Formulation, characterization, and in vitro antifungal efficacy of luliconazole-loaded nanostructured lipid carriers (LCZ-NLCs) against a panel of resistant fungal strains

Mohsen Nosratabadi  
Mazandaran University of Medical Sciences

Robab Ebrahimi Barogh  
Mazandaran University of Medical Sciences

Seyyed Mobin Rahimnia  
Mazandaran University of Medical Sciences

Pedram Ebrahimnejad  
Mazandaran University of Medical Sciences

Iman Haghani  
Mazandaran University of Medical Sciences

Javad Akhtari  
Mazandaran University of Medical Sciences

Zohreh Hajheydari  
Mazandaran University of Medical Sciences

Mahdi Abastabar  
abastabar@gmail.com

Mazandaran University of Medical Sciences

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Abstract

Luliconazole (LCZ) is a topical imidazole antifungal agent with broad-spectrum activity. However, LCZ faces challenges like low aqueous solubility, skin retention, and penetration, limiting its dermal bioavailability and effectiveness in drug delivery. This study aims to formulate, characterize, and assess the in vitro antifungal efficacy of luliconazole-loaded nanostructured lipid carriers (LCZ-NLCs) against resistant fungal strains. The LCZ-NLCs were synthesized using a modified emulsification-solvent evaporation method. Characterization included poly-dispersity index (PDI), zeta potential, entrapment efficiency (EE %), Field Emission Scanning Electron Microscopy (FESEM), Differential Scanning Calorimetry (DSC) analysis, and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) study. Additionally, in vitro drug release experiments, kinetic analysis of release data, cytotoxicity assays, and in vitro antifungal susceptibility tests were conducted. The results revealed that LCZ-NLCs exhibited nanoscale dimensions, uniform dispersion, and a favorable zeta potential. The encapsulation efficiency of LCZ in NLCs was around 90%. FESEM analysis showed spherical nanoparticles with consistent shape. ATR-FTIR analysis indicated no chemical interaction between LCZ and excipients. In vitro drug release experiments suggested that LCZ-NLCs significantly improved the drug's dissolution rate. Stability testing also showed consistent colloidal nanometer ranges in LCZ-NLCs samples. Also, cytotoxicity tests showed no toxicity within the tested concentration. Furthermore, in vitro antifungal susceptibility tests demonstrated potent antifungal activity of both LCZ and LCZ-NLCs against resistant fungal isolates. The study findings suggest that the LCZ-NLCs formulation developed in this research could be a promising topical treatment for superficial fungal infections, especially in cases of resistant infections.

