RESEARCH ARTICLE

Development of Luliconazole-Loaded Transethosomal Gel for Enhanced Antifungal Efficacy: Formulation, Characterization, and *In-vitro* Evaluation

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ABSTRACT

Luliconazole (LCZ) is an antifungal agent commonly used to treat skin infections, but its clinical efficacy is often hindered by its poor skin permeation and bioavailability. To address these limitations, a transethosomal gel formulation of LCZ was developed to enhance its topical delivery and therapeutic efficacy. Transethosomes, a novel vesicular carrier system, were employed to encapsulate LCZ, providing better penetration across the skin barrier. The gel formulation was prepared using Carbopol 934P as the gelling agent, with propylene glycol (PG) as a humectant. The developed LCZ-loaded transethosomal gel (TEG-3) was evaluated for various parameters, including physical appearance, pH, viscosity, spreadability, extrudability, swelling index, drug content, and in vitro drug release. The optimized TEG-3 formulation demonstrated favorable characteristics, such as sustained drug release (84.65% over 6 hours), high skin permeation, and excellent stability over three months of storage. Additionally, cytotoxicity studies indicated that the formulation was biocompatible, with cell viability over 90%, suggesting its potential as a safe and effective topical antifungal treatment. The results of this study suggest that the LCZ-loaded transethosomal gel formulation could be a promising candidate for enhanced topical drug delivery in antifungal therapy.

Keywords: Luliconazole, transethosomes, antifungal therapy, skin permeation, drug release, sustained release, topical drug delivery, Carbopol 934P, propylene glycol, swelling index.

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INTRODUCTION

Luliconazole (LCZ) is a broad-spectrum antifungal agent that has been extensively used for the treatment of various superficial fungal infections, including athlete's foot, ringworm, and jock itch. Despite its potent antifungal activity, the clinical efficacy of LCZ is limited by its low skin permeation and bioavailability when applied topically (Patel et al., 2019). Traditional formulations, such as creams and ointments, often fail to deliver sufficient amounts of the drug to the targeted site, reducing therapeutic efficacy and potentially leading to prolonged treatment durations[1].

To overcome these challenges, the development of advanced drug delivery systems, such as liposomes, ethosomes, and transethosomes, has gained significant attention in the field of dermatological drug delivery[2]. Transethosomes are nanocarriers composed of phospholipids, surfactants, and ethanol, designed to enhance drug permeation across the skin. These carriers have been shown to improve the delivery of both hydrophilic and lipophilic drugs, offering significant advantages over conventional formulations by enhancing skin penetration, controlling drug release, and improving stability[3].

The aim of this study was to develop and optimize a transethosomal gel formulation of LCZ to enhance its delivery through the skin and improve its antifungal efficacy. The gel was prepared using Carbopol 934P as a gelling agent, and various formulation parameters, including drug content, viscosity, spreadability, and drug release, were systematically evaluated. The optimized formulation was further subjected to *in-vitro* drug release studies, *ex vivo* permeation studies, cytotoxicity assays, and stability testing to assess its potential for clinical application. This study provides a promising approach for improving the topical delivery of LCZ, thereby enhancing its antifungal efficacy while minimizing side effects.

METHODOLOGY

Materials

Luliconazole (LCZ), a synthetic antifungal drug, was used as the active pharmaceutical ingredient in this study was gifted by Schon Pharmaceuticals, Indore. The gelling agent, Carbopol 934P, was purchased from SD Fine Chem Pvt. Ltd. Propylene glycol (PG) was sourced from Merck Chemicals. Sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate; both preservatives and triethanolamine were obtained from Sigma-Aldrich. All chemicals were of analytical grade.

Preparation of LCZ-Loaded Transethosomal Gel (TE Gel)

The LCZ-loaded transethosomal gel (TE gel) was prepared using transethosomes prepared by cold method in our previous studies.

