WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 9, 250-267. Review Article ISSN 2277– 7105

POTENTIAL TRANSDERMAL DELIVERY OF HERBAL DRUG VIA ETHOSOMAL SYSTEM FOR THE TREATMENT OF VARIOUS DISEASES

Alok Debnath*, Shaweta Sharma and P. K. Sharma

Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Plot No.2, Sector 17-A, Yamuna Expressway, Greater Noida, Gautam Budh Nagar, Uttar Pradesh 201310.

Article Received on
23 June 2020,

Revised on 14 July 2020,
Accepted on 04 Aug 2020,

DOI: 10.20959/wjpr20209-18349

***Corresponding Author**
Alok Debnath
Department of Pharmacy, School of medical and Allied Sciences, Galgotias University, Plot No.2, Sector 17-A,Yamuna Expressway, Greater Noida, Gautam Budh Nagar, Uttar Pradesh 201310.

ABSTRACT

Background: Ethosomes are transdermal drug delivery system and preferred over other liposomal drugs pertaining to high permeation through skin. The increased permeation is caused due to use of ethanol, which provides important benefits such as improved drug delivery, patient compliance, comfort, easy to administer. **Main Text:** Ethanol's have unique structure which helps in encapsulating highly lipophilic molecules such as cannabinoids, testosterone and minoxidil, and some cationic drugs such as propranolol, trihexaphenidyl, Cyclosporine, insulin, salbutamol etc. This types of enhanced drug delivery of bioactive molecules through skin by means of ethosomal carrier cause numerous challenges and opportunities for future development. This review is focused on Advantage, method of preparation, application, evaluation, herbal product and recent patents of Ethosomes. **Conclusion:** Ethosomes are more stable than other conventional liposomes. They are safe, have more efficacy and long-term stability, and can be manufactured easily. Ethosomes is a new trend of transdermal drug delivery and is successful in delivering through skin. In this system, side effects are minimized and it has better penetration ability. The ability to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides has opened new challenges and opportunities for development of novel improved therapies on the market. Considerable research has been conducted to characterize the novel non-invasive carrier. Such development may further increase the range of transdermal applications.

1. INTRODUCTION

Transdermal drug delivery system (TDDS) replaced oral drug delivery systems, as it eliminat es gastrointestinally interferences and presystemic metabolism. Thus only the lipophilic drugs having low molecular weight (< 500 Da) so they can pass through it.[1,2] Various methods have been adopted by different researchers to enhance the permeability of medicines through the skin, including chemical and physical improvement such as iontophoresis, sonophoresis, etc. The drug's permeability through stratum cornea barrier was also improved by liposomes, niosomes, transferosomes and ethosomes. Unlike classic liposomes [3], ethosomes, which are known for supplying drugs to the outside layers of skin, can increase permeation through the barrier of the stratum.[4,5] Ethosomes penetrate the skin quicker and have much greater transdermal flux than standard liposomes.[6,7] Transdermal delivery is an alternative to oral medicines and an alternative to hypodermic injections.[8-12]

2. ETHOSOMES

"Ethosomes are ethanolic liposomes".Ethosomes are non-invasive delivery carriers that reaches deep into the skin. The vesicles are used in cellular communication for transportation. It is also used for the long term to regulate the release frequency of drugs. The major advances in vesicle research is to find the vesicle derivative called Ethosomes.[9]

Ethosomes are modified established drug carrier liposomes. It includes elevated concentrations of phospholipids, alcohol and water. Ethosomes are made of phospholipids, ethanol, and water and are soft vesicles. Ethosomes are of different sizes ranges from nanometres (nm) to microns (µ). Ethosomes have higher transdermal flux.

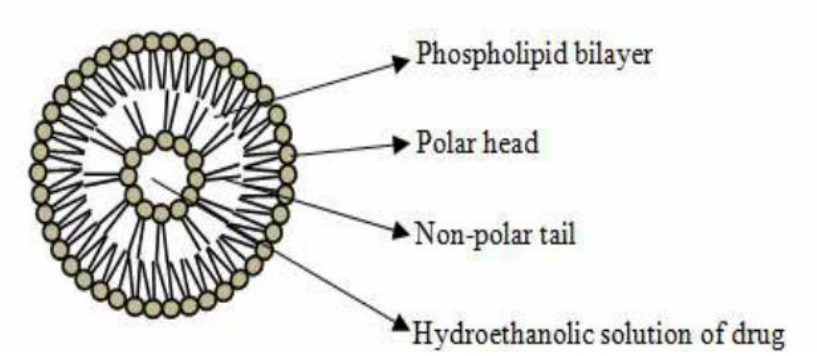

Fig 1: Diagram showing structure of Ethosomes.[7]

Barupal et al. (2010) prepared Ethosomes to explore dermal administration of aceclofenac, which confirms the high loading capacity and stability of ethosomes.

