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ETHOSOMES AS AMPHIPHILIC VESICLES FOR MODIFIED DRUG DIFFUSION TO SKIN

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ABSTRACT

Transdermal drug delivery system was 1st introduced more than 20 years ago. This system is a type of traditional drug delivery system in which drug under goes to the systemic circulation through the protective barrier i.e. skin. Skin is a major target and barrier for topical and transdermal drug delivery, but the low diffusion rate across the stratum corneum is a major hindrance. Various methods have been conducted to improve drug permeation rate temporarily via skin, such as by help of elastic vesicles or skin enhancers. Vesicular systems like ethosomes, are controversial methods for transdermal drug delivery due to their higher penetration rate through the skin due to their ethanolic content. Herbal Ethosomes are composed of phospholipids, API, Herbal ingredient, alcohol, polyglycol and water. The purpose of this article reviews is to focus on various aspects of Herbal ethosomes, including their mechanism of penetration, preparation, advantages, characterization, composition, preparation, and application, highlighting the potential for novel improved therapies.

KEYWORDS: Anti-fungal, Topical route, Ethosomes, Skin.

1.0. INTRODUCTION

Topical drug delivery system involves administering drugs through the skin for systemic distribution. This method offers several advantages, such as avoiding the gastrointestinal tract and providing controlled release, leading to improved patient compliance and reduced side effects. It is a non-invasive method and it allow the drug substance to enter the systemic circulation and provide desirable therapeutic effect.[1]

In point of view of pharmaceutical, Topical drug delivery route provides several benefits than other drug delivery routes, like- bypass of first-pass metabolism, reduction in administration frequency, low fluctuations in drug-plasma profile, improved efficacy and increased safety. Transdermal drug administration offers a convenient and safe alternative to parenteral therapy, reducing risks and discomfort, and enhancing patient compliance.[2]

The skin, the largest organ in our body, serves as both a protective barrier and a shield for our internal organs from the external environment. The skin plays a crucial role in protecting body organs, regulating body temperature, preventing dehydration, and regulating sensory receptors like touch and pain.[3] Human skin serves as a crucial barrier against endogenous substances like water and xenobiotic materials like chemicals and drugs, acting as the first line of defences in the human body. Skin consists of three primary layers are Epidermis, Dermis and Hypodermis (Figure 1).[4]

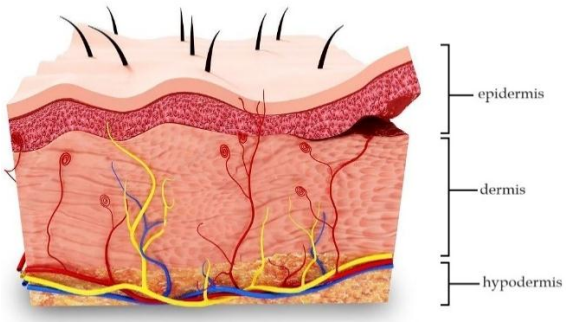

Fig. 1: Layers of skin.

Epidermis is act as a protective outer most layer or shield to the internal tissues of the body from the external environment. It has multiple layers, including- *Stratum Corneum*(The outermost layer of the epidermis, composed of dead corneocytes filled with keratin, provides skin strength and waterproofing), *Stratum Lucidum*(This layer, found in specific body parts like palms and feet, consists of clear, flattened cells densely packed with keratin), *Stratum Granulosum*(The epidermis layer, containing keratin granules and proteins, aids in keratinization, a process where epidermal cells become filled with keratin and die, forming the protective skin barrier).[5][6][7]

Dermis, the middle layer of the skin that placed between epidermis and hypodermis. It is composed of various tissues that provide mechanical support, elasticity, and nourishment. It contains collagen and elastic fibers, which provide strength and support to the skin, while allowing it to stretch and return to its normal shape. These fibers are crucial for maintaining

the skin's firmness and flexibility. The dermis, a vital part of the skin, contains blood vessels that supply oxygen and nutrients to skin cells, regulating body temperature and giving skin its pinkish hue. It also contains sensory receptors like Meissner's and Pacinian corpuscles, which detect touch, pressure, and vibration, transmitting these signals to the brain.[8][9][10][11]