Introduction

Fungal pathogens pose a serious threat to public health, affecting millions of people worldwide each year. Fungi are responsible for a wide range of human diseases, from superficial skin and mucosal lesions to severe, life-threatening mycoses\(^1\)\(^-\)\(^2\). It is worth noting that fungal infections are often categorized as superficial, subcutaneous, or systemic infections. Superficial infections, which are prevalent in areas with tropical, subtropical, and temperate climates, can be caused by dermatophytes, yeasts, and non-dermatophyte fungi\(^3\)\(^-\)\(^4\). Dermatophytosis is the most common form of superficial fungal infection, affecting approximately 20–25% of the world's population. The main dermatophytes that commonly infect humans belong to the genera Trichophyton, Microsporum, Epidermophyton, and Nannizzi, and mostly affect keratinized tissues such as the skin and its appendages, hair, and nails. The clinical manifestations of dermatophytosis can vary depending on where the infection is and the state of the host's immune system. Some common manifestations include tinea pedis, tinea capitis, tinea cruris, tinea corporis, and tinea unguium\(^4\)\(^-\)\(^5\). Superficial fungal infections can also be caused by a group of yeasts, mainly belonging to the genus Candida, especially Candida albicans. The clinical manifestations of superficial candidiasis are diverse and include oropharyngeal candidiasis, vaginal candidiasis, onychomycosis, and chronic mucocutaneous candidiasis\(^6\). Besides Candida species, various genera of non-dermatophytic molds such as Fusarium, Aspergillus, Scopulariopsis, and Acremonium can also cause superficial fungal infections known as Dermatomycosis\(^7\)\(^-\)\(^9\). Nowadays, the emergence of intrinsic and acquired antifungal resistance poses an increasing clinical challenge for most fungal pathogens\(^10\). Topical antifungals are commonly considered as the first-line treatment for superficial fungal infections. Luliconazole (LCZ) is an imidazole antifungal compound with a broad-spectrum activity against various fungal species. LCZ was first developed and approved in Japan in 2005 as a topical antifungal for the treatment of skin fungal infections. It was subsequently approved by the Food and Drug Administration (FDA) in 2013 for the treatment of tinea corporis, tinea pedis, and tinea cruris\(^12\)\(^-\)\(^14\). However, the lipophilic nature and low aqueous solubility of LCZ are significant concerns that restrict its dermal bioavailability and hinder its ability to penetrate the skin\(^15\). Nanostructured lipid carriers (NLCs) are an advanced type of drug delivery system that holds great promise in enhancing the delivery of antifungal drugs, particularly lipophilic drugs like LCZ. These lipid-based nanoparticles (NPs) consist of a solid lipid matrix and a liquid lipid, offering superior drug loading capacity and stability compared to other lipid-based delivery systems such as solid lipid nanoparticles (SLNs). Furthermore, nanostructured lipid carriers (NLCs) exhibit low toxicity, and their nanoparticles facilitate enhanced drug penetration into the stratum corneum\(^16\)\(^-\)\(^18\). The present study aimed to formulate, characterize, and evaluate the in vitro antifungal efficacy of LCZ-NLCs against a panel of resistant fungal strains. This could potentially shed light on the efficacy of LCZ-NLCs as a treatment option for such fungal infections.

Material and Methods

Materials

LCZ used in the study was provided by Nihon Nohyaku Co. Tween 80, Span 80, oleic acid, and chloroform were purchased from Merck (Germany). Glycerol monostearate (GMS) was obtained from Daejung Company (South Korea). Distilled water was purified using a Human Power 2 system (Human Co., Korea). Tetrazolium salt (MTT) was supplied by Sigma-Aldrich. Human foetal foreskin broblast (HFF2) was purchased from the National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). Sabouraud dextrose agar (SDA), RPMI medium (Merck, Germany), and morpholinepropanesulfonic acid (MOPS) from Sigma Chemical Co. (St. Louis, MO, USA) were used.

Preparation of LCZ-NLC NPs

The LCZ-NLCs were synthesized using a modified emulsification-solvent evaporation technique. In summary, a solution was prepared by dissolving 100 mg of LCZ, 100 mg of Span 80, 150 mg of GMS, and 50 mg of oleic acid in 500 µL of chloroform at a temperature of 60°C. The resulting solution was considered the organic phase. A volume of deionized water, not exceeding 10 mL, was utilized as the aqueous phase, which contained 100 mg of Tween 80. The aqueous phase was subjected to heating until it reached the same temperature as the organic phase. Subsequently, the aqueous phase was transferred dropwise into the heated organic phase using a high-shear homogenizer (Heidolph M 036170020, Germany) operating at 5000 rpm for a duration of 15 minutes. Subsequently, the homogenous emulsion underwent sonication at an amplitude of 40% for a duration of 5 minutes (Bandelin 3100, Germany). Following sonication, the emulsion was immediately cooled in an ice bath to facilitate the isolation of LCZ-NLCs. Afterward, the mixture was kept overnight to stirring at ambient temperature in order to eliminate any remaining organic solvent.
Characterization of LCZ-NLCs

Particle size, PDI, and Zeta Potential Evaluations

The poly-dispersity index (PDI) and diameter of NPs were estimated using dynamic light scattering (DLS) with a Zetasizer Nano ZS system (Malvern Instruments, Worcestershire: UK). The zeta potential of NLCs was determined using the laser Doppler electrophoresis technique\(^{19}\). Each measurement was done three times and data presented as mean ± standard deviation (SD).

