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REVIEW ARTICLE

A Holistic Review on Ethosome: A Promising Drug Delivery System for Topical Fungal Disease

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ABSTRACT

Topical drug delivery system has been used widely for various kinds of topical diseases. Topical therapy is good 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 systemic side effects. Fungal infections of the skin are one of the often faced dermatological diseases in worldwide. The earlier approaches that has been used to treat these diseases such as creams, ointment or jellies are not efficient in treatment of these diseases in terms of bioavailability and therapeutic effect, so to overcome this problem ethosomes have been arrived showing best results in the treatment of topical fungal diseases. Ethosomes are lipid vesicles which are soft and flexible. They consist of phospholipid,alcohol(ethanol,isopropyl alcohol),poly glycol(propylene glycol)and water. The most important feature of ethosome is to permeate intact through the skin due to its high deformability and also helps to transport the active substances or drug molecule more efficiently through the skin in terms of both quantity and depth. Ethosomes can provide effective intracellular delivery of hydrophilic, amphiphilic and lipophilic drug molecules. The size range of ethosomes varies from tens of nanometer to few microns. Ethosomal drug delivery system is a new novel tool for drug delivery with safety and efficacy. 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 focus on the various aspects including their mechanism of penetration, preparation, advantages, characterization and composition and the list of antifungal drugs which have been reported by various researchers.

Key words: Mycosis, Dermatophyte, Topical route, Ethosomes , Ethanol, Antifungal drugs.

INTRODUCTION

Topical Fungal infections of the skin are one of the often faced dermatological diseases in worldwide. The incidence of superficial fungal infections of skin, hair and nails has been increased. It has been estimated that about 40 million people have suffered from fungal infections in developing and under developed nations.[1] Dermatophyte infections of the feet represent the most common fungal infections due to the use of occlusive footwear. The incidences of superficial and deep skin fungal infections are rising .[2,3] Diseased skin is distinguished and characterized by increased permeability or reduced barrier function or altered lipid composition and organization of the stratum corneum.[4]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.[5] The susceptibility and vulherability to fungal infection has increased significantly in terms of frequency and also as a cause of morbidity and mortality.[6,7,8] The susceptibility is more for immunocompromised patients due to their impaired immune function. [9] Fungal infection is also termed as **mycosis.**

Mycosis (plural: mycoses)is a fungal infection of humans. Mycosis is very common and a variety of environmental and physiological condition helps in the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections therefore, mycosis often start in the skin. People are at risk of fungal infections when they are taking strong antibiotics for a long period of time because antibiotics kill not only damaging bacteria, but healthy bacteria as well. This alters the balance of

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microorganisms in the mouth, vagina, intestines and other places in the body, and results in an overgrowth of fungus. Individuals with weakened immune systems are also at risk of developing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people, also, are groups at risk.[10]mycosis is further classified in terms of tissue levels initially colonised, but our focus is for topical skin fungal disease. So they are explained below,

1. **Superficial mycosis**
   Superficial mycosis is limited to the outermost layers of the skin and hair. An example of such a fungal infection is *Tinea versicolor*, a fungus infection that commonly affects the skin of young people, especially the chest, back, and upper arms and legs. It does not usually affect the face. This fungus produces spots that are either lighter than the skin or a reddish-brown. This fungus exists in two forms, one of them causing visible spots. Factors that can cause the fungus to become more visible include high humidity, as well as immune or hormone abnormalities.[10,11]
2. **Cutaneous mycosis**
   Cutaneous mycoses extend deeper into the epidermis, and also include invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. Unlike the superficial mycoses, host immune responses may be evoked resulting in pathologic changes expressed in the deeper layers of the skin. The organisms that cause these diseases are called dermatophytes. The resulting diseases are often called ringworm (even though there is no worm involved) or tinea. Cutaneous mycoses are caused by *Microsporum*, *Trichophyton*, and *Epidermophyton* fungi, which together comprise 41 species.[10,11]

2.1. Dermatophytosis is a clinical condition caused by fungal infection of the skin in humans. The term "**ringworm**", commonly used to refer to such infections, is a misnomer, since the condition is caused by fungi of several different species and not by parasitic worms. The fungi that cause parasitic infection (dermatophytes) feed on keratin, the material found in the outer layer of skin, hair, and nails. These fungi thrive on skin that is warm and moist, but may also survive directly on the outsides of hair shafts or in their interiors. In pets, the fungus responsible for the disease survives in skin and on the outer surface of hairs.

It has been estimated that currently up to twenty percent of the population may be infected by ringworm or one of the other dermatophytoses. It is especially common among people who play sports involving skin to skin contact, wrestling in particular. Wrestlers with ringworm may be withheld from competition until their skin condition is deemed non-infectious by the proper authorities.[12]

2.1.1. Types of Dermatophyte infections

a) Athlete's foot (Tinea pedis)
Around one in five people in the UK have athlete's foot. It's caused by a fungus that grows in warm, damp areas of skin, such as between the toes. The fungal infection makes skin itchy, flaky and red. It also causes white cracks to appear, especially between the toes and on the side of the foot. Occasionally it causes blisters.

