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Formulation, Development and Evaluation of Invasomes Loaded Clotrimazole Gel for Fungal Treatment

Harshita Verma[1*], Dr. Pradeep Pal[2]

[1]Student, Mahakal Institute of Pharmaceutical Studies, 3VXW+P46, Dewas Road, Behind Air Strip, Datana, Madhya Pradesh 456664, India
[2]Associate Professor, Mahakal Institute of Pharmaceutical Studies, 3VXW+P46, Dewas Road, Behind Air Strip, Datana, Madhya Pradesh 456664, India

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***Corresponding author:** Harshita Verma
Student, Mahakal Institute of Pharmaceutical Studies, 3VXW+P46, Dewas Road, Behind Air Strip, Datana, Madhya Pradesh 456664, India

Abstract **Review Article**

The transdermal route is an important pathway for localized or systemic effects. The stratum corneum, the outer layer of the skin, is an essential skin permeation barrier for many drugs. To overcome this barrier, several techniques have been developed, including the use of the vehicle and nanocarriers to improve drug penetration. Recently, different types of nanocarriers have been designed to improve the dermal and transdermal delivery of medicines like 'INVASOME'. We made clotrimazole loaded invasome gel which is used in fungal treatment. The procedure used in formulation of invasomal gel is mechanical dispersion method. This preparation is evaluate by many parameters they are appearance, spreadability, solubility, in vitro drug diffusion. And also discuss application of invasome.

Keywords: Invasome Gel, Transdermal, Terpene, Vortex, Clotrimazole, Vesicle.

INTRODUCTION

The trasdermal delivery of drugs is rapidly increasing in the formulation development in enhancing the bioavailability of many drugs. When drugs are administered via transdermal route skin barrier is harmfully affected. In recent years, vesicular systems have been intensively studied as drug carrier systems for the dermal and transdermal administration of drugs. Traditional liposomal formulations, compared to conventional dosage forms, have shown in vitro an enhanced cutaneous drug accumulation allowing a reduction of the dose applied onto the skin. In the last two decades, new classes of lipid vesicles were introduced by different researchers. More recently, researchers investigated the novel vesicular systems called as invasomes. Briefly, invasomes contain not only phospholipids but also ethanol and terpenes, which make the vesicles deformable, and also serve as penetration enhancers [1]. This system has shown to improve skin penetration of hydrophilic and lipophilic drugs. A synergistic effect between terpenes and ethanol on the percutaneous absorption has been significantly observed. In this study, we investigated the ability of invasomes to increase the topical transport of CLOTRIMAZOLE in order to develop a topical formulation with enhanced CLOTRIMAZOLE skin delivery in order to achieve an effective fungal treatment [2]. Ethanol is a good penetration enhancer while terpenes have also shown potential to increase the penetration of many drugs by disrupting the tight lipid packing of the stratum corneum. The aim of the present study was to develop and characterize Clotrimazole-loaded invasomal drug carrier systems. Clotrimazole has been previously identified as a promising candidate for transdermal drug delivery [3].

- **Invasome Penetration Mechanism** - Terpenes and Ethanol Invasomes act as osmotic agents by promoting the deformability of vesicles, damaging the bilayer skeleton of SC, and increasing the permeability of invasomes. Some of the vesicles disintegrate, claiming to release their components such as terpenes, phospholipid segments. Non-collapsed invasive vesicles pass through the SC. Invasome can reach the internal regions of SC via follicular transport pathways. When the invasome reaches SC, many of them collapse, but smaller vesicles and flexible invasomes pass through deeper layers [5].

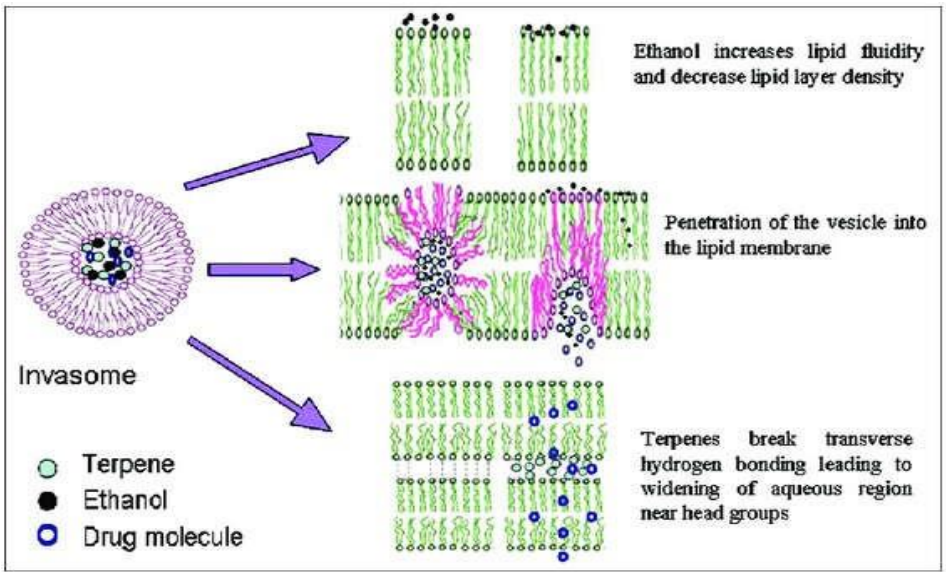

FIg: Invasome Penetration Mechanism

METHOD OF FORMULATION

- **Mechanical Dispersion Method -** Clotrimazole (10mg) was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.25 to 0.75% w/v) was added to ethanol and vortexed for 5 minutes [11, 12]. Drug and terpenes (0.25 to 0.75%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline (upto 10% w/v) was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

EVALUATION PARAMETER

- **Evaluation of Invasomes –**
- **Entrapment efficiency**
  Entrapment efficiency of Clotrimazole Invasomes formulation was determined using centrifugation method [5]. The entrapment efficiency of acyclovir in invasomes vesicle was determined by ultracentrifugation, 10mL of invasomes formulation were collect in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 260 nm using UV spectrophotometer.
- **Vesicle Size**
  Microscopic analysis was performed to determine the average size of prepared invasomes [6]. Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer.