Entrapment Efficiency (EE %) Measurement

The LCZ-NLCs dispersion was subjected to centrifuge at 20000 rpm (SIGMA; 3–30 KS: Germany) for a duration of 45 minutes. Subsequently, the supernatant was harvested and subjected to filtration using a polytetrafluoroethylene (PTFE) syringe filter with pores capturing 0.22 µm. Subsequently, the concentration of LCZ present in the filtrate (regarded as unencapsulated drugs) was assessed using UV-visible spectrophotometry (Jasco V630, Japan) at a wavelength of 295 nm\(^{20}\). In order to measure the EE% of NPs, the proportion of LCZ entrapped within the NLCs was calculated utilizing the following equation:

\[
\text{EE} \% = \frac{\text{Amount of drug in formulation} - \text{Amount of drug in supernatant}}{\text{Amount of drug in formulation}} \times 100
\]

Morphological Investigation

Field emission scanning electron microscopy (FESEM, TESCAN MIRA3) was employed for the visualization of the surfaces and shape of the LCZ-NLCs\(^{21}\).

Differential Scanning Calorimetry (DSC) Analysis

The LCZ, GMS, and freeze-dried LCZ-NLCs samples were analyzed through DSC with a Pyris 6 instrument (PerkinElmer, USA). The freeze-dried LCZ-NLCs sample was obtained by separating the NLCs through centrifugation. The drying process was performed using an alpha 1–2 Ldplus freeze dryer (Marin Christ, Osterode, Germany) operating under reduced pressure. Approximately 5 mg of the samples were placed in aluminum disks and sealed. Then preserved at a constant temperature of 20°C for 30 minutes. Following this, the samples were subjected to heating within a temperature range of 20 to 250°C. The heating process was carried out under an inert nitrogen gas atmosphere, with a heating rate of 20°C per minute. The calibration of the DSC instrument was performed using indium\(^{22}\).

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) study

The assessment of the chemical interaction between the components of the formulation was performed using a Cary 630 Fourier FTIR spectroscope with a diamond ATR attachment produced by Agilent Technologies Inc., California, USA. The ATR-FTIR spectroscopy technique was employed to analyze LCZ, LCZ-NLCs powder (in its freeze-dried form), GMS, oleic acid, Tween 80, and Span 80. Infrared spectra were acquired within the spectral range of 4000 – 400 cm\(^{-1}\), applying a resolution of 2 cm\(^{-1}\).

In vitro Drug Release Experiment

The study was performed using cellulose acetate-based membranes. The samples were placed within immersion cells, and a cellulose acetate membrane was securely fixed to the cells and sealed. The cells were then immersed in the United States Pharmacopeia (USP) dissolution apparatus II\(^{23}\). A dissolution media consisting of 300 ml of a 50% ethanol solution was chosen for the release test, and the temperature was maintained at 37°C with a rotating velocity of 100 rpm. Subsequently, the 5ml samples were extracted from the vessel and substituted with fresh media at various time intervals, specifically 1, 2, 4, 6, 8, 10, and 24 hours. Furthermore, the amount of LCZ in the solution that had been withdrawn was determined using a UV-visible spectrophotometer at a wavelength of 295 nm.

Kinetic analysis of the release data

Several kinetic models, including zero order, first order, Higuchi diffusion, Hixson-Crowell, and Korsmeyer-Peppas, were used to analyze the in vitro release data. The goal was to better understanding of the release kinetics represented by the formulations. The model exhibiting the highest R\(^2\) value was chosen as the most plausible elucidation\(^{24,25}\).

