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Research Article

Formulation and Evaluation of Carbopol Gel Containing Liposomes of Ketoconazole. (Part-II)

Rakesh P. Patel[1*], Hardik H. Patel [1] and Ashok H. Baria[2]

[1]Department of Pharmaceutics, S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, 382711, Mehsana, Gujarat, India.
[2]S. J. Thakkar Pharmacy College, Rajkot, Gujrat, India

ABSTRACT
The aim of this work was to prepare and evaluate the topical carbopol gel formulation containing ketoconazole encapsulated liposomes. Ketoconazole loaded liposomes were prepared by thin film hydration technique. The prepared liposomes were incorporated into 1% carbopol gel, and the systems were evaluated for *in-vitro* drug release, drug retention into skin and *in-vitro* antifungal activity. The *in-vitro* permeation of ketoconazole using wistar albino rat skin from liposomal gel was compared with that of plain drug gel and also with plain drug cream containing 2% w/w of ketoconazole. The release of ketoconazole from liposomal gel was much slower than from non liposomal formulations. Gel containing liposomal ketoconazole showed maximum antifungal activity after 30 hours over plain ketoconazole gel and cream formulations.

Keywords: Ketoconazole, Topical gel, Liposomes, Antifungal, *In-vitro release* study and Drug retention study

INTRODUCTION

Ketoconazole (KTZ) is a broad spectrum antifungal agent active against a wide variety of fungi and yeasts. It is readily but incompletely absorbed after oral dosing and is highly variable. Topically it is used in the treatment of candidal or tinea infections of the skin. Encapsulation of KTZ in liposomes may increase the half life providing prolonged drug delivery and minimize the commonly occurring side effects.

Liposomes have been widely used as drug carrier in topical treatment of diseases, especially in dermatology. They are capable to incorporate a variety of hydrophilic and hydrophobic drugs, to enhance the accumulation of drug at the administration site and to reduce side effects. Liposomes can provide sustained and/or controlled release of entrapped drug. Liposomal system allows for a high accumulation of drug in the skin, with relatively low permeation flux as compared to the conventional dosage form.

However major limitation of using liposomes topically is the liquid nature of preparation. That can be overcome by their incorporation in adequate vehicles where original structure of vesicles is preserved. It has already been shown that liposomes are fairly compatible with gels made from polymers derived from crosslinked poly acrylic acid, such as carbopol resins. Therefore, in the present investigation carbopol 934 was used for formation of gel and that was used as a vehicle for the incorporation of liposomes for topical delivery.

The objective of the present work is to prepare carbopol gel incorporating KTZ liposomes and to study the *in-vitro* drug release, skin retention and *in-vitro* antifungal study.

*** For Correspondence: Dr. Rakesh P. Patel**
S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mehsana-Gozaria Highway, PIN-382711, City: Mehsana, State: Gujarat, INDIA.
E-mail: raka_77us@yahoo.com

MATERIALS AND METHOD:

Materials

Soya lecithin (HiMedia Laboratories Pvt. Ltd., Mumbai), cholesterol (S.D. Fine Chem. Ltd., Mumbai), Tocopheryl acetate (Morvel Laboratories Pvt. Limited, Mehsana), Carbopol (Corel Pharma, Ahmedabad) and cellulose acetate membrane (Sartorious cellulose acetate membrane) were used in the study. All other chemicals and solvents were of analytical or pharmacopoeial grade. KTZ was received as a gift sample from Yash Pharmaceutical Ltd, Himmatnagar.

Preparation of carbopol gel and incorporation of liposomes into 1% carbopol gel

a) Preparation of carbopol gel

As a vehicle for incorporation of liposomes for topical delivery, a carbopol gel was made. Carbopol 934 (1 g) was dispersed in distilled water (88 g) by stirring at 800 rpm for 60 minutes. Then, propylene glycol (10 g) was added and the mixture was neutralised by dropwise addition of

Table 1: Physicochemical properties of gel formulations

Formulation	pH*	Viscosity (cPs)*	% Drug content *
Liposomal gel	5.40 ± 0.27	11309±214	98.40±0.71%
Plain gel	5.52 ± 0.15	10700±437	99.01±0.23%

* Values are represented as mean ± SD (n=3).