We can pick up athlete's foot if we walk bare foot on damp, contaminated floors such as communal shower facilities, swimming pools or saunas. If we don't wash our hands after we had touched a contaminated area, it can spread to other parts of the body.[13]

b) Nail infections (Tinea unguium)
Fungal nail infections usually start at the edge of the nail and spread slowly down to the base. They tend to take a long time to develop. They causes nail to discolour and become crumbly. The surrounding tissue may also thicken. Later, the nail can become so thick that it's painful to wear shoes. Toenails are usually affected more than fingernails.

We can get a fungal nail infection if you have athlete's foot and it spreads to our nails. We can also get an infection if our nails are weak, for example from a previous injury.[13]

c) Ringworm of the body (Tinea corporis)
This often affects exposed parts of the body, such as arms, legs or face, and causes a red, ring-shaped rash. Ringworm is contagious. we can be infected by coming into contact with somebody who already has ringworm or touching contaminated items, such as

clothing or bedding. Domesticated animals, such as sheep, cattle and pets can also carry the fungi that cause ringworm.[13]

d) Ringworm of the groin (Tinea cruris)

This is also called 'jock itch' because it's more common in young men. This is because the scrotum and thigh are in close contact and create conditions in which fungi can thrive. It can also affect women if they wear tight clothing. It can cause an itchy, red rash in groin and the surrounding area.

Like ringworm of the body, ringworm of the groin is contagious and can be passed on in the same ways. we may also get ringworm in our groin if we have athlete's foot and touch our groin after touching our foot without washing your hands.[13]

e) Ringworm of the scalp (Tinea capitis)

This can occur at any age, but mostly affects children before they reach puberty. Ringworm can affect any part of your scalp but it usually get patches of it. Symptoms can be similar to those of ringworm on your groin and body and your scalp will look scaly and feel itchy. It may also develop pus-filled areas on your scalp, called 'kerions'. During the infection hair may fall out and leave bald areas but this usually grows back once the infection has been treated.

We can get ringworm on our scalp by sharing a contaminated comb or clothing used by somebody with the infection.[13]

3. Superficial Candidiasis

candidiasis is an infection caused by *candida*,a yeast like fungus candidiasis usually affects the skin and mucous membrane(soft,moist areas around body openings,like mouth and anus) children of any age develop *candida* paronychia,an infection of skin around the nails. girls and womens may develop *candida* vulvovaginitis,an infection of vagina and area around vaginal opening.it can reach to deep tissues and even in systemic circulation in absence of proper medication.[14,15]

4. Causes of fungal skin infections [13]

- are overweight
- don't dry your skin fully after bathing
- come into contact with a person or animal with a fungal skin infection
- come into contact with contaminated items, for example, clothes, towels and bedclothes
- walk barefoot in shower and pool areas
- wear tight clothing that doesn't allow sweat to evaporate
- have poorly controlled diabetes
- have recently taken a course of antibiotics
- have a weakened immune system, for example, HIV/AIDS

As we know that topical therapy is good choice for the treatment for cutaneous infections due to its various advantages such as targeting of drugs to the site of infection and reduction of the risk of systemic side effects and also because it is a non-invasive procedure for drug delivery. The drug given topically by pass the hepatic first pass metabolism so it helps to overcome the problem of drug degradation by digestive enzymes and also discomfort associated with parenteral drug administration .[16,17]

Antifungal drugs are generally used as conventional cream and gel preparations in topical treatment. The efficacy of that treatment depends on the penetration of drugs through the target layers of the skin. But antifungal drug in the form of cream, ointment or jellies alone are not efficient in terms of both bioavailability and therapeutic effect for the treatment of the topical fungal disease because of stratum corneum barrier [16,17]

Skin is one of the most accessible organ of human body for topical administration and main route of topical drug delivery system. Number of medicated products is applied to the skin or mucous membrane that either enhances or restores a fundamental function of a skin or pharmacologically alters an action in the underlined tissues.It is necessary to understand the anatomy,physiology, physicochemical properties of the skin to utilize the phenomenon of percutaneous absorption successfully.[18]

SKIN ANATOMY AND PHYSIOLOGY

The skin is one of the most extensive and readily accessible organ of the human body and the skin as a route of drug delivery offers many advantages over traditional drug delivery systems including lower fluctuations in plasma drug levels,

avoidance of gastrointestinal disturbances and first-pass metabolism of the drugs, and high patient compliance. One of the greatest disadvantages to transdermal drug delivery is the skin's low permeability that limits the number of drugs that can be delivered by this route. The skin offers an excellent barrier to molecular transport, as stratum corneum is barrier to the passage of most of the drugs, except for lipophilic and low molecular weight drugs. For transdermal and topical drug delivery system to be effective, the drug must obviously be able to penetrate the skin barrier and reach the target site.[19]