Cytotoxicity assays

HFF2 cells, derived from human foetal foreskin fibroblast, were grown in a RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS), and 100 IU/mL penicillin and 100 mg/mL streptomycin (Gibco). The cells were cultured at 37°C in a humidified atmosphere consisting of 5% CO2 and 95% air. Each well of a 96-well plate was seeded with 10,000 HFF2 cells in 100 µl of RPMI medium supplemented with 10% FBS. After allowing the cells to adhere for 24 hours, a series of doubling dilutions of LCZ-NLCs and Free drug in the concentration range of 0.5 to 8µg/ml were added to three replicate wells. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay, as described below. After a 2-day incubation period, 10 µl of MTT (5 mg/ml stock solution) was added to each well, and the plates were further incubated at 37°C for 4 hours. Following this, the medium was removed, and the formazan blue, produced within the cells, was dissolved by adding 200 µl of dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using a microplate reader (ELx800, BioTek Instruments, Inc., Winooski, VT). Cell viability data was presented as the mean ± standard deviation (SD) of two independent experiments, each performed in three replicates. The percentage of viability, compared to the control wells (where the average optical density of untreated cells was considered as 100% viability), was determined by analyzing the concentration-response curves using linear regression analysis.
Fungal Strains

A collection of 62 resistant fungal strains, including clinical Trichophyton indotineae isolates (n = 13), clinical and environmental Aspergillus fumigatus isolates (n = 22), clinical Fusarium solani isolates (n = 15) and clinical Candida albicans isolates (n = 12) were included in the present study. The isolates were obtained from the reference culture collection of the Invasive Fungi Research Center (IFRC) at the Mazandaran University of Medical Sciences as part of ongoing surveillance and routine diagnostic testing. All isolates were previously identified through the sequencing of internal transcribed spacer ribosomal DNA (ITS-rDNA), β-tubulin, and translation elongation factor (TEF1) regions26–28. The high minimum inhibitory concentrations of Trichophyton indotineae isolates to terbinaine (MICs > 4 µg/ml), Aspergillus isolates to voriconazole and itraconazole (MICs > 16 µg/ml), Fusarium isolates to itraconazole (MICs > 16 µg/ml) and Candida isolates to fluconazole (MICs > 64 µg/ml) were previously demonstrated using reference broth microdilution according to CLSI methods29–32. All fungal isolates were sub-cultured on Sabouraud Dextrose Agar (SDA) (Merck, Germany) supplemented with 0.02% chloramphenicol and incubated at 30°C for up to 5–10 days for proper sporulation.

Antifungal Susceptibility Testing (AFST)

In this study, we evaluated the in vitro antifungal activity of LCZ, NLCs, as well as LCZ-NLCs against a panel of resistant fungal strains. LCZ was dissolved in DMSO (Dimethyl Sulfoxide), and then a two-fold dilution was obtained in RPMI 1640 medium. Antifungal susceptibility testing was performed by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) M38-A3 and M60 documents for filamentous and yeasts fungi, respectively33,34. The final concentration of LCZ in the wells ranged from 0.001 to 1 µg/ml. The LCZ-NLCs formulations were freshly synthesized and applied for antifungal susceptibility testing within a week. For filamentous fungi, conidial suspensions were prepared using saline containing 0.05% Tween 80. The suspensions were then adjusted spectrophotometrically at a wavelength of 530 nm to achieve optical densities ranging from 69–70% transmission for Fusarium solani isolates, 65–70% for Trichophyton indotineae isolates, and 80–82% for Aspergillus fumigatus isolates. Subsequently, the suspensions were diluted 1:50 in RPMI 1640 medium to obtain a final inoculum between 0.4 × 10^-4 × 10^6 CFU/ml for Fusarium solani isolates, 0.5 × 10^3 – 3 × 10^3 CFU/ml for Trichophyton indotineae isolates, and 0.4 × 10^4 × 10^4 CFU/ml for Aspergillus fumigatus isolates. For the Candida albicans isolates, the inoculum was prepared using sterile saline with a transmission of 75–77% at 530 nm. The final size of the inoculum fell within the range of 1×10^5–5×10^5 CFU/ml. The two times test inoculum concentration (1–5 × 10^3 CFU/mL) was achieved by diluting the yeast stock suspension 1:10 with sterile distilled water and then 1:100 with RPMI medium. The 96-well plates were incubated at 35°C, and the results were visually read after 24, 48, and 96 hours for yeast, lamentous, and dermatophyte isolates, respectively. The minimum inhibitory concentration (MIC) was determined as the lowest concentration that completely inhibited the growth of fungi.