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Table 1: Gel formulations using optimized transethosomes

Components	TEG-1	TEG-2	TEG-3	TEG-4	TEG-5	TEG-6	TEG-7
LCZ TEs (% w/w)	1	1	1	1	1	1	1
Carbopol 934P (% w/w)	0.5	0.75	1	1.15	1.25	1.5	1.75
PG (% w/w)	5	6	8	5	6	6	5
Sodium methyl hydroxybenzoate (% w/w)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium propyl hydroxybenzoate (% w/w)	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water (ml)	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

Carbopol 934P was selected as the gelling agent, and its concentration was varied from 1% to 4% w/w. Propylene glycol (PG) was used as a humectant and plasticizer at concentrations ranging from 5% to 12% w/w. Sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate were incorporated as preservatives. The final pH of the formulation was adjusted to 5.0-7.0 using triethanolamine. The optimized LCZ-loaded transethosome (TE) was incorporated at 1% w/w into the Carbopol 934P mixture under continuous stirring. The formulation process involved the following steps:

Carbopol 934P Dispersion: Carbopol 934P was dispersed in distilled water using a mechanical stirrer. The dispersion was allowed to swell overnight to ensure proper hydration and stabilization.

Incorporation of LCZ-Loaded TEs: 1% w/w of the optimized LCZ-loaded TEs was added to the Carbopol 934P mixture under continuous stirring.

Addition of Excipients: Propylene glycol, preservatives, and other excipients were added to the gel.

pH Adjustment: The pH of the final gel was adjusted to 5.0-7.0 using triethanolamine.

Figure 1: TEG-3 gel formulation containing luliconazole loaded transethosomes

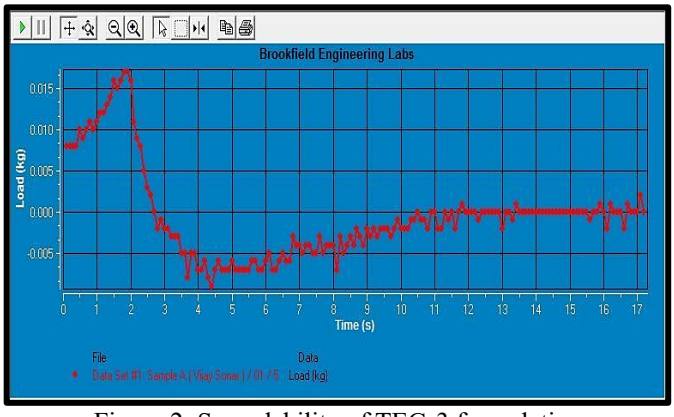

Figure 2: Spreadability of TEG-3 formulation

Table 2: pH test of TE gel formulations

S. No.	TE gel formulations	pH (mean ± SD)
1	TEG-1	5.34 ± 0.12
2	TEG-2	6.35 ± 0.59
3	TEG-3	5.85 ± 0.32
4	TEG-4	6.58 ± 0.59
5	TEG-5	6.25 ± 0.32
6	TEG-6	5.35 ± 0.32
7	TEG-7	6.75 ± 0.32

Formulation Table

Seven formulations (TEG-1 to TEG-7) were prepared to study the effects of varying Carbopol 934P and PG concentrations on gel properties. The compositions are shown in table 1.

Characterization of LCZ Transethosomal Gel

Physical Appearance

The physical characteristics of the formulated TE gels were evaluated visually for parameters such as color, homogeneity, consistency, phase separation, and grittiness. The assessment was carried out according to the methods described in previous studies on topical formulations.

pH Analysis

The pH of each gel formulation was determined using a digital pH meter (Mettler Toledo). One gram of the gel was dissolved in 100 mL distilled water, and the pH was recorded after stabilization of the electrode. The pH of the gel was measured in triplicate to ensure accuracy. The method follows standard protocols for gel formulation analysis[4].

Viscosity Analysis

The viscosity of the gel was determined using a Brookfield viscometer by selecting appropriate spindle number and rpm was adjusted accordingly.

