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Development and Evaluation of Antifungal Efficacy of Ketoconazole Pharmacosome

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Research Article

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Abstract

Pharmacosomes are neutral colloidal, lipid vesicular drug delivery system having both negative & positive charge that enhance the bioavailability of drugs by enhacing their solubility both in aqueous as well as non-aqueous phase, additionally reduce gastrointestinal toxicity of drug. Ketoconazole is an imidazole derivative agent which is used both in the treatment of topical or systematic fungal infections with fungi static activity against dermatophytes, yeasts and other pathogenic fungi. Ketoconazole is Biopharmaceutical classification system (BCS) class II drugs that display pH dependent dissolution and absorption. In this present research work Pharmacosome of Ketoconazole was prepared in different ratios of Ketoconazole-PC complex (1:1, 1:1.5 and1:2) using conventional solvent evaporation method. FT-IR spectra showed no significant untoward interaction, Vesicles shape and morphology was carried out with Phase contrast microscopy (PCM) and Scanning electron microscopy (SEM), In-vitro permeation study and anti-fungal activity were duly examined. Drug content in the formulations (1:1) (1:1.5) and (1:2) were found 92.5%, 94.3% and 89.7% respectively. FT-IR conformed proper formation of drug- Pharmaosome complex. Particle size distribution were found to be regular and of spherical shape. *In-vitro* permeation study showed 78.03%, 89.05% and 55.27% drug release as per respectively formulations (KTP1, KTP2 and KTP3). Pharmacosomes of ketoconazole show improvement in antifungal activity than ketoconazole (Pure).

Keywords: Pharmacosomes; Vesicles; Ketoconazole; Phospholipids

Introduction

Optimum therapeutic outcomes require not only proper drug selection but also effective drug delivery [1]. The most common and traditional way to take medications are oral route and Parenteral route. However, this route of administration suffers from some significant drawback's likely poor bioavailability due to hepatic first pass metabolism and the tendency to produce rapid blood level spikes (both high and low), leading to a need for high and/or frequent dosing, drug degradation in gastrointestinal tract due to enzymes, pH etc [2].

To overcome these difficulties there is a need for the development of new drug delivery system; which will enhance the therapeutic efficacy and safety of drugs by more precise (i.e., target specific), spatial and temporal placement within the body by which decreasing both the size and frequency of doses. New drug delivery system is also essential for the delivery of novel, genetically engineered pharmaceuticals (i.e., peptides, proteins) to their site of action, without incurring significant immunogenecity or

biological inactivation [3,4]. In recent years it has been shown that the skin is a useful route for drug delivery to the systemic circulation. Transdermal drug delivery system includes all topically administered drug formulations intended to deliver the active ingredients into the circulation. Transdermal drug delivery system (TDDS) provides a means to sustain drug release as well as reduce the intensity of action and thus reduce the side effects associated with its oral therapy. The fundamental aspect of transdermal drug delivery is to deliver drug across epidermis to attain systemic & sustain effect. These follow controlled as well as sutained delivery of drug across the skin.

Pharmacosomes are neutral colloidal, lipid vesicular drug delivery system having both negative & positive charge that enhance the bioavailability of drugs by enhacing their solubility in aqueous as well as non-aqueous phase, additionally reduce gastrointestinal toxicity of drug. In pharmacosomes drugs are covalently bound to lipids & exist as colloidal. They are appropriately described just as "pharmacosomes" by cause of the association of a drug (pharmakon) to a carrier/ delivery system (soma). These zwitteric drug-lipid complexes are stable and more bioavailable with low interfacial tension between the system and the GI fluid, thereby facilitating membrane, tissue, or cell wall transfer, in the organism.

As a novel drug delivery system pharmacosome offer following advantages-

- A substantial and controlled drug loading.
- Increased drug entrapment efficiency.
- Enhanced bioavailability of drug with low lipid and water solubility.
- Covalent connection provides improved stability and effectiveness.
- No drug leaking.
- Suitable for medicines that are both lipophilic and hydrophilic.
- Direct drug delivery to the infection site is possible.
- Reduction of side effects, therapy costs, and toxicity.

The salient features of pharmacosomes are, increased entrapment efficiency, easy removal of unentrapped drug from the formulation, no loss of drug due to leakage, no problem of drug incorporation and no influence of uncaptured volume and drug-bilayer interaction on entrapment efficiency [5]. A part from other methods used for modifying the solubility, the complexation with phospholipids has been found to show improvement in both absorption as well as permeation of the active constituent [6]. Ketoconazole is an imidazole derivative agent which is used both in the treatment of topical or systematic fungal infections with fungistatic activity against dermatophytes, yeasts and other pathogenic fungi [7]. Ketoconazole is Biopharmaceutical classification system (BCS) class II drugs that display pH dependent dissolution and absorption. Ketoconazole is lipophilic and insoluble in water [8].

Material

Ketoconazole was provided from Helios Pharmaceuticals Baddi (H.P.) as gift sample and Phospholipid (PC) was purchased from Sigma Aldrich chemical Pvt. Ltd. India.