Hypodermis The hypodermis, also known as subcutaneous tissue or subcutis, is the innermost layer of the skin, composed of adipose and connective tissue, and serves as a cushioning layer that insulates the body. Some of the main functions of hypodermis like- Insulation, Energy storage, Protection.[12][13]

2.0. ETHOSOMES

Touitou et al.'s 1996 invention of ethosomes is a significant advancement in vesicular delivery. Ethosomes are soft and malleable vesicles that used for drug delivery through the transdermal route. They have hydrophilic, lipophilic, or amphiphilic characteristics and can range from nano-meters to micro-meters in size. Ethosomes are modified forms of liposomes and have high ethanolic content that allowing the drug to reach deep in skin layers and enters into systemic circulation. The Ethosomes system consists of phospholipids, high alcohol concentrations (ethanol and isopropyl alcohol), and water (Figure 2). The high ethanol concentration makes Ethosomes unique as it disrupts skin lipid bilayer organization, enhancing vesicles' ability to penetrate the stratum corneum when incorporated into a vesicle membrane.

Herbal ethosome technology has significantly improved the bioavailability of popular herbs like Sophora alopecuroides, Cannabis sativa, and Glycyrrhiza glabra, enabling the development of various treatments for various diseases.[14][15][16][17][18]

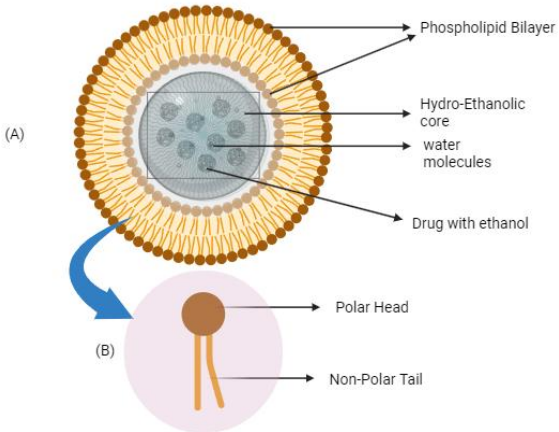

Fig. 2: (A) Ethosome content, (B) Phospholipid.

2.1. ETHOSOMES COMPOSITION

Ethosomes are vesicular carriers made of hydroalcoholic or hydro/alcoholic/glycolic phospholipids, with high alcohol concentrations or their combination. Different additives are used in these ethosomes.[19][20] The formulation is represented in table-1.

Table 1: Materials used in the formulation of Ethosomes.[21]

CLASS	EXAMPLE	USES
Phospholipid	Soya phosphatidyl choline, Egg phosphatidyl choline, Dipalmityl phosphatidyl choline, Distearryl phosphatidyl choline	Vesicles forming component.
Polyglycol	Propylene glycol, Transcutol RTM	As a skin penetration enhancer.
Alcohol	Ethanol, Isopropyl alcohol	For providing the stability to vesicle membrane. As a penetration enhancer.
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123, Rhodamine Red fluorescene isothiocynate 6-carboxy fluorescene	For characterization study.
Vehicle	Carbopol D 934	As a gel former.

2.1.1. ADVANTAGES OF ETHOSOMES DRUG DELIVERY SYSTEM

- Large molecules, such as peptides and protein molecules, can be delivered.
- The study focuses on the enhanced permeation of drugs through the skin for transdermal drug delivery.
- The ethosomal drug delivery system is widely utilized in the pharmaceutical, veterinary, and cosmetic sectors.
- The ethosomal drug is administered in semisolid form, such as gel or cream, which results in high patient compliance.
- The Ethosomal system is a passive, non-invasive solution that is currently available for immediate commercialization.
- This method offers a simpler approach to drug delivery compared to more complex methods like Iontophoresis and Phonophoresis.
- The formulation of this product is made from non-toxic raw materials.[22][23]

2.1.2. DISADVANTAGES OF ETHOSOMES DRUG DELIVERY SYSTEM

- Poorly shelled ethosomes can clump together, causing precipitation.

