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FORMULATION, DEVELOPMENT AND EVALUATION OF TRANSFERSOMES GEL OF TERBINAFINE HYDROCHLORIDE FOR EFFECTIVE TREATMENT OF TOPICAL FUNGAL DISEASE

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ABSTRACT

Occurrence of skin fungal infections is increasing nowadays and their presence is more prominent in patients suffering from immune compromised diseases like AIDS. Skin fungal infections are a major cause of visits by patients to dermatology clinics. Treatment approaches include both topical and oral antifungal agents. The topical route is generally preferred due to the possible side effects of oral medication. Advances in the field of formulation may soon render outdated conventional products such as creams, ointments and gels. Several carrier systems loaded with antifungal drugs have demonstrated promising results in the treatment of skin fungal infections. The aim of the present study was to investigate the potential of transfersomal gel formulations for transdermal delivery of terbinafine hydrochloride and to evaluate the effect of lipid concentration, ethanol concentration, drug concentration and stirrer time. Characterization of transfersomes performed by vesicle size, surface charge, entrapment efficiency and stability study. Characterization of transfersomes containing gel performed by the measurement of viscosity, pH measurements, drug content, extrudability study, spreadability and in vitro drug diffusion study. It was found that viscosity of prepared gel was 3652±15cps, % assay was 98.85±0.23, extrudability was 145±6gm and spreadability was found that 12.25±0.35 (g.cm/sec) respectively. The prepared gel containing terbinafine hydrochloride-loaded transfersomal formulation was optimized and can be use for topical preparation for its antifungal affect. The results were obtained which showed that transfersomal gel was a promising candidate for transdermal delivery with targeted and prolonged release of a drug. It also enhances skin permeation of many drugs.

Key words: Fungal infections, Transfersomal gel, Terbinafine hydrochloride, Franz diffusion cell.

INTRODUCTION:

Fungi are parasitic microorganisms which can affect the skin and mucous membrane along with generation of systemic infections of various internal organs (Kim., 2016). Fungal infections of skin or mucous membrane, in majority, promote visits of victims to dermatologists (Hainer, 2003). It has been reported that 20%-25% of human population show presence of skin fungal infections (Gupta et al., 2005). Incidences of occurrence of skin fungal infection are very high in immune compromised patients (Gretzula & Penneys; 2005). Skin fungal infections are categorized into superficial, cutaneous and subcutaneous depending upon the level of tissue invasion (Bseiso et al., 2015). When attack of invading fungi is limited to outermost skin layers only then generated infection is called superficial fungal infection. Tinea versicolor, white piedra and tinea nigra are examples of superficial fungal infections. Superficial fungal infection leads to increase in the skin pH along with mild scaling, redness and inflammation at the invading site. The barrier nature of skin becomes poor in such a state (Hawkins & Smidt., 2014). Invasion of parasitic fungus into deeper epidermal skin layer develop cutaneous fungal infection. This infection is also known as dermatomycoses and it may have involvement of skin appendages like nails and hairs (Gupta et al.,1998). Dermatomycoses can also instigate cellular immune response developing pathological variations in patients (Watanabe; 2008). Various fungi generating dermatomycoses come under three genera, namely *Epi-dermophyton*, *Trichophyton* and *Microsporum.* Tinea faciei, tinea barbae, tinea capitis and tinea manuum are the examples of cutaneous fungal infections (Nenof et al., 2014). Furthermore, extension of fungal infection to dermal or subcutaneous region results subcutaneous fungal infection. It is caused by fungi namely *Sporothrix schenckii* and *Candida albicans* (Elgart., 2014). This fungal infection is characterized by either ulcerated or infiltrated nodular lesions in the infected areas (Patel et al.,2011). Maduramycosis and chro momycosis are other examples of subcutaneous fungal infections (Arenas; 2012). Poor skin penetration of hydrophilic antifungal drugs and high dosing frequency of conventional antifungal formulations reduce their effectiveness against skin fungal pathogens (Akhtar et al., 2015). Therefore, several nanocarrier systems have been investigated by pharmaceutical scientists to fulfil these criteria and considerations for topical delivery of antifungal drugs (Kumar et al., 2014).Transfersomes are flexible or deformable vesicles and hence also called as elastic vesicles. Gregor Cevc in 1991 introduced the concept and term of elastic vesicles. Since then, extensive work is going on worldwide on these elastic vesicles under different titles like flexible vesicles, ethosomes, etc. Transfersome is derived from the Latin word transferre, meaning to carry across and the Greek word soma, meaning body. A transfersome carrier is an artificial vesicle that resembles the natural cell vesicle. Thus it is suitable for both targeted and controlled drug delivery. Functionally, it may be described as lipid droplet with such deformability that permits its easy penetration through the pores much smaller than the droplet size. Transfersome is a highly adaptable and stress-responsive complex aggregate. On topical application, the carrier search and exploits hydrophilic pathways i.e.