Statistical Analysis

The data is presented in the form of the mean ± standard deviation (SD) of three separated measurements. The data that was collected underwent statistical analysis using analysis of variance (ANOVA) and the Tukey-Kramer post-hoc test. A p-value less than 0.05 revealed a significant difference.

Results and Discussion

Characterization of LCZ-NLCs

The DLS method was used to assess the mean particle size, PDI, and zeta potential of the LCZ-NLCs formulation. The obtained data are shown in Table 1. The LCZ-NLCs exhibited nanoscale dimensions, uniform dispersion, and favorable zeta potential, contributing to their stability. The EE% of LCZ in NLCs was approximately 90%.

| Formulation | Particle Size (nm) | PDI || Zeta Potential (mv) | EE (%) |
|-------------|--------------------|-----|----------------------|--------|
| LCZ-NLC     | 202.43 ± 8.25      | 0.225 ± 0.024 | -11.80 ± 0.600 | 89.30 ± 3.70 |
|             | Polydispersity Index | Entrapment Efficiency |

Morphological Study

The FESEM images of LCZ-NLCs (Fig. 1) displayed nanoparticles that are almost spherical and have a uniform shape, with similar diameter and size consistency compared to the results obtained from the DLS technique (Table 1). The variations in size between the DLS and FESEM results can be attributed to various factors, including the measurement approach, sample setting, instrument drawbacks, and sample properties. The measurement approach is particularly significant in influencing size variation. DLS measures the hydrodynamic size of particles based on Brownian motion, whereas FESEM directly captures the size of individual particles using an electron beam, resulting in a better estimation.

ATR-FTIR Spectra Analysis

The ATR-FTIR analysis of LCZ showed absorption at 3114, 3075, and 3040 cm^-1 (aromatic C–H stretching), 2518 cm^-1 (S-H stretching), 2201 cm^-1 (C≡N stretching), 1700 cm^-1, 1653, and 1638 cm^-1 (C=C alkene stretching), 1634 cm^-1 (C=N stretching), 1556 cm^-1 (C=C aromatic stretching), and 1101 and 758 cm^-1 (C–Cl stretching)35. The ATR-FTIR spectrum of pure oleic acid showed two bands at 2923 and 2852 cm^-1 (symmetric C–H stretching and the
asymmetric C–H stretching, respectively). The intense band at 1707 cm\(^{-1}\) is corresponding to asymmetric C = O stretching and the band at 1285 cm\(^{-1}\) attributing to the C–O stretching. Bands exhibited at 1461 – 932 cm\(^{-1}\) represent the O–H stretching. The typical infrared characteristic absorption bands observed in GMS including: 3299 – 3236 cm\(^{-1}\) (O–H stretching), 2956–2850 cm\(^{-1}\) (C–H symmetric and asymmetric stretching), 1729 cm\(^{-1}\) (C = O stretching), 1470 cm\(^{-1}\) (C–H bending), and 1176 cm – 1 (C–O bending)\(^{37}\). The ATR-FTIR spectrum of Tween 80 demonstrated the main peaks at 3502 cm\(^{-1}\) (O–H stretching), 2922 cm\(^{-1}\) (C–H asymmetric stretching), 2859 cm\(^{-1}\) (C–H symmetric stretching), 1735 cm\(^{-1}\) (C = O stretching), and 1093 cm\(^{-1}\) (C–O stretching). The ATR-FTIR spectrum of Span 80 revealed the peaks at 3401 cm\(^{-1}\) (O–H stretching), 2923 cm\(^{-1}\) (C–H asymmetric stretching), 2854 cm\(^{-1}\) (C–H symmetric stretching) and 1739 cm\(^{-1}\) (C = O stretching). As shown in Fig. 2, there was no chemical interaction between LCZ and the excipients because the results exhibited no significant shifting in the characteristic peaks position of the substances.