Human skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue .It is the most relevant route for absorption of drug. As we know that skin is the largest organ covering approximately about 1.8 $m^{2\ total}$ surface area of human. The dermal–epidermal junction is undulating in section, ridges of the epidermis, known as rete ridges, project into the dermis. The junction provides mechanical support for the epidermis and acts as a partial barrier against exchange of cells and large molecules. Below the dermis is a fatty layer, the panniculus adiposus, usually designated as 'subcutaneous'. This is separated from the rest of the body by a vestigial layer of striated muscle, the panniculus carnosus. There are two main kinds of human skin. Glabrous skin(non-hairy skin), found on the palms and soles, is grooved on its surface by continuously alternating ridges and sulci in individually unique configurations known as dermatoglyphics .It is characterized by a thick epidermis divided into several well-marked layers, including a compact stratum corneum, by the presence of encapsulated sense organs within the dermis, and by a lack of hair follicles and sebaceous glands. Hair-bearing skin, on the other hand, has both hair follicles and sebaceous glands but lacks encapsulated sense organs. There is also wide variation between different body sites. For example, the scalp with its large hair follicles may be contrasted with the forehead, which has only small vellus-producing follicles, albeit associated with large sebaceous glands. The axilla is notable because it has apocrine glands in addition to the eccrine sweat glands, which are found throughout the body.[20]

The major barrier that is stratum corneum is the outermost layer of the epidermis.It consists of 10 to 25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers.[21] It has been shown that the stratum corneum is the main barrier to penetration through the skin. When a topical formulation is placed on the skin, the active drug is required to penetrate through the stratum corneum into the viable tissue. The limiting factor for these processes is the slow diffusion through the dead horny layer of skin. Stratum corneum behaves as a hydrophobic membrane. The rates of permeation of skin by low and high molecular weight organic non-electrolytes are mostly determined within the stratum corneum.The molecular structures and appearance of the molecules can be examined using molecular modeling computer programs. Under normal conditions, the main route is observed through the intercellular spaces or lipid bilayers. The diffusional path length is therefore much longer than simple thickness of the stratum corneum (20-30 mm). The penetration through skin is also affected by several biological factors such as skin age, body site, skin condition and diseases, water content of the skin or hydration. The intercellular spaces contain structured lipids/proteins and a diffusing molecule has to cross a variety of lipophilic and hydrophilic domains before reaching to the stratum corneum and viable epidermis junction.[22]The further skin details are as follows

I. Epidermis

The epidermises consist of stratified squamous epithelium. The main cells of the epidermis are known as keratinocytes, which synthesise the protein keratin. Protein bridges called desmosomes connect the keratinocytes, which are in a constant state of transition from the deeper layers to the superficial. The four separate layers of the epidermis are formed by the differing stages of keratin maturation. The epidermis varies in thickness from 0.05 mm on the eyelids to 0.8±1.5 mm on the soles of the feet and palms of the hand. Moving from the lower layers upwards to the surface, the four layers of the epidermis are:

- stratum basale (basal or germinativum cell layer)
- stratum spinosum (spinous or prickle cell layer)
- stratum granulosum (granular cell layer)
- stratum corneum (horny layer).

In addition, the stratum lucidum is a thin layer of translucent cells seen in thick epidermis. It represents a transition from the stratum granulosum and stratum corneum and is not usually seen in thin epidermis.

Together, the stratum spinosum and stratum granulosum are sometimes referred to as the Malphigian layer.[23]

I.I. Stratum basale

The innermost layer of the epidermis which lies adjacent to the dermis comprises mainly dividing and non-dividing keratinocytes, which are attached to the basement membrane by hemi desmosomes. As keratinocytes divide and differentiated, they move from this deeper layer to the surface. Making up a small proportion of the basal cell population is the pigment (melanin) producing melanocytes. These cells are characterised by dendritic processes, which stretch between relatively large numbers of neighbouring keratinocytes. Melanin accumulates in melanosomes that are transferred to the adjacent keratinocytes where they remain as granules. Melanin pigment provides protection against ultraviolet (UV) radiation, chronic exposure to light increases the ratio of melanocytes to keratinocytes, so more are found in facial skin compared to the lower back and a greater number on the outer arm compared to the inner arm. The number of melanocytes is the same in equivalent body sites in white and black skin but the distribution and rate of production of melanin is different. Intrinsic ageing diminishes the melanocyte population. Merkel cells are also found in the basal layer with large numbers in touchsensitive sites such as the fingertips and lips. They are closely associated with cutaneous nerves and seem to be involved in light touch sensation.[23]

