The Pharma Innovation Journal 2018; 7(12): 355-362

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(12): 355-362

www.thepharmajournal.com
Received: 05-10-2018
Accepted: 07-11-2018

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A review on Ethosome: A novel drug delivery system for topical fungal disease

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Abstract
Topical drug delivery system has been used broadly for various types of topical diseases. Topical therapy is excellent choice for the treatment of the cutaneous infections due to its advantages such as targeting of drugs to the site of infection and reduction of the risk of complete side effects. Fungal infections of the skin are one of the frequently faced dermatological diseases in worldwide. The previous antifungal drugs that have been used to treat these diseases such as creams, gels and ointment are not capable in treatment of these diseases in terms of bioavailability and therapeutic effect. The effectiveness of that treatment depends on the penetration of drugs through the target layers of the skin at the efficient concentrations, so to overcome this problem Ethosomes have been arrived showing best results in the treatment of topical fungal diseases. However, *stratum corneum*, the outermost layer of the skin, is an effective barrier for penetration of drugs into deeper layers of the skin. Ethosomes are lipid vesicles which are soft and flexible. They consist of phospholipid, alcohol, poly glycol and water. An alcohol acts as permeation enhancer and increase permeability of skin. The most important feature of Ethosome is to infuse intact through the skin due to its high deformability and also helps to transport the drug molecule more efficiently through the skin in terms of both quantity and depth. Ethosomes can provide effective intracellular delivery of hydrophilic, lipophilic and amphiphilic drug molecules. The size range of Ethosomes varies from tens of nanometer to few microns. Ethosomes have become an area of research interest, because of its special characters such as enhanced skin permeation, improved drug delivery and increased drug entrapment efficiency. This review on Ethosomes drug delivery is to spotlight on the various aspects including their advantages, preparation, characterization, mechanism of penetration, composition and application of Ethosomes.

Keywords: Topical drug delivery, Ethosomes, Antifungal, Phospholipids, Intracellular delivery

Introduction
The occurrence of topical fungal infections of skin, nails and hair has been increased in worldwide. It has been estimated that about 40 million people have suffered from fungal infections in developing and under developed country. The development of fungal infections can be rapid and stern due to compromising with immune function [1, 2]. Dermatophyte infections of the feet signify the most common fungal infections due to the use of occlusive foot wear. The incidences of superficial and deep skin fungal infections are increasing [3, 4]. Diseased skin is distinguished and characterized by increased permeability or reduced barrier function or altered lipid composition and organization of the stratum corneum [5]. The human skin is susceptible to fungal growth under warm and humid conditions. Due to their keratinophilic and keratinolytic nature they are able to use cutaneous keratin as a nutrient and thus producing the infection [6]. The susceptibility and liability to fungal infection has increased significantly in terms of frequency and also as a cause of morbidity and mortality [7-9]. *Dermatophytes* are one of the most frequent causes of o*nchomycosis and tinea*. Candidal infections are also among the most widespread superficial cutaneous fungal infections [10]. Even, *candida* can invade deeper tissues as well as the blood which leads to lif threating systemic candidiasis, when the immune system is weakened [11]. Topical therapy is excellent choice for the treatment for cutaneous infections due to its various advantages such as targeting of drugs to the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment, high patient compliance and also because it is a non-invasive procedure for drug delivery. The effectiveness of the topical antifungal treatment depends on the penetration of drugs through the target tissue. Therefore, the effective drug concentration levels should be achieved in the skin.

In topical administration of antifungals, the drug substances should pass the *stratum corneum*, which is the outermost layer of the skin, to reach lower layers of the skin, particularly into *viable epidermis*. In this situation, the formulation may play a major role for penetration of drugs into skin [12]. Different type of topical effective antifungal compounds has been used in the treatment of a variety of dermatological skin infections. The main classes of topical antifungals are polyenes, azoles and ally lamine/benzyl amines. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels, lotions and sprays alone are not competent in terms of both bioavailability and therapeutic effect for the treatment of the topical fungal disease because of stratum corneum barrier [13, 14]. Development of other approaches for topical treatment of fungal infections of skin encompasses new carrier systems for approved and investigational compounds. Delivery of antifungal compounds into skin can be enhanced with the carrier including Ethosomes.