Method

Ketoconazole pharmacosomes were prepared by acidification of ketoconazole with hydrochloric acid that causes availability of active hydrogen for complexation. It was then extracted into chloroform and subsequently recrystallized. Salt form of drug and lipid are dissolved in a volatile organic solvent dichloromethane. Thereafter, solvent is evaporated using rotatory vacuum evaporator in round bottom flask which leaves a thin film of solid mixture deposited on the walls of flask (Table 1). Then dried film hydrated with aqueous medium & readily gives a vesicular suspension [9].

Ingredients	Formulations		
	KTP1	KTP2	KTP3
Ketoconazole: Soya lecithin	1:01	01:01.5	01:02.0
Dicholoromethane	20	20	20

Table 1: Composition of Pharmacosome.

Formulation of Pharmacosomal Ketoconazole Gel

Gel of pharmacosomal formulations were mechanically dispersed to carbopol 934 (1% w/w) on mechanical stirrer and allowed stirring for one hour. Finally, triethanolamine (2%) and methyl paraben (0.02%) was added and pH was adjusted.

Result & Discussion

Drug & Excipients Incompatibility Study

Fourier-Transform Infrared Spectroscopy: The FTIR spectra were obtaining by using an FTIR spectrophotometer. IR spectra for ketoprofen, Phospholipid (soya-lecithin), mixture of Ketoprofen, phospholipid and formulation were obtained on an IR spectrometer in the transmission mode with the wave number region 3500-500 cm^{-1} which shows in Figures 1- 4 respectively.

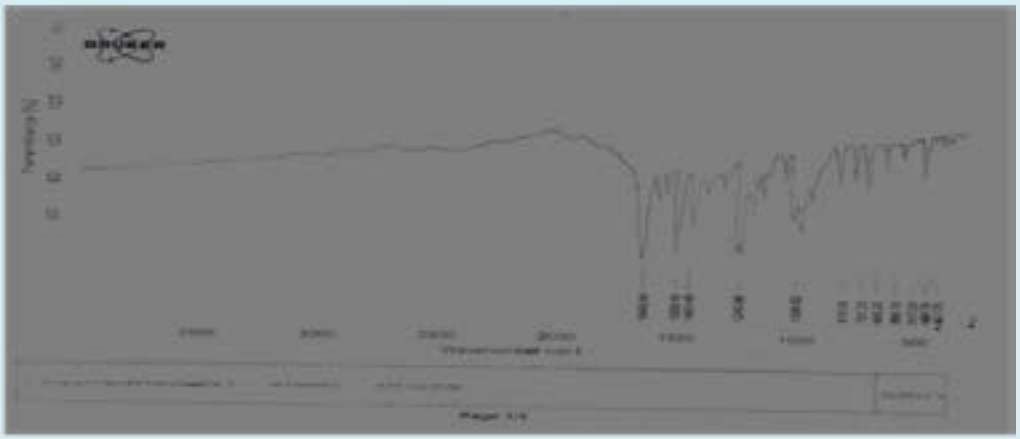

Figure 1: IR Spectra of Ketoconazole (Pure).

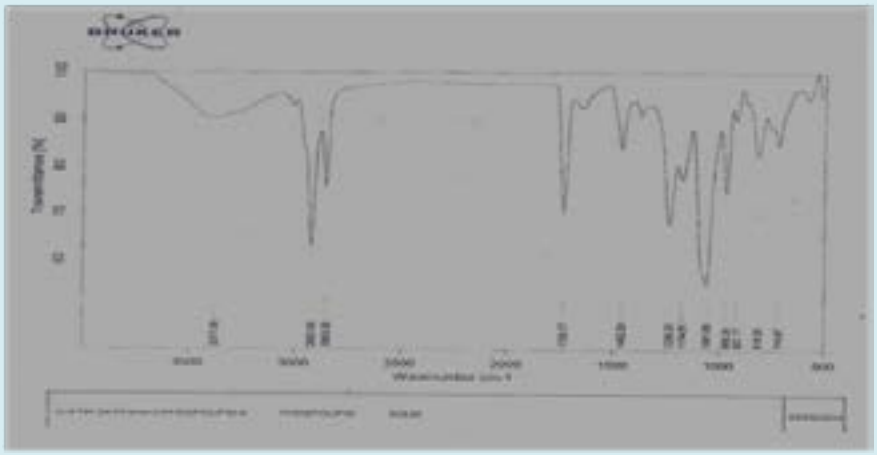

Figure 2: IR Spectra of Phosphlipid.

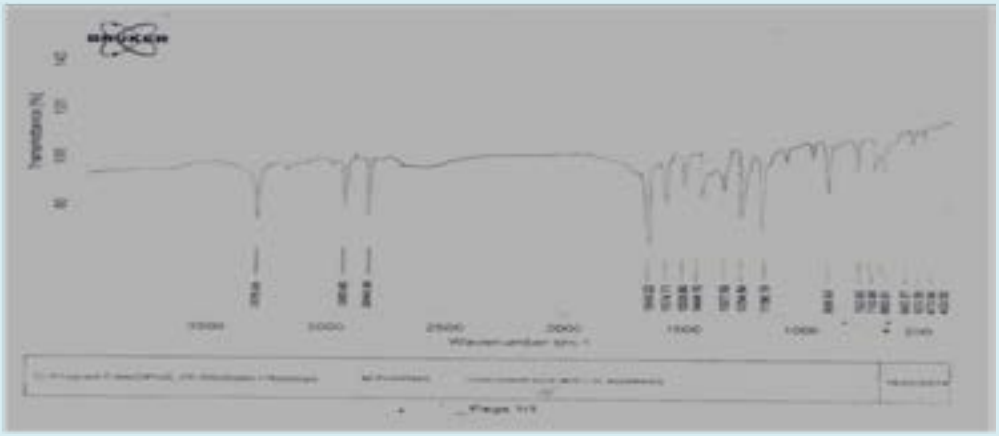

Figure 3: Ketoconazole and Phospholipid mixture.