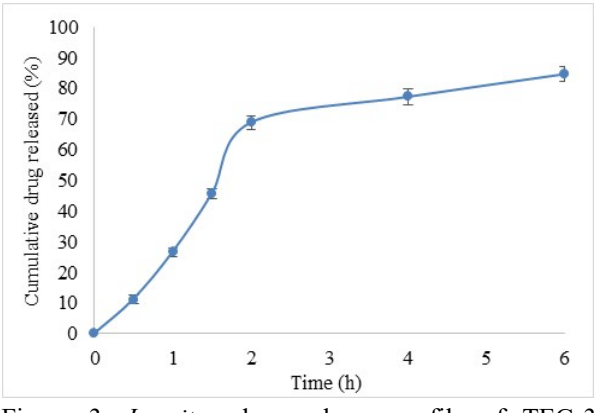

Figure 3: *In vitro* drug release profile of TEG-3 formulation

Table 3: Viscosity of TE gel formulations

S. No.	TE gel formulations	Viscosity (cP) (mean ± SD)
1	TEG-1	22,100 ± 605
2	TEG-2	28,050 ± 650
3	TEG-3	40,065 ± 761
4	TEG-4	49,855 ± 783
5	TEG-5	55,110 ± 942
6	TEG-6	65,050 ± 1100
7	TEG-7	70,075 ± 1351

Viscosity measurements are critical for evaluating the flow properties of the gel and its suitability for topical application[5].

Drug Content Determination

The drug content was determined by dissolving 100 mg of the gel in a mixture of methanol (5 mL) and phosphate buffer pH 7.4 (20 mL). The solution was filtered, and 1 mL of the filtrate was transferred to a 10 mL volumetric flask, where the volume was made up with phosphate buffer. The drug content was analyzed using a UV-Visible spectrophotometer at 297 nm. This method is consistent with established procedures for determining the drug concentration in gel formulations[6].

Table 4: Percent drug content of TE gel formulations

S. No.	TE gel formulations	Drug content (%) (mean ± SD)
1	TEG-1	97.65 ± 1.11
2	TEG-2	98.31 ± 1.19
3	TEG-3	98.89 ± 1.12
4	TEG-4	97.89 ± 2.12
5	TEG-5	96.32 ± 1.34
6	TEG-6	97.12 ± 1.27
7	TEG-7	96.34 ± 1.22

Table 5: Spreadability

S. No.	TE gel formulations	Spreadability (g.cm/sec) (mean ± SD)
1	TEG-1	11 ± 0.5
2	TEG-2	9.54 ± 0.4
3	TEG-3	7.29 ± 0.2
4	TEG-4	6.50 ± 0.18
5	TEG-5	6.30 ± 0.25
6	TEG-6	5.65 ± 0.2
7	TEG-7	4.85 ± 0.14

Spreadability

The significance of the spreadability test is described by the gel's capacity to spread when applied to the skin. Spreadability was determined using the slip-and-drag (parallel-plate) method, where two glass slides were used to assess how easily the gel layers separated under an applied load. In this method, a predefined amount of the gel was placed between the slides, and a fixed weight of 200 g was applied on the upper slide to allow uniform spreading. After a defined time, the weight was removed, and the time (in seconds) required for the slides to separate was recorded. Spreadability was calculated using the following equation:

$S = M \times L/T$

Where, S = Spreadability (g·cm/s)

M = Applied weight (g)

L = Length or diameter of spread gel (cm)

T = Time required for plates to separate (s)