- The drug's solubility in both lipophilic and aqueous environments is sufficient to reach dermal microcirculation and access the systemic circulation.
- Skin irritation or dermatitis can occur due to excipients and enhancers of drug delivery systems.
- Ethosomal administration is not intended for rapid drug input but rather for slow, sustained drug delivery.
- When ethosomes are transfers from the organic to the aqueous layer results in product loss.
- The drug's molecular size must be reasonable enough for percutaneous absorption.[24]

2.2. MECHNISM OF DRUG PERMEATION

Ethosomes offer increased drug permeation into the stratum corneum, but the absorption mechanism is unclear. It is likely due to the ethanol effect and the ethosomes effect, which occur in two phases (Figure 3,4).

2.2.1. Ethanol Effect- Ethanol enhances skin penetration by entering intercellular lipids, increasing fluidity of cell membrane lipids and decreasing density of lipid multilayers, a well-known mechanism of its penetration enhancement effect.[25]

2.2.2. Ethosomes Effect- Ethosomes' increased cell membrane lipid fluidity leads to increased skin permeability, allowing them to easily penetrate deep skin layers, fuse with skin lipids, and release drugs into the skin's deep layers.[26]

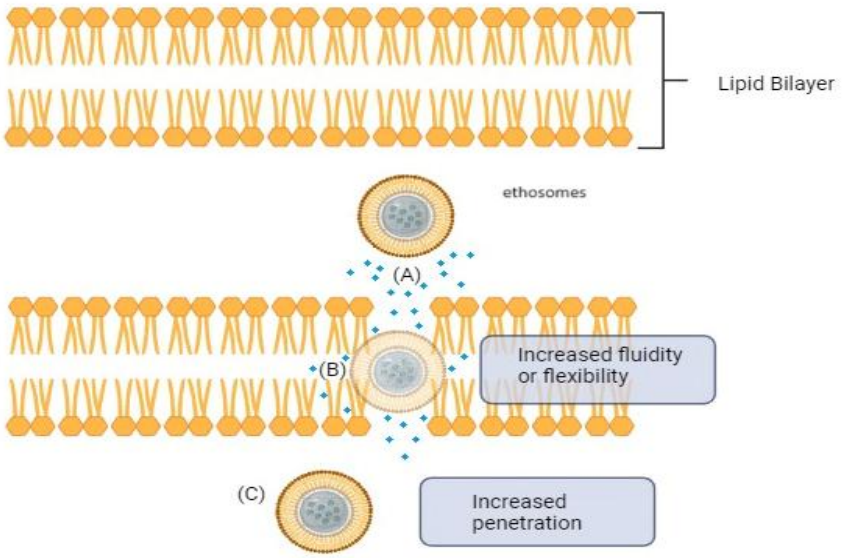

Fig.3: proposed mechanism of action ethosomal drug permeation.

