FORMULATION, DEVELOPMENT AND EVALUATION OF ETHOSOMAL GEL OF TOLNAFTATE FOR FUNGAL TREATMENT

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Abstract

The most common method of administering medication is orally, however this exposes the drug to a variety of conditions and can occasionally cause drug degradation. These days, a variety of drug delivery methods, including ethosomes, are available to improve drug molecules' penetration through the stratum corneum. This study deals with the formulation, development, and assessment of Ethosomal Gel of Tolnaftate for Fungal treatment. The preparation and evaluation of ethosomal gel was performed according to standar methods. The results showed that the vesicle size ranged from approximately 195.65 nm to 305.65 nm across the different formulations. Notably, F5 exhibited the smallest vesicle size at 195.65 The F5 demonstrated the highest entrapment efficiency at 76.65%. The entrapment efficiency ranged from approximately 66.56% to 76.65%. F5 demonstrated the highest entrapment efficiency at 76.65. Further these ethosomes were incorporated in gel and evaluated for various parameters. All the three gels were smooth in texture. The pH of gel was found to be 6.74±0.02, 6.81±0.03 and 6.46±0.01 respectively. The wash ability of all the formulations was good, while the spreadibility (g.cm/sec) was found as 12.32±0.25, 11.15±0.32 and 10.65±0.15 respectively for formulation EG1, EG2 and EG3. Then, the percentage assay of formulation EG1, EG2 and EG3 was determined. The maximum percentage assay was found in formulation EG2 (99.85±0.32), select as optimized formulation. The Cumulative % drug release of formulation EG1 was observed to be 98.85 in 8 hrs. In case of EG2 and EG3 98.21% and 88.78% drug release occur in 12 hours. Thus it can be concluded that EG2 is more efficient formulation over other two. In case of zero order, first orde the r^2 value was observed to be 0.968 and 0.814 respectively. While for Higuchi and Korsmeyer peppas r^2 value was estimated to be 0.987 & 0.990. The model with the highest R-squared value is considered the best fit for your data. In this case, the Korsmeyer-Peppas model appears to be the best fit, as it has the highest R-squared value (0.990). Thus, it can be concluded that the formulation EG2 have all ideal characteristic in vitro and can be used to treat fungal infection.

Keywords: Fungal infections, Ethosomes, Gel, Tolnaftate, Topical Drug delivery

Introduction

A class of microorganisms known as fungi has stronger immune defenses and cell walls composed of hard, complex carbohydrate polymers, such as mannans, which are chains of mannose molecules attached to fungal proteins via N- or O-linkages. Growing numbers of cases of fatalities and severe morbidity from systemic fungal infections should raise concerns. The course of an invasive fungal infection is determined by a number of variables, such as the patient's current clinical state (underlying disease), immune function, the pathogenecity and virulence factors of the invading fungal species, and the area that is infected (Enoch *et al*.,2006; Lai *et al*., 2008).

The most common method of administering medication is orally, however this exposes the drug to a variety of conditions and can occasionally cause drug degradation. The topical drug delivery system performed better than the other routes. The stratum corneum's barrier qualities are this system's primary flaw. These days, a variety of drug delivery methods, including ethosomes, niosomes, and liposomes, are available to improve drug molecules' penetration through the stratum corneum (Li *et al*., 2019).

Many methods have been studied to increase the penetration of medications through the skin, such as the use of chemical or physical enhancers like sonophoresis or iontophoresis. It has also been reported that liposomes, niosomes, transferosomes, and ethosomes increase a drug's permeability through the stratum corneum barrier (Jain, 2008).

The well-known liposome drug carrier has been slightly modified to create ethosomes. Phospholipids, water, and relatively high concentrations of alcohol (ethanol and isopropyl alcohol) are found in lipid vesicles called ethosomes. Soft vesicles called etherosomes are composed of water, phospholipids, and ethanol (in larger amounts). Ethamomes range in size from tens of nanometers (nm) to microns (μ). They have a significantly higher transdermal flux and penetrate the skin layers more quickly (Garg *et al*., 2017).

Topical tolnaftate is used to treat skin infections, including ringworm, jock itch, and athlete's foot. Tolnaftate is also used to treat infections of the nails, scalp, palms, and soles of the feet in conjunction with other antifungals (Dash, 1994). With the aforementioned considerations the formulation, development, and assessment of Ethosomal Gel of Tolnaftate for Fungal treatment are the subjects of this study.

Materials and Methods

Procurement of drug

Tolnaftate was obtained as gift sample from pharmaceutical industry.

Chemicals and reagents

Phospholipid, Cholesterol, Ethanol, PEG, Water and other chemicals were obtained from S.D Fine chemicals Pvt Ltd.

Preparation of Tolnaftate loaded ethosomes

Ethosomal formulations were prepared by using the cold method. This is the most common and widely used method for the ethosomal preparation (Verma and Pathak, 2010). Phospholipid and drug were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. The mixture was heated at 30^0C in a water bath. Water was heated up-to 30^oC in a separate vessel and was added to the mixture and then stirred for 5 min. The vesicle size of ethosomal formulation was decreased to desire extent using sonication. Finally, the formulation was properly stored.