Ethosomes are ethanolic liposomes. Ethosomes (Fig 1) can be defined as non-invasive delivery carriers that enable drugs to reach deep into the skin layers or the systemic circulation. They are soft and flexible nano vesicles. They posses exclusive structure which makes them capable of overcoming the natural skin barrier and delivering drugs through the skin layers. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water [15]. Size of vesicles varies from 10 of nano to micrometers [16]. The synergistic effects of combination of phospholipids and high concentration of ethanol in formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers [17]. Being non-invasive it is responsible for disturbing the organization of skin lipid bilayer [18]. Release of drug could be result of fusion of Ethosome system with skin lipids and drug release at various points along the penetration pathway [19]. Delivery of drugs through Ethosomes can be modulated not only for enhanced skin permeation but localizes the drug at the site of action which enables drugs to reach the deep skin layers. They can efficiently entrap various kinds of molecules such as hydrophilic, lipophilic or amphiphilic [20, 21]. In Ethosomes high drug loading is possible because of reasons like solubility of many drugs in ethanol and high degree of lamellarity in vesicles [19]. High concentration of ethanol makes them flexible as well increases the penetrating power because it increases the thermodynamic activity of due to evaporation of ethanol and enhances penetration due to reduction in barrier property of stratum corneum [22]. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug secured from immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was the finding of a vesicle derivative, known as an Ethosomes [23]. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux. Despite these potentials it is much partial for its stability but the article summarizes marketed formulations that are being successfully used in various countries and opportunities to deal with them.

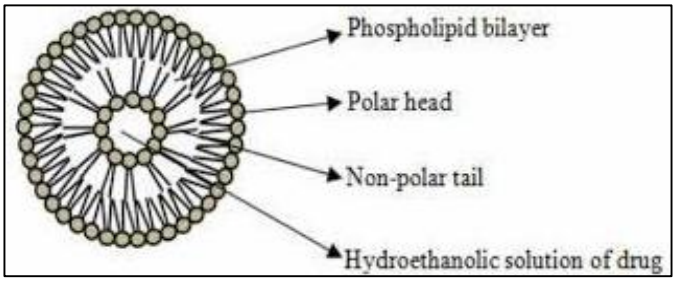

Fig 1: Structure of Ethosomes [46]

Structure of skin

In order to appreciate the mechanism of Ethosome-based drug delivery system, we must first understand the structure of skin and the layers that make it up. The main function of skin is to give the protection from water loss. Physical protection is provided by outer most layer of skin and when undamaged, obstructs most bacteria, viruses, and other foreign substances from entering the body. The skin (Fig 2) consists of mainly three layers epidermis, dermis and subcutaneous tissue. Stratum corneum is outermost layer of epidermis and is seen on the surface of the skin. The main cells of the epidermis are the keratinocytes, which synthesize the protein keratin. The keratinocytes develop at the bottom and rise to the top, where they are shack from the surface as dead cells. It consists of 10-25 layers of dead, elongated, fully keratinized corneocytes, which embedded in matrix of lipid bilayers. Stratum corneum is main barrier to penetration through the skin. When topical formulation is placed on skin active drug is required to penetrate through the stratum corneum into viable tissue. The keratinocytes in the stratum corneum are dead squamous cells that are no longer multiplying [24]. The dermis consists is mostly made of dense irregular connective tissue and is much thicker than the epidermis. The dermis is liable for the tensile strength of skin. Its main roles are to regulate temperature and to supply the epidermis with nutrient-saturated blood. Much of the body's water supply is storage within the dermis. The subcutaneous layer lies below the dermis. The subcutaneous layer is mainly composed of fat and connective tissue. It performs as a protective bolster and helps to insulate the body by monitoring heat gain and heat loss. Not all authors regard this layer a part of the skin, but it definitely has a strong impact on the way the skin looks [24, 25]. Many chemical enhancers have been investigated e.g. liposome, niosome, transferosomes and Ethosome. Liposome is unstable and poor skin permeation. Noisome also has poor permeability. To overcome problem of permeability recently introduced new vesicular carrier system i.e. Ethosomes.