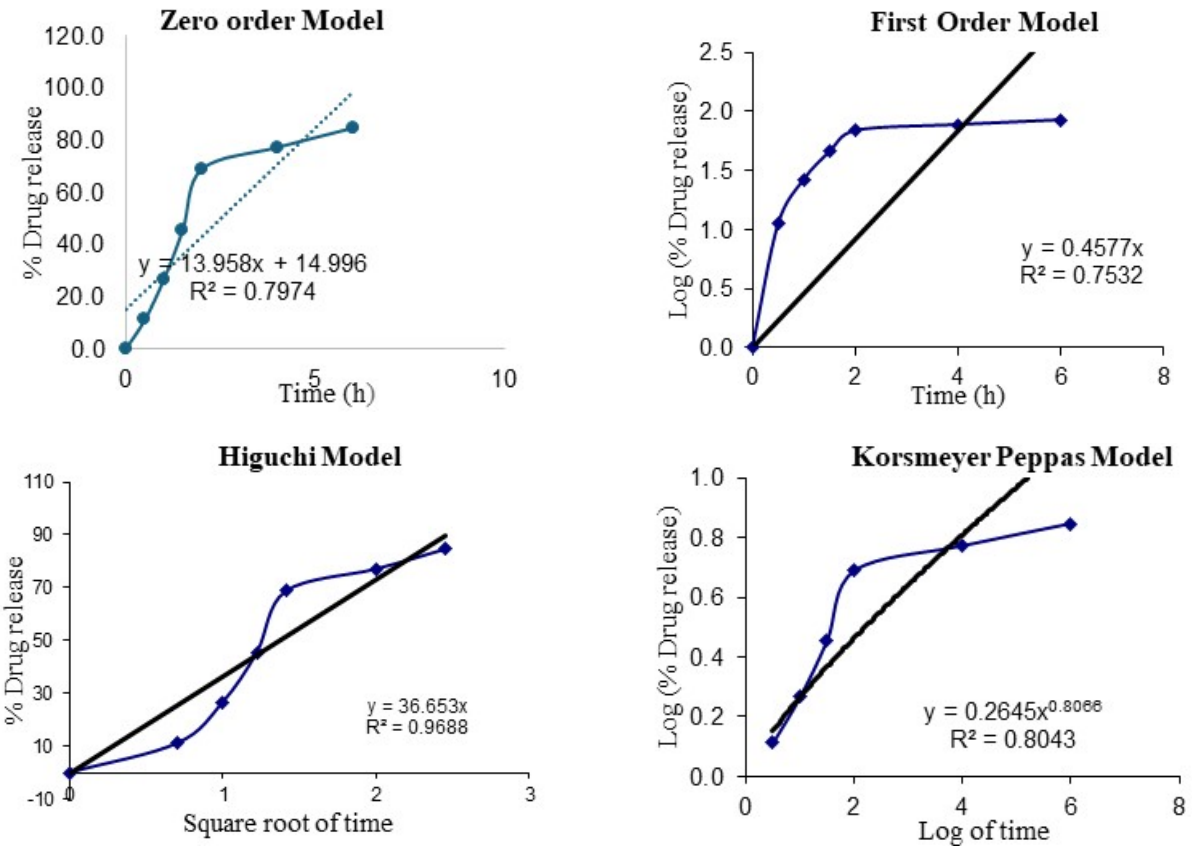

Figure 4: Release kinetics plots of TEG-3 formulation

The test is widely used to evaluate the spreadability of topical gels[7].

Extrudability

The extrudability of the gel was assessed by applying weight to expel more than 5.0 mm of gel from collapsible tubes in 10 seconds. The formula used to calculate extrudability is:

$$Extrudability = \frac{\text{Weight in gram to extrude the gel}}{\text{Area (in cm}^2\text{)}}$$

Extrudability tests are important for assessing the ease of gel application[8].

Swelling Index

The dynamic swelling behavior of the gel was studied using the tea bag method. A weighed sample of gel was placed in a pre-weighed cellophane tea bag and immersed in phosphate buffer pH 5.5 at 37°C for 2 hours. The swelling capacity was calculated as

Swelling capacity $(g.g^{-1}) = (W_t - W_o - W_c)/W_o$

where, W_0 is the weight of the dry gel, W_t is the weight of the swollen gel along with the wet cellophane tea bag and W_c is the weight of the wet cellophane tea bag[9,10].

***In-vitro* Drug Release Studies**

An *in vitro* release study was conducted using a dialysis membrane with a molecular weight cutoff of 12–14,000 Da (Sigma-Aldrich, St. Louis, MO, USA). Dialysis bags were cut open, and one end was securely knotted to prevent leakage. The optimized transethosomal gel was placed into the dialysis bags, which were then sealed to compare their release patterns. During the process dialysis bag was immersed in 250 mL of buffer solution pH 7.4 in a beaker and this beaker was subjected to magnetic stirring at a rate of 100 revolutions per minute and maintained at a temperature of 37 ± 0.2 °C. Samples (5 mL) were withdrawn from the medium at 0, 1, 2, 4, and 6 hours, and each time an equal volume of fresh buffer was added to maintain sink conditions. The amount of released drug in the buffer was determined using a UV-vis spectrophotometer at λmax = 297 nm. Each analysis was performed in triplicate, and the average value was recorded[11].

Mathematical Modeling of Drug Release Kinetics

The drug release data were analyzed using several kinetic models: zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The goodness of fit was determined using the coefficient of determination (R^2) and residual sum of squares (RSS). The model that best described the release kinetics was chosen, providing insights into the release mechanism. The kinetic model equations of first-order, zero-order, Higuchi model, and Korsmeyer–Peppas model of the drug release kinetics were used to determine drug release patterns. Regarding the Korsmeyer–Peppas model, if "n" is less than or equals 0.45, this is referred to as Fickian diffusion. If "n" is greater than 0.45, it denotes non-Fickian diffusion. However, when "n" is between 0.45 and 0.89, this is referred to as the anomalous system.