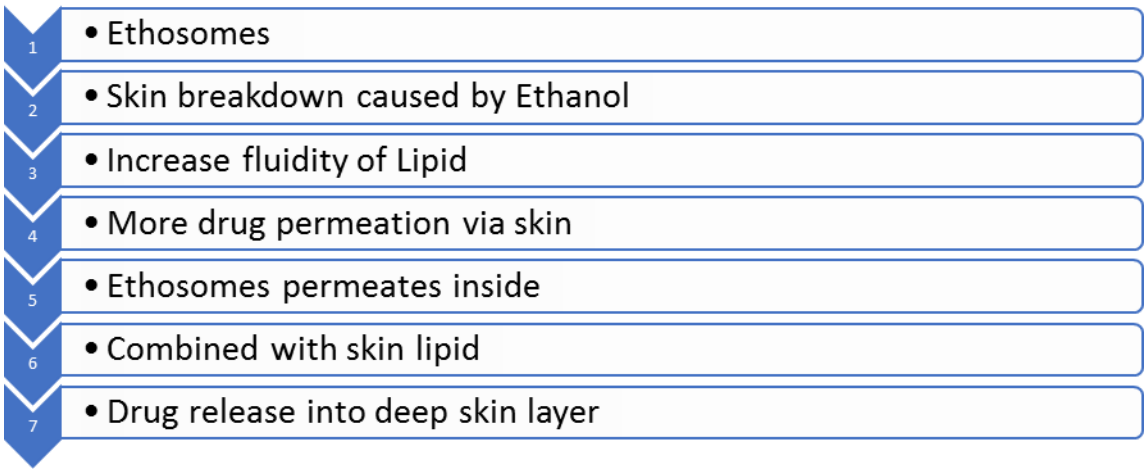

Fig. 4: Mechanism of Action of Ethosomes.

2.3. METHODS FOR PREPARATION OF ETHOSOMES:

Two simple and convenient methods are available for formulating and preparing ethosomes, without the need for sophisticated instruments or complex processes.

2.3.1. Cold method- The most common method for preparing Ethosomal formulation involves mixing phospholipid, drug, and other lipid materials, adding propylene glycol, heating to 30°C, stirring for 5 minutes, and adjusting vesicles size using sonication or extrusion methods. The formulation is then stored under refrigeration and can be further refined using sonication or extrusion methods (Figure 5).[27][28][29]

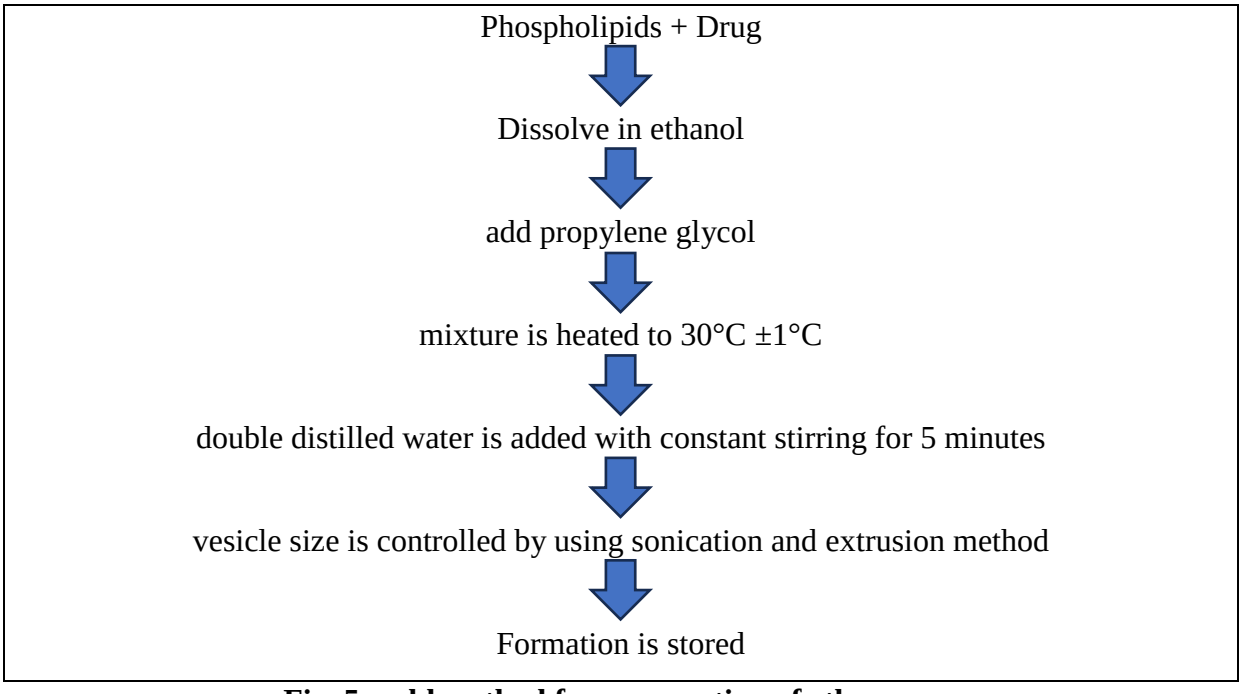

Fig. 5: cold method for preparation of ethosomes.

