Designed Gel Loaded with Anti-Fungal Element of Fluconazole-Sesame Oil Encapsulated Nanoparticles: Optimization and Analysis of Anti-Fungal Performance

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ABSTRACT

Oral candidiasis is a common fungal infection, with Candida albicans being the causative agent in over 95% of cases. Fluconazole is commonly used to treat oral candidiasis, but conventional oral delivery can cause side effects. This study aimed to develop a fluconazole-loaded sesame oil nanotransfersome formulation (FS-NTF) embedded in hyaluronic acid hydrogel (HA-FS-NTF) to improve localized treatment. An optimal FS-NTF formulation was developed using a Box-Behnken design evaluating the effects of lecithin, fluconazole, and sesame oil amounts on vesicle size, entrapment efficiency, antifungal activity, and ulcer index. The optimized FS-NTF showed 140 nm vesicle size, 70% entrapment efficiency, 14.5 mm fungal inhibition zone, and ulcer index of 1. The HA-FS-NTF exhibited suitable rheological properties for oral delivery. In vitro release was 85% in 3 hours, significantly higher than fluconazole suspension (12%) and gel (43%). Ex vivo permeation across sheep buccal mucosa was also markedly enhanced for HA-FS-NTF (400 µg/cm2) versus gel (294 µg/cm2) and suspension (122 µg/cm2). HA-FS-NTF showed superior antifungal activity with 14.33 mm inhibition zone and ulcer index of 0.67 in rats versus other formulations. Overall, fluconazole delivery is enhanced using nanotransfersomes in hyaluronic acid hydrogel, representing a promising approach for localized oral candidiasis treatment.

Keyword: *fluconazole; nanotransfersome; Box-Behnken design; rheology; permeation; ulcer index*

1. Introduction

Oral candidiasis, caused by overgrowth of Candida species on the oral mucosa, is a very common fungal infection seen in both acute and chronic presentations. More than 150 Candida species have been identified, but around 95% of oral candidiasis cases are caused specifically by Candida albicans [1,2]. Oral candidiasis can range from mild to severe infections that are resistant to treatment and prone to recurrence.

The first-line pharmacotherapy for oral candidiasis is fluconazole, a fluorinated bis-triazole antifungal agent. Fluconazole exhibits efficacy against C. albicans and various other Candida species in both immunocompromised and immunocompetent patients [3]. Its mechanism of action involves inhibition of 14-α-demethylase, an enzyme required for ergosterol biosynthesis in the fungal cell wall. Conventional oral administration of fluconazole can produce side effects like abdominal discomfort, nausea, vomiting, and liver toxicity [4]. Localized delivery of fluconazole directly to the oral mucosa could minimize systemic side effects and development of resistance, providing more effective therapy for oral candidiasis [3]. However, common topical dosage forms like oral sprays, rinses, gels, and creams are limited by inaccurate dosing, short residence time, and performance variability [4,5]. Alternate local delivery options such as lozenges and paints also have drawbacks like brief drug contact due to salivary washout and poor patient compliance [4,5].

Novel formulations that can provide sustained fluconazole release directly at the oral mucosal site are needed to overcome the limitations of current local delivery methods. Various approaches have been investigated to achieve more effective fluconazole delivery, including mucoadhesive oral films [3], mucoadhesive nanoparticles [4], hydrogels [6], and biomembranes [7]. This study aimed to develop a new fluconazole formulation combining the sustained release of nanotechnology systems with mucoadhesion for localized oral candidiasis treatment. Fluconazole-loaded sesame oil nanotransfersomes (FS-NTF) were formulated and optimized using Box-Behnken statistical design. The optimized FS-NTF was then embedded in hyaluronic acid hydrogel (HA-FS-NTF) to provide mucoadhesive properties. Sesame oil was incorporated in the nanotransfersomes based on its known antifungal effects [8]. Hyaluronic acid can also exhibit antifungal effects by inhibiting fungal enzymes [9,10], in addition to facilitating ulcer healing due to its hydration and anti-inflammatory properties [11]. It was hypothesized that the HA-FS-NTF formulation would show superior antifungal efficacy and healing of oral candidiasis lesions. The formulation was characterized for vesicle size, entrapment efficiency, rheology, drug release, permeation, antifungal activity, and ulcer healing in animal models.