I.II. Stratum spinosum

As basal cells reproduce and mature, they move towards the outer layer of skin, initially forming the stratum spinosum. Intercellular bridges, the desmosomes, which appear as `prickles' at a microscopic level, connect the cells. Langerhans cells are dendritic, immunologically active cells derived from the bone marrow are found on all epidermal surfaces but are mainly located in the middle of this layer. They play a significant role in immune reactions of the skin, acting as antigen-presenting cells.[23]

I.III. Stratum granulosum

Continuing their transition to the surface the cells continue to flatten, lose their nuclei and their cytoplasm appears granular at this level.[23]

I.IV. Stratum corneum

The final outcome of keratinocyte maturation is found in the stratum corneum, which is made up of layers of hexagonal-shaped, non-viable cornified cells known as corneocytes. In most areas of the skin, there are 10±30 layers of stacked corneocytes with the palms and soles having the most. Each corneocyte is surrounded by a protein envelope and is filled with water-retaining keratin proteins. The cellular shape and orientation of the keratin proteins add strength to the stratum corneum. Surrounding the cells in the extracellular space are stacked layers of lipid bilayers . The resulting structure provides the natural physical and water-retaining barrier of the skin. The corneocyte layer can absorb three times its weight in water but if its water content drops below 10% it no longer remains pliable and cracks. The movement of epidermal cells to this layer usually takes about 28 days and is known as the epidermal transit time.[23]

II.I. Dermoepidermal junction/basement membrane

This is a complex structure composed of two layers. Abnormalities here result in the expression of rare skin diseases such as bullous pemphigoid and epidermolysis bullosa. The structure is highly irregular, with dermal papillae from the papillary dermis projecting perpendicular to the skin surface. It is via diffusion at this junction that the epidermis obtains nutrients and disposes of waste. The dermoepidermal junction flattens during ageing which accounts in part for some of the visual signs of ageing.[23]

III.I. Dermis

The dermis varies in thickness, ranging from 0.6 mm on the eyelids to 3 mm on the back, palms and soles. It is found below the epidermis and is composed of a tough, supportive cell matrix. Two layers comprise the dermis:

- a thin papillary layer
- a thicker reticular layer.

The papillary dermis lies below and connects with the epidermis. It contains thin loosely arranged collagen fibres. Thicker bundles of collagen run parallel to the skin surface in the deeper reticular layer, which extends from the base of the papillary layer to the subcutis tissue. The dermis is made up of fibroblasts, which produce collagen, elastin and structural proteoglycans, together with immunocompetent mast cells and macrophages. Collagen fibres make up 70% of the dermis, giving it strength and toughness. Elastin maintains normal elasticity and flexibility while proteoglycans provide viscosity and hydration. Embedded within the fibrous tissue of the dermis are the dermal vasculature, lymphatics, nervous cells and fibres, sweat glands, hair roots and small quantities of striated muscle.[23]

IV.I. Subcutis

This is made up of loose connective tissue and fat, which can be up to 3 cm thick on the abdomen.[23]

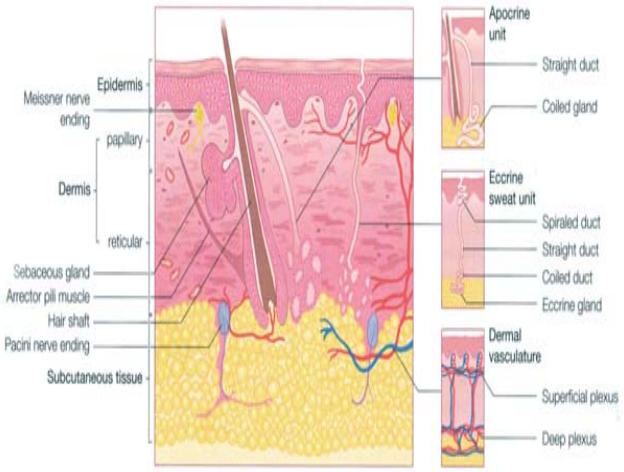

Fig 1: Structure of skin [24]

SKIN ABSORPTION PATHWAYS:

Skin absorption pathways can be divided into the transport:

1. Epidermal route (across the intact SC)
2. Trans-follicular (shunt pathway) Absorption (along the skin appendages)

The physicochemical properties of the drug as well as the nature of the formulation are the main factors influencing the choice of pathway.[25]

a) Epidermal route

For drugs, which mainly cross-intact horny layer, two potential micro routes of entry exists, The Trans-cellular (intra-cellular) route and paracellular route.[25]

- **The Transcellular (Intracellular) route**

Transcellular pathway means transport of molecules across epithelial cellular membrane. These include passive transport of small molecules, active transport of ionic and polar compounds and endocytosis and transcytosis of macromolecules. Under normal conditions the transcellular route is not considered as the preferred way of dermal invasion the reason being the very low permeability through the corneocytes and the obligation to partition several times from the more hydrophilic corneocytes into the lipid intercellular layers in the stratum corneum and vice versa. The transcellular pathway can gain an importance when a penetration enhancer is used, for example, urea which increases the permeability of the corneocytes by altering the keratin structure.[25]