It was concluded that –High drug loading capacity and concentration of both ethanol and phospholipid are essential factors to get better entrapment efficiency

Table 1: Ingredients required for formulation of Ethosomes.[13]

Class of Polymer	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol, Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorence(FITC) 6-Carboxy fluoresence	For Characterization study
Vehicle	Carbopol 934	Gel forming agent

3. DRUG PENETRATION THROUGH ETHOSOMES

The unique feature of ethosomes is its ability to penetrate through the stratum corneum pertaining to high ethanol concentration. Moreover, it can also move deeper into small spaces through the loosely packed lipid multilayer while the conventional vesicles are unable to do that inspite of having similar stability.[14,15]

Ethanol interact with the polar heads of lipid layers, reduces its tightness, and increase the flexibility of layer. It also aids in permeation by disturbing the stratum corneum barrier.[16,17]

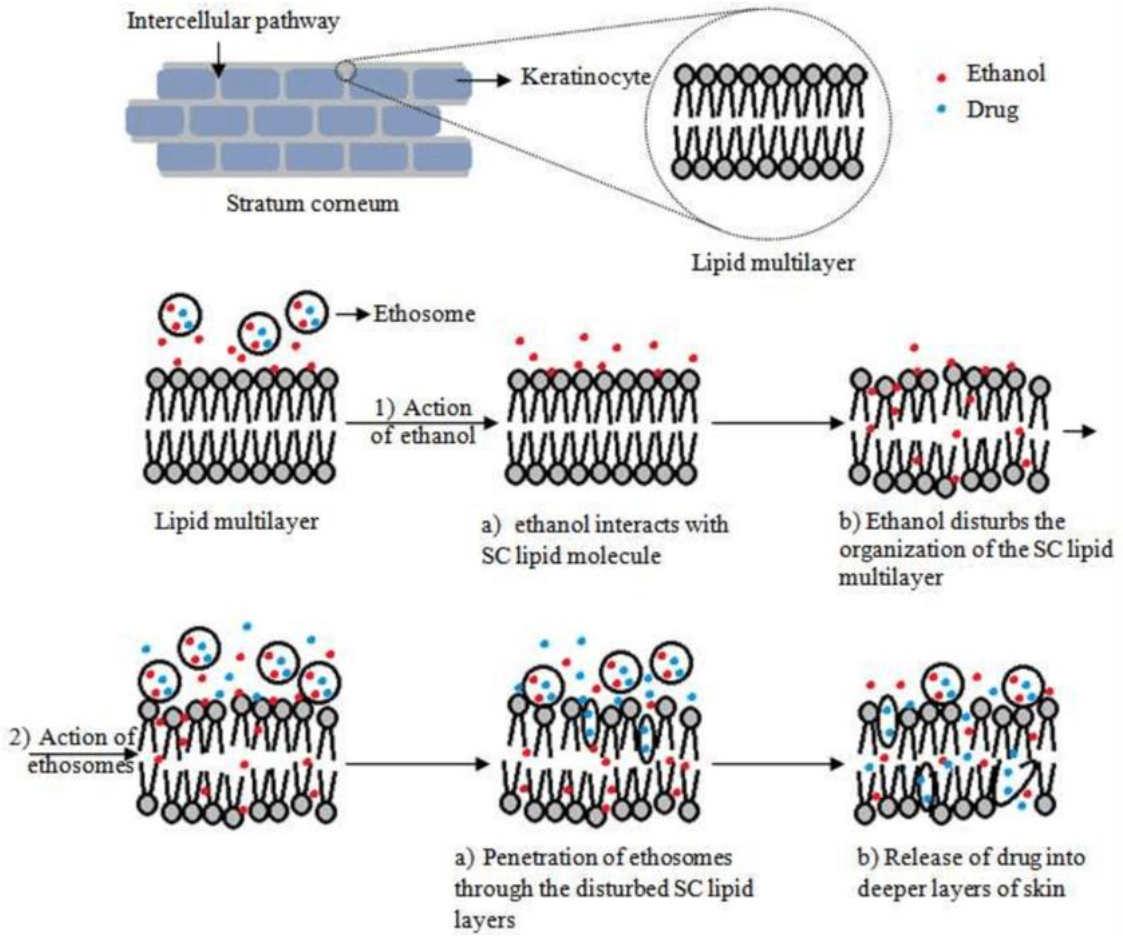

Fig 2: Mechanism of Skin Permeation and Drug delivery by Ethosomes (R Rakesh et al).

Table 2: Comparison between Liposomes, Transferosomes and Ethosomes.[18]

Characters	Liposome	Transferosmes	Ethosomes
Vesicles	Bilayer lipid vesicle	2nd generation elastic lipid vesicle carriers	3rd generation elastic lipid vesicle carrier
Composition	Phospholipids and cholesterol	Phospholipids and edge activator	Phospholipids and ethanol
Characteristics	Microscopic spheres vesicles	Ultra flexible liposome	Elastic liposome
Flexibility	Rigid in nature	High deformability	High deformability and elasticity
Permeation Mechanism	Diffusion/Fusion/Lipolysis	Deformation of vesicle	Lipid perturbation
Extent of skin permeation	Penetration rate is very less	Can easily penetrate through paracellular space	Can easily penetrate through paracellular space
Route of administration	Oral, parenteral, topical, and transdermal	Topical and transdermal	Topical and transdermal

4. METHODS OF PREPARATION

Ethosomes are prepared by two methods viz. cold and hot techniques. Classical mechanical dispersion and active membrane methods are also reported by some researchers cold method is widely used for the preparation of ethosomes.