***Ex Vivo* Permeation Studies**

Ex vivo permeation was studied using the Franz diffusion cell method, in which both the transethosomal gel and plain gel were applied to goat skin (obtained from a slaughterhouse), as it closely resembles human skin. The skin was first collected and washed with saline solution. After removing fatty tissues, the skin was carefully cut into circles to fit the circumference of the Franz cell. The Franz diffusion cell apparatus has a surface area of 1.73 cm^2 and a receptor volume of 12 mL. A phosphate buffer solution at pH 7.4 was added to the receptor compartment as the simulated skin medium. The cell was maintained at 37 °C with constant stirring. Approximately 1 g of each gel was spread onto the skin. Samples were withdrawn from the receptor compartment at 0.5, 1, 2, 4, 6, and 8 hours, and immediately replaced with an equal volume of phosphate buffer solution to maintain sink conditions. The samples were analyzed at λmax = 297 nm using a UV-vis spectrophotometer to determine the total permeated drug. The drug permeability data were then plotted against time[12].

***In vitro* Cytotoxicity Analysis**

The cytotoxicity of LCZ transethosomal gel was evaluated on L929 fibroblast cells using the MTT assay. Cells were exposed to various concentrations of the gel for 24 hours, and cell viability was determined by measuring absorbance at 540 nm. The percentage of cell inhibition was calculated using the following formula:

$$\text{Cell inhibition}(\%) = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

This method is widely used to assess the cytotoxicity of pharmaceutical formulations (Yadav et al., 2021).

Stability Studies

Accelerated stability studies were conducted according to ICH Q1A (R2) guidelines under the following conditions:

- Condition 1: 2-8°C (refrigerated)
- Condition 2: 25°C/60% Relative Humidity (RH)
- Condition 3: 30°C/65% RH

Samples were withdrawn at 0, 1, 2, and 3 months, and physical appearance, pH, viscosity, and drug content were evaluated. Stability testing followed standard protocols for pharmaceutical formulations[13].

RESULTS AND DISCUSSION

Physical Appearance

The formulated transethosomal gels (TEGs) exhibited excellent homogeneity and a smooth, uniform texture. Visual examination revealed that all batches of TEGs were translucent, with no visible abrasive particles or phase separation. The transparency of the gels suggests the effective incorporation of transethosomes into the gel matrix, ensuring that the drug and other excipients were uniformly dispersed. This uniformity is critical for ensuring consistent performance across different batches and achieving the intended therapeutic effect. Similar results were observed by Sharma et al. (2017), who reported the successful formulation of smooth and transparent transethosomal gels.

pH Analysis

The pH of the gel formulations ranged from 5.3 to 6.8 (**Table 2**), which is suitable for topical application. The formulations within this pH range help maintain the skin's integrity and prevent irritation. The pH values of the formulations were stable across different batches, indicating the efficacy of the pH adjustment process.

Table 6: Stability study of TEG-3 formulation

Test	Initial	Condition	Time		
			1 M	2 M	3 M
Description	Transparent, odorless translucent gel	2 to 8°C	NC	NC	NC
		25°C/60% RH	NC	NC	NC
		30°C/75% RH	NC	NC	NC
pH	6.73 ± 0.25	2 to 8°C	6.71± 0.14	6.65 ± 0.18	6.87 ± 0.24
		25°C/60% RH	6.74 ± 0.09	6.61 ± 0.17	6.78 ± 0.14
		30°C/75% RH	6.63 ± 0.21	6.82 ± 0.25	6.69 ± 0.08
Viscosity (cps)	40,065 ± 761	2 to 8°C	40,090 ± 800	39,960 ± 580	39,912 ± 690
		25°C/60% RH	40,000 ± 700	39,910 ± 660	39826 ± 725
		30°C/75% RH	40,050 ± 650	39,900 ± 735	39750 ± 565
Drug content (%)	98.89 ± 1.12	2 to 8°C	98.81 ± 1.21	98.75 ± 1.05	98.64 ± 1.54
		25°C/60% RH	98.74 ± 1.38	98.19 ± 1.65	97.84 ± 1.25
		30°C/75% RH	98.72 ± 0.84	98.11 ± 1.62	97.42 ± 1.43

Viscosity Analysis

The viscosity of the TE gel formulations ranged from 22,100 to 70,000 cP, indicating variations in their consistency and flow behavior (**Table 3**). Among all batches, TEG-3 exhibited an optimal viscosity of 40,065 ± 761 cP, placing it within the mid-range of the tested formulations.