- **The Paracellular (Intercellular) route**

Paracellular pathway means transport of molecules around or between the cells. Tight junctions or similar situations exist between the cells. The principal pathway taken by a permeant is decided mainly by the partition coefficient (log k). Hydrophilic drugs partition preferentially into the intercellular domains, whereas lipophilic permeants (o/w log k >2) traverse the stratum corneum via the intracellular route. The intercellular route is considered to be the predominantly used pathway in most cases especially when steady-state conditions in the stratum corneum are reached. Substance transport occurs in the bilayer-structured continuous intercellular lipid domain within the stratum corneum. Although this pathway is very tortuous and therefore much longer in distance than the overall thickness of the stratum corneum (~20 μm) and has been estimated as long as 500 μm. The intercellular route is considered to yield much faster absorption due to the high diffusion coefficient of most drugs within the lipid bilayer.[25]

b) Trans-follicular (shunt pathway) Absorption (along the skin appendages)

Skin appendageal route comprises transport via eccrine sweat glands, apocrine sweat glands and hair follicles with their associated sebaceous glands. These routes circumvent penetration through the stratum corneum and are therefore known as “shunt”

routes. The skin's appendages offer only secondary avenues for permeation. Sebaceous and eccrine glands are the only appendages, which are seriously considered as shunts bypassing the stratum corneam since these are distributed over the entire body. Though eccrine glands are numerous, their orifices are tiny and add up to a miniscule fraction of the body's surface. Moreover, they are either evacuated or so profusely active that molecules cannot diffuse inwardly against the glands output. For these reasons, they are not considered as a serious route for percutaneous absorption. However, the follicular route remains an important avenue for percutaneous absorption since the opening of the follicular pore, where the hair shaft exits the skin, is relatively large and sebum aids in diffusion of penetrants. Partitioning into sebum, followed by diffusion through the sebum to the depths of the epidermis is the envisioned mechanism of permeation by this route. Vasculature sub serving the hair follicle located in the dermis is the likely point of systemic entry. Absorption across a membrane, the current or flux is and terms of matter or molecules rather then electrons, and the driving force is a concentration gradient (technically, a chemical potential gradient) rather then a voltage drop. A membranes act as a "diffusional resistor." Resistance is proportional to thickness (h), inversely proportional to the diffusive mobility of matter within the membrane or to the diffusion coefficient (D), inversely proportional to the fractional area of a route where there is more than one (F), and inversely proportional to the carrying capacity of a phase.[25]

R = h/FDK

Where; R =Resistance of diffusion resistor
F = Fractional area
H = Thickness
D = diffusivity
K = Relative capacity

Hair follicles: Hair follicles with their associated sebaceous glands are present all over the skin surface with the exception of lips, palms, and soles. Furthermore, hair follicles intersperse down to the subcutis offering permeation pathways deep into the skin. The density of hair follicles varies with species and body site. The sebaceous glands produce the sebum, which lubricates and protects the skin and is involved in the regulation of the pH on the skin surface.[25]

Eccrine glands: Eccrine glands can be found on the entire body surface of humans except for the lips, external ear canal, clitoris, and labia minora. These glands play an important role in thermoregulation which is necessary for fluid and electrolyte homeostasis. They secrete a milky or oily odorless liquid which produces the characteristic body smell after metabolism through surface bacteria of the skin.[25]

Apocrine glands: The apocrine glands are limited to specific body regions and are also coiled tubes. These glands are about ten times the size of the eccrine ducts extend as low as the subcutaneous tissues and are paired with hair follicles.

This Trans-follicular (shunt pathway) route is considered to be of minor importance because of its relatively small area, approximately 0.1 % of the total skin area. In contrast, in the initial stages of a skin absorption process and in the case of large hydrophilic compounds and ions invasion through the appendages may play a considerable role. Recent studies also report that the appendages route may be involved in the absorption of liposomes, nanoparticles, and cyclodextrin-inclusion complexes.[25]

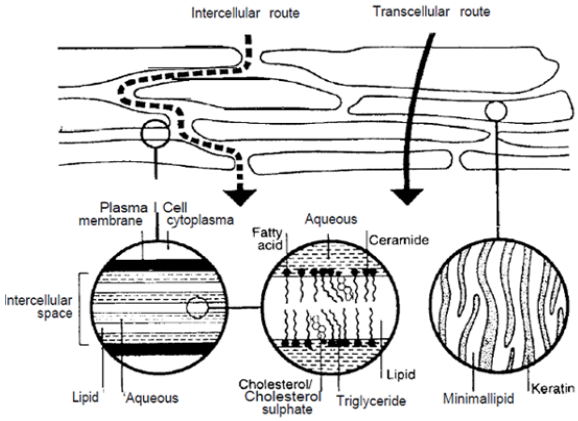

Fig 2: Possible micro routes for drug penetration across human skin [25]

FUNCTIONS OF SKIN [26]

1. Provides a protective barrier against mechanical, thermal and physical injury and noxious agents.
2. Prevents loss of moisture.
3. Reduces the harmful effects of UV radiation.