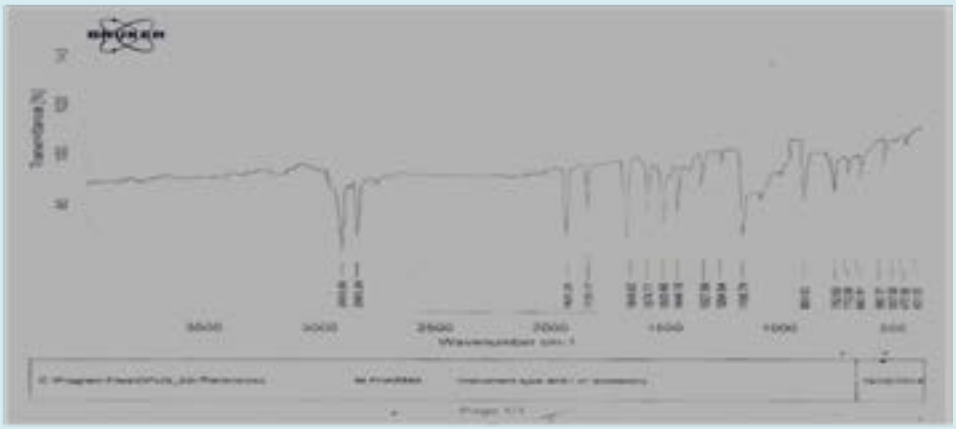

Figure 4: Pharmacosome Formulation.

Differential Scanning Calorimetry (DSC) Study

DSC thermogram of Ketoconazole showed endotherm at 153.49°C (Figure 5). DSC thermogram of soya lecithin showed endotherm at 22.32°C (Figure 6). DSC thermogram of mixture of Ketoconazole And soya lecithin showed endotherm at 151.84°C (Figure 7). DSC thermogram of Ketoconazole Pharmacosome showed endotherm at 150.63°C (Figure 8).

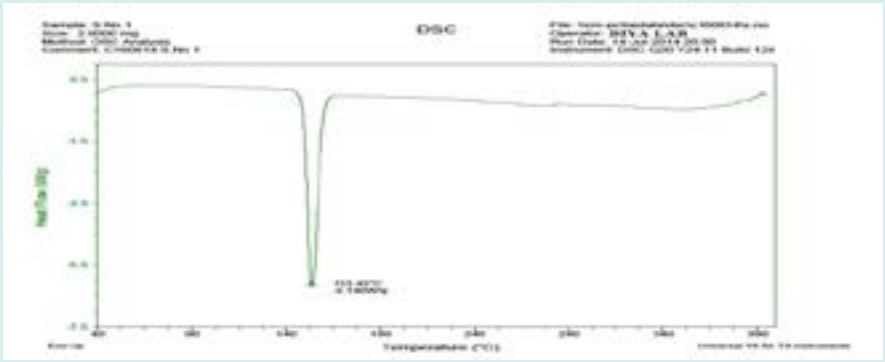

Figure 5: DSC thermogram of Ketoconazole (Pure).

