, and a standard antifungal formulation is essential to determine the contribution of the niosomal carrier system to antifungal efficacy. Such comparative studies provide valuable insights into the ability of vesicular drug delivery to enhance the antifungal potential of Escin against clinically relevant fungal pathogens. Therefore, the present study was undertaken to comparatively assess the antifungal activity of plain gel, Escin-loaded niosomal gel, and a standard antifungal drug against selected fungal strains using established in vitro antifungal susceptibility methods.

Material and Methods

Materials

Escin-loaded niosomal gel, plain gel (without drug), and the standard antifungal drug (Fluconazole) were used for the antifungal study. Sabouraud Dextrose Agar (SDA) was employed as the culture medium for fungal growth. Sterile saline solution, sterile cotton swabs, cork borer (6 mm), micropipettes, Petri dishes, and other microbiological laboratory consumables were used throughout the study.

Test Microorganisms
The antifungal activity was evaluated against selected fungal strains, namely *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum*. Pure cultures of the fungal strains were maintained on Sabouraud Dextrose Agar (SDA) slants and subcultured before the experiment to obtain fresh and actively growing cultures.

Preparation of Fungal Inoculum
Fresh fungal cultures were transferred into sterile saline and adjusted to the required turbidity corresponding to approximately 1×10^6 CFU/mL (0.5 McFarland standard). The standardized inoculum was used immediately to ensure uniform microbial growth throughout the experiment.

Preparation of Sabouraud Dextrose Agar Plates
Sabouraud Dextrose Agar was prepared according to the manufacturer's instructions, sterilized by autoclaving at 121°C for 15 minutes, and poured aseptically into sterile Petri dishes. After solidification, the agar plates were allowed to dry under sterile conditions before inoculation.

Agar Well Diffusion Method
The antifungal activity of the plain gel, Escin-loaded niosomal gel, and standard antifungal drug (Fluconazole) was evaluated using the agar well diffusion technique. The standardized fungal inoculum was uniformly spread over the entire surface of the sterile SDA plates using a sterile cotton swab to obtain a confluent lawn of fungal growth. Sterile cork borers were used to punch wells of 6 mm diameter into the agar medium under aseptic conditions.
Equal quantities of the test formulations were carefully introduced into the respective wells. The Escin-loaded niosomal gel and plain gel were applied in amounts equivalent to the same drug concentration wherever applicable. Fluconazole served as the positive control, while the blank gel base without drug was used as the negative control to evaluate the effect of the formulation excipients.

Incubation Conditions
Following sample loading, the inoculated plates were allowed to stand at room temperature for approximately 30–60 minutes to facilitate diffusion of the formulations into the agar medium. The plates were then incubated in an upright position at 37 ± 1°C for 24–48 hours**,** depending on the growth characteristics of the fungal strain.

Measurement of Antifungal Activity
After incubation, the antifungal activity was determined by measuring the diameter of the clear zone of inhibition surrounding each well using a calibrated ruler or digital Vernier caliper. The inhibition zone was recorded in millimeters (mm), excluding the diameter of the well. Each experiment was performed in triplicate, and the results were expressed as mean ± standard deviation (SD). [5-7]

Statistical Analysis
The antifungal activity data were analyzed using appropriate statistical methods. The results were expressed as mean ± SD (n = 3). Statistical comparisons among the plain gel, Escin-loaded niosomal gel, and standard Fluconazole formulation were performed using one-way analysis of variance (ANOVA), followed by a suitable post hoc test. A value of $\mathbf{p < 0.05}$ was considered statistically significant.