4. Acts as a sensory organ.
5. Helps regulate temperature control.
6. Plays a role in immunological surveillance.
7. Synthesises vitamin D3 (cholecalciferol).

ADVANTAGES OF TOPICAL DRUG DELIVERY SYSTEM[26]

- Avoidance of first pass metabolism.
- Convenient and easy to apply.
- Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes, gastric emptying time etc.
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input.
- Avoids fluctuation in drug levels, inter- and intrapatient variations.
- Ability to easily terminate the medications, when needed.
- A relatively large area of application in comparison with buccal or nasal cavity
- Ability to deliver drug more selectively to a specific site.
- Avoidance of gastro-intestinal incompatibility.
- Providing utilization of drugs with short biological half-life, narrow therapeutic window.
- Improving physiological and pharmacological response.
- Improve patient compliance.
- Provide suitability for self-medication.

DISADVANTAGES OF TOPICAL DRUG DELIVERY SYSTEM [26]

- Skin irritation of contact dermatitis may occur due to the drug and/or excipients.
- Poor permeability of some drugs through the skin.
- Possibility of allergenic reactions.
- Can be used only for drugs which require very small plasma concentration for action
- Enzyme in epidermis may denature the drugs
- Drugs of larger particle size not easy to absorb through the skin

Due to above disadvantages of topical drug delivery system a novel therapeutic tool have been arrived called as “Ethosomes”.

ETHOSOMES

Ethosomes are soft and flexible vesicle system mainly composed of phospholipids, water, poly glycol(propylene glycol) and a high concentration of alcohol(ethanol, isopropyl alcohol), usually about 20% to 40%.Due to flexible lipid bilayer structure, ethosomes are easily deformable and permeate deep into the skin, thus enhancing drug delivery. Moreover, they increase drug deposition in the skin and enhance its permeability in scar tissue.[27,28]

The size range of ethosomes may vary from tens of nanometers (nm) to microns(μ) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux . Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic,or amphiphilic. Ethosomal formulation provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs.[27,29]

Ethosomes are mainly used for the delivery of drugs through transdermal route. The transdermal delivery is the most important and common route of drug administration. The main factor which limits the application of transdermal route for drug delivery is the permeation of drugs through the skin.[4] Human skin has selective permeability for drugs. Lipophilic drugs can pass through the skin but the drugs which are hydrophilic in nature can’t pass through it. Water soluble drugs either show very less or no permeation. To improve the permeation of drugs through the skin various mechanisms have been investigated and ethosomes are one of the successful mode to enhance permeability drug through the stratum corneum barrier.[30,31]

Ethosomes are non invasive delivery carriers that enable drugs to reach the deep skin layers. These are soft, malleable vesicles discovered for enhanced delivery of active agents. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for having property of disturbing the skin lipid bilayer organization therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum.[32]

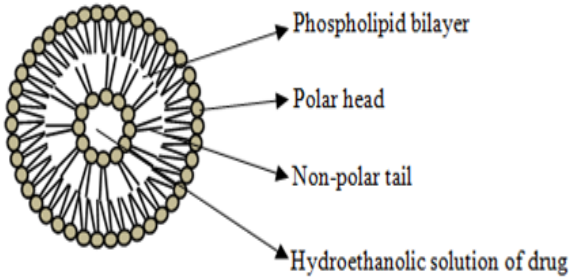

Fig 3: Structure of ethosome [16]

ADVANTAGES [33]

A. Enhanced permeation of drug through skin for transdermal drug delivery.
B. Delivery of large molecules (peptides, protein molecules] is possible.
C. It contains non-toxic raw material in formulation.
D. High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
E. The ethosomal system is passive, non-invasive.
F. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
G. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated Methods.

LIMITATION [34]

A. In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water.
B. Loss of product during transfer form organic to water media.

COMPOSITION [34]

Table 1: Composition of ethosomes

INGREDIENTS	EXAMPLES	USED AS
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearylphosphatidyl choline	used as vesicle forming agent
Poly glycol	Propylene glycol	used as skin penetration enhancer.
Alcohol	Isopropyl alcohol Ethanol	used as skin penetration enhancer and in providing softness to vesicle membrane
Other phospholipid	Cholestrol	stabilizer

MECHANISM OF PENETRATION

The mechanism of penetration of the ethosomes involves two simultaneous mechanisms of ethanol effet 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.[35]

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.[19]

Ethosome 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 gets fused with skin lipids and releases the drugs into deep layer of skin.[19]