Cold method

In a coated container, phospholipid are dissolved in ethanol at room temperature. P.G (propylene glycol) and other polyols are added while stirring and this mixture is heated at 30^0 C. Drug is dissolved in aqueous or organic media according to its hydrophilic/lipophilic characteristics. Resulted ethosomal suspension was stirred and cooled at room temperature for further 5 minutes. Vesicle structure can be modulated by extrusion or sonication method. Prepared formulation must be stored at under cooling condition.[19, 20]

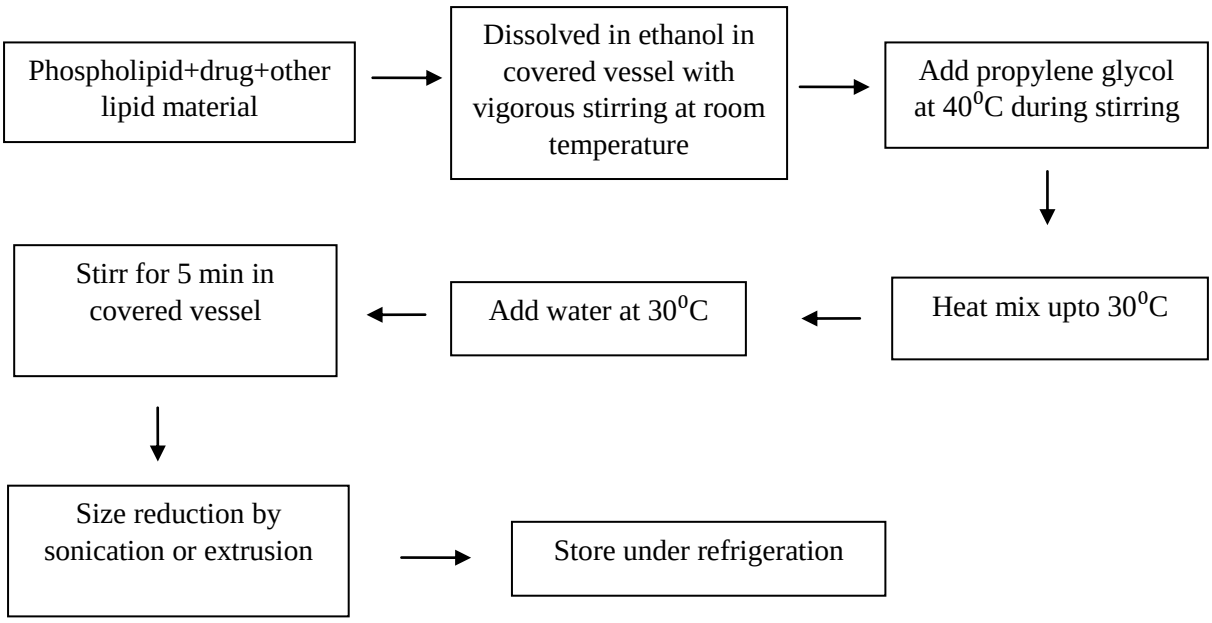

Fig 3: Cold method for the preparation of Ethosomes.

Hot method

Phospholipid are spread in water to form a colloidal solution and heated on water bath at 40^0 C. Ethanol and glycols are also heated at same temperature in a separate vessel. Organic phase was added to aqueous phase at once when temperature reaches at 40^0 C and heating was continued for another 5 min Resultant ethosomal suspension is cooled at room temperature. Drug is dissolved in one of the phase according to the hydrophilic or lipophilic properties. Vesicle modulations are performed by the method of sonication or extrusion. [21]

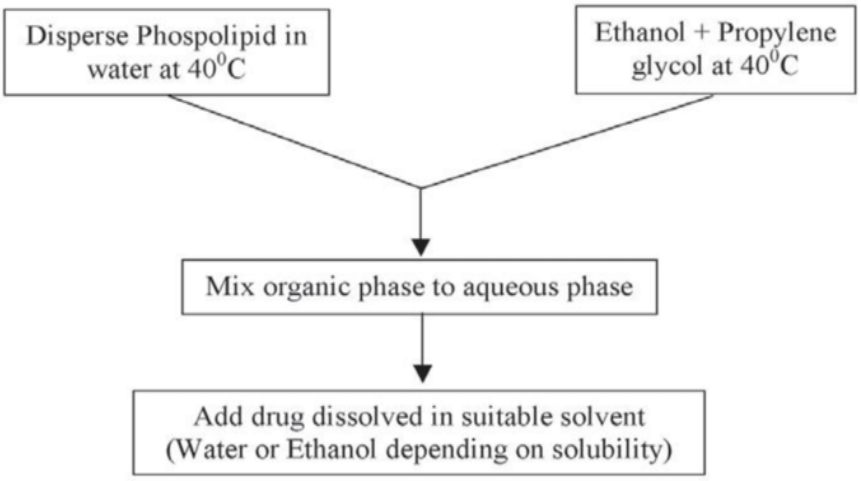

Fig 4: Hot method for the preparation of Ethosomes.