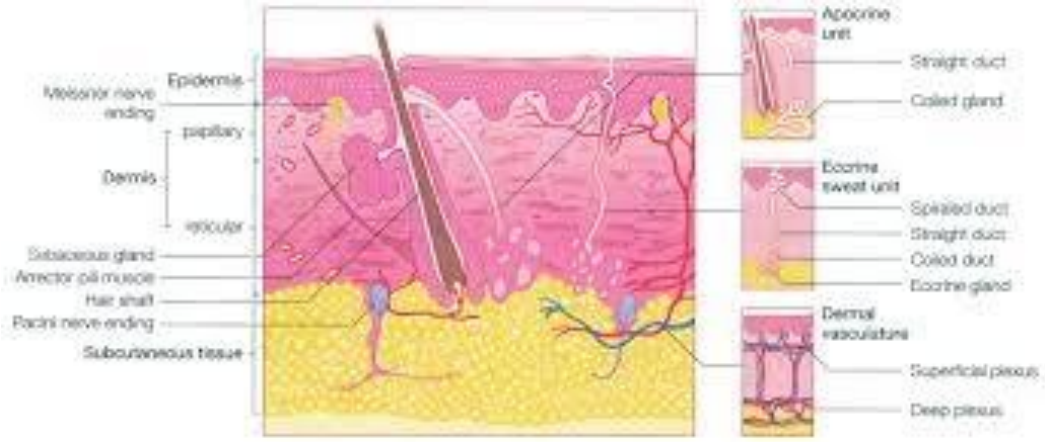

Fig 2: Structure of skin [46]

Potential advantage of Ethosomal drug delivery system

In comparison to other transdermal & dermal delivery systems, Ethosomal drug delivery systems contain several advantages. Few advantages are;

1. Delivery of large molecules (peptides, protein molecules) is possible.
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5. High patient compliance: The Ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
6. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization
8. Drugs entrapped in Ethosome having different physicochemical characterstics and olecular size are showing high degree of permeation compare to other nano-carriers.
9. Ethosomes show highest transdermal flux enhances the permeation of drug through deeper layers of skin.
10. Due to intense research toxicological profiles of the Ethosome components are well-evaluated and documented in the scientific literature thus the Ethosome technology has no large-scale drug development risk
11. Ethosomes improve skin delivery under occlusive and non-occlusive conditions.
12. Ethosomal system act as delivery system for a fluorescent probe (quantum dots) to the skin, in terms of quantity and depth.
13. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes [26].

Disadvantages of Ethosomal drug delivery

1. Drugs that require high blood levels cannot be administered –limited to only potent drugs (daily dose - 10mg or less)
2. Poor practical yield.
3. Ethosomes with poor shells may clump together and leads to precipitation.
4. Transfer of Ethosomes from organic to aqueous layer leads to loss of product
5. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.
6. Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
7. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
8. Adhesive may not adhere well to all types of skin.
9. May not be economical.
10. Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.
11. In case if shell locking is ineffective then the Ethosomes may coalescence and fall apart on transfer into water [27-32].

Composition of Ethosomes

Ethosomes exhibit lipid bilayer like liposomes; however they differ from liposomes in terms of composition (high content of ethanol). The Ethosomes are composed of hydroalcoholic or hydro/glycolic phospholipid in which the concentration of alcohol is relatively high. Ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine, phosphatidic acid, phosphatidylserine, Phosphatidyl ethanolamine, phosphatidyl glycerol, phosphatidyl inositol, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Some preferred phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1-1% can also be added to the preparation. Alcohol, such ethanol and isopropyl alcohol; among glycols, propylene glycol and Transcutol are generally used which may range from 20 to 50% in the final product. In addition to non-ionic surfactants (PEG-alkyl ethers) and cationic lipids (cocoamide, POE alkyl amines, dodecylamine, cetrimide etc) can be combined with the phospholipids in the preparations [33]. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%. Various additives which are used for formulation of Ethosomes are listed in the Table 1.