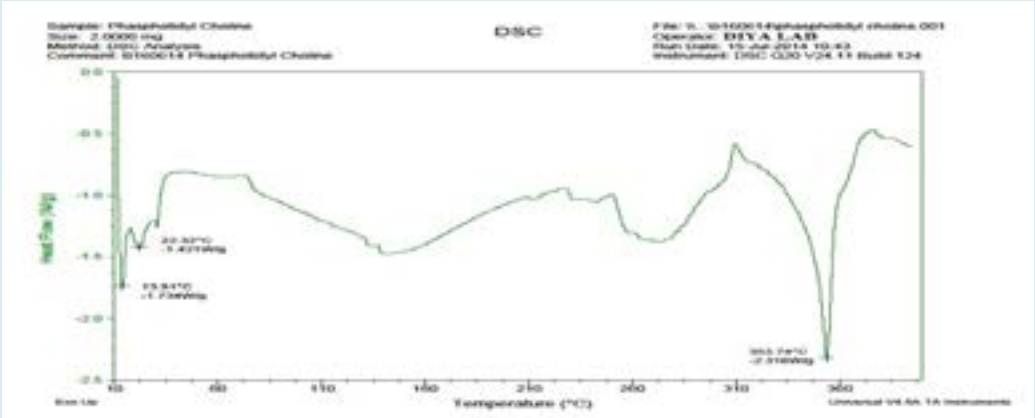

Figure 6: DSC thermogram Phospholipid

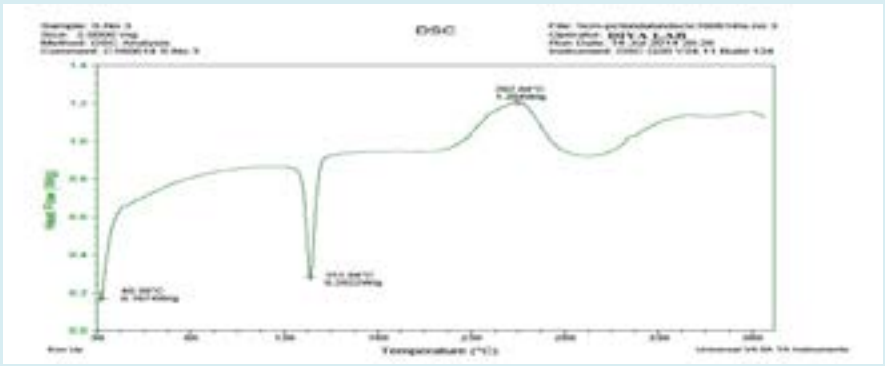

Figure 7: Ketoconazole + Phosphotidylcholin.

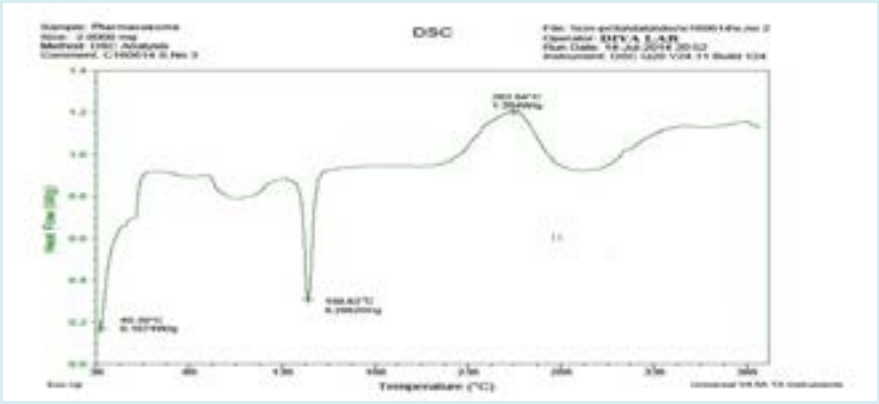

Figure 8: DSC Thermogram of Ketoconazole Pharmacosomes

Size Potencial

Zeta potential is an important parameter that affects the aggregation of vesicles and depicts the physical stability of vesicular systems and it can be measured by Zeta meter [10].

The effect of phospholipids concentration on the size and distribution of pharmacosomes vesicles was investigated by using Malvern Zetasizer. In current investigation, it was observed that there was not much difference in zeta potential of different Pharmacosome formulation (Figures 9- 11).

Figure 9: Distribution plot of KTP1.

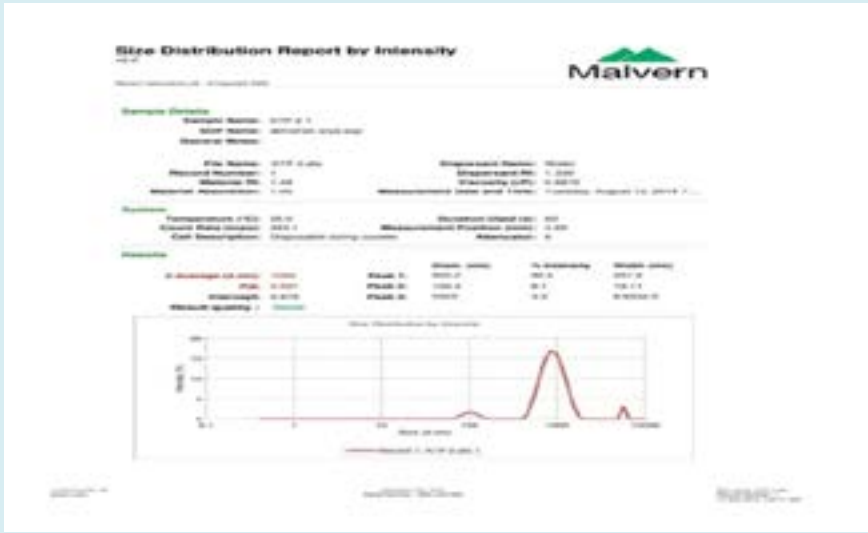

Figure 10: Distribution plot of KTP2.