'pores' in the skin, which it opens wide enough to permit it to pass through with its drug cargo, deforming itself to accomplish this without losing its vesicular integrity. The vesicle is both self-regulating and self-optimizing due to its interdependency on local composition and shape of the bilayer. This allows the transfersome to cross different transport barriers efficiently. Transfersome penetrates the stratum corneum either via intracellular route or the transcellular route (Cevc; 2004; Sivannarayana et al., 2012). As a new broad-spectrum antifungal drug, terbinafine is one of the most widely used antifungal drugs clinically (Sivannarayana; 2008). It has obvious inhibitory and killing effect for onychomycosis caused by dermatophytes after orally administrated more than three months. Terbinafine is lipophilic and could appropriately permeate through corneous layer into nail plate. Taking advantage of transfersomes as carrier for terbinafine could significantly improve drug transmittance through skin. In this case, external preparation for the treatment of severe onychomycosis will become a reality. Simultaneously, it will provide evident basis for transfersomes as transdermal drug carrier. In the present investigation, we attempted to develop and optimize transferosomal gel containing terbinafine for improved transdermal permeation.

MATERIALS AND METHODS

Materials

Terbinafine hydrochloride was obtained as a gift sample from Macleods Pharmaceuticals, Mumbai. Soya PC, span 20 was purchased from Himedia Laboratory, Mumbai. Ethanol, chloroform and carbopol-934 purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Demineralized and double distilled water was prepared freshly and used whenever required. All other reagents and chemicals used were of analytical grade.

Methods

Preparation of Terbinafine hydrochloride loaded transfersomes

In a round bottom flask, required amounts of Soya PC and surfactant were dissolved in ethanol by shaking. The thin film was created via rotary evaporation for 15 minutes at 25°C, 600mm/hg pressure, and 100rpm in a rotary evaporator. After that, the solvent was evaporated using a nitrogen gas stream (Anish et al., 2019). To remove any residual solvent, the lipid film was put in a desiccator for at least 12 hours. Terbinafine hydrochloride was dissolved in 10ml, 7.4 pH which was heated to 55^0C. The film was then hand-shook for half an hour to hydrate it with the hot buffer. The mixture was then shaken in an orbital shaker for half an hour. Following that, the transferosomes were examined under a microscope. The transferosomal suspension was kept at 40°C in the refrigerator.

Optimization of transfersomes formulation

Transfersomes formulation optimized based on the evaluation of mentioned strategy procedure

resting on the source of average vesicle size and (%) entrapment efficiency (EE). In the transfersomal formulation, the ratio of lipid and surfactant in the transferosomal formulation was adjusted by using several ratios such as 9:1, 8:2, 7:1, and 6:2 percent ratios, while all other parameters remained fixed. the ethanol content was optimized by taking their different quantity such as 5, 10, 15 and 20 and all other parameters were kept remain constant. Drug concentration optimized by taking different concentration of drug such as 1, 1.5, and 2.0% w/v and prepared their formulation and all other parameters such as Soya PC, stirrer time kept remain constant. Stirrer time was optimized by stirring the formulation for different time, i.e., 5, 10, and 15 min.