Table 1: Different additive employed in formulation of Ethosomes [34-38]

Class	Example	Uses
Phospholipids	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	It influences on the size, entrapment efficacy, zeta potential and penetration properties of the vesicles.
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Polyglycol	Propylene glycol, Transcutol RTM	As a skin penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Edge activators	N-DMSO, Tween [22], Span	Enhances skin permeability
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate(FITC) 6 – Carboxy fluorescence	For characterization study
others	Dicetyl phosphate	Prevent aggregation of vesicles
Vehicle	Carbopol D-934, HPMC	As a gel former

Formulation aspects of Ethosomes

Effects to be considered for Ethosomal formulation are composition of lipid, Optimized ratio of additive mixture, Charge of the vesicle, Entrapment efficiency of drug and type of skin barrier. The crucial factors giving an optimized formulation are entrapment efficiency, vesicle size and transdermal flux and less influencing factors are phospholipid, ethanol and sonication time. Entrapment efficiency is fraction of total drug entrapped in Ethosomal system. It is deciding parameter for determining Polydispersibility index. Vesicle size precisely depends upon concentration of ethanol as it increases with decrease in ethanol concentration and increases with Phospholipid concentration. Concentration ratio between phospholipid and ethanol and water is needed to be optimized.

Methods of preparation

Ethosomes can be prepared and formulated by four methods. All methods are sound simple and convenient because no need of complex processes or sophisticated instruments.

1. Classic method

The phospholipid and drug are dissolved in ethanol and heated to 30°C±1°C in a water bath. Double distilled water is added in a fine stream to the lipid mixture, with constant stirring at 700rpm, in a closed vessel. The resulting vesicle suspension is homogenized by passing through a polycarbonate membrane using a hand extruder for three cycles [39].

2. Mechanical dispersion method

Soya phosphatidylcholine is dissolved in a mixture of chloroform: methanol in round bottom flask (RBF). The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form a thin lipid film on wall of the RBF. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vacuum overnight. Hydration is done with different concentration of hydroethanolic mixture containing drug by rotating the RBF at suitable temperature [39].

3. Cold method

This is the most common method utilized for the preparation of Ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 300C in a water bath. The water heated to 300C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of Ethosomal formulation can be decreased to desire extend using sonication [40] or extrusion [41] method. Finally, the formulation is stored under refrigeration [42].

4. Hot method

In this method phospholipid is dispersed in water by heating in a water bath at 400C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400C. Once both mixtures reach 400C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of Ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method [43].

Mechanism of drug penetration

The mechanism of penetration of the Ethosomes involves two simultaneous mechanisms of ethanol effect and Ethosome effect on the stratum corneum lipid bilayer Because of the use of ethanol in the preparation of the Ethosomes, the deformability of the vesicles is increased. The high alcohol content is expected to partially extract the stratum corneum lipids. These processes are responsible for increasing inter and intracellular permeability of Ethosomes. The ultradeformable vesicles can move in the path of the disordered stratum corneum and finally release drug in the deeper layers of the skin [44].

1. Ethanol effect
2. Ethosomes effect

1. Ethanol effect

Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosomes effect

Increased cell membrane lipid fluidity caused by the ethanol of Ethosomes results increased skin permeability. So the Ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin [45].

Characterization of Ethosomes [46]

1. Vesicle shape

Visualization of Ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Visualization by electron microscopy reveals a Ethosomal formulation exhibited vesicular structure 300-400 nm in diameter. The vesicles seem to be alleable as

evident by their imperfect round shape.

2. Vesicle size and Zeta potential
Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).

3. Entrapment efficiency
The entrapment efficiency of drug in Ethosomes can be measured by the ultracentrifugation technique. The chemical nature of the lipid is an important factor in determining the EE of drug in the Ethosomes because lipid which forms bilayer structure hold the drug perfectly. On the other hand, the imperfection of the lipid structure could offer space to accommodate the drug. The vesicles are separated in a high speed cooling centrifuge at 20,000 rpm for 90 minutes in the temperature maintained at 4°C. Separate the sediment and supernatant liquids determine the amount of drug in the sediment by lysing the vesicles using methanol.

% Entrapment = Actual content/Theoretical content x 100
4. Surface morphology study
Different types of lipids influence the surface morphology or shape of the particles. lipid microparticle suspensions were deposited on metallic stubs then placed in liquid nitrogen and dried under vacuum. The freeze-dried microparticles were coated uniformly with gold. It is characterized for morphology and surface properties using a scanning electron microscope.