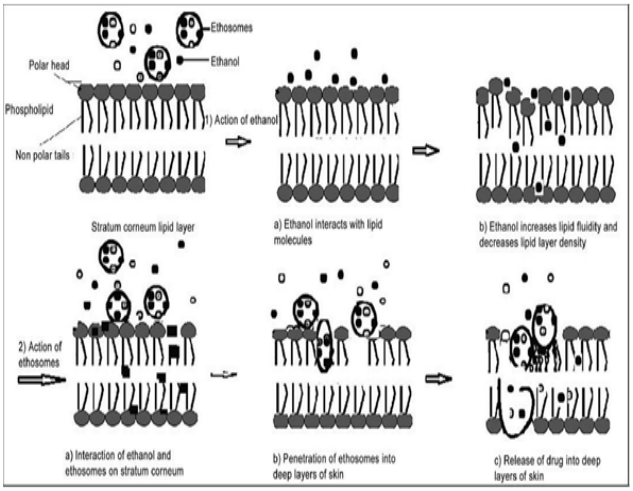

Fig 4: Mechanism of penetration of ethosome through stratum corneum [31]

INTERACTION BETWEEN SKIN AND ETHOSOME

The enhanced delivery of drug molecule using ethosomes can be understood by studying the interaction between ethosomes and skin lipids. A mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the 'ethanol effect', whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosomes effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of drug in deep layers of the skin.[32]

ETHANOL-AS PENETRATION ENHANCER

Substances or a chemical that reversibly reduce the barrier resistance of the stratum corneum are known as chemical penetration enhancers. Ethanol

is one of the most commonly used permeation enhancers. There are many mechanisms that has been proposed for permeation enhancing action of ethanol. As a solvent, ethanol can be added in the formulation to enhance the solubility of the drug. Ethanol is a volatile solvent and will rapidly evaporate at skin temperature. Ethanol loss from the formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. In addition, ethanol helps to alter the solubility properties of the stratum corneum, facilitating improved drug partitioning. The effect of ethanol is concentration dependent. The effect of ethanol on skin water content have been observed that formulations containing high levels of alcohol were capable of dehydrating the skin, which help to explain the concentration dependant action of ethanol.[32]

METHODS OF PREPARATION

A. 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.[19]

B. Mechanical dispersion method[25]

Soya phosphotidylcholine 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.[19]

C. Cold method

This is the most common method utilized for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials(were dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mechanical stirrer. Propylene glycol was added during stirring. Stirring is continued for about 30-35 minutes This mixture is heated to 30°C in a water bath. The water heated to 30°C in a separate vessel is added to the mixture in the form of fine stream , which is then stirred for 5 min in a covered vessel.The vesicle sizes of ethosomes can be reduced by using sonication . Finally, formulation is stored under refrigeration.[16]

D. Hot method

In this method, phospholipid is dispersed in water by heating in a water bath at 40°C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40°C. Once both mixtures reach 40°C, 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 desired extent using probe sonication or extrusion method.[17]

METHODS OF CHARACTERIZATION [34,36]

a. Visualization

Visualization of ethosomes can be done using transmission electron microscopy and electron microscopy as well as scanning electron microscopy. Visualization by electron microscopy reveals an ethosomal formulation exhibited vesicular structure 300-400 nm in diameter.

b. 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.

c. 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.

% Entrapment = Actual content/Theoretical content x 100

d. Interaction study by using DSC and FTIR

Interaction study between the lipid and drug can be determined by usind dsc.The transition temperature (Tm) of the vesicular lipid systems is determined by using the Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland).The transition temperature was measured by using the aluminium crucibles at a heating rate 10 degree/minute within a temperature range from 20º-300ºC. Ineraction study can also be done by ftir.

e. 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). The size of ethosomes ranges between tens of nanometers to microns and is influenced by the composition of the formulation. Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. In general, particles could be dispersed with proper stability when the absolute value of zeta potential was above 30mV due to the electric repulsion between particles.

f. 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.

g. Surface Tension Activity Measurement

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

h. Penetration and Permeation Studies

Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM) .

i. Stability of ethosome

The ability of ethosomal formulations to retain the drug was checked by keeping the preparations at different temperatures, *i.e.*25±2°C (room temperature), 37±2°C and 45±2°C for different periods of time. The stability of ethosomes can also be determined quantitatively by monitoring size and morphology of the vesicles using DLS and TEM.

THERAPEUTIC APPLICATIONS OF ETHOSOMES [17]

a) In the treatment herpetic infection- 5% acyclovir ethosomal preparation compared herpetic infections.

b) Transcellular Delivery - Ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

c) Ethosomes are used in pilosabeceous targeting - 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.

d) Transdermal Delivery of Hormones- Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects.The risk of failure of treatment is known to increase with each pill missed.

e) Delivery of Anti-Arthritis Drug -Topical delivery of anti-arthritis drug is a better option for its site-specific delivery andovercomes the problem associated with conventional oral therapy.

f) Delivery of Antibiotics - Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents.Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues.Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of\skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.

