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FORMULATION AND CHARACTERIZATION OF ANTI-FUNGAL ETHOSOMAL GEL FOR EFFECTIVE TOPICAL FUNGAL TREATMENT

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Abstract

This study focuses on the formulation and characterization of an anti-fungal ethosomal gel containing Tioconazole for effective topical treatment. Ethosomal formulations (F1-F6) were examined microscopically, revealing uniform vesicle sizes ranging from 159.85 to 220.32 µm across different formulations. Formulations F4 and F6 exhibited high entrapment efficiencies of 74.65%, indicating effective encapsulation of Tioconazole within the ethosomal vesicles. Gel formulations (EF1-EF3) demonstrated excellent homogeneity (+++), texture, and spreadability (9.23-12.32 gm.cm/sec), with good extrudability and washability. pH values (6.78-6.92) were suitable for topical application, ensuring skin compatibility. Viscosity (2985-3365 cps) and high assay percentages (97.36%-99.15%) confirmed formulation consistency and potency. The cumulative drug release profile from EF2 showed sustained release kinetics over 10 hours, reaching 96.65% drug release. Regression analysis (Zero order, First order, and Pappas plot) of EF2 indicated high correlation coefficients (R^2 = 0.9281-0.9729), supporting predictable and controlled drug release. Overall, this ethosomal gel formulation demonstrates potential for enhanced therapeutic efficacy in topical fungal treatment.

Key Words: Ethosomal gel, Tioconazole, anti-fungal treatment, vesicle size, entrapment efficiency, sustained release, topical application, formulation characterization

Introduction

Fungal infections, particularly those affecting the skin and mucous membranes, pose significant challenges due to their prevalence and potential for chronicity. Dermatophytic infections, candidiasis, and other fungal conditions can severely impact quality of life and require effective treatment strategies. Conventional topical formulations often face limitations such as poor drug penetration and retention at the site of infection, leading to suboptimal therapeutic outcomes and potential resistance development (Chen et al., 2019; Elsayed et al., 2006).

In recent years, ethosomal formulations have emerged as promising vehicles for enhancing the delivery of antifungal agents (Sahu et al., 2021; Touitou et al., 2001). Ethosomes are phospholipid-based nanocarriers characterized by their ability to encapsulate drugs and penetrate through the lipid-rich skin barrier more effectively than conventional liposomes. This enhanced delivery capability is attributed to their high flexibility and lipid composition, which allows them to fuse with the stratum corneum and release the encapsulated drug at the site of action.

Tioconazole, a broad-spectrum antifungal agent belonging to the imidazole class, has shown efficacy against various fungal pathogens. Its incorporation into ethosomal formulations offers the potential to improve therapeutic outcomes by increasing drug bioavailability and enhancing tissue penetration (Chen et al., 2019; Sahu et al., 2021).

Moreover, ethosomal gels provide a convenient topical dosage form that ensures prolonged contact time and controlled release of the drug, optimizing treatment efficacy while minimizing systemic side effects (Elsayed et al., 2006; Touitou et al., 2001).

This study aims to formulate and characterize an ethosomal gel containing Tioconazole for effective topical fungal treatment. The formulation will be evaluated for physicochemical properties such as vesicle size, entrapment efficiency, zeta potential, pH, viscosity, and drug release kinetics. Stability studies will also be conducted to assess the formulation's robustness under various storage conditions.

By leveraging the advantages of ethosomal technology, this research seeks to contribute to the development of advanced topical therapies capable of addressing the challenges posed by fungal infections more effectively. The insights gained from this study may pave the way for the development of innovative antifungal formulations with improved patient compliance and therapeutic outcomes.

Material and Methods

Material

The ethosomal gel formulation containing Tioconazole was developed using key materials sourced from reputable suppliers: Tioconazole from Bioplus Life Science, Soya Phosphatidyl Choline from Ash Chemie India, and phosphate buffer components (Disodium Hydrogen Phosphate, Di potassium Hydrogen Orthophosphate, Sodium Chloride) from S. D. Fine Chem. Ltd. Solvents including Methanol, Ethanol, and Chloroform from Qualigens Fine Chemicals were crucial for drug and lipid dissolution. Carbopol 934p from S. D. Fine Chem. Ltd. served as a gelling agent, while preservatives (Methyl Paraben, Propyl Paraben) and Propylene Glycol from the same supplier ensured stability and efficacy of the formulation. This approach aimed to enhance drug delivery, stability, and therapeutic efficacy against fungal infections.