5. Transition temperature
The Transition temperature (T) of vesicular lipids is measured in duplicate by DSC in an aluminum pan at a heating rate of 10°C per min within a temperature range from 20º-300ºC., under a constant nitrogen stream

6. Drug content
Drug content of the Ethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.

7. Surface tension measurement
The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

8. Stability studies
The ability of Ethosomal preparations to retain the drug (i.e., drug-retentive behavior) can be checked by keeping the preparations at different temperatures, i.e., 25 ± 2°C (room temperature, RT), 37 ± 2°C and 45 ± 2°C for different periods of time (1, 20, 40, 60, 80 and 120 days). The Ethosomal preparations were kept in sealed vials (10 ml capacity) after flushing with nitrogen. The stability of Ethosomes was also determined quantitatively by monitoring size and morphology of the vesicles using DLS and TEM.

9. Skin permeation studies
Confocal laser scanning microscopy (CLSM) method is used to determine the depth of penetration from Ethosomes. The Ethosomes shows significantly higher skin deposition possibly due to combined effect of ethanol and phospholipid thus providing a mode for dermal and transdermal delivery.

10. Degree of deformability and turbidity
The degree of deformability of the Ethosomal preparation can be performed by extrusion method
and the turbidity of the preparation can be performed by using nephelometer.

11. Phospholipid-ethanol interaction
The Phospholipid-ethanol interaction was studied by using Proton decoupled 31P-NMR and Differential Scanning calorimetry.

12. In vitro drug release study and Drug Deposition study
In vitro drug release study and drug deposition of Ethosomal preparation can be performed by Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion.

Evaluation of Ethosome [47]
1. Filter membrane-vesicle interaction study by scanning electron microscopy
Vesicle suspension (0.2 mL) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS (phosphate buffer saline solution), (pH 6.5). The filters were removed after 1 hour and prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% vol/vol in water). Finally, filters were coated with gold and examined in SEM.

2. Skin permeation studies
The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue with a scalpel. The excised skin was placed on aluminums foil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm2 and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C. The receptor compartment contained phosphate buffer saline solution (10 mL of pH 6.5). Excised skin was mounted between the donor and the receptor compartment. Ethosomal formulation (1.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1, 2, 4, 8, 12, 16, 20 & 24 hour
time intervals and analyzed by high performance liquid chromatography assay.

3. Vesicle-skin interaction study by TEM and SEM
From animals ultra-thin sections were cut (Ultracut, Vienna, Austria), collected on form var coated grids and examined under transmission electron microscope. For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope.

4. Vesicle-skin interaction study by fluorescence microscopy
Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5-µm thick sections were cut using

microtome (Erma optical works, Tokyo, Japan) and examined under a fluorescence micro Cytotoxicity Assay MT-2 cells (Tlymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India) containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L Lglutamine at 37°C under a 5% CO2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540nm.

5. Drug uptake studies

The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 µL RPMI medium was added. Cells were incubated with 100 µL of the drug solution in phosphate buffer saline solution (pH 7.4), Ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay**.**

6. HPLC Assay

The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay.

Statistical analysis

Statistical significance of all the data generated was tested by employing ANOVA followed by student zed range test. A confidence limit of $P <$. 05 was fixed for interpretation of the results using the software PRISM (Graph Pad, Version 2.01, San Diego, CA).

Researches done on Ethosome carrying antifungal drug

Various researches have been done on Ethosome carrying antifungal drug Table-2.

Table 2: Researches done on Ethosome carrying antifungal drugs [48-56]

S. No	Name of the drugs used for research	Name of researchers
1	Fluconazole	Bhalaria M.K *et al* 2009
2	Ketoconazole	Sheer A *et al* 2011
3	Amphotericin B	Devi M *et al* 2011
4	Clotrimazole	SamnaniA *et al* 2012
5	Voriconazole	Song C.K *et al* 2012
6	Econazole nitrate	Pathak K *et al* 2012
7	Nystatin	Devanna N *et al* 2013
8	Terbinafine Hcl	Ozer O *et al* 2013

Therapeutic applications

Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layers of the skin and hence appear to be vesicles of choice for transdermal drug delivery of hydrophilic and impermeable drugs through the skin. Various therapeutic applications of Ethosomes are as shown in table 3.