A viscosity that is too low (as seen in TEG-1 and TEG-2) may result in a gel that spreads excessively, lacks structural integrity, and may run off the skin after application, compromising therapeutic effectiveness. Conversely, very high viscosity values (observed in TEG-4, TEG-5, TEG-6 and TEG-7) can make the gel too stiff, reducing spreadability and making application difficult for the patient.

In contrast, TEG-3 achieves an ideal balance between these extremes, offering sufficient thickness to ensure good

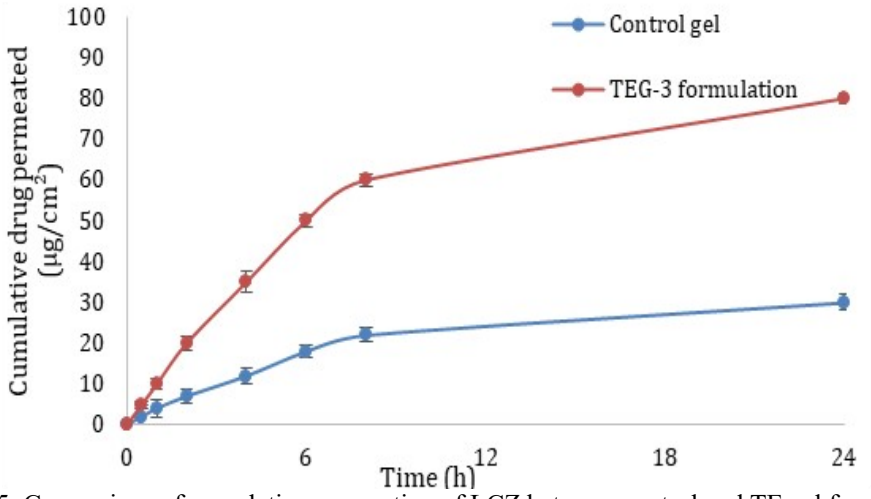

Figure 5: Comparison of cumulative permeation of LCZ between control and TE gel formulation

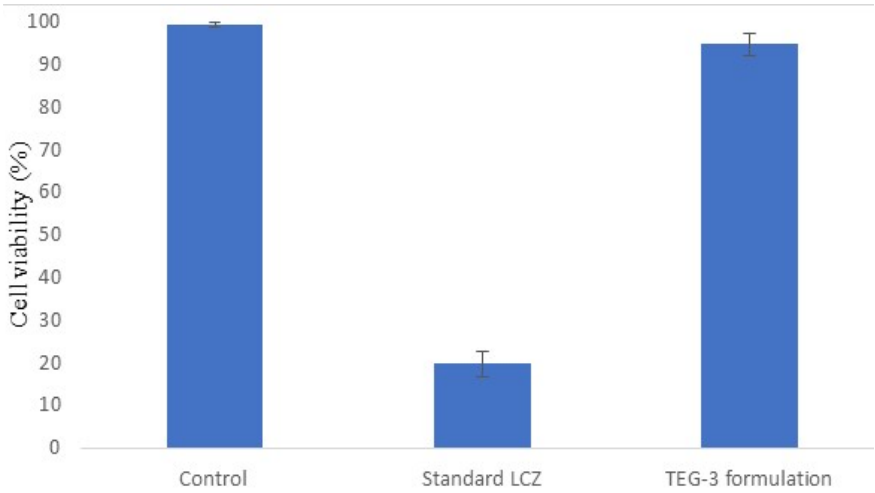

Figure 6: Cell viability studies of control, standard LCZ and TEG-3 formulation on L929 cells

retention on the skin while still allowing smooth and effortless spreading.