2.3.2. Hot method- The method involves heating phospholipid in water bath at 40°C to form a colloidal solution, then mixing ethanol and propylene glycol at 40°C in separate vessel. The organic phase is added to the aqueous solution, and the drug is dissolved in water or ethanol based on its hydrophilic/hydrophobic properties. The vesicle size of the Ethosomal formulation can be adjusted using probe sonication or extrusion methods (Figure 6).[29][30]

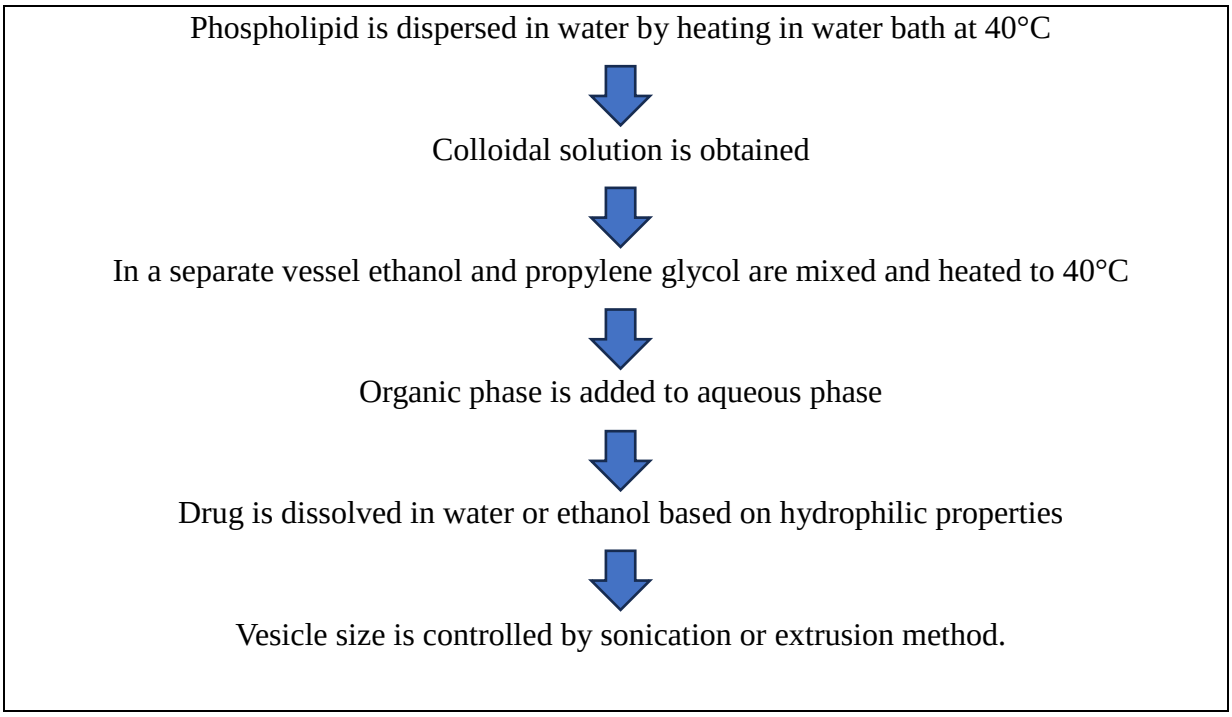

Fig. 6: Hot method for preparation of ethosomes.

2.4. CHARACTERISATION OF ETHOSOMES

2.4.1. Visualisation-

Ethosomes can be visualized using transmission electron microscopy (TEM) and scanning electron microscopy (SEM).[31]

2.4.2. Vesicle size and zeta potential-

The ethosome suspension's zeta potential can be measured using a Zeta meter, and particle size can be detected using dynamic light scattering and photon correlation spectroscopy.[32][33]

2.4.3. PH measurement

The pH of the formulation was measured using a pH meter by immersing the glass electrode in the semisolid formulation.[34]

2.4.4. Transition temperature

Differential scanning calorimetry can be used to determine the transition temperature of vesicular lipid systems.[35]