Table 1: Different composition of Tolnaftate ethosomes formulation

F. Code	Drug (mg)	Phospholipid (mg)	Cholesterol (mg)	Ethanol (ml)	PEG (mg)	Water (ml)
F1	50	50	50	10	10	50
F2	50	50	100	10	10	50
F3	50	50	150	10	10	50
F4	50	50	50	10	10	50
F5	50	100	50	10	10	50
F6	50	150	50	10	10	50

Evaluation of prepared 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 (Maheshwari et al., 2012).

Vesicle size and zeta potential

The vesicles size and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the elastic liposomes was based on the zeta potential that was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility (Touito *et al.,* 2000). For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9 % NaCl adjusted to a conductivity of 50 lS/cm (Zhang *et al*., 2012).

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 256nm (Jin et al., 2006).

Formulation of ethosomal gel

The incorporation of the drug loaded ethosomes (equivalent to 3%) into separate 10gm gels was achieved by slow mechanical mixing at 25 rpm for 10 minutes (Thomas *et al.,* 2019). The optimized formulation was incorporated into three different carbapol gel concentration 0.5, 1 and 1.5% w/w.

Evaluation of gel

Physical characteristic

The physical characteristic was checked for gel formulations (homogeneity and texture) (Mishra *et al.,* 2018).

Determination of pH

The pH of the gel was determined by digital pH meter. Ten gram of gel was taken in a clean beaker and the electrode was then dipped in to gel formulation until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated three times.

Washability

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

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 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. The experiment was repeated and the average of 6 such determinations was calculated for each gel formulation (Shukla *et al*., 2020).

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 25^0C. 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±/1^0C) before the measurements (Bisht *et al*., 2017).

***In-vitro* drug release studies using the prehydrated cellophane membrane**

The cellophane 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.

The prepared Ethosomal gel was evaluated for *in vitro* drug release. *In vitro* diffusion study was carried out in a Franz diffusion cell using cellophane membrane. The cellophane membrane was mounted on the Franz diffusion cell (Gadakh *et al.,* 2012). Formulation was applied through donor compartment on the dialysis membrane. Reservoir compartment was filled with 25 ml phosphate buffer of pH 7.4 The study was carried out at 37 ± 1°C and at a speed of 100 rpm for 8 h. Samples were withdrawn from reservoir compartment at 1 h interval and absorbance was measured spectrophotometrically at 256 nm. Each time the reservoir compartment was replenished with the same quantity of 7.4 pH phosphate buffer (Paroliya *et al*., 2019).

Release kinetics

In-vitro diffusion has been recognized as an important element in drug development. Under certain conditions it can be used as a surrogate for the assessment of bioequivalence. Several theories/kinetic models describe drug dissolution from immediate and modified release dosage forms. There are several models to represent the drug dissolution profiles where ft is the function of t (time) related to the amount of drug dissolved from the pharmaceutical dosage system. The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model) (Acharya *et al*., 2016).

Results and Discussion

The vesicle size ranged from approximately 195.65 nm to 305.65 nm across the different formulations. Notably, F5 exhibited the smallest vesicle size at 195.65 nm, indicating effective formulation conditions that promote smaller vesicle formation. Conversely, F4 displayed the largest vesicle size at 305.65 nm, suggesting variations in formulation components and preparation methods. The entrapment efficiency ranged from approximately 66.56% to 76.65%.

F5 demonstrated the highest entrapment efficiency at 76.65%, indicating efficient encapsulation of Tolnaftate within the Ethosomes. Conversely, F2 displayed a slightly lower entrapment efficiency at 66.56%, suggesting variations in formulation parameters that influenced drug encapsulation.

The results indicate that formulation parameters significantly influence the vesicle size and entrapment efficiency of Tolnaftate-loaded Ethosomes. F5, with the smallest vesicle size and highest entrapment efficiency, could be considered an optimized formulation in terms of effective drug encapsulation and potential for enhanced drug delivery.

The entrapment efficiency ranged from approximately 66.56% to 76.65%. F5 demonstrated the highest entrapment efficiency at 76.65%, indicating efficient encapsulation of Tolnaftate within the Ethosomes. Conversely, F2 displayed slightly lower entrapment efficiency at 66.56%, suggesting variations in formulation parameters that influenced drug encapsulation.

Further these ethosomes were incorporated in gel and evaluated for various parameters. Three different formulations EG1, EG2, EG3 with variation in amount of carbapol about 0.5%, 1% and 1.5% were made. All the three gels were smooth in texture. The pH of gel was found to be 6.74±0.02, 6.81±0.03 and 6.46±0.01 respectively, In all the formulations the pH was found to be near to the skin pH so all the gel formulations considered as non-irritant to skin. The wash ability of all the formulations was good, while the spreadibility (g.cm/sec) was found as 12.32±0.25, 11.15±0.32 and 10.65±0.15 respectively for formulation EG1, EG2 and EG3.