2. Materials and Methods

2.1. Materials

Fluconazole was obtained from TADA lab India (Himachal Pradesh) Tween 20® was acquired from NISH Pharma (India). Lecithin and sesame oil were procured from Center Drug Hub (India, Himachal Pradesh). HPLC-grade solvents were from Center Drug Hub (India, Himachal Pradesh). Other chemicals were analytical grade.

2.2. Optimization of FS-NTF Using Box-Behnken Design

A Box-Behnken design was utilized to optimize the FS-NTF using Design-Expert® v12.0.6.0 software. Three independent factors (lecithin concentration, fluconazole amount, sesame oil amount) were evaluated at three levels each. Higher and lower levels were selected based on previous studies [10,13]. The software generated 19 formulations with different factor combinations per a response surface design. The formulations were prepared and analyzed for four dependent variables: vesicle size, entrapment efficiency, antifungal activity based on zone of inhibition, and ulcer index in rats. Experimental data was entered into the software and analyzed using ANOVA to understand the effects of variables on the responses. Polynomial equations were generated to correlate the dependent and independent variables. Perturbation plots, contour plots, and 3D surface plots were constructed to illustrate the variable effects. The best fitting model was identified from the adequate precision ratios and predicted/adjusted R2 values for each response.

2.2.2. Preparation of FS-NTF

The FS-NTF was prepared by thin film hydration technique [14]. Accurately weighed amounts of Tween 20®, lecithin, fluconazole and sesame oil were dissolved in chloroform:methanol (1:1 v/v) in a round bottom flask. The organic solvents were removed by rotary evaporation at 45°C and 80 rpm to form a thin film. The film was hydrated with phosphate buffered saline (pH 7.4) at room temperature to form multilamellar vesicles, which were probe sonicated for 15 min to produce unilamellar FS-NTF. The FS-NTF was collected after extrusion through polycarbonate membranes.

2.2.3. Characterization of FS-NTF

The vesicle size was measured by dynamic light scattering after diluting the FS-NTF 10 times with purified water. The size was measured using a Microtrac® particle size analyzer at 25 ± 1°C.

The entrapment efficiency was determined indirectly [15] by freeze drying the FS-NTF, dispersing in methanol, separating the supernatant, and analyzing by UV spectrophotometry at 261.6 nm. The entrapment efficiency percentage (EE%) was calculated using the measured free drug concentration and total drug added initially.

2.2.4. Preparation of HA-FS-NTF Hydrogel

The optimized FS-NTF was incorporated into hyaluronic acid hydrogel prepared at 2% w/v hyaluronic acid and 0.5% w/v Gantrez® S-97 crosslinker in phosphate buffered saline (pH 4.5), based on preliminary tests. The hydrogel was refrigerated overnight to ensure uniform drug distribution as confirmed by content uniformity testing.

2.2.5. Rheological Evaluation of HA-FS-NTF

The viscosity of blank hydrogel and HA-FS-NTF was measured using a Brookfield viscometer at 25 ± 5°C. The shear rate, shear stress and viscosity were recorded at shear rates of 2-60 s-1 to determine the flow pattern. The minimum and maximum viscosity, Farrow's constant and flow behavior were calculated.

2.2.6. In Vitro Drug Release Study

The in vitro drug release from HA-FS-NTF was compared with fluconazole suspension and gel using USP Type I dissolution apparatus with dialysis membrane. One gram of each formulation was tested in phosphate buffered saline (pH 6.8) at 37 ± 0.5°C and 50 rpm for 3 hours. Samples were collected at regular intervals, filtered and analyzed by UV spectrophotometry at 261.6 nm.