Table 1 Optimization of ratio of lipid concentration

Formulation code	Soya PC: Span 20 (% w/v)	Ethanol	Drug (% w/v)
F1	9:1	10	1
F2	**8:2**	**10**	**1**
F3	7:1	10	1
F4	6:2	10	1

Table 2 Optimization of ethanol concentration

Formulation code	Soya PC: Span 20 (% w/v)	Ethanol	Drug (% w/v)
F5	8:2	5	1.0
F6	**8:2**	**10**	**1.0**
F7	8:2	15	1.0
F8	8:2	20	1.0

Table 3 Optimization of drug concentration

Formulation code	Soya PC: Span 20 (% w/v)	Drug (% w/v)	Ethanol (ml)
F9	**8:2**	**1.0**	**10**
F10	8:2	1.5	10
F11	8:2	2.0	10

Table 4 Optimization of Stirrer duration

Formulation code	Soya PC: Span 20 (% w/v)	Drug (% w/v)	Stirrer duration (min)
F12	**8:2**	**1.0**	**5**
F13	8:2	1.0	10
F14	8:2	1.0	15

Characterization of Terbinafine hydrochloride loaded transfersomes

Microscopic observation of prepared transferosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared transferosomes formulation.

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK).

Zeta potential

The zeta potential was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. 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.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded transfersomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 min. The transfersomes along with encapsulated drug were separated at the bottom of the tubes. Plain transfersomes without drug 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 282 nm.

$$\% \, Entrapment \, Efficiency = \frac{Therotical \, drug \, content - Practical \, drug \, content}{Therotical \, drug \, content} \times 100$$

***In vitro* drug diffusion study**

The dialysis diffusion approach was used to perform in vitro drug release of prepared transfersomes utilizing the dissolution test apparatus. The dissolving media was phosphate buffer pH 7.4. The dialysis technique was carried out utilizing a cellulose acetate dialysis membrane with a molecular weight cutoff of 12,000–14,000 moles. This membrane ensures drug penetration while retaining transfersomal vesicles. Before usage, the membrane was soaked in fake tears for 12 hours. A glass cylinder with a length of 8 cm and a diameter of 1 cm was filled with four ml of transfersomal dispersion, and a dialysis membrane was threaded to the mouth of the cylinder. Each glass cylinder was attached to the shaft of the dissolution apparatus (USP Dissolution tester, Labindia DS 8000) and descended down into a 100 ml beaker containing 50 ml of as dissolution medium without touching the bottom surface of the beaker. The beaker was then placed into

vessels of dissolution apparatus that contained about 100 ml of water to keep temperature at 34 ± 0.5°C. The glass cylinders were adjusted to rotate at a constant speed of 20 rpm. One ml of dissolution medium was withdrawn at predetermined time intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 h). To maintain a consistent volume, the samples were changed with new dissolving media. The concentrations of drugs in samples were measured spectrophotometrically at 282nm, the wavelength of the drug. The release tests were done in triplicates, with the mean and standard deviation reported. At the end of each sampling period, samples are removed and replaced with equal quantities of fresh receptor fluid.

Preparation of Gel base

Carbopol 934 (1%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was agitated continuously at 800 rpm for 1 hour before 10 ml of propylene glycol was added. To eliminate air bubbles, the gel was diluted to 100 ml and sonicated for 10 minutes on a bath sonicator. Final pH of the gel base was adjusted to 6.8. Transferosomal preparation corresponding to 0.1 %w/w of terbinafine hydrochloride was incorporated into the gel base to get the desired concentration of drug in gel base.

Characterization of Transfersomes containing Gel

Measurement of Viscosity

Viscosity measurements of prepared topical transfersomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm; viscosity.

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted.