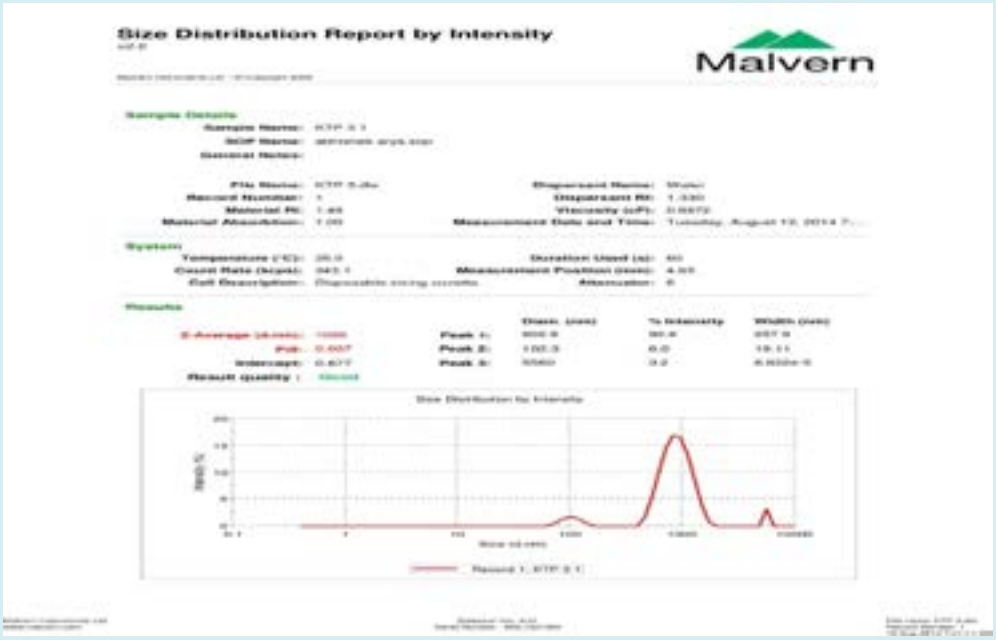

Figure 11: Distribution plot of KTP3

Size and Shape Analysis

The Pharmacosomal dispersion was spread on glass slide and Photomicrographs were taken using video camera connected to microscope with the help of computer installed with special software proved the multi-lamellar vesicular structure and spherical shape of Ketoconazole Pharmacosomes (Table 2). The examination of prepared formulations revealed the predominance of spherical shaped vesicles (Figures 12-16). The vesicles were uniform and appeared to be multilayered. Visual observation of Pharmacosomes confirmed the multi-lamellar vesicular structures.

Size range			Formulations					
			KTP1		KTP2		KTP3	
Eye piece Micro meter division	Calibrated stage micrometer (µm)	Average size (d)	No.of vesicles (n)*	% Vesicles	No.of vesicles (n)*	% Vesicles	No.of vesicles (n)	% Vesicles
0-1	0 - 1.25	0.625	80	40%	75	37.50%	65	32.50%
2-Jan	1.25 - 2.5	1.875	85	42.50%	90	45%	92	46%
3-Feb	2.5 - 3.75	3.125	20	10%	25	12.50%	27	13.50%
4-Mar	3.75 – 5	4.375	8	4%	6	3%	10	5%
5-Apr	5 - 6.25	5.625	4	2%	2	1%	4	2%
6-May	6.25 - 7.5	6.875	3	1.45%	2	1%	2	1%

Table 2: Size distribution of Ketoconazole Pharmacosomes of formulation KTP1.

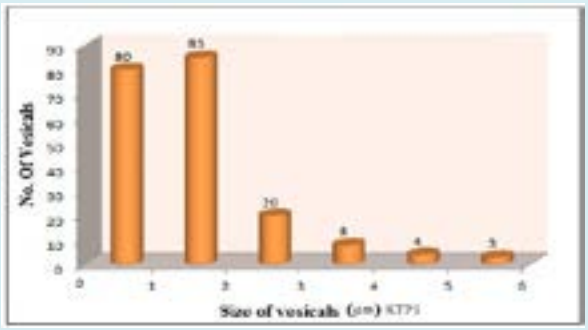

Figure12: Size distribution of KTP1.

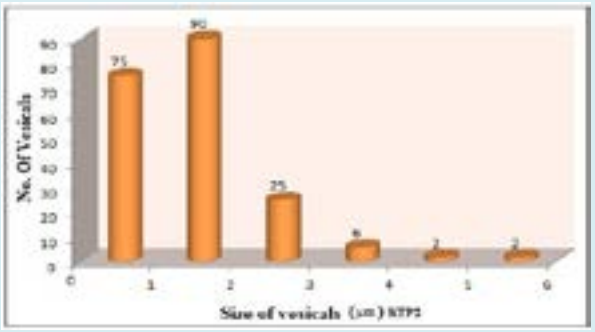

Figure13: Size distribution of KTP2.

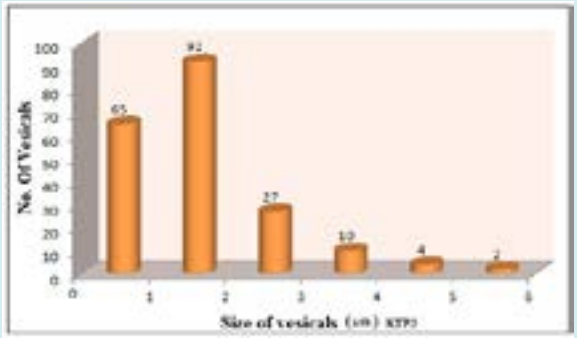

Figure 14: Size distribution of KTP3.

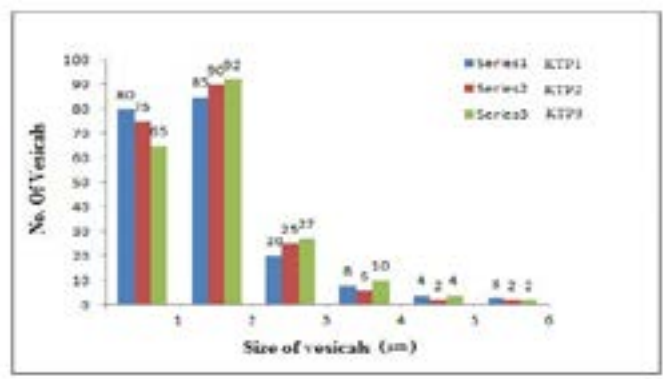

Figure 15: Size distribution of KTP1, KTP2, KTP3.

