SYNOPSIS OF THE THESIS ENTITLED

ETHOSOMES: A NOVEL APPROACH FOR TOPICAL AND TRANSDERMAL DELIVERY OF ANTI FUNGAL DRUGS

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India is a country with vast diversity in the climatic and weather conditions. The climate is generally hot, humid and tropical type. This type of climate tends to favour the growth of variety of microorganisms and disease causing pathogens. Many studies have reported the occurrence of various diseases and infections caused by such organisms. About 10% of annual funding for health research is spent on health problems that account for 90% of global disease burden. Fungal infection is one such disease common among the Indians. It has been observed that people tend to neglect the fungal infections greatly and majority doesn’t consider it as a disease and prefers to go for unconventional treatments. Around the world fungal infections have recently emerged as a growing threat to human health. Fungi usually make their homes in moist areas of the body where skin surfaces meet between the toes, in the genital area, and under the breasts. Many fungi that infect the skin (dermatophytes) live only in the topmost layer of the epidermis (stratum corneum) and do not penetrate deeper.

Even though there are several treatments against fungal infections, clinicians are particularly concerned that the increasing use of antifungal drugs may lead to drug-resistant fungi, especially in settings such as hospitals where nosocomial (hospital-acquired) infections are a growing problem. Recent studies have documented resistance of Candida species to fluconazole and other azole and triazole drugs, which are used widely to treat patients with systemic fungal diseases. Among anti fungal agents Ketoconazole and Miconazole, both imidazoline derivatives are most commonly used in India. They are normally well tolerated with relatively safer profile. But anaphylactic reactions, cardio-respiratory toxicity of Miconazole and alteration of hepatic function by Ketoconzole have been fatal in some cases. Apart from this, half-life for these drugs are shorter hence need frequent dosing to attain the desired therapeutic effect. The pharmacological studies proved that Miconazole has lower bioactivity. Since most of the fungal infections are appearing over the skin, the treatment regimen of these infections always comprises external application formulations such as creams, ointments, lotions. Conventionally both these drugs are administered as topical creams. However, skin tends to be the strongest barrier for the entry of drug entities and hence it is essential to design a drug delivery system that would deliver the medicament into the skin layers (cutaneous delivery), or through the skin and into the systemic circulation (percutaneous absorption). With topical dosage forms, great attention has been devoted towards newer formulations which can ensure
maximum localization of drug within the affected area to enhance the local effect or increase the penetration through the stratum corneum and viable epidermis for systemic effects\textsuperscript{5}. Among many such formulations, the use of vesicular systems, such as liposomes, niosomes have great importance. The effectiveness of these vesicular systems depends on their physicochemical properties. One of the major drawbacks of liposomal vesicles is their larger size and rigid lipid bilayer, which reduces its skin penetration ability and restrict it only as a localized drug reservoir. The role of permeation enhancer can be useful in these circumstances, especially ethanol which is considered as one of the best permeation enhancers but the existence of ethanol at high concentration with liposomal vesicles may be difficult. But novel delivery system ethosomes represent a lipid vesicular carrier system embodying ethanol in relatively high concentration and are very efficient in delivering drugs into and across the skin. It was found that ethosomes could penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to the systemic circulation\textsuperscript{6}. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, vesicles have the ability to penetrate the stratum corneum. Also because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure, giving it more freedom and ability to squeeze through small places such as the openings created in disturbing the stratum corneum lipid\textsuperscript{7}. This ‘ethanol effect’ is followed by the ‘ethosome 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 the drug in deep layers of the skin. The interdigitated, malleable ethosome vesicle can forge paths in the disordered stratum corneum\textsuperscript{8}.

Based on the advantages of ethosomes, there is a need for the development of ethosomal formulation containing Ketoconazole and Miconazole for enhanced penetration through the skin, reducing the dosing frequency, minimizing adverse effects, and for better patient benefit.
The present investigation includes

1. Development of ethosomes containing Ketoconazole, Miconazole using different concentrations of ethanol.
2. Characterization of developed ethosomes for size, shape, entrapment efficiency and In-vitro release pattern.
3. DSC to understand the possible interactions between drug and other excipients.
4. Accelerated stability studies to identify the effect of temperature on vesicle stability.
5. Comparison of release pattern between ethosomal creams and non-ethosomal creams
6. In-vivo anti fungal study on rabbits to understand the wound healing activity of the developed product.

The method mentioned by Touitou et al was adapted for the preparation of drug encapsulated ethosomes. Concentrations of ethanol were varied and phospholipid concentration was fixed. A high speed mixer at 700 rpm was used for 5 minutes interval at 40°C. Propylene glycol was used as additional permeation enhancer at a fixed concentration for all the formulations. The delivery systems were characterized by various in-vitro and in-vivo methods. In-vitro evaluation includes size distribution, shape, entrapment efficiency, DSC study, stability studies and in-vitro drug release characteristics. In-vivo evaluation includes the wound healing activity on selected New Zealand rabbits and reduction in microbial load using plate count method.