2.4.5. Drug entrapment-

The ultracentrifugation technique is used to measure the entrapment efficiency of ethosomes.[36]

2.4.6. Drug content

The drug can be quantified using a modified high-performance liquid chromatographic method and a UV spectrophotometer.[37]

2.4.7. Surface tension measurement

The Du Novy ring tensiometer is a ring method utilized to measure the surface tension of a drug.[38]

2.4.8. Skin permeation studies

The ethosomal preparation's penetration into skin layers can be assessed using Confocal Laser Scanning Microscopy (CLSM).[39]

2.4.9. Stability measurement

The stability of ethosomes was assessed through TEM visualization and DLS size determination at different intervals following vesicle preparation.[40]

2.5. EVALUATION OF ETHOSOMES

2.5.1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy

The process involves applying a vesicle suspension to a 50 nm filter membrane in diffusion cells, with the upper side exposed to air and the lower side in phosphate buffer saline solution. After 1 hour, filters are fixed in Karnovsky's fixative and dehydrated with graded ethanol solutions. The filters are then coated with gold and examined in SEM.[41]

2.5.2. Skin Permeation Studies

The study involved removing the hair from test animals and separating the abdominal skin from the connective tissue. The skin was then placed on aluminium foil and the dermal side was gently teased off for any adhering fat or subcutaneous tissue. The diffusion cell and receptor cell volume were maintained at 32°C ± 1°C, and the receptor compartment

contained phosphate buffer saline solution. The skin was mounted between the donor and receptor compartments, and an ethosomal formulation was applied to the epidermal surface. Samples were withdrawn at different intervals and analysed using a high-performance liquid chromatography assay.[22][42]

2.5.3. Stability Studies

The stability of vesicles was assessed by storing them at 4°C ± 0.5°C, and their size, zeta potential, and entrapment efficiency were measured after 180 days.

2.5.4. Vesicle-Skin Interaction Study by TEM and SEM

Ultra-thin sections from animals were cut, collected, and examined under a transmission electron microscope. After dehydration, the sections were mounted on stubs and coated with gold palladium alloy. The sections were then examined under a scanning electron microscope.[43]

2.5.5. Vesicle-Skin Interaction Study by Fluorescence Microscopy

The study used fluorescence microscopy for TEM and SEM analysis, using paraffin blocks and 5-µm thick sections cut using a microtome. Cytotoxicity was assessed using MT-2 cells propagated in Dulbecco's modified Eagle medium containing 10% fetal calf serum, penicillin, streptomycin, and glutamine. Cytotoxicity was measured as the cytotoxic dose 50 (CD50), resulting in a 50% reduction of absorbance at 540 nm.[44]

2.5.6. Drug Uptake Studies

The study involved transferring a drug into MT-2 cells in 24-well plates, adding 100 µL of RPMI medium. The cells were then incubated with the drug solution in phosphate buffer saline solution, Ethosomal formulation, or marketed formulation, and its uptake was determined using HPLC assay.[45][46]

2.5.7. HPLC Assay

The study determined the amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cells using HPLC assay. The drug was eluted in a C-18 column at room temperature and monitored at 271 nm using a SPDM10A VP diode array UV detector. The coefficient of variance ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968.[47]

2.6. APPLICATION OF ETHOSOMES AS A DRUG CARRIER

2.6.1. Delivery of Anti-Viral Drugs

Zidovudine is an antiviral agent that can increase transdermal flush and prolong release, acting on acquired immunodeficiency virus.[48]

2.6.2. Transdermal Delivery of Hormones

Hormone administration in the oral cavity can cause issues like high metabolism, low bioavailability, and dose-dependent side effects. Ethosomes can reduce these issues and improve drug permeation through the skin [e.g.: Testosterone (Testoderm patch)].[48][49]

2.6.3. Delivery of Anti-Arthritis Drug:

Lodzki et al. prepared a CBD ethosomal formulation for transdermal delivery, demonstrating increased skin penetration and activity of the drug for treating rheumatoid arthritis.[50]