Table 3: Application of Ethosomes as a drug carrier

Drug	Results
NSAIDS (Diclofenac)	Selective delivery of drug to desired side for prolong period of time
Acyclovir	Increase skin permeation Improved in biological activity two to three times Improved in Pharmacodynamic profile
Insulin	Significant decrease in blood glucose level Provide control release
Trihexyphenidyl hydrochloride	Improved transdermal flux Provide controlled release Improved patient compliance Biologically active at dose several times lower than the currently used formulation
DNA	Better expression of genes Selective targeting to dermal cells
Antibiotic Cannabidol Erythromycin	Improved skin deposition Improved biological activity Prolonging drug action
Bacitracin	Improved dermal deposition Improved intracellular delivery Increased bioavailability
Anti-HIV agents Zidovudine Lamivudine	Improved transdermal flux Improved in biological activity two to three times Prolonging drug action Reduced drug toxicity Affected the normal histology of skin
Azelaic acid	Prolong drug release
Ammonium glycyrrhizinate	Improved dermal deposition exhibiting sustained release Improved biological anti-inflammatory activity

Conclusion

Topical treatment of the cutaneous infections has been preferred due to its superiorities over oral treatment such as avoidance of systemic adverse effects, targeting of the drug on the site of infection and high patient compliance. On the other hand, adequate drug concentrations in target layers of the skin should be provided to ensure the efficacy of topical treatment. Thus, delivery of antifungals to target region of the skin is a great challenge in terms of therapeutic aspect. So Ethosome is a promising drug delivery system against various topical fungal diseases in terms of both bioavailability and Pharmacotherapeutic effect. The results of all researches done on Ethosome carrying antifungal drugs proves to have better efficiency, minimum therapy time and reduced drug dose. So Ethosome can become a versatile and compatible tool for various antifungal drugs and good candidate for transdermal drug delivery of antifungal drugs.

References

1. Ameen M. Epidemiology of Superficial Fungal Infections, Clinical Dermatology. 2010; 28(2):197-201.
2. Havlickova B, Friedrich M. Epidemiological Trends in Skin Mycoses Worldwide, Mycoses. 2008; 51(4):2-15.
3. Donnelly R, McCarron P, Tunney M. Antifungal photodynamic therapy. Micro biol. Res. 2008; 163:1-12.
4. Segal E, Elad D. Fungal vaccines and immunotherapy. J Med Mycol. 2006; 16:134-151.
5. Bouwstra JA, Honeywell-Nguyen PL, Gooris GS, Ponec M. Structure of the skin barrier and its modulation by vesicular formulations. Prog Lipid Res. 2003; 42:1-36.
6. Garcia ME, Blanco JL. Principales enfermedades fu'ngicas que afectan a los animal's dome' sticos. Rev. Iberoam. Micol. 2000; 70:S2-S7.
7. Elias PM. Epidermal lipids, barrier function and desquamation. J Invest. Dermatol. 1983; 8:44s-49s.
8. Shao P, Huanga L, Hsueh P. Recent advances and challenges in treatment of invasive fungal infections. Int. J Antimicrob Agent. 2007; 30:487-495.
9. Ramsdale M. Programmed cell death in pathogenic fungi. Biochim Biophys Acta. 2008; 1783:1369-1380.
10. Zhang AY, Camp WL, Elewski BE. Advances in Topical and Systemic Antifungals, Clinical Dermatol- ogy. 2007;