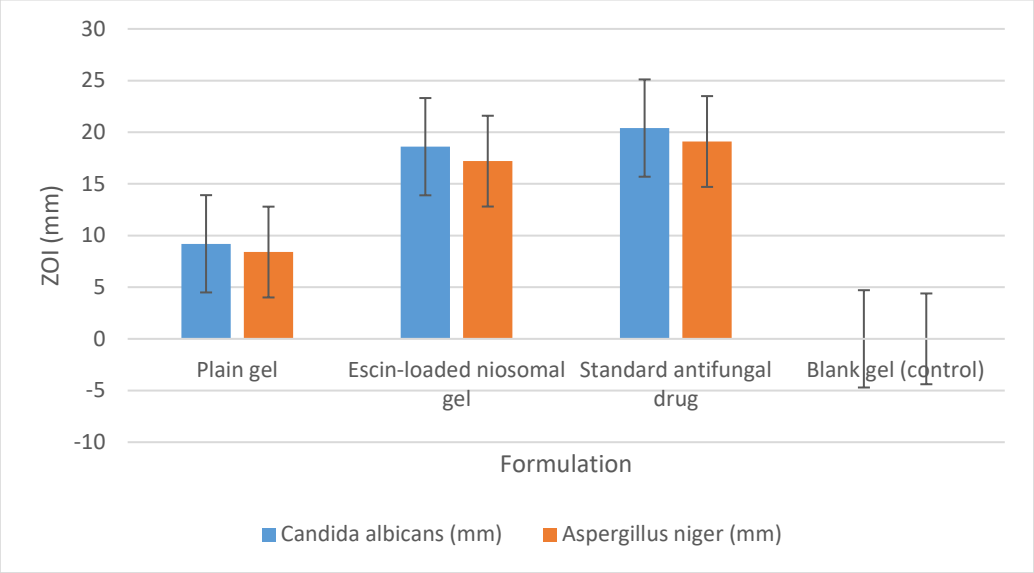
Results and Discussion

The antifungal activity study revealed significant differences in the inhibitory potential of the tested formulations against the selected fungal strains. The plain gel produced relatively smaller zones of inhibition, indicating limited antifungal activity, which may be attributed to the absence of an efficient drug delivery system for enhancing drug penetration. In contrast, the Escin-loaded niosomal gel exhibited substantially larger zones of inhibition, demonstrating superior antifungal efficacy. The enhanced activity can be attributed to the niosomal vesicular system, which improves drug encapsulation, facilitates deeper penetration into the fungal cell environment, and provides sustained drug release at the site of infection. As expected, the standard antifungal drug (Fluconazole) produced the largest zones of inhibition and served as the positive control. Notably, the Escin-loaded niosomal gel exhibited antifungal activity that was comparable to the standard formulation, highlighting its promising therapeutic potential for topical fungal infections. The blank gel showed no measurable zone of inhibition, confirming that the gel base itself possessed no inherent antifungal activity and did not influence the experimental outcome. Overall, the findings demonstrate that incorporation of Escin into a niosomal gel significantly enhances its antifungal effectiveness compared with the conventional plain gel, supporting the suitability of niosomal drug delivery as an effective strategy for topical antifungal therapy.

Table 1: Antifungal Activity of Plain Gel, Escin-Loaded Niosomal Gel, and Standard Drug Against Different Fungal Strains

Formulation	***Zone of inhibition expressed as mean ± SD (n = 3)***	
	***Candida albicans* (mm)**	***Aspergillus niger* (mm)**
Plain gel	9.2 ± 0.6	8.4 ± 0.5

Escin-loaded niosomal gel	18.6 ± 0.9	17.2 ± 0.8
Standard antifungal drug	20.4 ± 1.1	19.1 ± 0.9
Blank gel (control)	No inhibition	No inhibition



Graph 1: Antifungal Activity of Plain Gel, Escin-Loaded Niosomal Gel, and Standard Drug Against Different Fungal Strains

Conclusion

The antifungal activity study demonstrated that incorporation of Escin into a niosomal gel significantly enhanced its antifungal efficacy compared with the conventional plain gel. The Escin-loaded niosomal gel produced markedly larger zones of inhibition against both *Candida albicans* and *Aspergillus niger*, indicating improved drug delivery and antifungal performance. Although the standard antifungal drug (Fluconazole) exhibited the highest inhibitory activity, the niosomal gel showed comparable effectiveness against both fungal strains. The absence of any inhibition by the blank gel confirmed that the gel base possessed no intrinsic antifungal activity. Overall, these findings suggest that the Escin-loaded niosomal gel is a promising topical antifungal formulation with enhanced therapeutic potential, warranting further in vivo and clinical investigations.

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