Evaluation of Invasomes containing gel –

- **Measurement of viscosity**
  Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.
- **pH measurements**
  pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted [7].
- **Drug content**
  Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol [8]. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max} 260 nm.
- **Extrudability study**
  Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load [9]. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.
- **Spreadibility**
  Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined

by method reported by Multimer *et al.,* [10]. An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan.To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the topplate to cover a distance of 10 cm upon adding 80 g of weight was noted. Good spreadibility show lesser time to spread.

- **In-vitro drug diffusion study**
  The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion [8]. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.
- **Stability Studies -**
  Stability study was carried out for drug loaded invasomal gel at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25- 28 ± 2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any viscosity and % assay.

PHARMACEUTICAL APPLICATION OF INVASOME

- **Immunosuppressive drug delivery-** Immunosuppression is the primary approach to treating autoimmune diseases. Also, it is useful for the clinical application of existing immunosuppressive agents that have been suffered from various drug side effects. The topical applications of CTZ can be a suitable alternative for the management of psoriasis and other dermatological diseases [13].
- **Anticancer drug delivery -** From its inception, cancer treatment is still a challenging field in the era of biomedical science. Due to the ineffectiveness of currently engaged therapeutic strategies, a large number of deaths have been occurring each year. There is an urge to develop an advanced substitute to resolve cancer ineffective treatment issues. Interestingly, the temoporfin is a potent (second-generation) photosensitizer. It shows high tumor selectivity and residual photosensitivity of only 2 weeks. Thus, this could be an effective anticancer agent in photodynamic therapy of early or recurrent carcinomas [13].
- **Delivery of vitamin analog -** Isotretinoin is a vitamin A analog and used to treat eosinophilic pustular folliculitis. In recent attempts, Dwivedi and co-investigators have been revealed the synthesis of isotretinoin invasome using the mechanical dispersion method [13].
- **Used in alopecia treatment -** There is a tremendous need for progress in engaged therapies to efficient alopecia treatment. The literature says that the use of invasomes yields long-term effects that are self-satisfactory. Today, patients also use alternative and complementary therapies to try to find a safe, natural, and efficient cure for hair restoration [12]. In this context, the FDA approved the finasteride, a 5α reductase inhibitor, and now this is commonly preferred for the treatment of alopecia. Despite this, there are needs to develop a novel carrier for the delivery of finasteride across the dermis layer. Herein authors have been prepared the invasome of finasteride using the combination of a terpene (limonene, nerolidol, and carvone: 0.5%, 1.5%, and 1%, accordingly) through mechanical dispersion [13].
- **Delivery of anti-acne agent-** Acne is a widespread skin disorder worldwide in recent times. Dapsone is an exceptionally active pharmaceutical ingredient for leprosy treatment. It has significant potential for acne therapy due to its antiinflammatory action. As essential for the delivery of topical drugs, an effective carrier must be established for the delivery of dapsone to the specific site. In consequence, El-nabarawi *et al*., prepared the dapsone-loaded invasome by a film hydration technique using terpene (limonene, cineole, citral, or fenchone) and phosphatidylcholine for the treatment of mild to moderate acne [13].
- **Treatment of erectile dysfunction -** Erectile dysfunction is an inability to start or maintain the required penile erection during satisfying sexual intercourse and millions of men's population are affected by erectile dysfunction. Whereas about 30 million of the men's population has been added to the erectile dysfunction each year, and only 2 lakhs men pursue treatment from a physician. Therefore, there is an urge to investigators from the suffered population to treat erectile dysfunction. Owing to this, there is a need to deliver the drug efficiently using a suitable carrier which can overcome the limitations of previously engaged techniques [14]. Avanafil is a selective phosphodiesterase type 5 inhibitor (FDA approved), generally used for oral administration in the treatment of erectile dysfunction. Although, the oral bioavailability of the avanafil is challenging due to poor aqueous solubility, extensive presystemic metabolism. Additionally, there is a chance of alteration of the

absorption of drugs in the presence of food. In 2019, Ahmed et al. were prepared the avanafil-loaded invasomes for the treatment of erectile dysfunction [13].

- **Effective Permeability of Drug into Cells** - Gauhar R. Qadri et al., (2015) has worked to prepare Invasome of israpidine for enhanced transdermal delivery against hypertension formulation, characterization and in vivo pharmacodynamic study. It was observed that prepared isradipine-loaded invasomes delivers ameliorated flux, reasonable entrapment efficiency, and more effectiveness for transdermal delivery [14].

CONCLUSION

One such technique is the formulation of invasomes, which could be a promising tool for delivering drugs through the skin and can provide better skin permeation than liposomes. Several pharmaceutical active ingredients and chemical molecules are highly potent, but they are less active therapeutically. They can be targeted by using invasomes as a novel carrier because of its exceptional and tunable properties. Invasomes have been made their victorious entry into the pharmaceutical application research arena in 2002 with the development of clotrimazole-loaded invasomes. Invasomes significantly improved the pharmacokinetic parameters since drug encapsulation. Moreover, the efficacy of the invasome dosage form depends upon the penetration rate, the ability to deliver the actives to the targeted site and low toxicity, etc.

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