MARKETED FORMULATIONS OF ETHOSOMES [29,32]

Table 2: List of marketed formulation of ethosome

Name of product	Uses	Manufacturer
Cellutight EF	Topical cellulite cream, contains a powerful combination of ingredients to increase metabolism and break down fat	Hampden Health, USA
Decorin cream	Anti-aging cream, treating, repairing, and delaying the visible aging signs of the skin including wrinkle lines, sagging, age spots, loss of elasticity, and hyperpigmentation	Genome Cosmetics, Pennsylvania, US
Nanominox	First minoxidil containing product, which uses ethosomes. Contains 4% Minoxidil, well-known hair growth promoter that must be metabolized by sulfation to the active compound	Sinere, Germany
Noicellex	Topical anti-cellulite cream	Novel Therapeutic Technologies, Israel
Skin genuity	Powerful cellulite buster, reduces orange peel	Physonics, Nottingham, UK
Supravir cream	For the treatment of herpes virus, formulation of acyclovir drug has a long shelf life with no stability problems, stable for at least three years, at 25°C. Skin permeation experiments showed that the cream retained its initial penetration enhancing properties even after three years	Trima, Israel

RESEARCHES DONE ON ETHOSOME CARRYING ANTIFUNGAL DRUG

Various reasearches have been done on ethosome carrying antifungal drug. In 2009 Bhalaria M.K *et al* ,prepared and characterized fluconazole encapsulated ethosomes and compared with liposomes.Drug encapsulated ethosomes were prepared and optimized by "Hot" method technique.they concluded that fluconazole encapsulated ethosomes are better in terms of entrapment efficiency, vesicle size, in vitro release and duration of therapy when compared with liposomes.[14]

In 2011 Sheer A *et al* , prepared Ketoconazole entrapped ethosomal carriers. They studied effect of different concentration of lecithin (1.0, 1.5, 2.5, 3.0% w/w) and ethanol (25, 30,35, 40% w/w) on different properties of ethosomal formulations. The size of the vesicles was found to have increased with increasing lecithin concentration. Also, it was observed that the size of the vesicles decreased with increasing ethanol concentration. At last they concluded that ethosomes were a promising candidate for transdermal delivery of Ketoconazole and the results proves that ethosomes are safe and very efficient drug carrier for systemic as well as topical delivery of drug.[37]

In 2011 Devi M *et al*, prepared Amphotericin B (AmB) loaded vesicular systems such as liposomes, ethosomes, transfersomes, incorporated in dermatological base, and assess their comparative potential to deliver the drug for treatment of infection.Vesicular carriers were characterized for encapsulation efficiency, shape, size and size distribution, *in vitro* release kinetics, storage stability and *in vitro* antifungal activity. AmB loaded ethosome showed good results.[38]

In 2012 Samnani A *et al*, prepared Clotrimazole loaded ethosomes were, optimized and characterized for vesicular shape and surface morphology, vesicular size, size distribution, entrapment efficiency, and stability. Clotrimazole loaded ethosomes showed good result.[39]

In 2012 Song C.K *et al* , Describes a novel carrier, transethosome, for enhanced skin delivery of voriconazole. Transethosomes (TELs) are composed of phospholipid, ethanol, water and edge activator (surfactants)or permeation enhancer (oleic acid). Characterization of the TELs was based on results from recovery, particle size, transmission electron microscopy (TEM), zeta potential, elasticity studies and *in vitro, in vivo* study. So here they concluded that the novel carrier TELs could serve as an effective dermal delivery for voriconazole.[40]

In 2012 Pathak K *et al*, Developed nanovesicles of econazole nitrate (EN) and formulating them as a suitable dermatological gel for improved therapeutic efficacy, better dispersity, and good storage stability. The results collectively suggest that because of the controlled drug release, better antifungal activity, and good storage stability, EN ethosomal gel has tremendous potential to serve as a topical delivery system.[41]

In 2013 Devanna N *et al*, Prepared Nystatin loaded ethosomes and characterized in terms of morphology (size, shape, Texture), % entrapment efficiency, *in vitro* drug release,diffusion behavior, etc. They optimized Formulations on the basis of entrapment efficiency and release pattern.The results shown that ethosomal delivery systems are effective tool in enhancing the transdermal delivery of nystatin.[42]

In 2013 Ozer O *et al* . Prepared Terbinafine-HCl loaded liposome and ethosome formulations and also gel form of these formulations were prepared. The formulations were characterized and in vitro and ex vivo release studies were performed. they explain the use of Terbinafine hydrochloride (TBF-HCl) as an active substance for treatment of

onychomycosis. Onychomycosis is a fungal infection which is the most common disease of nail plate. Here ethosome loaded with drug showed good result than liposome.[43]

Table 3: Researches done on ethosome carrying antifungal drugs [14,37-43]

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	Samnani A *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

CONCLUSION

A conclusion can be drawn from the above review that ethosome is a promising drug delivery system against various topical fungal diseases in terms of both bioavailability and pharmacotherapeutic effect. The results of all reasearches 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.

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