Drug Content Determination

The drug content in the transethosomal gels was determined to be between 96.32% and 98.89% of the labeled amount (**Table 4**). The TEG-3 formulation exhibited the highest drug content (98.89%), which reflects the accurate encapsulation of LCZ in the transethosomes during the formulation process. Consistent drug content across different batches further validates the reproducibility and reliability of the formulation process.

Spreadability

The spreadability values of the TE gel formulations ranged from 4.85 to 11 g.cm/s (**Table 5**), indicating variability in

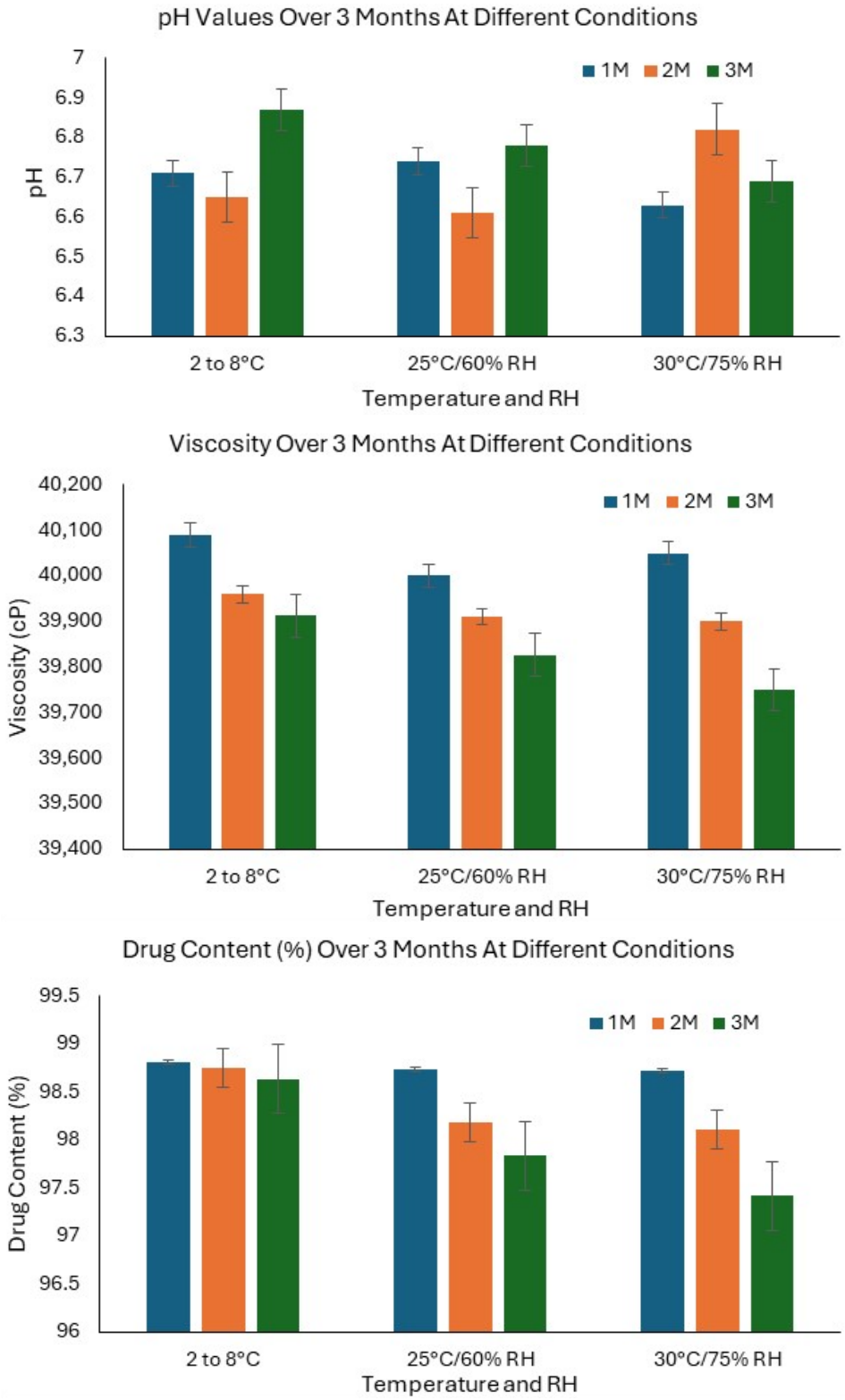

Figure 7: Graphical representation of stability data at three different conditions which was assessed for three months by analysis of drug content, pH, and viscosity

their ease of application. Although TEG-1 showed the highest spreadability (11 ± 0.5 g.cm/s), excessively high spreadability may indicate a gel that is too thin or less viscous, which can compromise retention on the application site. In contrast, TEG-3 demonstrated an optimal spreadability value of 7.29 ± 0.2 g.cm/s, representing a balanced consistency that allows the gel to spread smoothly without being overly fluid.