The developed ethosomes were spherical and stable. As per findings average vesicle size reduced with increase in ethanol concentration from 20% to 40%. Further increase in ethanol concentration may increase the size but also cause the damage to lamilarity. Since both drugs do not possess any surface active property, the reason for reduction in average vesicle size may be due to the presence of ethanol.

The ability of the ethosomal vesicles for entrapping drug was investigated using ultracentrifugation method. Both drugs showed good retention in ethanol present in ethosomal core. Entrapment efficiency was significantly high with
ethosome containing 30% ethanol concentration. Further increase in ethanol concentration reduced the size as well as entrapment efficiency\textsuperscript{11}.

Accelerated stability study was performed for a period of eight weeks on developed ethosomes, which was in accordance with ICH guidelines. The developed formulations were observed for any change in appearance, colour, drug content and entrapment efficiency. There was no significant physical change observed with developed ethosomes. Changes in drug content and entrapment efficiency were also negligible for ethosomes. The ethosomes with 30% ethanol concentration were found to be more stable during the eight week long stability study.

The in-vitro skin permeation study was carried out on rat skin using a franz diffusion cell. The skin permeation study was performed for 72 hour duration. From the obtained data, the percentage drug diffused from ethosomal vesicles containing 30% ethanol concentration had better release profile than the rest. The unreleased portion of the drug was substantially less in ethosomal vesicles containing 30% ethanol concentration\textsuperscript{12}. Based on results obtained from physical characterisations, entrapment efficiency, stability profile and in-vitro release study, it was evident that 30% ethanol concentration may be the optimum concentration for developing ethosomes containing Ketoconazole and Miconazole.

The possible interactions between drug and excipients were studied using DSC method. Best ethosomal formulation (with 30% ethanol concentrations) was subjected for DSC study. The absence of melting endotherm of drug and shifting of phospholipid bilayer component endotherm suggested significant interaction of drugs with lipid bilayers, which leads to enhanced entrapment efficiency of drugs in ethosomal core\textsuperscript{13}.

The ethosomal formulation with 30% ethanol concentration was incorporated in water miscible cream base and compared against similarly prepared non-ethosomal creams for in-vitro release and in-vivo antifungal activity. Improved release data were observed in case of ethosomal creams during the 72 hours of the study. The possible reason for improved drug release profile from ethosomal creams may be due to the presence of ethanol and other penetration enhancers at optimum concentration. In case of non-ethosomal creams, significantly lower percentage of drug release was observed.
at all points of time. This may be due to the absence of any penetration enhancers within the developed formulation. Apart from that more than 50% of incorporated drug were found to be unreleased in non-ethosomal creams. Based on results obtained it was clearly evident that cream containing ethosomal vesicles had clear edge over non-ethosomal creams.

The in-vivo antifungal activity of developed ethosomal creams was evaluated and compared with non-ethosomal creams\textsuperscript{14}. The in-vivo studies were conducted on selected healthy white New Zealand rabbits with approval from institutional animal ethical committee (Reg. No:APTUS/IAEC/234). Animals were inoculated with selected strains of \textit{C.albicans}. All tested samples demonstrated clear antifungal activity but at different levels. On the 18\textsuperscript{th} day of evaluation, the mycological cure rate with ethosomal creams were 83\% and 75\% respectively for Ketoconazole and Miconazole. The results obtained for ethosomal creams were considerably superior to non-ethosomal creams of both the drugs. This was further substantiated by the data obtained from measuring microbial load for \textit{C.albicans} at the site of inoculum using plate count method. On 18\textsuperscript{th} day, microbial load for \textit{C.albicans} was significantly lowered for rabbits treated with ethosomal creams.

The skin irritation levels were substantially low in case of animals treated with ethosomal creams. Most of the anti fungal creams contain some kind of permeation enhancers which normally cause skin irritation and limit their use. But during this study it was observed that ethosomes with penetration enhancers like ethanol and propylene glycol did not cause skin irritation. In fact ethosomal creams successfully reduced the level of skin irritation caused during fungal infection, which makes ethosomes as one of the best vehicles for delivering antifungal drugs like Ketoconazole and Miconazole through topical or transdermal route.

Based on the study, it can be concluded that ethosomal formulations are excellent carriers for antifungal agents for topical and transdermal delivery. They can be stored at room temperature and possess excellent stability, safety and efficacy profile. Further study may be required to explore the scope of ethosomal technology in drug delivery.
References