Classic mechanical dispersion method

Phospholipid is dissolved into an organic solvent or its blend in a round bottom flask (RBF). Retire the organic solvent from the wall of the RBF by means of a rotary vacuum evaporator above fat transition temperature. Through keeping the content in vacuum overnight, traces of solvent from the deposited lipid film should be removed. The lipid film is then hydrated with the hydroethanol solution of the drug by turning the vial at the appropriate temperature and then cooling down at room temperature. Ethosomes must be kept under cooling.[22]

5. BENEFITS OF ETHOSOMAL DRUG DELIVERY SYSTEM

1. Large molecules can be supplied (peptides, protein molecules).
2. The formulation includes non-toxic raw material.
3. Ethosomes enhances the permeation of drug through skin.
4. Now a day in Pharmaceutical, Veterinary, Cosmetic fields, ethosomal preparations are widely used.
5. Patient compliance is better than other products.
6. This method is simpler than other complicated methods such as Iontophoresis and Phonophoresis.
7. The Ethosomal system is passive and non-invasive and can be sold immediately.[23]

6. CHARACTERIZATION OF ETHOSOMES[24]

There are different type of methods of characterization of Ethosomes, they are as follows-

- **Physical Characterization:** Different software are used to find out is it prepared in an economical way or not. (Motic Image plus,)&Malvern Zetasizer.

- **Visualization:** TEM & SEM are used.[14]
- **Vesicle size:** Dynamic Light Dispersing (DLS) and Photon Correlations Spectroscopy (PCS) are used to determine the size of the particles.
- **Entrapment Efficiency:** Ultracentrifugation technique is used to find out entrapment efficiency.
- **Transition Temperature:** Differential Scanning Calorimetry (DSC) is used to find out the transition temperature in vesicle system.[23]
- **Surface Tension:** The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.[23]
- **Vesicle stability:** DLS & TEM are used to find out the vesicle stability.[25]
- **Drug content:** HPLC is used to determine the drug content.[26]
- **Penetration & Permeation studies:** The penetration and permeation studies are based on Confocal Laser Scanning Microscopy (CLSM).[27]

7. APPLICATIONS OF ETHOSOMES[28]

- Ethosomes have different type of applications and can be used for various purposes in drug delivery system. Ethosomes can be used in place of liposomes. Ethosomes have better penetration ability thus it is used in transdermal drug delivery. Ethosomes are used by skin penetration in hydrophilic and impermeable medicines. Different types of drugs are given with the help of ethosomal carrier.[25]
- Ethosomes are used in cosmeceuticals, which increases the stability of the product and lower the skin irritation caused due to cosmetic chemicals, but the main factor is the composition and the vesicles sizes.[29]
- Ethosomes are used for anti-arthritis drug because it overcomes the conventional oral therapy problems.
- Ethosomes are used in transdermal delivery of hormones as it by pass the first pass metabolism.
- Ethosomes are used in Herpetic infection in which 5% acyclovir is used.
- Ethosomes are used to deliver the drug molecules, which is difficult to administer with other conventional dosage forms, but conventional transdermal product, have poor permeation. So formulation of these product increase the permeation and efficacy of the product.[30]

- Ethosomes are used in transcellular delivery.[14] A study demonstrated better intracellular uptake of bacitracin, DNA and erythromycin using CLSM and FACS techniques in different cell lines. Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from Ethosomes as compared to the marketed formulation suggested that ethosomes were attractive clinical alternative for anti-HIV therapy.

8. RECENT ADVANCES IN ETHOSOMAL SYSTEM

Table 3: Patents related to ethosomal drug delivery system.

S.No.	Title	Patent No.	Year
1.	Chinese medicinal Ethosomes herpes gel patch for treating zoster	CN103536700 (A)	2014
2.	Leflunomide Ethosomes composition and its preparation method	CN103800277	2014
3.	Ethosomes gel film coating agent with multiple wound repair effects.	CN103893394 (A)	2014
4.	Bullatacin Ethosome gel	CN102552147 (A)	2012
5.	Daptomycin Ethosome preparation	CN103006562 (A)	2013
6.	Ethosome preparation of male hormone medicaments.	CN102406605 (A)	2012
7.	Lidocaine Ethosomes	CN102813624 (A)	2012
8.	Paclitaxel ethosome gel	CN102579323(A)	2012
9.	Progesterone ethosome,	CN102397255(A)	2012
10.	Acyclovir Ethosomes	CN102133183 (A)	2011
11.	Podophyllotoxin Ethosomes	CN102144972(A)	2011

Rakesh R et al (2012) examined the feasibility of transdermal cromolyn-sodium delivery using a novel lipid-vein carrier ethosome. *In-vitro* drug releasing study reveal that optimized cellophane-based ethosomal formulations showed improved and prolonged drug delivery compared to standard liposomes. The results showed that ethosomes could be a successful carrier for cromolyn sodium transdermal delivery.[31]