2.2.7. Ex Vivo Permeation Study

Ex vivo permeation of HA-FS-NTF, fluconazole gel and suspension was evaluated across sheep buccal mucosa using Franz diffusion cells at 32 ± 2°C. The donor samples were placed over the mucosa between the donor and receptor chambers. The receptor chamber contained phosphate buffered saline (pH 6.8) at 410-430 rpm. Samples were collected at regular intervals and analyzed by HPLC [16]. The cumulative drug permeation per unit time and area was plotted to calculate permeability parameters like steady state flux, permeability coefficient and diffusion coefficient. The percent permeation and total amount diffused were calculated.

2.2.8. Antifungal Activity Study

The inhibitory activity against C. albicans was evaluated by disk diffusion method using Sabouraud dextrose agar plates. Filter paper disks soaked with FS-NTF or ethanol (control) were placed on inoculated plates and incubated for 48 hours before measuring the zone diameters.

2.2.9. Ulcer Index Study in Rats

The ulcer healing efficacy was assessed in immunocompromised rats following an established model [17]. Oral candidiasis was induced and treatment was given with HA-FS-NTF, fluconazole suspension, blank hydrogel, hydrogel with fluconazole, and hydrogel with FS-NTF without sesame oil for 3 days. The oral ulcer scores were recorded based on a defined scoring table.

2.2.10. Statistical Analysis

Results are expressed as mean ± standard deviation. Statistical comparisons were made using paired t-test at $p < 0.05$ significance level.

3. Results and Discussion

3.1. Optimization of FS-NTF

Tween 20® was selected as the edge activator to impart flexibility and enhance penetration of the nanotransfersomes through the mucosa [18]. Sesame oil was incorporated based on its reported antifungal effects against C. albicans [8]. The Box-Behnken design was implemented to optimize the formulation parameters.

3.2. Effect of Variables on Responses

3.2.1. Effect on Vesicle Size

The quadratic model best fit the vesicle size data. Lecithin amount (factor A) and fluconazole amount (factor B) significantly influenced vesicle size based on the p-values (<0.05) and positive coefficient values in the polynomial equation. Increasing both factors increased vesicle size due to more phospholipid bilayers and greater drug encapsulation. Contour and 3D plots also showed an enhancing effect of lecithin and fluconazole amounts on vesicle size.

3.2.2. Effect on Entrapment Efficiency

The quadratic model optimally fit the entrapment efficiency data. The lecithin and sesame oil amounts (factors A and C) positively impacted entrapment efficiency based on the p-values (<0.05) and positive coefficient values. In contrast, increasing fluconazole amount (factor B) reduced entrapment efficiency due to limitations in incorporating higher drug levels. Contour and 3D plots demonstrated that fluconazole amount increased entrapment efficiency initially but reduced it at higher levels due to saturation.

3.2.3. Effect on Antifungal Activity

The zone of inhibition data best fit a quadratic model. The fluconazole amount (factor B) exhibited the most significant positive effect on antifungal activity based on the p-value (<0.05) and highest positive coefficient value. Lecithin amount (factor A) had an insignificant negative effect, while sesame oil amount (factor C) showed a minor positive influence. The contour and 3D plots revealed negligible impact of sesame oil amount on antifungal activity.

3.2.4. Effect on Ulcer Index

A linear model best described the ulcer index results. Fluconazole and sesame oil amounts (factors B and C) both significantly decreased the ulcer index according to the p-values (<0.05) and negative coefficient values. The sesame oil amount (factor C) exerted a more prominent ulcer healing effect than fluconazole based on the higher coefficient value. This agreed with the greater impact of sesame oil amount versus fluconazole amount observed in the contour and 3D plots.

3.3. Optimization of FS-NTF and HA-FS-NTF Preparation

The optimized FS-NTF was prepared using 75 mg lecithin, 60 mg fluconazole and 75 mg sesame oil. The observed vesicle size, entrapment efficiency, antifungal activity and ulcer index matched the predicted values, confirming the validity of the Box-Behnken optimization approach. Embedding the optimized FS-NTF in the hyaluronic acid hydrogel provided a mucoadhesive system for localized oral delivery.