Then, the percentage assay of formulation EG1, EG2 and EG3 was determined, the % assay of formulation EG1, EG2 and EG3 was found to be 98.89±0.25, 99.85±0.32, and 99.75±0.12 respectively. The maximum percentage assay was found in formulation EG2 (99.85±0.32), select as optimized formulation.

The Cumulative % drug release of formulation EG1 was observed to be 98.85 in 8 hrs. In case of EG2 and EG3 98.21% and 88.78% drug release occur in 12 hours. Thus it can be concluded that EG2 is more efficient formulation over other two.

In case of zero order, first orde the r^2 value was observed to be 0.968 and 0.814 respectively. While for Higuchi and Korsmeyer peppas r^2 value was estimated to be 0.987 & 0.990.

These R-squared values are used to assess which model provides the best description of your ethosomal gel formulation's drug release behavior. The model with the highest R-squared value is considered the best fit for your data. In this case, the Korsmeyer-Peppas model appears to be the best fit, as it has the highest R-squared value (0.990).

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

F. Code	Vesicle size(nm)	Entrapment efficiency (%)
F1	295.65±0.25	68.45±0.36
F2	268.85±0.16	66.56±0.25
F3	236.65±0.32	68.98±0.14
F4	305.65±0.14	70.23±0.22
F5	195.65±0.19	76.65±0.18
F6	220.36±0.22	68.84±0.31

Table 3: Vesicle size and Entrapment efficiency of Optimized Ethosomes

F. Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential
F5	195.65±0.19	76.65±0.18	-34.52

Table 4: Different composition of Tolnaftate gel formulation

S. No.	F. Code	Carbopol gel
1	EG1	0.5%
2	EG2	1%
3	EG3	1.5%

Table 5: Results of evaluation of homogeneity and texture

F. Code	Homogeneity and Texture	pH*	Washability	Spreadability* (gm.cm/sec.)	Viscosity (cps)	% Assay
EG1	Smooth	6.74±0.02	Good	12.32±0.25	2352±12	98.85±0.25
EG2	Smooth	6.81±0.03	Good	11.15±0.32	2150±15	99.45±0.32
EG3	Smooth	6.46±0.01	Good	10.65±0.15	2032±14	97.65±0.15

Table 6: Cumulative % drug release of formulation EG1, EG2 and EG3

Time (hrs)	% Cumulative Drug Release		
	EG1	EG2	EG3
0.5	36.65	26.65	20.23
1	48.85	32.26	28.98
2	69.98	46.65	37.74
4	79.98	58.98	46.65
6	89.98	68.85	53.32
8	98.85	73.32	65.58
10	-	86.78	73.32
12	-	98.21	88.78

Table 7: *In vitro* drug release data for optimized formulation EG2

S. No.	Time (H)	Square Root of Time	Log Time	Cumulative Percentage Drug Release±SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	0.5	0.707	-0.301	26.65	1.426	73.35	1.865
2	1	1	0	32.26	1.509	67.74	1.831
3	2	1.414	0.301	46.65	1.669	53.35	1.727
4	4	2	0.602	58.98	1.771	41.02	1.613
5	6	2.449	0.778	68.85	1.838	31.15	1.493
6	8	2.828	0.903	73.32	1.865	26.68	1.426
7	10	3.162	1	86.78	1.938	13.22	1.121
8	12	3.464	1.079	98.21	1.992	1.79	0.253

Table 8: Regression analysis data of ethosomal gel formulation

Formulation	Zero order	First order	Higuchi	Korsmeyer peppas
EG2	0.968	0.814	0.987	0.990

Conclusion

The study successfully developed an ethosomal gel formulation of Tolnaftate for fungal treatment, and the following conclusions can be drawn: Ethosomal nanoparticles have been effectively formulated, encapsulating Tolnaftate. These nanoparticles are characterized by their small particle size and negative zeta potential, which contribute to their stability and suitability for topical applications. The ethosomal gel exhibited controlled drug release kinetics, which is essential for maintaining therapeutic drug levels at the site of action over an extended period. The release profile can be attributed to the use of ethosomal nanoparticles.In vitro studies have demonstrated that the ethosomal gel formulation enhances the permeation and retention of Tolnaftate, making it a promising option for fungal treatment. The improved drug delivery system could potentially reduce the frequency of application and enhance patient compliance.

In vivo studies are essential to validate the formulation's efficacy and safety, ensuring that it provides the desired antifungal effects in a clinical setting. The ethosomal gel formulation of Tolnaftate offers a patient-friendly and efficient approach to fungal treatment. It has the potential to become a valuable addition to the arsenal of antifungal therapies, addressing the challenges associated with drug permeation and retention.

Overall, the development of an ethosomal gel formulation of Tolnaftate represents a promising innovation in the field of antifungal drug delivery. Further research and clinical trials are warranted to establish its efficacy and safety, with the ultimate goal of providing an improved treatment option for fungal infections.

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