Saxena Gk et al (2010) worked on the transdermal supply of hydrophobic medication through ethosomes. Results suggested that Ethosomes can boost the transdermal flux and prolong release and serve as an appealing route for continuous stavudine delivery.[32]

Nikalje AP et al (2012) focused on the review of Ethosomes as a novel tool for transdermal drug delivery. The study showed the preparation, benefits, structure, the characterisation and assessment of Ethosomes. Ethosomal carrier allow the creation of new, enhanced therapies.[33]

Zhen Z et al. (2012) proposed the study to characterize a novel transdermal delivery system i.e. Ethosomes containing 5-fluorouracil. Ethosomes with the use of Confocal Laser Scanning Microscopy have been evaluated for penetration in the skin and the Hyper Tropical Scar (HS). After a 24-hour implementation, the intensity of fluorescence was highest in Ethosome-Scar followed by Ethosome-Skin. The resulted proposed HS as an efficient carrier for ethosomes.[34]

Ferrara et al (2004) have focused on the comparative preformulation evaluation of Ethosomes and liposomes of Azelaic acid. The findings showed that the release rate from the ethosomes was faster than the liposomal structures. It has been found that Ethosomes with the largest ethanol level released faster than others.[35]

Xingyan Liu et al (2011) developed a stable formulation with good entrapment efficiency, release rate, and transdermal absorption. The study revealed that ligustrazine patches could improve absorption and bioavailability of drug in comparison to standard administration of ligustrazine.[36]

Sarat CC et al (2012) examined the comparative assessment sonicated and un-sonicated Ketoconazole encapsulated Ethosomes Drug encapsulated ethosomes were prepared using "Hot" method technique. For Ethosomes comprising 30 percent sonic ethanol in vitro drug release of Ketoconazole was enhanced. Evaluation trials revealed improved characteristics with growing levels of ethanol and vesicles subjected for sonication through advanced Ethosomes of ketoconazole.[37]

9. HERBAL ETHOSOMES

Table 4: Formulation of herbal ethosome.

Botanical Source	Formulation	Biological activity	Active Ingredients	Drawback of traditional Dose	Application of emulsion formulation	Method of preparation
Glycyrrhiza glabra	Ammonium Glycyrrhizinate Ethosomes	Anti-Inflammatory	Glycyrrhizic Acid	Poor permeability	Increase of *in vitro* percutaneous permeation and significantly enhanced anti-inflammatory activity	Solvent dispersion method
Tripterygium wilfordi	Triptolide	Anti-inflammatory	Diterpene Tricpoxide	Poor water solubility and toxicity	High entrapment efficiency, good percutaneous permeability	combining filming rehydration method ultrasonic method
Podophyllum hexandrum	Podophyllotoxiin	Purgative,antirheumatic,antiviral and antitumor	Etoposide and Teniposide	Slow pharmacological action	Higher entrapment efficiency and enhance its therapeutic effect	Solvent dispersion method
Sesbania grandiflora	Sebania Ethosomes	Anti -microbial	Leucocyanidine and cyaniding	Poor permeability	Enhanced transdermal permeation	Solvent dispersion method
Sophora alopecuroides	Sophora Ethosomes	Antiendotoxic,anti-cancer and anti-inflammatory	Sophocarpine, matrine, oxymatrine, Sophoridine	Low percutaneous penetration and bitter taste	Enhanced drug delivery and stability	Transmembrane pH gradient active loading method
Sophora flavesens	Matrine Ethosomes	Cardioprotective,Anti-inflammatory	Matrine and oxymatrine alkaloids	Lower bioavailability	Improve percutaneous permeation	Solvent dispersion method

9.1. Sea buckthorn leaf

SBT are collected from its region such as Leh, Ladakh, India then it is authenticated and see that if they were analytical standard. It is extracted by cold percolation method to prepare 75% ethanolic extract of dried SBT leaves.[38]

9.2. Green tea leaves

Green tea leaves have many benefits such as it contain polyphenol from flavonoid groups and it is catechin type. Catechin can help in the treatment of damage skin and it has anti-inflammatory and antioxidant activity.[39]

9.3. Glycyrrhiza glabra

It is a perennial herbaceous agent that has been used for thousands of years as flavouring in food and medicinal products. The root of Licorice has been commonly used in the globe since ancient times to treat cough. The substance includes active substances, such as glycyrhizin, glycyrrhtinic acid, flavonoids and chalcones. Because of their steroid-like structure, the primary active compounds are the glycyrrhizin and glycyrrhetinic acid and are powerful inhibitors of cortisol metabolism. Cough, colds, asthma and COPD have been treated by using the root of this plant.[40] Glycyrrhizine is the main active substance produced by plant G, triterpenic glycoside. In an Ovalbumin-induced experimental asthma mouse model, glycyrrhizine alleviated asthma allergic. It also decreased IgE concentrations of OVA specifics and the IgG2a complete serum upregulation. These findings suggested that IgE-stimulating cytokines decreased glycyrhizine interfered with manufacturing.