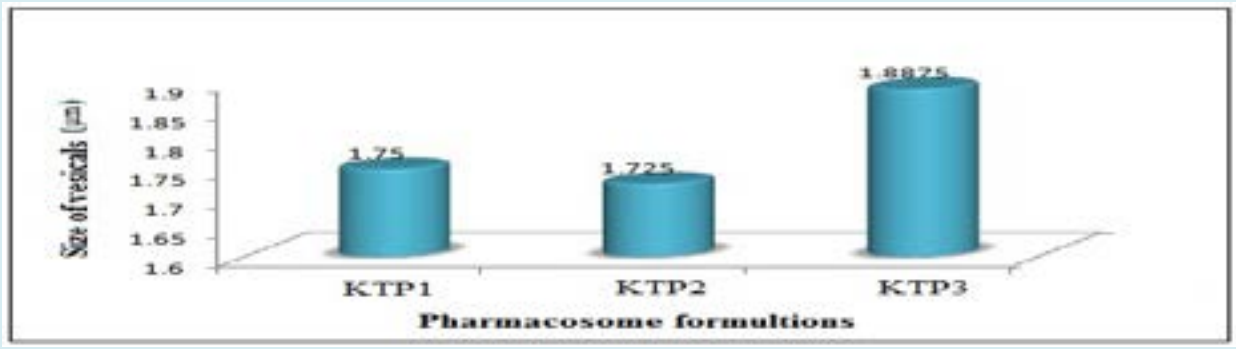

Figure 16: Average Size distribution.

Visualization of Vesicles

The vesicles were uniform and appeared to be multilayered. Visual observation of Pharmacosomes confirmed the multi-lamellar vesicular structures. Photomicrographs were taken using video camera connected to microscope with the help of computer installed with special software proved the multi-lamellar vesicular structure and spherical shape of Ketoconazole Pharmacosomes (Figures 17 & 18). These vesicles of Phospholipid might be validated by another investigation that is scanning electron microscopy (SEM).

Figure 17: Pharmacosomal photomicrograph of Ketoconazole vesicles.

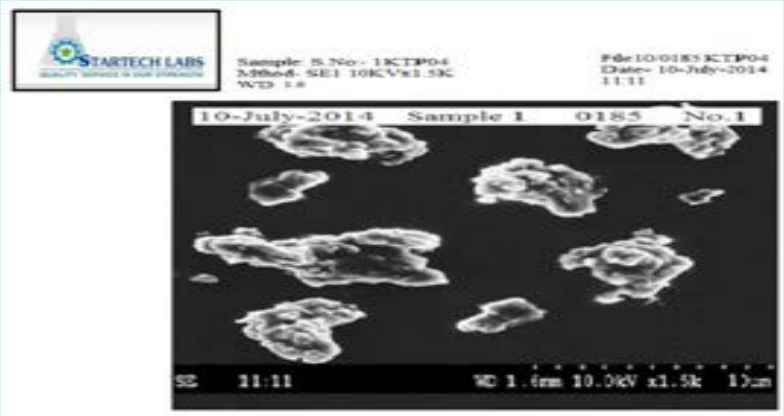

Figure 18: SEM of Ketoconazole Pharmacosome.

Drug Content Uniformity

To determine the drug content in ketoconazole-PC complex, complex equivalent to 100 mg was weighed and added to a volumetric flask with 100 ml of pH 7.4 phosphate buffer saline. The drug & phospholipid complex dilution was allowed to stir continuously for 24 hr using magnetic stirrer. After getting homogeneous dispersion dilutions were made and measured by using UV spectrophotometer at λ max 260nm [11].

Solubility

Solubility analysis is essential due to complexation of ketoconazole & ketoconazole phospholipid which is performed by flask shake method in saline buffer & n-octanol at pH 7.4. 50 mg of ketoconazole (and 50 mg equivalent in case of complex) was taken in a 100 ml conical flask. 50 ml of pH 7.4 phosphate buffer saline was added and then stirred for 15 minutes. The dispersion of drug in phospholipid was taken in a 250 ml separating funnel with 50 ml octanol and allowed to shake well afterward this is allowed to stand for 30 minutes [12]. Amount of drug solubulize was determined by using UV spectrophotometer at λmax 260 nm.

In-Vitro Skin Permeation Studies

In-vitro skin permeation study or in vitro skin diffusion study have been extensively studied using franz diffusion cell and cellulose acetate membrane. The study may be used as an indirect measurement of drug solubility especially in preliminary assessment of formulation factors and manufacturing methods that are likely to influence bioavailability (Table 3). All formulations had propylene glycol as skin penetration enhancer in a definite concentration [13].

The objectives in the developments of in-vitro diffusion tests are to show the release rates and extent of drug release from dosage form. Skin permeation study was carried out for 72 hours duration [14], all results were shown on table and represented graphically (Figure 19).

KTP1	Dilution Factor	Drug Released KTP1	Drug Released KTP2	Drug Released KTP3
0	10	0	0	0
1	10	1.45	6.72	0
2	10	15.04	20.99	1.88
4	10	22.39	29.52	6.03
6	10	35.05	42.03	17.85
12	10	44.4	54.02	30.66
24	10	55.57	60.43	37.56
36	10	59.79	70.28	42.83
48	10	65.85	77.46	46.77
60	10	71.04	84.26	50.22
72	10	78.03	89.05	55.27

Table 3: In-vitro drug release for Ketoconazole Pharmacosomes.