Drug content

Accurately weighed equivalent to 100 mg of topical transfersomal gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 ml of filtered solution was taken in 10 ml capacity of volumetric flask and volume was made upto 10ml with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 282nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on

application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadability

Spreadability of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadability, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 6cm upon adding 20g of weight was noted. Good spreadability show lesser time to spread (Nimker et al., 2017).

$$Spreadibility\,(g.cm/sec) = \frac{Weight\ tide\ to\ Upper\ Slide \times Lenth\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

RESULTS AND DISCUSSIONS

The absorption maxima of terbinafine hydrochloride were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Labindia UV 3000+) using concentration range of 5-25µg/ml terbinafine hydrochloride in 7.4phosphate buffers. Terbinafine hydrochloride showed a linear relationship with correlation coefficient of 0.999 in the concentration range of 5-25µg/ml in phosphate buffer pH 7.4. All the data of preformulation study were found similar as given in standard monograph which confirmed that the drug was authenticated and pure in form and it could be used for formulation development of terbinafine hydrochloride loaded transfersomes. Optimization of the transfersomes to generate the formulation code was done using the strategy as reflected in Table 1 optimization of lipid concentration, Table 2 optimization of ethanol concentration, Table 3 optimization of drug concentration and Table 4 optimization of stirrer time. It was observed that the vesicles dimension of transfersomes was increased with raising the concentration of phosphatidylcholine and ethanol. There was no noteworthy difference observed in average vesicle size with increasing the drug concentration, but with increase in the stirrer time the size of vesicle decreased from 135.65to 112.20after 15 min of stirring. Considering the EE, it was observed that the percent drug entrapment increased with escalating the concentration of ethanol and on escalating the time of stirring. The resulted formulation code F-12 was considered as the optimized formulation. The average vesicle size of optimized formulation (F-12)

observed as 135.65nm, zeta potential observed as -35.45mV and %EE was found as 76.65% Table 5, 6. Prepared gel of transfersomes loaded with terbinafine hydrochloride (TG-12) was prepared and evaluated for viscosity, pH, % drug content, extrudability, spreadability and drug release study (Table 7). It was found that viscosity of prepared gel TG-12 was 3652±15cps, % assay was 98.85±0.23%, extrudability was 145±6g and spreadability (g.cm/sec) was found that 12.25±0.35 (g.cm/sec) respectively.

Table 5: Results of characterization of optimized formulation of transfersomes

Formulation Code	Average vesicle size (nm)	% entrapment efficiency
F1	345.65	65.54
F2	268.98	72.45
F3	265.63	63.32
F4	324.32	67.78
F5	265.45	65.58
F6	256.65	75.65
F7	274.45	64.45
F8	255.65	63.32
F9	182.23	76.65
F10	226.65	65.45
F11	278.65	69.98
F12	135.65	76.65
F13	125.45	68.85
F14	112.20	61.12

Table 6: Characterization of Optimized formulation of transfersomes

Characterization	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F-12	135.65	76.65	-35.45

Table 7: Characterization of gel based formulation containing Terbinafine hydrochloride loaded Transfersomes

Formulation	Viscosity* (cps)	Assay* (%)	Extrudability* (g)	Spreadability* (g.cm/sec)
TG-12	3652±15	98.85±0.23	145±6	12.25±0.35

*Average of three determination

CONCLUSION

Transfersomes were prepared and optimized on the base of average vesicle size and % drug entrapment. The optimized formulation was further incorporated with gel base (Carbopol gel) and characterized for their viscosity, pH, % drug content, extrudability, spreadability and drug release study. Optimized formulation (F-12) of transfersomes resulted in average vesicle size as 135.65 nm, zeta potential as -35.45mV and % EE as 76.65%. Prepared gel of optimized formulation viscosity was 3652±15cps, % assay was 98.85±0.23%, extrudability was 145±6g and spreadability (g.cm/sec) was found that 12.25±0.35 (g.cm/sec) respectively. It can be concluded that prepared gel containing terbinafine hydrochloride-loaded transfersomal formulation was optimized and can be of use for topical preparation for its antifungal effect.

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