9.4. Zingiber officinale

Zingiber officinale is a dietary component frequently referred to as ginger. Plant rhizome is used for cold, asthma and bronchitis treatments.[40] Antibacterial activity is present in the ethanolic extract from the ginger rhizome to control respiratory pathogens. Fresh ginger is antiviral in the formation of plaque on airway epithelium, caused by human pulmonary syncytial virus.[41] The ginger-enhanced diet showed a reduced serum level of inflammatory cytokines IL-1, IL-6 and TNF-α in a research of patients living with acute respiratory distress syndrome.[42] In addition, Inhibited the production of LPS stimulated macrophages by Gingerol, an active component of ginger from TNF-α, IL-1β and IL-12.[40,43]

9.5. Piper longum

Piper longum (family Pipperaceae) is a significant for traditional medication used in Asia and the Pacific islands, for the treatment of tuberculosis and respiratory tract diseases.[44] In the therapy of asthma infantile, the fruits and roots of this plant are used.[45, 46] In experimental animal research, the alcoholic extract of P. longum fruit showed immunomodulatory potential.[47] Piperine is a significant isolated alkaloid in P. longum fruits and has been reported to inhibit the release into an ovalbumin-induced asthma model of Th-2 mediated cytokines, eosinophilic infiltrations and airway hyper responsiveness.[45]

9.6. Solanum xanthocarpum

The annual herb-based plant is Solanum xanthocarpum, which in traditional Indian medicine is often referred to as kantkari. It is mentioned as an asthma and bronchitis therapy in Ayurveda. Beer juice has reportedly been helpful for sore throat. Solanum xanthocarpum has been used in the medical scheme of Siddha, especially in the southern portion of India, for the therapy of respiratory diseases. Powder treatment of entire S crops. The distinct parameters of asthmic lung function trials were enhanced by xanthocarpum.[48]

9.7. Tylophora indica

Tylophora indica (family Apocynaceae), a permanent climbing plant used in the Ayurveda Medication System. The plant leaves have been used widely to treat multiple allergic and inflammatory conditions such as bronchial asthma, bronchitis, and bipolar fever. The potential of Indica is anti-asthmic and anti-allergic.[49]

Plastic extracts inhibited the response of Schultz-Dale and systemic anaphylaxis in pigs.[50] The cellular immune responses were inhibited in experimental designs by the indica leaves.[51]

Tylophorin is a significant T-plant alkaloid. In experimental research, anti-inflammatory, antiasthmatic, and antianaphylactic potential have been recorded.[52]

9.8. Bryophyllum pinnatum or Kalanchoe integra

The Crassulaceae family showed multiple pharmacological events, such as anthelmintic, immunosuppressive, wound healing, hepatoprotective, anti-inflammatory, antidiabetic, anti-oxide, antimicrobial, analgesic, antipyretic and anti-pyretic activities. Boiled leaf extracts are helpful for the control of acute or chronic bronchitis, pneumonia, bronchial asthma or

palpitation in respiratory illnesses. Flavonoids and Tannins had positive impacts in bronchial asthma management, and scientists have demonstrated anti-histamine and anti-anaphylactic impacts of Kalanchoe species.[39] It could thus be a potential nutraceutical for the management of airway remodelling of bronchial asthma, with anti-inflammatory activity.[53]

10. STABILITY OF ETHOSOMES

Ethosomes have much more stability than other conventional liposomes.[54] Leakage is the major problem associated with ethosomes as well as they fuse together to grow large vesicles.[55]

11. FUTURE ASPECTS OF ETHOSOMES

In ethosomal preparation, special focus done in skin delivery of protein and other macro particles. Novel Therapeutic technology (NTT) increase in demand for biopharmaceutical products for treating deep skin infection, herpes, hormone deficiency, inflammatory disorder, atopic dermatitis and erectile dysfunctioning.

CONCLUSION

Ethosomes are more stable than other conventional liposomes. They are safe, have more efficacy and long-term stability, and can be manufactured easily. Ethosomes is a new trend of transdermal drug delivery and is successful in delivering through skin. In this system, side effects are minimized and it has better penetration ability. The ability to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides has opened new challenges and opportunities for development of novel improved therapies on the market. Considerable research has been conducted to characterize the novel non-invasive carrier. Such development may further increase the range of transdermal applications.

REFERENCES

1. 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.
2. 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.
3. 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.