Methods

Preparation of Ethosomes of Tioconazole

Soya PC (0.5 to 1.5% w/v) was dissolved in ethanol (5-10% v/v) and heated up to 30 ± 1°C in a water bath in a closed vessel [50]. Distilled water or drug solution in distilled water (0.5% w/v solution) containing SDS (50mg), which is previously heated up to 30 ± 1°C, was added slowly in a fine stream to the above ethanolic lipid solution with continuous mixing using a magnetic stirrer at 900 rpm. Mixing was continued for another 5 minutes and finally, the vesicular dispersions resulted was left to cool at room temperature (25 ± 1°C) for 45 minutes (Das; 2018) . Different ethosomal dispersions and their composition are shown in table 1.

Table 1: Different Composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid (% w/v)	Ethanol (% w/v)	PEG (%w/v)	SDS (mg)	Water (%w/v)
F1	500	0.5	5	20	50	100
F2	500	0.5	10	20	-	100
F3	500	1.0	5	20	50	100
F4	500	1.0	10	20	-	100
F5	500	1.5	5	20	50	100
F6	500	1.5	10	20	-	100

Evaluation of Tioconazole loaded Ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (Cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation (Touitou et al., 2000).

Vesicle size and zeta potential

Vesicle size and zeta potential of the Ethosomes were measured by photon correlation spectroscopy using a horiba scientific, nanoparticle analyzer instrument.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium (Li et al., 2012). About 1 ml of the drug loaded ethosomes dispersion was placed in the eppendorf tubes and centrifuged at 10,000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without Tioconazole was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 244nm. **Formulation of ethosomal loaded gel**

The incorporation of the drug loaded ethosomes (equivalent to 0.5%) into gels was achieved by slow mechanical mixing at 25 rpm (REMI type BS stirrer) for 10 minutes (Zhang et al., 2018). The optimized formulation was incorporated into three different Carbopol gel concentration 0.5, 1 and 2% w/w.

Table 7.4: Composition of different gel base

S. No.	Formulation	Carbopol (%)
1.	EF1	0.5
2.	EF2	1
3.	EF3	2

Evaluation of gel

Physical characteristic

The **physical** characteristic was checked for gel formulations (homogeneity and texture) and observations were shown in Table 2.

Determination of pH

The pH of the gel was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained (Zhai et al., 2015). And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes (Mbah et al., 2019). The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Assay

Weight equivalent to 10 mg of ethosomal gel dissolved in 5 ml methanol in 10 ml volumetric flask, sonicate it for 10 min and volume make up to 10 ml and dilute suitably to 10µg/ml and take the absorbance at 244 nm and calculate using calibration curve of linearity (Ascenso et al., 2015).

Spreadability

Two glass slides of standard dimensions (6×2) were selected. The gel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the gel formulation between the two slides was traced uniformly to form a thin layer.

The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted

(Behl et al., 1980). The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation (Table 7.5).

$$\text{Spreadability} = \frac{m.l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 grams)

l= length of glass slide (6cms).

t = time taken is seconds.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25^{0}C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25±/1^{0}C) before the measurements.

***In-vitro* drug release studies using the semipermeable membrane Preparation of semi permeable membrane for the diffusion studies:**

The semipermeable membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies (Ghafourian et al., 2004; Bounoure et al., 2008).

The prepared ethosomes delivery system was evaluated for *in vitro* drug release. The drug release studies were carried out using modified franz diffusion cell. The dissolution study was carried out in 24 ml dissolution medium which was stirred at 50 rpm maintained at 37±0.2°C.

Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to

10ml by PBS (pH 7.4). The samples withdrawn were assayed spectrophotometrically at 244nm for Tioconazole and using UV visible spectrophotometer. The release of Tioconazole was calculated with the help of Standard curve of Tioconazole.

Results and Discussion

The microscopic examination of ethosomal formulations revealed uniform vesicle sizes, ensuring consistency in formulation across different codes (F1-F6) Figure 1. Vesicle sizes ranged from 159.85 to 220.32 μm, with formulations F4 and F6 showing higher entrapment efficiencies of 74.65% and 74.65%, respectively. This indicates effective encapsulation of Tioconazole within the ethosomal vesicles, which is important for sustained drug release and enhanced therapeutic efficacy table 2.