**DSC Thermogram Investigation**

According to Fig. 3, melting point of pure LCZ and GMS can be seen as an endothermic sharp peak in DSC thermogram of each component. DSC thermogram of pure LCZ had a sharp endothermic peak around 153°C which is corresponding to the melting point of LCZ that is in agreed with previous studies\(^{38}\). Thermogram of GMS showed an endothermic peak with the midpoint of around 65°C corresponding to melting point of GMS\(^{35}\). In brief, the characteristic peaks of pure LCZ and pure GMS disappeared, which showed an effective incorporation of LCZ in the lipid that led to NLC preparation and drug encapsulation.

**In vitro Drug Release and Kinetic**

An *in vitro* release test was carried out to observe the release behavior of LCZ-NLC (1% w/v) in comparison to LCZ aqueous dispersion (1% w/v), and their release patterns are presented in Fig. 4. The results indicated that the NLC carrier released over 50% of the active substance within the first 10 hours of the test. Additionally, LCZ-NLC exhibited a higher drug release percentage (73%) compared to its aqueous dispersion (36%) over 24 hours, which was statistically significant (*P* < 0.05). These findings suggest that the NLC formulation of LCZ significantly improved the drug's dissolution rate and could be considered an effective vehicle for the delivery of LCZ. The Korsmeyer-Peppas model had the greatest coefficient of determination (R\(^2\)) among all five drug release kinetics models. Consequently, the release of drugs from LCZ-NLCs complied with the Korsmeyer-Peppas model. The drug release exponent (n) in the Korsmeyer-Peppas model signifies the mechanism of drug release. The Fickian diffusion occurs when the n value is less than 0.43. Non-Fickian transport is seen when the n value ranges from 0.43 to 1.00. A value of n = 1.00 denotes case II transport, whereas n values more than 1.0 imply super case II transport\(^{40,41}\). In the current study, n = 0.83 which indicated a non-Fickian release mechanism.

**Stability Studies**

Stability testing was conducted by assessing the zeta potential, entrapment efficiency, particle size, and PDI during a three-month period at temperatures of 4 and 25°C (Table 2). The findings revealed that the LCZ-NLC samples stored at both temperatures exhibited consistent colloidal nanometer ranges. There were no significant changes in size, PDI, entrapment efficiency, and zeta potential observed (*P* > 0.05) for the LCZ-NLC samples stored at 4 and 25°C. However, based on the results, it can be inferred that 25°C may be more suitable storage temperature for LCZ-NLC.

<table>
<thead>
<tr>
<th>Storage condition</th>
<th>Time (month)</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>EE%</th>
<th>Organelleptic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial (LCZ-NLC)</td>
<td>0</td>
<td>202.43 ± 8.25</td>
<td>0.225 ± 0.024</td>
<td>-11.80 ± 0.600</td>
<td>89.30 ± 3.70</td>
<td>Stable-milky</td>
</tr>
<tr>
<td>4°C</td>
<td>1</td>
<td>203.99 ± 9.15</td>
<td>0.226 ± 0.020</td>
<td>-11.87 ± 0.602</td>
<td>88.87 ± 3.10</td>
<td>stable-milky</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>204.12 ± 11.20</td>
<td>0.228 ± 0.018</td>
<td>-11.99 ± 0.610</td>
<td>88.5 ± 3.22</td>
<td>stable-milky</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>205.78 ± 7.91</td>
<td>0.231 ± 0.015</td>
<td>-12.00 ± 0.645</td>
<td>88.09 ± 3.44</td>
<td>stable-milky</td>
</tr>
<tr>
<td>25°C</td>
<td>1</td>
<td>202.79 ± 9.17</td>
<td>0.225 ± 0.022</td>
<td>-11.92 ± 0.671</td>
<td>89.02 ± 2.10</td>
<td>stable-milky</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>203.00 ± 11.00</td>
<td>0.226 ± 0.029</td>
<td>-12.20 ± 0.701</td>
<td>88.60 ± 2.46</td>
<td>stable-milky</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>203.68 ± 12.99</td>
<td>0.228 ± 0.027</td>
<td>-12.21 ± 0.710</td>
<td>87.88 ± 3.88</td>
<td>stable-milky</td>
</tr>
</tbody>
</table>

