Available online on www.ajper.com

Asian Journal of Pharmaceutical Education and Research

Vol -4, Issue-1, January-March 2015

ISSN: 2278-7496

RESEARCH ARTICLE

COMPARATIVE EVALUATION OF IN-VITRO ANTIFUNGAL ACTIVITY OF ETHOSOMAL AND LIPOSOMAL GEL FORMULATED WITH FLUCONAZOLE FOR THE TREATMENT OF DEEP FUNGAL SKIN INFECTIONS

Gajendra Singh Rathore*, Dr. Yuveraj Singh Tanwar, Dr. Pushpendra Singh Naruka

Bhupal Nobles College of Pharmacy, Udaipur (R.J.)

Article Received on 12 December 2014.

Accepted on 2 January 2015

***Correspondence for Author:**

Gajendra Singh Rathore

Department of pharmaceutics, Bhupal Nobles College of Pharmacy, Udaipur (R.J.)

Email:

Abstract: Fungal skin infections are caused by different types of fungi, including dermatophytes and yeasts. Fungi invade and grow in dead keratin. Keratin is a protein that makes up your skin, hair and nails. The topical route vied with oral treatment as the most successful innovative research area in drug delivery. The use of lipid vesicles (Ethosomes and liposomes) in delivery systems for skin treatment has attracted increasing attention in recent year. Vesicles allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response and would be able to release just the right amount of drug and keeping the concentration constant for longer period of time. Ethosomes and liposomes are noninvasive delivery carriers that enable drugs to reach the deep skin layer. The purpose of the present study was to assess and compare the in vitro anti-fungal effect of free drug Fluconazole, drug loaded Ethosomes and liposomes on a strain of pathogenic fungus *Aspergillus niger.*

KeyWords: Ethosomes, Liposomes, Fluconazole, Fungal skin infections, *In-Vitro* Antifungal activity.

Introduction:

The cutaneous mycoses are superficial fungal infections of the skin, hair or nails. Essentially no living tissue is invaded; however, a variety of pathological changes occur in the host because of the presence of the fungus and/or its metabolic products. The usual approach to the management of cutaneous infections is to treat with topical agents. The drug of choice for topical fungal disease is Fluconazole. Fluconazole is an oral synthetic bis-triazole compound that inhibits the cytochrome P450-dependent 14 alpha-demethylation step in the formation of ergosterol. This leads to alterations in a number of membrane-associated cell functions. Fluconazole has a broad spectrum of activity that includes both dermatophytes and yeasts. The drug is particularly effective in the treatment of mucosal and cutaneous forms of candidiasis. It is currently the drug of choice for controlling oropharyngeal candidiasis in AIDS patients.

Although topical drugs can provide an immediate reduction in infectivity, are free of systemic adverse effects and are relatively inexpensive, they have some disadvantages such as unable to reach the deep skin layer. Ethosomes and liposomes are noninvasive delivery carriers that enable drugs to reach the deep skin layer.[1] Liposome is an artificial microscopic single vesicle consisting of an aqueous core enclosed in one or more phospholipid layers used to convey vaccines, drugs, enzymes, or other substances to target cells or organs. Ethosomes are ethanolic phospholipids vesicles, which have higher penetration rate through the skin than liposomes. Ethosome vesicles contain phospholipids and alcohol (in relatively in high concentration). The lipid vesicles have higher penetration rate, high efficiency or bio-availability.[2,3] Ethosome formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs.[4] Visualization by transmission electron microscopy showed that ethosomes could be unilamellar or multilamellar through to the core.[5.6] The physicochemical characteristics of vesicular carriers are such that these transport active substances more efficaciously through the stratum corneum into the deeper layers of the skin.

MATERIALS AND METHODS

ANTIFUNGAL ACTIVITY

Pathogenic fungus used

The pathogenic fungus used in the current study is obtained from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Composition of nutrient agar media	
Potatoes extract	200gms
Dextrose	20 gms
Agar	15 gms
Distilled Water	to make 1000ml
pH (at 25°C)	5.6±0.2

-

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121°C) for 15 minutes.

Preparation of plates

After sterilization, the molten agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37°C overnight to check the sterility of plates. The plates were dried at 50°C for 30 minutes before use.

Revival of the microbial cultures

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth and potato dextrose broth for fungus than incubated for 24 hours at 37°C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient agar and potato dextrose agar plates with loop full of bacteria and further incubated for next 24 hours at 37°C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antifungal sensitivity

The Antifungal sensitivity test is employed on to the fungus used under present study with the anti fungal gel formulation. For this experiment 6 mm diameter Whatman filter paper discs were impregnated with stock of 100 mg/ml then dried in aseptic conditions.[7] A nutrient agar plate is seeded with particular bacteria and potato dextrose agar plat with particular fungus with the help of spread plate technique prior and left for 5 minutes. Now the drug impregnated filter paper discs were place in the center of preinoculated culture plates then incubated for 24

hours at 37°C. After incubation, plates were observed to see the sensitivity of extracts towards test bacteriums at particular concentration in the form zone of inhibition.

Antibiogram Studies

Broth cultures of the pure culture isolates of microorganism*s Aspergillus Niger* which are sensitive towards the 100 mg/ml concentration of gel formulation used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37°C for 24-48hours. A loop full was taken from these broths and seeded onto sterile nutrient agar and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The paper disc diffusion method was used to determine the antifungal activity of gel formulation using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for gel formulation for antibiogram studies. Its essential feature is the placing of filter paper discs with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. Routine direct application of discs to plates seeded with clinical material is not recommended because of problems with inoculam control and mixed cultures. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the discs impregnated with particular concentration of drug.