Gel formulations (EF1-EF3) exhibited excellent homogeneity and texture (+++), indicating uniform distribution of components. Spreadability ranged from 9.23 to 12.32 gm.cm/sec, ensuring ease of application, while all formulations showed good extrudability and washability, essential for patient compliance and efficacy table 3.

pH values (6.78-6.92) of the gel formulations (EF1-EF3) were within acceptable ranges for topical applications, ensuring skin compatibility. Viscosity values (2985-3365 cps) indicated suitable consistency for easy application and prolonged contact on the skin. High assay percentages (97.36%-99.15%) confirmed the formulations' potency and uniformity in drug content table 4.

The cumulative drug release profile over 10 hours from EF2 demonstrated sustained release kinetics, with percentages increasing steadily from 26.54% at 0.5 hours to 96.65% at 10 hours. This sustained release pattern is beneficial for maintaining therapeutic drug levels over an extended period, enhancing treatment efficacy table 5.

Regression analysis (Zero order, First order, and Pappas plot) of EF2 indicated high correlation coefficients (R^2 = 0.9281-0.9729), suggesting that the drug release followed a predictable and controlled pattern. This supports the reliability and consistency of the ethosomal formulation in delivering Tioconazole effectively table 6.

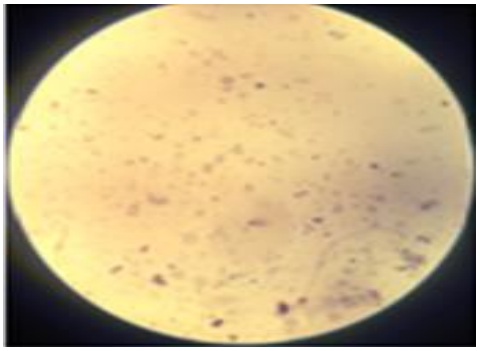

Figure 1: Microscopic observation of prepared ethosomes formulation

Table 2: Result for Vesicle size and Entrapment efficiency of drug loaded Ethosomes

Formulation Code	Vesicle size (μ)	% Entrapment Efficiency
F1	220.32±0.25	69.98±0.45
F2	192.23±0.32	70.32±0.32.
F3	185.65±0.15	66.74±0.14
F4	159.85±0.36	74.65±0.22
F5	173.32±0.22	68.98±0.36
F6	181.41±0.14	74.65±0.25

Table 3: Results of Homogeneity, Extrudability, Spreadability of gel formulation

Code	Homogeneity and Texture	Spreadability (gm.cm/sec.)	Extrudability	Washability
EF1	**+++**	12.32±0.15	**+++**	Good
EF2	**+++**	10.15±0.20	**+++**	Good
EF3	**+++**	9.23±0.35	**+++**	Good

+++ Good ++ Average

Table 4: Results of pH, Viscosity and % Assay

Code	pH*	Viscosity* (cps)	% Assay*
EF1	6.85±0.15	3365±16	98.32±0.32
EF2	6.78±0.16	3245±10	99.15±0.25
EF3	6.92±0.26	2985±15	97.36±0.15

***Average of three determinations**

Table 5: Cumulative % drug release of Tioconazole from optimized ethosomes gel formulation EF2

S. No.	Time (hrs)	% Cumulative drug release ethosomal gel
1	0.5	26.54
2	1	43.25
3	2	55.65
4	4	68.54
5	6	74.65
6	8	88.98
8	10	96.65

Table 6: Regression Analysis Data of Ethosomal Formulation

Formulation	Zero order	First order	Pappas plot
EF2	$R^2 = 0.9281$	$R^2 = 0.9303$	$R^2 = 0.9729$

Conclusion

In conclusion, the ethosomal gel formulation of Tioconazole demonstrated high drug entrapment efficiency, favorable physicochemical properties, and sustained drug release kinetics over 10 hours. The formulations exhibited excellent homogeneity, spreadability, and met criteria for pH, viscosity, and drug assay, indicating suitability for topical application. Regression analysis confirmed predictable drug release patterns. Overall, these findings suggest the formulation's potential as an effective treatment option for topical fungal infections, pending further clinical validation.

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