25(2):165-183.
11. Vermaand P, Pathak K. Nanosized Ethanolic Vesi- cles Loaded with Econazole Nitrate for the Treatment of Deep Fungal Infections through Topical Gel Formula- tion, Nanomedicine. 2012; 8(4):489-496.
12. Lee CM, Maibach HI. Deep Percutaneous Pene- tration into Muscles and Joints, Journal of Pharmaceu- tical Science. 2006; 95(7):1405-1412.
13. Rakesh R, Anoop KR. Ethosome for transdermal and topical drug delivery. Int J Pharm Sci. 2012; 4(3):17-24.
14. Sivakranth M, Ara PA, Krishnaveni C, Venkatesh E. Ethosomes a novel vesicular drug delivery system. Int J Adv Pharm. 2012; 2(1):16-27.
15. Touitou E. Composition of applying active substance to or through the skin.US Patent US patent 5 540 934.1996.
16. Patel N.Ethosomes: A promising tool for transdermal delivery of drug. Pharma Info. Net, 2007, 5(3)
17. Gannu P Kumarn, Pogaku R Nonionic. Surfactant vesicular systems for effective drug delivery-an overview. Acta Pharmaceutica Sinica B. Elsevier Publication. 2011; 1(4):208–219.
18. Madhulika P, Deependra S, Manju S. Novel colloidal carriers for psoriasis: Current issues, mechanistic insight and novel delivery approaches. Journal of Controlled Release. Elsevier Publication. 2013; (170):380-395.
19. Rahul G. Maheshwari S, Rakesh T, Piyush S, Gajanan D, Abhishek T, Rakesh P, *et al*. Ethosomes and ultradeformable liposomes for transdermal delivery of clotrimazole: A comparative assessment. Saudi Pharmaceutical Journal. Elsevier Publication. 2012; (20):161-170.
20. Verma D, Fahr A. Synergistic penetration effect of ethanol and phospholipids on the topical delivery of Cyclosporin A. Journal of Control Release, Elsevier Publication. 2004; (97):55-66.
21. Bhalaria M, Naik S, Misra N. Ethosomes A novel delivery system for antifungal drugs in the treatment of topical fungal diseases. Indian Journal of Experimental Biology. 2009; (47):368-375.
22. Vaibhav D, Dinesh M, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. European Journal of Pharmaceutics and Bio pharmaceutics. Elsevier Publication. 2007; (67):398-405.
23. .Manosroi A, Jantrawut P, Khositsuntiwong N, Manosroi W, Manosroi J. Novel Elastic Nano vesicles for Cosmeceutical and Pharmaceutical Applications, Chiang Mai Journal of Science. 2009; 36(2):168-178.
24. Cosco D, Celia C, Cilurzo F, Trapasso E, Paolino D. Colloidal carriers for the enhanced delivery through the skin. Expert Opin. Drug Deliv. 2008; 5(7):737.
25. Ro BI, Dawson TL. The role of sebaceous gland activity and scalp micro oral metabolism in the etiology of seborrheic dermatitis and dandru. J Invest. Dermatol. 2005; 10(3):194.
26. Gangwar S, Singh S, Garg G. Ethosomes A Novel Tool for Drug Delivery through the Skin, Journal of Pharmacy Research. 2010; 3(4):688-691.
27. Jain H, Patel J, Joshi K, Patel P, Upadhyay UM. Ethosomes A Novel Drug Carrier, International Journal of Clinical Practice. 2011; 7:1:1-4.
28. Upadhyay N, Mandal S, Bhatia L, Shailesh S, Chauhan P. A Review on Ethosomes: An Emerging Approach for Drug Delivery through the Skin, Recent Research in Science and Technology. 2011; 3(7):19-24.
29. Sivakranth M, AnjumaAra P, Krishnaveni C, Venkatesh E. Ethosomes A Novel Vesicular Drug Delivery System, International Journal of Advances in Pharmaceutical Research. 2012; 2(1):16-27.
30. Kumar R, Aslam MD, Tripathi A, Prasad D, Chaudhary V, Jain V, *et al*. Ethosomes: Novel Vesicular Carriers in Transdermal Drug Delivery, Journal of Global Pharma Technology. 2010; 2(6):1-7.
31. Rathore AR, Khambete H, Jain S. Preparation and Characterization of Repaglinide Loaded Ethosomal Gel for the Treatment of NIDDM, International Journal of Pharmaceutical and Biological Archives. 2013; 4(2):385-390.
32. Shahwal V, Samnani A, Dubey B, Bhowmick M. Ethosomes An Overview, International Journal of Biomedical and Advance Research. 2011; 2:161-168.
33. Elsayed Mustafa MA, *et al*. Deformable liposomes and Ethosomes: mechanism of enhanced skin delivery. Int j pharm. 2006; 322:60-66.
34. Maestrelli F, Capasso G, Gonzalez-Rodriguez ML, Rabasco AM, Ghelardini C, Mura P. Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded Ethosomes. J Liposome Res. 2009; 19(4):253260.
35. S.Rani Nakka David, Chong Fui Pin, Formulation and invitro evaluation of Ethosomeas vesicular carrier for enhanced topical delivery of isotretinoin, International Journal of Drug Delivery, 2013; 5: 28-34.
36. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: characterization and in vitro/in vivo evaluation. *Colloids Surf B Biointerfaces*. 2012; 92: 299–304.
37. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary Ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. Arch Pharm Res. 2012; 35(1):109-117.
38. Dave V, Kumar D, Lewis S, Paliwal S. Ethosome for enhanced transdermal drug delivery of aceclofenac. *Int J Drug Deliv*. 2010; 2(1):81-92.
39. Tyagi LK, Kumar S, Maurya SS, Kori ML. Ethosomes novel vesicular carier for enhanced transdermal drug delivery system. Bulletin Pharm Res. 2013; 3(1):6-13.
40. Jain S, Umamaheshwari RB, Bhadra D, Jain NK. Ethosomes: A Novel Vesicular Carrier for Enhanced Transdermal Delivery of a Anti-HIV Agent, Indian Journal of Pharmaceutical Sciences. 2004; 66:72-81.
41. Verma DD, Fahr A. Synergistic Penetration Effect of Ethanol and Phospholipids on the Topical Delivery of Cyclosporin, Journal of Controlled Release, 1997, 55-66.
42. Touitou E. Composition of Applying Active Substance to or Through the Skin, US patent. 1998; 5:540-934.
43. Touitou E. Composition of Applying Active Substance to or Through The Skin, US patent. 1996; 5:716-638.
44. Kumar R, Aslam MD, Tripathi A, Prasad D, Chaudhary V, Jain V, et al. Ethosomes novel vesicular carriers in transdermal drug delivery. J Global Pharma Tech. 2010; 2(6):1-7.
45. Heeremans JLM, Gerristen HR, Meusen SP, Mijnheer FW, Gangaram RS, Panday G, et al. Crommelin DJA., The preparation of Tissue Type Plasminogen Activator (T- PA) containing liposomes: Entrapment Efficacy and Ultracentrifugation Damage, Journal of Drug Targeting.