Table 2: Percentage cumulative drug release and flux from different formulations*

Time (hours)	PKG		PKC		LKG	
	% CDR	Flux (µg cm^{-2} hr^{-1})	% CDR	Flux (µg cm^{-2} hr^{-1})	% CDR	Flux (µg cm^{-2} hr^{-1})
0	0	0	0	0	0	0
1	17.15±0.90	366.94±0.98	19.63±0.60	409.08±0.85	5.65±0.34	117.56±0.84
2	24.90±1.45	295.77±1.93	35.30±1.17	329.03±2.01	10.20±0.12	100.78±0.47
3	34.47±0.72	239.60±0.89	41.30±0.91	283.36±1.18	14.57±0.43	94.89±1.06
4	42.30±0.91	210.56±1.02	47.24±0.78	245.52±0.98	18.84±0.11	85.76±0.43
6	54.70±1.05	174.16±1.21	61.20±1.41	205.53±2.36	22.70±0.51	78.90±1.22
8	61.20±1.11	155.93±1.80	72.00±0.99	178.02±1.72	26.89±0.17	68.93±0.64
10	71.60±1.67	145.74±2.16	84.05.±0.72	161.51±0.91	30.30±0.28	61.70±0.75
12	78.50±0.49	134.40±0.75	88.40±0.60	148.66±0.90	33.85±0.38	57.81±0.97

* Values are represented as mean ± SD (n=3).

triethanolamine. Mixing was continued until a transparent gel appeared, while the amount of the base was adjusted to achieve a gel with pH 5.5.

Table 3: Percentage of drug retained in skin after 12 hr

Test formulation	% Drug retained*
Plain gel (PKG)	12.62 ± 0.83
Plain cream (PKC)	5.34 ± 1.14
Liposomal gel (LKG)	36.87 ± 1.21

* Values are represented as mean ± SD (n=3).

b) Incorporation of liposomes of optimized batch into carbopol gel

KTZ liposomes were prepared by by thin film hydration technique using soya lecithin, cholesterol and drug in different weight ratios (Patel et al., 2009). Liposomes containing KTZ (separated from the unentrapped drug) were mixed into the 1% (w/w) Carbopol gel with an electrical mixer (25 rpm, 2 min), the amount of liposomes of optimized batch (F_4) (Patel et al., 2009) added into the gel, such that the prepared gel have 2% w/w KTZ concentration (20 mg drug per 1gm of gel). Plain drug gels (2% w/w) were made under the same conditions. Instead of liposomes, those samples contained free KTZ were incorporated.

Evaluation of liposomal topical gel

a) Physical examination

The prepared gel formulations were inspected visually for their color, homogeneity, consistency, and spreadability.

b) pH

The pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter (Systronics, 361-micro pH meter) (Mohamed, 2004).

c) Viscosity

Viscosity of prepared gels were measured by Brookfield-DV-II+Pro Viscometer. Apparent viscosity measured at 25°C and rotating the spindle at 1.5 rpm.(Mohamed, 2004).

d) Content uniformity

Gel formulations (100 mg) was dissolved in methanol and filtered and the volume was made to 100 ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 222 nm using Shimadzu – 1700 UV Visible spectrophotometer. Drug content was

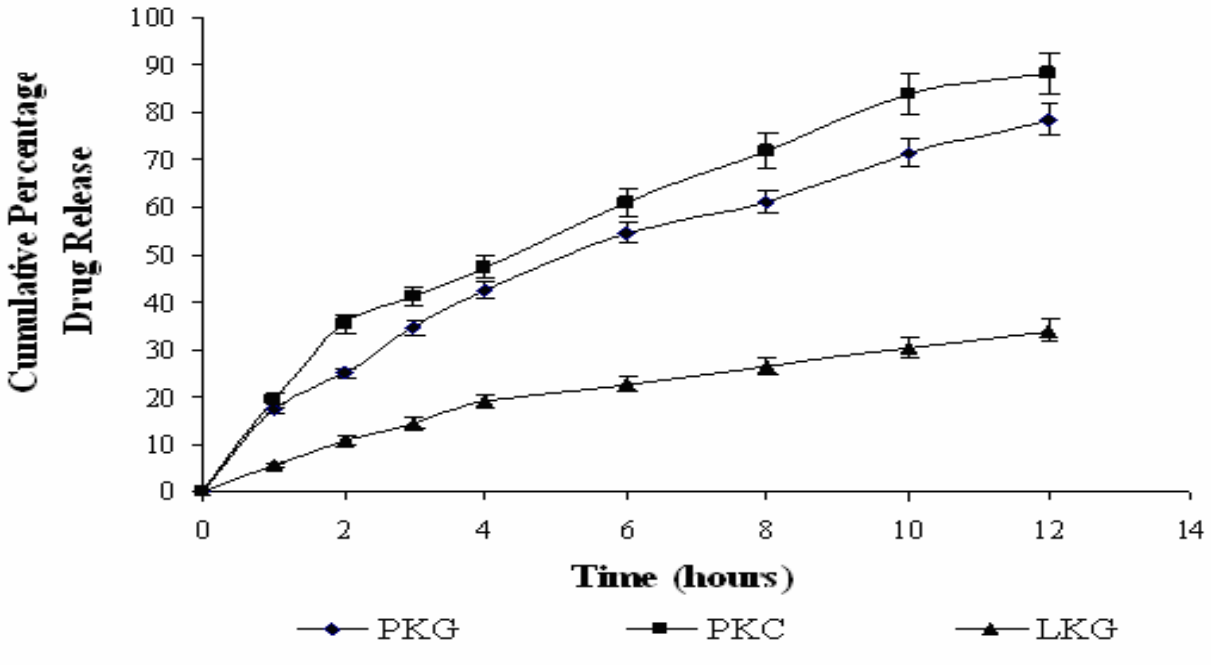

Figure 1: Cumulative percentage drug release from different formulations i.e. from liposomal gel, plain gel and plain cream. All the values are shown as mean ± SD (n=3).