3.4. Rheological Properties of HA-FS-NTF

The rheograms exhibited typical shear-thinning behavior for the blank hydrogel and HA-FS-NTF, with viscosity decreasing as shear rate increased. Incorporating FS-NTF increased viscosity compared to blank hydrogel, which could prolong retention in the oral cavity during activities like talking, eating, or smiling [19]. Both formulations demonstrated pseudoplastic flow based on Farrow's constant values above 1. The thixotropic and pseudoplastic properties make the HA-FS-NTF suitable for oral delivery applications.

3.5. In Vitro Drug Release Kinetics

The in vitro release of fluconazole from HA-FS-NTF was 85% in 3 hours, markedly higher than the fluconazole suspension (12%) and gel (43%). The nanotransfersome carrier with small vesicle size and high drug solubilization likely enhanced the drug release across the dialysis membrane. Hydration and swelling of the hydrogel matrix also facilitated uniform drug release. These findings agree with previous results showing improved release of hydrophobic agents from nanotransfersomes [20].

3.6. Ex Vivo Permeation Parameters

The ex vivo fluconazole permeation across sheep buccal mucosa was greatest for HA-FS-NTF (400 μg/cm2) compared to fluconazole gel (294 μg/cm2) and suspension (122 μg/cm2) after 3 hours. The steady state flux, permeability coefficient, diffusion coefficient and enhancement factor values were all superior for HA-FS-NTF. The nanotransfersomes likely improved drug delivery across the mucosa through para- and transcellular pathways because of their ultraflexible nature and tiny size [18,21]. The edge activator may have also fluidized the lipid bilayers to facilitate permeation [22]. Faster permeation of fluconazole to deeper mucosal layers is essential to treat invasive oral candidiasis infections.

3.7. Antifungal Activity against C. albicans

The zone of inhibition assay tested the susceptibility of C. albicans to the fluconazole formulations. The HA-FS-NTF showed the largest inhibition zone diameter (14.33 mm), significantly higher than hydrogel with fluconazole or FS-NTF without sesame oil. This demonstrated the synergistic antifungal effects provided by the fluconazole, sesame oil and hyaluronic acid components in combination. The sesame oil appeared critical for optimal antifungal activity based on the reduced efficacy without it.

3.8. Oral Ulcer Healing in Rats

The oral ulcer index score measured the therapeutic efficacy of the fluconazole formulations in rats with experimentally-induced oral candidiasis. The HA-FS-NTF treatment group showed the lowest ulcer score (0.67) compared to the other groups, indicating superior healing of the oral lesions. The fluconazole hydrogel had slightly better efficacy versus fluconazole suspension, likely due to the added antifungal effects of hyaluronic acid [9,10]. Removing sesame oil from the FS-NTF hydrogel clearly diminished its therapeutic efficacy, confirming sesame oil's integral role. Overall, the HA-FS-NTF formulation produced optimal oral ulcer healing activity owing to the combined benefits of the fluconazole, sesame oil and hyaluronic acid.

4. Conclusions

In summary, the Box-Behnken optimization approach was successfully implemented to develop an optimal fluconazole-loaded sesame oil nanotransfersome formulation (FS-NTF). Embedding the optimized FS-NTF in hyaluronic acid hydrogel yielded a mucoadhesive system (HA-FS-NTF) ideal for localized oral delivery. The HA-FS-NTF displayed suitable rheological properties, enhanced drug release and permeation kinetics, and superior antifungal and ulcer healing efficacy compared to fluconazole suspension and gel formulations. These positive outcomes can be attributed to the synergistic effects of the fluconazole, sesame oil and hyaluronic acid components. Fluconazole delivery for oral candidiasis treatment is significantly improved using nanotransfersomes loaded in crosslinked hyaluronic acid hydrogel. This represents a promising formulation strategy for localized therapy of fungal infections in the oral cavity.

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