**Cytotoxicity**

The results of the cytotoxicity testing of the free drug and drug-loaded nanoparticles indicate that no toxic effects were observed within the studied concentration range and at the 48-hour time point (Fig. 5). In other words, the survival rate did not differ significantly between all tested doses and the control group. The figure below illustrates the results of the drug's impact on cells when exposed to both the free drug and drug-loaded nanoparticles, compared to the control group.

**Antifungal Activity**

According to CLSI susceptibility testing methodology documents, the MIC50, MIC90, GM-MIC and MIC ranges were calculated. Table 3 shows the detailed results for *in vitro* antifungal susceptibility profile of LCZ and LCZ-NLCs against tested isolates. *Trichophyton indotineae* strains demonstrated MIC90 and GM values of 0.016 and 0.004 µg/ml, respectively, for both LCZ and LCZ-NLCs. *Aspergillus fumigatus* isolates exhibited MIC90 and GM values of 0.032 and 0.007...
μg/ml for LCZ, and 0.016 and 0.005 μg/ml for LCZ-NLCs. *Fusarium solani* strains displayed MIC90 and GM values of 0.064 and 0.015 μg/ml for LCZ, and 0.032 and 0.011 μg/ml for LCZ-NLCs. *Candida albicans* strains showed MIC90 and GM values of 0.125 and 0.056 μg/ml for LCZ, and 0.064 and 0.040 μg/ml for LCZ-NLCs. Additionally, it is worth noting that the formulation of NLCs without LCZ did not exhibit any inhibitory antifungal effect against all isolates. Although the LCZ-NLCs formulation exhibited lower MIC values for some of isolates, the *in vitro* MIC and GM values were largely similar between LCZ and LCZ-NLCs. In the present study, both of LCZ and LCZ-NLCs demonstrated potent *in vitro* antifungal activity against resistant fungal isolates that these findings are consistent with the results of previous studies. The low aqueous solubility and poor skin penetration of luliconazole pose challenges to its dermal bioavailability and topical delivery. To overcome these limitations and enhance its therapeutic efficacy, it is crucial to explore alternative formulations or delivery systems for luliconazole. These approaches aim to improve the drug’s dermal bioavailability, retention, and penetration from the site of skin application. Numerous studies have been conducted on the use of various formulations, such as nanocrystals loaded hydrogel, lyotropic liquid crystalline nanoparticles, solid lipid nanoparticles, niosomal gel, etc. to enhance the delivery of luliconazole. The results obtained from the study conducted by Kumar et al. demonstrated that the luliconazole nanocrystal (LNC) incorporated hydrogel prepared in their research exhibited significant potential in enhancing the topical delivery of luliconazole when compared to conventional formulations. In another study, Yang et al. developed a luliconazole Nanoemulsion as a promising opthalmic delivery system for the treatment of fungal keratitis. In recent years, nanostructured lipid carriers (NLCs) have emerged as advanced and improved carrier systems compared to traditional systems like microemulsions and liposomes. NLCs present several advantages such as higher drug loading capacity, large surface area, and small size, contribute to their potential as a promising option for drug delivery. However, there is limited literature available on the topical use of LCZ-NLCs. Bagel et al. developed and evaluated luliconazole-loaded NLCs incorporated into a gel for the treatment of dermatophytosis. Their findings demonstrated that the NLC formulation exhibited high effectiveness against *Trichophyton rubrum*, as evidenced by the diameter of the inhibition zone, when compared to a commercially available cream.