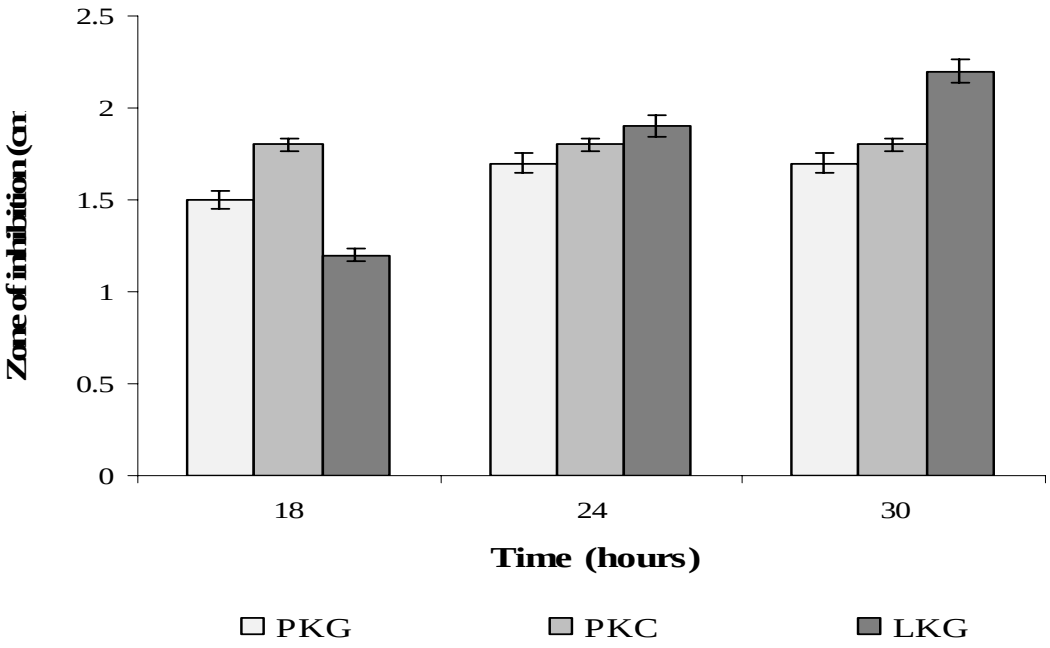

Figure 2: Evaluation of *in vitro* antifungal activity of plain drug gel, plain drug cream and liposomal gel. All the values are shown as mean ± SD (n=3).

determined from calibration curve for KTZ (Reddy et al., 2006).

e) *In-vitro* drug release study

An essential parameter in the evaluation of drug delivery is the rate at which the drug is released from the carrier. Skin permeation studies with KTZ-containing liposomal formulations (LKG) were carried out. The results obtained were compared with plain drug gel (PKG) and also with plain drug cream (PKC) containing equivalent amounts of KTZ (Bhatia et al, 2004).

Modified Franz diffusion cell was used for these studies. Full thickness abdominal skin of male wistar albino rats weighing 140 to 200 g was used for the skin permeation and the deposition studies**.** Briefly, to obtain skin, animals were sacrificed. Hair from the abdominal region was carefully removed and an excision in the skin was made. The dermal side of the skin was thoroughly cleaned of any adhering tissues (Satturwar et al., 2005). Dermis part of the skin was wiped 3 to 4 times with a wet cotton swab soaked in isopropanol to remove any adhering fat material (Bhatia et al, 2004). The skin specimens were cut into appropriate size after carefully removing subcutaneous fat and washing with normal saline (Wen et al., 2006). Skin was mounted in a modified Franz diffusion cell, kept at 37ºC. The known quantity (500 mg gel containing 10 mg of the KTZ) was spread uniformly on the skin on donor side. (Reddy et al., 2006). pH 5.0 acetate buffer containing 20% methanol (to maintain sink condition) was used as the acceptor medium, from which samples were collected at regular intervals during 12 hours and replaced with the same amount of buffer to maintain the receptor phase at 22 ml (Pierre et al., 2001; Siewert et al., 2003).