4. 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.
5. Kumar R., Aslam MD., Tripathi A., Prasad D., Chaudhary V., Jain V., Mishra SK., Singh R., Ethosomes: Novel Vesicular Carriers in Transdermal Drug Delivery, Journal of Global Pharma Technology, 2010; 2(6): 1-7.
6. 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): 385390.
7. Shahwal V., Samnani A., Dubey B., Bhowmick M., Ethosomes: An Overview, International Journal of Biomedical and Advance Research 2011; 2: 161-168.
8. Guy RH, Hadgraft J, editors. New York: Marcel Dekker; 2003. Transdermal Drug Delivery.H. Razavi, S. Janfaza, J. Paramed. Sci. Spring., 2015; 6(2): 38-43.
9. Williams A. London: Pharmaceutical Press; 2003. Transdermal and Topical Drug Delivery.Xiao-yu L, Yue-feng R, Wen-quan L. Study on transdermal penetration of ethinyl estradiol ethosome gel. Chinese Pharm J., 2006; 41: 284-286.
10. Prausnitz MR, Mitragotri S, Langer R. Current status and future potential of transdermal drug delivery. Nat Rev Drug Discov, 2004; 3: 115–124.
11. Autepp, Kamble Ms, Chaudhari Pd, Bhosale Av. A Comprehensive Review On Ethosomes. International Journal Of Research And Development In Pharmacy And Life Sciences, 2012-13; 2(1): 218-224.
12. Choi MJ, Maibach HI. Elastic vesicles as topical/transdermal drug delivery systems. International Journal of Cosmetic Science, 2005; 27(4): 211–221.
13. Anju Dhiman et al., Potential Phytotherapeutic Agents in Design of Ethosomes: A Review, Journal of Pharmaceutical and Scientific Innovation, Sept.-Oct., 2012; 26-30.
14. Tauitou E et al., Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties, J Con Release, 2000; 65: 403-413.
15. Carl, S., Jakob, T.M., Annette, G., Klaus, E.A., Ann-Therese, K., Charlotte, A.J., Marica, B.E., A Study of The Enhanced Sensitizing Capacity of A Contact Allergen In Lipid Vesicle Formulation, Toxicol Appl Pharmacol, 2011; 252: 221-227.
16. Jain S, Bhandra D, Jain S and Jain N K. TransfersomesA Novel carrier for effective transdermal drug delivery controlled and novel drug delivery 1st Edition, CBS Publishers and Distributors, New Delhi, 1997; 426-451.

17. Bronaugh RL, Maibach HI, editors. Edn. 4th. New York: Marcel Dekker; 2005. Percutaneous Absorption.
18. Sudhakar CK, Jain S, Charyulu RN. A Comparison Study of Liposomes, Transfersomes and Ethosomes Bearing Lamivudine. International Journal of Pharmaceutical Sciences and Research, Oct 1, 2016; 7(10): 4214.
19. Shumilov M, Touitou E. Buspirone transdermal administration for menopausal syndromes, in vitro and in animal model studies. International journal of pharmaceutics, Mar 15, 2010; 387(1-2): 26-33.
20. Bhalaria Mk, Naik S, Misra An.Ethosomes: A Novel Delivery System For Antifungal Drugs In The Treatment Of Topical Fungal Diseases. Indian Journal Of Experimental Biology, 2009; 47: 368-375.
21. Sheer A, Chauhan M. Ethosomes as vesicular carrier for enhanced transdermal delivery of Ketoconazole-Formulation and Evaluation. IJPI's Journal of Pharmaceutics and Cosmetology, 2011; 1(3): 1-4.
22. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. Journal of controlled release, Nov 6, 2007; 123(2): 148-54.
23. Patel S. Ethosomes: A promising tool for transdermal delivery of drug. Pharma Info. Net., 2007; 5(3).
24. Celia C, Cilurzo F, Trapasso E, Cosco D, Fresta M, Paolino D. Ethosomes® and transfersomes® containing linoleic acid: physicochemical and technological features of topical drug delivery carriers for the potential treatment of melasma disorders. Biomedical microdevices, Feb 1, 2012; 14(1): 119-30.
25. Van den Bergh BA, Swartzendruber DC, Bos- Van der Geest A, Hoogstraate JJ, Schrijvers AH, Boddé HE, Junginger HE, Bouwstra JA. Development of an optimal protocol for the ultrastructural examination of skin by transmission electron microscopy. Journal of microscopy, Aug, 1997; 187(2): 125-33.
26. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. Biomaterials, Sep 1, 2000; 21(18): 1879-85.
27. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. Journal of Investigative Dermatology, Jul 1, 2004; 123(1): 168-76.
28. Arshady R, editor. Microspheres microcapsules & liposomes: preparation & chemical applications. Citus Books, 1999.