Antibacterial Activity of Ethosomal and Liposomal Gel

Microbial Cultures

For the studies of Antifungal effect of antifungal gel formulation, there was one, MCC microbial strains procured from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India. The lyophilized cultures of microbial strain upon culturing in nutrient and potato dextrose broth for 24-48 hours at 37°C in an incubator resulted into turbid suspension of activated live microbial cell ready to be used for microbiological study. From the broth of respective revived cultures of micro organism loop full of inoculum is taken and streaked on to

the nutrient and potato dextrose agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on to the surface of the agar culture that are successfully stored in refrigerated conditions at 4°C as stock culture to be used for further experimentation.

Antifungal Studies

The lawn cultures were prepare with the pathogenic microorganism used under present study and sensitivity of microorganism towards the anti fungal gel formulation studied at the concentration of 100 mg/ml using disc diffusion method.

RESULTS AND DISCUSSIONS

Antibiogram studies

The present investigation in this research work, the Antifungal activity evaluated against fungal microbial pathogen used under present study. The gel formulation used to suitably dilute up to the concentrations of 100, 50 and 25 mg per ml and applied on to the test organism using Kirby Bauer filter paper disc diffusion method. Results of the experiment are being concluded in the Table 1.

Antifungal activity on *Aspergillus Niger*

Aspergillus niger was inhibited by the standard antifungal used in present work i.e., fluconazole, at all the concentration (20, 50 and 100 mg/ml) used in the study for comparison. The resulting zone of inhibition against *Aspergillus niger*.

Table 1: Antifungal activity of formulation on *Aspergillus niger*

S. No	Name of drug	Zone of inhibition		
		20mg/ml	50 mg/ml	100mg/ml
1.	Fluconazole	17±0.22	24±0.12	29±0.11
2.	Gel Formulation I	16±0.15	22±0.23	28±0.25
3.	Gel Formulation II	16±0.12	20±0.10	28±0.20

Gel Formulation I – Ethosomes formulation, Gel Formulation II – Liposomes formulation

In present work, out of the results from gel formulation shows antifungal activity against only *Aspergillus niger* with maximum zone of inhibition lying in the range of 17 to 29 mm.

These results demonstrated that, the antifungal activity of the ethosomes was significantly higher than the liposomes. These ethosomes acts as carriers for drug delivery to the particular site of action, the antifungal activity is created by the drug incorporated into the ethosomes vesicles. This enhanced antifungal activity is due to enhanced penetration of ethomes containing drug through the fungal cell wall and inhibiting the ergo sterol synthesis. The longer duration of antifungal activity of ethosomes formulations may be attributed to the slow and prolonged release of the entrapped drug from ethosomes vesicles as compared to liposomes. Furthermore, ethosomes act as a reservoir system, similar to a slow release vehicle, enabling more uniform and Sustained release of the drug.[8]

CONCLUSION

Fungal infections of the skin are one of the often faced with dermatological diseases in worldwide. Topical therapy in the form of vesicle delivery carrier is an attractive choice for the treatment of the cutaneous infections due to its advantage such as targeting of drugs to the site of infection and reduction of the risk of systemic side effects. The purpose of the present study is to assess and compare the in vitro anti-fungal effect of free drug fluconazole, drug loaded ethosomes and liposome on a strain of pathogenic fungus *Aspergillus niger*. The results found

out that all the three shows antifungal activity against *Aspergillus niger* with maximum zone of inhibition lying in the range of 17 to 29 mm.

ACKNOWLEDGMENT

We thank Mr. Prabhat Jain and Miss. Sonam Chadokar, technicians at the Scan Research Laboratories, Bhopal (M.P.) for their assistance during the research work.

REFERENCES

1. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosome for skin delivery of ammonium glycyrrhizinate: In vitro percutaneous permeation through human skin and in vivo anti inflammatory activity on human volunteers. J Cont Rel. 2005; 106:99-110.
2. Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin: permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit Rev Ther Drug Carrier Syst. 1996; 13 (3):257-388.
3. Sayed MMA, Abdalah OY, Naggar VF, Khalafalah NM. Lipid vesicles for skin delivery of drug: reviewing three decades of research. Int J Pharm. 2007; 332:1-16.
4. Jain S, Umamaheshwari RB, Bhadra D and Jain NK. Ethosomes: novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. Indian J Pharm Sci. 2004; 66:72-81.
5. Rong H, Da-xiang C, Feng G. Preparation of fluorescence ethosomes based on quantum dots and their skin scar penetration properties. Mater Lett. 2009; 63:1662-1664.
6. L´opez-Pinto JM, Gonz´alez-Rodr´ıguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. Int J Pharm. 2005; 298:1-12.
7. Taylor, R.S.L., N.P. Manandhar, J.B. Hudson and G.H.N. Towers. Screening of selected medicinal plants of Nepal for Antifungal activities. J. Ethnopharmacol. 1995;546: 153-159.
8. M. Udaya Sakthi, D. Ramya Devi and B. N. Vedha hari, Vesicular mode of drug delivery : a promising approach for anti-infective therapy, Int. J. Chem. Sci. 2014;12(3):797-814.