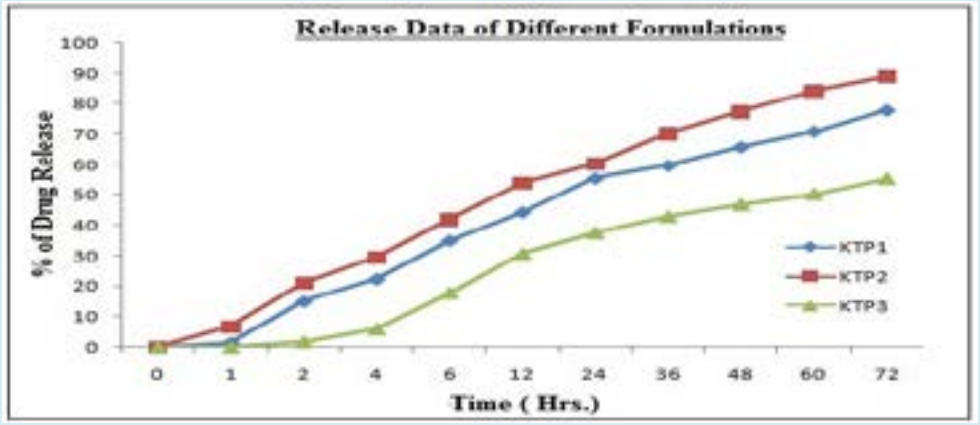

Figure 19: Release Data of Different Formulation.

Stability Studies

Stability play a vital role in a drug delivery system that is performed to evaluate total drug content as well as drug entrapment at room temperature (30± 2°C) and refrigeration temperature (4±20C). Phospholipid may get detoriate at higher temperature & it is major component of pharmacosome formulation thus its stability at higher temperature (more than 60°C) is not permissible [15]. The ability of vesicles to retain the drug was assessed by keeping the Pharmacosome suspension at different temperature (Table 4). Ketoconazole based Pharmacosomes formulations were evaluated for loss in percentage drug content (Figure 20).

KTP1	Drug content in %									
	Initial		After 2 Weeks		After 4 weeks		After 6 Weeks		After 8 week	
	4±2 ^{0}C	27± 2^{0}C	4±2 ^{0}C	27± 2^{0}C	4±2^{0}C	27±2 ^{0}C	4±2^{0}C	27±2 ^{0}C	4±2^{0}C	27±2 ^{0}C
KTP1	100	100	100	98.61 ±0.22	99.45±0.16	97.86±0.24	98.75±0.21	96.92±0.21	97.61±0.16	95.86±0.21
KTP2	100	100	100	99.75±0.20	99.85±0.21	98.93±0.19	99.12±0.25	98.14±0.21	98.65±0.19	97.65±0.25
KTP3	100	100	100	98.45±0.19	99.35±0.18	97.71±0.16	98.55±0.18	96.55±0.16	97.48±0.18	95.61±0.21

Table 4: Stability Data of Pharmacosomes Formulation

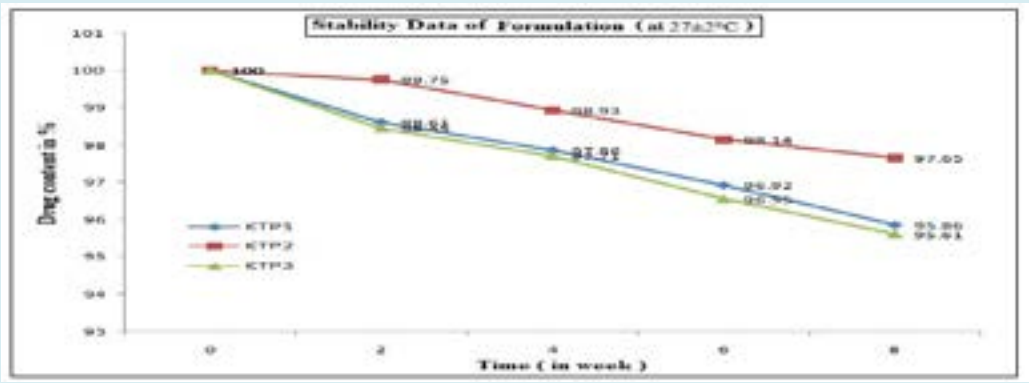

Figure 20: Loss in percentage drug content during stability study of Formulation

Anti-Fungal Activity

For antifungal activity a culture media was prepared by using 16.25 gm of sabouraud dextrose agar which was transferred in a 500 ml of conical flask and 250 ml of purified water and some amount of heat is applied to dissolve it completely. This prepared culture media was sterilized for 15 min at 121°C at 15 lb pressure in autoclave for about

20 min (Table 5). After cooling the culture media fungal strain (Candida albicans) was inoculated in medium which is transferred to the three petridishes. After solidification of inoculated media in petridishes three cups are bored in each petridishes with help of sterile bore of 6 mm. In this standard solution of ketoconazole & pharmacosome of ketoconazole were placed and allowed to incubate for 72 hours at in incubators (Figures 21-23). After 72 hours percentage zone of inhibition was evaluated which confers positive or negative growth [16].