1995; 3:301.
46. Roshni Dhurve, Neha Kashyap, Ashwani Mishra. Anupam Kumar Pathak A Holistic Review on Ethosome: A Promising Drug Delivery System for Topical Fungal Disease International Journal of Pharmaceutical & Biological Archives. 2014; 5(5):13-26.
47. Ghule Arpan Ramakrishna, Shinkar Dattatraya Manohar, Saudagar Ravindra Bhanudas Ethosomes. Carrier for Enhanced Transdermal Drug Delivery System, J. Adv. Pharm. Edu. & Res. 2014; 4(4):380-387.
48. Mishra AN, Bhalaria MK, Sachin S. Ethosome a novel delivery system for antifungal drug in treatment of topical fungal diseases. Indian J Exp Bio. 2009; 47:368-375.
49. Akiladevi D, Basak S. Ethosomes a noninvasive approach for transdermal drug delivery. Int j Current Pharma Res. 2010; 2(4):1-4.
50. Sheer A, Chauhan M. Ethosomes as vesicular carrier for enhanced transdermal delivery of Ketoconazole - Formulation and Evaluation. J Pharma Cos. 2011; 1(3):1-14.
51. Devi M, Kumar MS, Mahadevan N. Amphotericin-B Loaded Vesicular Systems for the Treatment of Topical Fungal Infection. Int J Rec Adv Pharm Res. 2011; 4:37-46.
52. Samnani A, Shahwal V, Bhowmick M, Joshi A, Dubey BK. Design and evaluation of ultradeformable soft elastic nano vesicle Ethosomes of clotrimazole for dermal delivery. Int J Bio Med Adv Res. 2012; 3(2):111-117.
53. Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S, Kim DD. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: Characterization and in vitro/in vivo evaluation. Colloids and Surfaces B: Biointerfaces. 2012; 92:299-304.
54. Verma P, Pathak K. Nanosized ethanolic vesicles loaded with econazole nitrate for the treatment of deep fungal infections through topical gel formulation. Nanomedicine. 2012; 8(4):489-96.
55. Srikanth K, Gupta RM, Devanna N. Ethanolic vesicles of nystatin for enhanced transdermal drug delivery *in vitro* and *ex vivo* evaluation. Indo American J Pharm Res. 2013; 3(9):7316-7324.
56. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary Ethosomes and Transferases of terbinafine hydrochloride a comparative study. Arch Pharm Res. 2011; 35(1):109-117.