### Table 3

<table>
<thead>
<tr>
<th>Fungal isolates</th>
<th>Susceptibility (n) at MIC (µg/ml) of:</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>
| **Trichophyton indotiniae**  
(n = 13)  | LCZ    | 3   | 2    | 2    | 2    | 3    | 1    |       | 0.004 | 0.016 | 0.001–0.064 | 0.004 | 0.016 |
|        | LCZ-NLC | 3   | 2    | 2    | 2    | 3    | 1    |       | 0.004 | 0.016 | 0.001–0.032 | 0.004 | 0.016 |
| **Aspergillus fumigatus**  
(n = 22)  | LCZ    | 2   | 3    | 4    | 6    | 3    | 2    | 1    | 1    | 0.008 | 0.032 | 0.001–0.0125 | 0.007 | 0.008 |
|        | LCZ-NLC | 2   | 6    | 3    | 6    | 3    | 1    | 1    |       | 0.008 | 0.016 | 0.001–0.064 | 0.005 | 0.008 |
| **Fusarium solani**  
(n = 15)  | LCZ    | 1   | 1    | 3    | 6    | 1    | 2    | 1    |       | 0.016 | 0.064 | 0.002–0.125 | 0.015 | 0.016 |
|        | LCZ-NLC | 1   | 2    | 4    | 5    | 3    |       |       |       | 0.016 | 0.032 | 0.002–0.032 | 0.011 | 0.016 |
| **Candida albicans**  
(n = 12)  | LCZ    | 2   | 3    | 4    | 2    | 1    |       |       |       | 0.064 | 0.125 | 0.016–0.25  | 0.056 | 0.064 |
|        | LCZ-NLC | 4   | 2    | 5    | 1    |       |       |       |       | 0.048 | 0.064 | 0.016–0.25  | 0.040 | 0.064 |

### Conclusion

The use of NLCs is intended to enhance the solubility, permeability, and bioavailability of LCZ, potentially resulting in a reduced frequency of application and, subsequently, a decrease in local side effects. The findings of study indicated that the LCZ-NLCs formulation developed in this study could be considered a promising topical treatment for superficial fungal infections, particularly in cases of resistant infections. It is also important to note that the safety and efficacy of LCZ-NLCs should be evaluated through animal experiments and clinical trials, which will provide more detailed information about potential side effects.

### Declarations

#### Author Contribution

Authors’ Contribution


### Data Availability
All data associated with the project are presented in the manuscript or as supplementary material.

**Authors' Contribution**

Conceptualization: M.A

Formal analysis: M.A, P.E, J.A

Investigation: M.N, R.E.B, Z.H, I.H

Methodology: M.N, R.E.B, S.M.R

Project administration: M.A, P.E, I.H

Software: S.M.R, R.E.B

Writing–original draft: M.N, S.M.R, J.A

Writing–review & editing: M.A, I.H, P.E

**Competing interests**

All the authors of this article declare that they have no conflict of interest.

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**References**


Figures

![Image of FESEM nanoparticles](image)

**Figure 1**

The FESEM image of LCZ-NLC nanoparticles.
Figure 2

ATR-FTIR spectra of freeze-dried LCZ-NLC, Luliconazole, GMS, Oleic acid, Span 80, and Tween 80.
Figure 3
The DSC thermogram of GMS, freeze-dried LCZ-NLC, and Luliconazole.

Figure 4
Cumulative drug release (%) over time for LCZ-NLC and LCZ aqueous dispersion.
In-vitro release profiles of LCZ from NLC vehicle and its aqueous dispersion in ethanol 50 %. The data present as mean±SD (n=3). The difference was significant during 24 hours (P<0.05).

Figure 5

The results of comparing the effects of the highest concentration of the drug (8 µg/ml) and its nanoparticle formulation at the same concentration with the control group. A: Free luliconazole, B: luliconazole (NLC), C: control (no treatments).