This intermediate spreadability suggests that TEG-3 offers the best combination of ease of application and adequate firmness, ensuring uniform distribution on the skin while maintaining sufficient viscosity for prolonged residence time and effective drug delivery (**Figure 2**).

Extrudability

The extrudability of TEG-3 was determined to be 482.43 g/cm². A lower extrudability value indicates that the gel has a good consistency for application, as it will not flow too easily when dispensed from the container. This ensures that the formulation stays in place once applied to the skin. The value of 482.43 g/cm² is within the desired range for a topical formulation.

Swelling Index

The swelling index of the TEG-3 formulation was found to be 4.90 ± 0.17 g/g, which is higher than that of the plain gel (3.72 ± 0.14 g/g). This increase in swelling index can be attributed to the presence of transethosomes in the gel formulation. The lipid bilayer of transethosomes allows for better water absorption, resulting in a greater swelling index compared to the blank gel. The swelling behavior is important as it can influence the release rate of the drug, as a higher swelling index can lead to a more sustained release.

***In-vitro* Drug Release Studies**

The *in vitro* drug release studies of TEG-3 demonstrated that 84.65% of the LCZ was released after 6 hours (**Figure 3**). This slow and sustained release is desirable for enhancing the therapeutic effect of the drug over an extended period. The sustained release pattern is primarily attributed to the transethosomal carrier, which regulates the diffusion of the drug through the gel matrix. The controlled release ensures that LCZ is delivered effectively to the target site without causing irritation or side effects due to rapid drug release.

Mathematical Modeling of Release Kinetics

The release data of TEG-3 were fitted to different kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models (**Figure 4**). The Higuchi model showed the best fit, indicating that the drug release from the formulation was predominantly diffusion-controlled. The Korsmeyer-Peppas model also suggested that both diffusion and erosion mechanisms were involved in the release process, as indicated by the release exponent (n) value between 0.5 and 1.0. This result showed that transethosomal formulations typically follow a diffusion-controlled release mechanism, with some contribution from erosion.

***Ex-vivo* Permeation Studies**

The *ex vivo* permeation study (**Figure 5**) showed that TEG-3 significantly enhanced the permeation of LCZ when compared to the control gel. The TEG-3 formulation exhibited a four-fold higher cumulative permeation, which can be attributed to the nano-sized transethosomes that facilitate deeper penetration of the drug into the skin. The increased permeation is a key benefit of using transethosomes, as it helps improve the bioavailability of the drug.

***In vitro* Cytotoxicity Analysis by MTT Assay**

The cytotoxicity of TEG-3 was evaluated using the MTT assay, and the formulation demonstrated a cell viability of 94.67%, which is above the 70% threshold for non-cytotoxicity (ISO 10993-5:2009). The high cell viability indicates that TEG-3 is biocompatible and safe for use on human skin. These findings suggest that TEG-3 could be a promising formulation for topical antifungal therapy.

Stability Studies

Stability studies conducted at different temperature and humidity conditions (**Table 6**) demonstrated that TEG-3 was stable over a three-month period. No significant changes in the physical appearance, pH, viscosity, or drug content were observed under any of the storage conditions. This stability is crucial for ensuring the long-term efficacy and safety of the formulation. We found that transethosomal formulations remained stable over extended storage periods under various conditions.

CONCLUSION

The developed LCZ-loaded transethosomal gel (TEG-3) exhibited favorable characteristics, including optimal viscosity, good spreadability, enhanced skin permeation, and sustained drug release. The formulation showed biocompatibility, minimal cytotoxicity, and excellent stability under various storage conditions. These results suggest that TEG-3 is a promising candidate for the topical delivery of LCZ, offering potential for enhanced antifungal therapy.

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