Sample	%Concn. of drug	Load of *Candida albicans*			
		0	6	10	18
Normal control	Nil	TNTC	998	625	330
KTP (Test)	2%	TNTC	425	150	5
Ketoconazole (Standerd)	2%	TNTC	556	230	38

Table 5: Microbial load determination by plate count method.
Note: TNTC = Too Numerous To Count

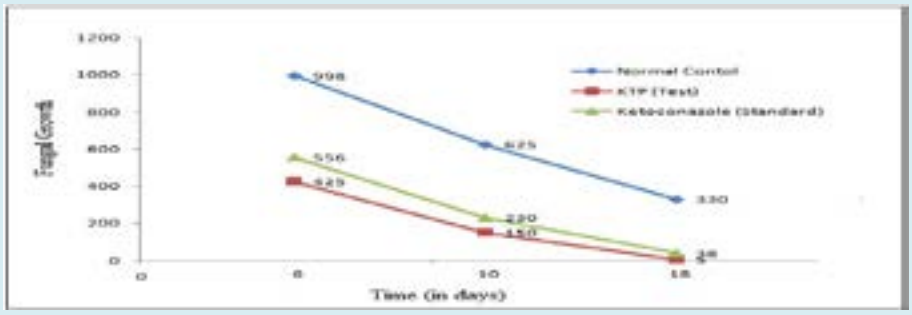

Figure 21: Fungal growth inhibition Data.

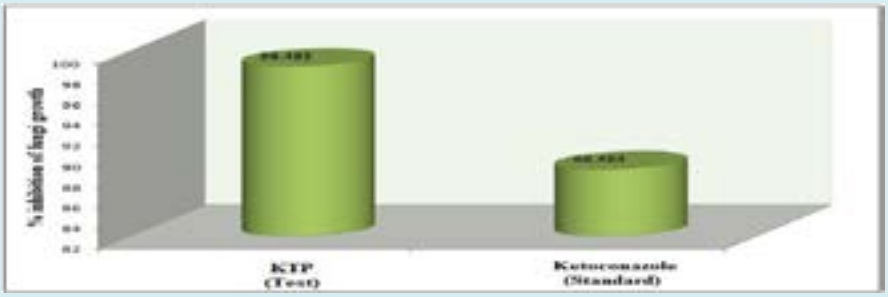

Figure 22: Percentage Inhibition of fungal growth.

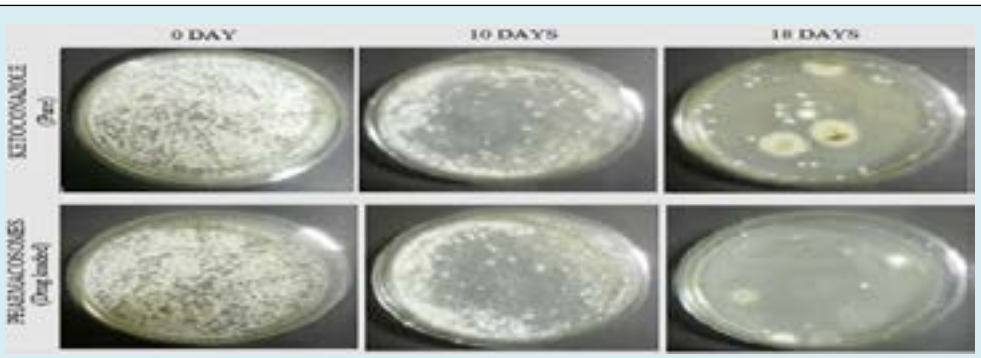

Figure 23: Anti-fungal activity of the Ketoconazole and Pharmacosomal Formulation.

Conclusion

The aim of the Formulation and Evaluation of Pharmacosomes of Ketoconazole was to find out and various formulation (KTP1, KTP2, KTP3) were developed by using suitable ratio of ketoconazole and phospholipid complex. The physicochemical investigations of FT-IR showed no interaction between drug and selected excipients. In thermographs showed changed position of peaks that confirmed the formation of complex in the pharmacosomes. DSC data of the prepared complex showed a noticeable reduction in the enthalpy as well as the melting point. Particle size distribution (Zeta Potential) was found good. Pharmacosomes of ketoconazole were found to be spherical shaped. *In-vitro* Permeation study was found 78.03%, 89.05% and 55.27% drug release as per respectively formulations (KTP1, KTP2 and KTP3). Antifungal studies also showed the good results of formulation. Thus, the objective of the present work of formulation and evaluation of Ketoconazole Pharmacosomes has been achieved with success.

Future Prospect

Pharmacosomes is a potential approach in vesicular drug delivery system which exhibits several advantages over conventional vesicular drug delivery systems. Pharmacosomes carrier opens new challenge and opportunities for the development of noel improved therapies. Applying this approach to more addition drug the advantages of pharmacosomes exploited. Till date this approach requires some more effort for study the non-bilayer and the mechanism of action of vesicle system. In future lot of work will be done for improving solubility permeability or avoid the problem associate with drug, Phyto constituents like GI disturbance and route of drug delivery system etc. The developed formulations are expected to improve the patient compliance, form better dosage regimen and provide optimum maintenance therapy to patients.

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