f) Skin retention study

After conducting the permeation study, the authors carefully removed the skin mounted on the Franz diffusion cells. The remaining formulation adhering to the skin was scraped with a spatula (Agarwal & Katare, 2002). Cleaned with cotton soaked in acetate buffer (pH 5.0) and then gently dried by pressing between two tissue papers (Pierre et al., 2001). The cleaned skin piece was mashed, and 50 ml of methanol was added to the meshed mass and mechanically shaken in a water shaker bath at 37 ºC for 1 hour for the complete extraction of the drug (Agarwal & Katare, 2002). The filtrate was removed and the drug content in filtrate was determined spectrophotometrically at 222 nm using a UV spectrophotometer.

g) *In-vitro* antifungal activity using cup-plate method

In-vitro antifungal activity was carried out by cup plate (or cylinder plate) method. The cylinder plate method depends upon diffusion of antifungal from a vertical cylinder through a solidified agar layer in a petridish or plate to an extent such that growh of added micro-organism is prevented entirely in a zone around the cylinder containing anti fungal. Liposomal suspension after the removal of free drug was incorporated in carbopol 934 gel base. Evaluation of *in-vitro* antifungal activity was carried out by cup plate method. The over night grown culture of *Candida albicans* was inoculated into the sterilized agar media plates. After solidification, wells were cut into the media and fixed with 100 mg of the specimens to be tested using PKC, PKG and LKG. The plates were incubated at room temperature and the width of zone of inhibitions resulting after drug diffusion into media were measured (Satturwar et al., 2001).

RESULT AND DISCUSSION

Physical examination of gel

The prepared LKG and PKG formulations were white viscous creamy preparations with a smooth and homogeneous appearance. They were easily spreadable with acceptable bioadhesion and fair mechanical properties.

pH

The pH values of prepared LKG and PKG were 5.40±0.27 and 5.52±0.15, respectively.

Viscosity

The measured viscosity of prepared LKG and PKG were 11309±214 cPs and 10700±437 cPs, respectively.

Content uniformity

Drug content of prepared LKG and PKG were 98.40±0.71% and 99.01±0.23%, respectively.

***In- vitro* drug release study**

The *in-vitro* release profile is an important tool that predicts in advance how a drug will behave *in- vivo*. The *in-vitro* permeation of KTZ using wistar albino rat skin from LKG was compared with that of PKG and PKC containing 2% w/w of KTZ. The permeation of KTZ was calculated in terms of the % cumulative drug released and flux (n=3) at each sampling time points during 12 hours study.

From the results shown in table 2 it can be concluded that cumulative permeation of KTZ was significantly greater from PKC and PKG than from LKG. Plot of % cumulative drug release vs. time (hours) is shown in figure 2. The release of KTZ from LKG is much slower than from non liposomal formulations. KTZ from liposomes showed release of about 34% after 12 hours. The lower flux value of liposomal gel is suggestive of prolong drug release. Multilamellar liposomal formulation produced sustained release of drug because of the presence of several lipid bilayers that release the drug slowly over prolonged period of time.

Skin retention study

Liposomal encapsulation of KTZ shows drug reservoir effect in skin so *in-vitro* skin deposition of KTZ was also calculated. Results of *in-vitro* skin deposition are recorded in table 3. From results shown in table 3 it can be concluded that liposomal encapsulation showed more drug retention compared with plain drug gel and plain drug cream. The higher drug skin retention incase of liposomal gel may be due to, creation of reservoir effect for drug in skin due to deposition of other components of liposomes with drug into the skin and thereby increasing the drug retention capacity into the skin.

***In-vitro* antifungal activity using cup-plate method**

On the basis of *in vitro* characterization studies, KTZ liposomal formulations were further evaluated for *in -vitro* antifungal activity by cup-plate method. Results of the antifungal activity of LKG formulation compared to PKG and PKC formulations. Results of *in- vitro* antifungal activity are shown in figure 2. LKG exhibit maximum antifungal activity after 30 as compared to PKG and PKC formulations. Gel formulation containing liposome loaded with KTZ showed greater and prolonged action than formulations containing KTZ in non-liposomal from.

CONCLUSION

Liposomes containing KTZ (separated from the unentrapped drug) were mixed into the 1% (w/w) Carbopol gel, the amount of liposomes of optimized batch (F_4) added into the gel, such that the prepared gel having 2% w/w KTZ concentration (20 mg drug per 1gm of gel). Plain drug gels (2% w/w) were made under the same conditions. Instead of liposomes, those samples contained free KTZ were incorporated. Cumulative permeation of KTZ was significantly greater from PKC and PKG than from LKG. The release of KTZ from liposomal gel was much slower than from non liposomal formulations. Liposomal encapsulation showed more drug retention compared with plain drug gel and plain drug cream. The higher drug skin retention incase of liposomal gel may be due to, creation of reservoir effect for drug in skin due to deposition of other components of liposomes with drug into the skin and thereby increasing the drug retention capacity into the skin.

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