29. Manosroi A, Jantrawut P, Khositsuntiwong N, Manosroi W, Manosroi J. Novel elastic nanovesicles for cosmeceutical and pharmaceutical applications. Chiang Mai J Sci., May 1, 2009; 36(2): 168-78.
30. Jain S, Jain P, Umamaheshwari RB, Jain NK. Transfersomes—a novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. Drug development and industrial pharmacy, Jan 1, 2003; 29(9): 1013-26.
31. Rakesh R, Anoop KR. Formulation and optimization of nano-sized ethosomes for enhanced transdermal delivery of cromolyn sodium. Journal of pharmacy & bioallied sciences, Oct, 2012; 4(4): 333.
32. Chauhan P, Tyagi BK. Herbal novel drug delivery systems and transfersomes. Journal of Drug Delivery and Therapeutics, May 14, 2018; 8(3): 162-8.
33. Parashar T, Sachan R, Singh V, Singh G, Tyagi S, Patel C, Gupta A. Ethosomes: A recent vesicle of transdermal drug delivery system. International journal of research and development in pharmacy and life sciences, Feb, 2013; 2(2): 285-92.
34. Zhang Z, Wo Y, Zhang Y, Wang D, He R, Chen H, Cui D. In vitro study of ethosome penetration in human skin and hypertrophic scar tissue. Nanomedicine: Nanotechnology, Biology and Medicine, Aug 1, 2012; 8(6): 1026-33.
35. Liu X, Liu H, Liu J, He Z, Ding C, Huang G, Zhou W, Zhou L. Preparation of a ligustrazine ethosome patch and its evaluation in vitro and in vivo. International journal of nanomedicine, 2011; 6: 241.
36. Mulani H, Bhise K. QbD Approach in the formulation and evaluation of Miconazole Nitrate loaded ethosomal cream-o-gel. Int. Res. J. Pharm. Sci., 2017; 8: 1-37.
37. Geetha S, Ram MS, Singh V, Ilavazhagan G, Sawhney RC. Anti-oxidant and immunomodulatory properties of seabuckthorn (Hippophae rhamnoides)—an in vitro study. Journal of Ethnopharmacology, Mar 1, 2002; 79(3): 373-8.
38. Cruz EA, Reuter S, Martin H, Dehzad N, Muzitano MF, Costa SS, Rossi-Bergmann B, Buhl R, Stassen M, Taube C. Kalanchoe pinnata inhibits mast cell activation and prevents allergic airway disease. Phytomedicine, Jan 15, 2012; 19(2): 115-21.
39. Ram A, Balachandar S, Vijayananth P, Singh VP. Medicinal plants useful for treating chronic obstructive pulmonary disease (COPD): current status and future perspectives. Fitoterapia, Mar 1, 2011; 82(2): 141-51.
40. San Chang J, Wang KC, Yeh CF, Shieh DE, Chiang LC. Fresh ginger (Zingiber officinale) has anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines. Journal of ethnopharmacology, Jan 9, 2013; 145(1): 146-51.

41. Shariatpanahi ZV, Mokhtari M, Taleban FA, Alavi F, Surmaghi MH, Mehrabi Y, Shahbazi S. Effect of enteral feeding with ginger extract in acute respiratory distress syndrome. Journal of critical care, Apr 1, 2013; 28(2): 217-e1.
42. Tripathi S, Maier KG, Bruch D, Kittur DS. Effect of 6-gingerol on pro-inflammatory cytokine production and costimulatory molecule expression in murine peritoneal macrophages. Journal of Surgical Research, Apr 1, 2007; 138(2): 209-13.
43. Mathur V, Satrawala Y, Rajput MS. Physical and chemical penetration enhancers in transdermal drug delivery system. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm., Aug 23, 2014; 4(3).
44. Dahanukar SA, Karandikar SM, Desai M. Efficacy of Piper longum in childhood asthma. Indian Drugs, Sep 10, 1984; 21(9): 384-8.
45. Savithramma N, Sulochana C, Rao KN. Ethnobotanical survey of plants used to treat asthma in Andhra Pradesh, India. Journal of Ethnopharmacology, Aug 15, 2007; 113(1): 54-61.
46. Sunila ES, Kuttan G. Immunomodulatory and antitumor activity of Piper longum Linn. and piperine. Journal of ethnopharmacology, Feb 1, 2004; 90(2-3): 339-46.
47. Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. A pilot study on the clinical efficacy of Solanum xanthocarpum and Solanum trilobatum in bronchial asthma. Journal of ethnopharmacology, Aug 1, 1999; 66(2): 205-10.
48. Ganguly T, Badheka LP, Sainis KB. Immunomodulatory effect of Tylophora indica on Con A induced lymphoproliferation. Phytomedicine, Jan 1, 2001; 8(6): 431-7.
49. Ganguly T, Sainis KB. Inhibition of cellular immune responses by Tylophora indica in experimental models. Phytomedicine, Jan 1, 2001; 8(5): 348-55.
50. Licciardi PV, Underwood JR. Plant-derived medicines: a novel class of immunological adjuvants. International immunopharmacology, Mar 1, 2011; 11(3): 390-8.
51. Gopalakrishnan C, Shankaranarayanan D, Nazimudeen SK, Kameswaran L. Effect of tylophorine, a major alkaloid of Tylophora indica, on immunopathological and inflammatory reactions. The Indian journal of medical research, Jun, 1980; 71: 940.
52. Julius AG, Charles A, Samuel NE, Ansong AD, Mahmood S, Samuel A, Kwame BN, Patrick A, Edwin N. Nutraceutical with Anti-Inflammatory Activity for the Management of Airway Remodeling in Bronchial Asthma: Kalanchoe integra Var. Crenata (Andr.) Cuf Leaf Extract. Pharmacology & Pharmacy, Mar 4, 2014; 2014.

53. Goti A, Patel V. Ethosomally Entrapped Clotrimazole: A View to Improve Therapeutic Response of Antifungal drug. InAAPS National Biotechnology Conference, Jun 23, 2007; (82).
54. Rakesh R, Anoop KR. Ethosomes for transdermal and topical drug delivery. Int J Pharm Pharm Sci., 2012; 4(3): 17-24.
55. Bansal S, Kashyap CP, Aggarwal G, Harikumar SL. A comparative review on vesicular drug delivery system and stability issues. Int J Res Pharm Chem., 2012; 2(3): 704-13.