2.6.4. Cosmaceutical Applications of Ethosomes

The use of ethosomes in cosmaceuticals extends the stability of cosmetic chemicals and reduces skin irritation, particularly for percutaneous sweetening in elastic forms. The compositions and sizes of vesicles are crucial factors for obtaining these benefits in cosmaceutical applications, particularly in elastic forms.[51]

2.6.5. Delivery of Antibiotics

Ethosomes penetrate the epidermis, bringing drugs into deeper skin layers and suppressing infection. Bacitracin and erythromycin-loaded ethosomal formulations for dermal and intracellular delivery were developed, revealing their penetration into cellular membranes and release of entrapped drug molecules.[52]

2.6.6. Delivery of Anti-Parkinsonism Agent

Dayan and Touitou developed an ethosomal formulation of trihexyphenidyl hydrochloride (THP), a psychoactive drug used in Parkinson's disease treatment. The study found that the ethosomal-THP formulation had better skin permeation potential, making it suitable for better Parkinson disease management.[53]

2.6.7. Delivery of problematic drug molecules

Oral delivery of large biogenic molecules like peptides, proteins, and insulin is challenging due to their degradation in the GIT tract, making transdermal delivery a better alternative.

However, conventional transdermal formulations have poor permeation, and ethosome formulations significantly increase permeation and therapeutic efficacy.[54]

Table no. 2: Effective transdermal delivery of Therapeutic active via novel vesicular carriers.

Drug	Therapeutic Indication	Vesicular Carrier	Inference
Celecoxib	Inflammation	Transfersomes	Therapeutically effective delivery for the treatment of rheumatoid arthritis.[55]
Itraconazole	Fungal infection	Liposomes	Enhanced transdermal permeation for the effective treatment of topical infection.[56]
Nystatin	Fungal infection	Transfersomes	Higher drug accumulation in the skin.[57]
Ceramide	Skin care (moisturizer)	Liposomes	Enhanced permeation of ceramides *via* liposomes.[58]
Cinnamic acid	Cancer	Transfersomes	Enhanced transdermal delivery of cinnamic acid and these vehicles penetrate the skin in the complete form.[59]
Lidocaine Hydrochloride	Pain and itching	Liposomes	Effective drug release. Safe, non-toxic, can penetrate the skin effectively.[60]
Curcumin	Cancer	Transfersomes	Higher permeation of drug from transfersomal gel.[61]
Cisplatin	Cancer	Transfersomes	Better drug penetration.[62]
Felodipine	Hypertension	Transfersomes	358.42% relative bioavailability of felodipine versus oral administration, supported by the outcomes of confocal laser scanning microscopic studies that suggested rapid drug permeation.[63]
Indomethacin	Rheumatoid arthritis	Liposomes	Enhanced transdermal delivery for the treatment of rheumatoid arthritis.[64]
Diclofenac	Inflammation	Elastic Liposomes	Better efficacy observed in elastic liposomes in contrast to conventional carriers.[65]
Clotrimazole	Fungal infection	Elastic Liposomes	Sustained release and higher skin permeation with enhanced anti-fungal activity.[67]
Clonazepam	Depression	Liposomes	Improved skin permeability.[66]
Propranolol Hydrochloride	Hypertension	Elastic Liposomes	Efficient in improvising drug delivery in contrast to rigid vesicular carriers.[68]

3.0. DISCUSSION AND CONCLUSION

New possibilities and challenges are emerging in the development of advanced therapies thanks to ethosomal carriers. Ethosomes, flexible and deformable vesicles, are promising vehicles for drug delivery. Their ease of preparation, safety, and effectiveness make them

attractive candidates. Ethosomes can be tailored to enhance the skin penetration of drugs. Compared to liposomes or hydroalcoholic solutions, ethosomes have proven to be more successful in delivering drugs to the skin. It is evident that ethosomes offer superior skin penetration compared to liposomes. Ethosomes can significantly overcome the epidermal barrier, which has been the primary obstacle in transdermal drug delivery systems. Using ethosomes as drug carriers offers benefits in treating skin diseases. Ethosomes enhance the penetration of drugs through the skin and enable delivery to deeper layers, improving the effectiveness of treatments.

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