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Preparation and evaluation of topical hydrogel containing ketoconazole-loaded bilosomes

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ABSTRACT

Ketoconazole (KET) is an antifungal drug that was used for the first time as loaded bilosome that incorporated into Carbopol hydrogels to enhance topical skin application and improve therapeutic efficacy. KET-bilosome which contained span 60: cholesterol: SDC at milligrams weight (350:60:15) was prepared using ultra-sonication method. Then, the bilosome formulation was incorporated into the hydrogel of Carbopol 934 and 940 as a gelling agent. All hydrogels were evaluated for their physical appearance, pH, KET content, viscosity and *in-vitro* release. The optimum KET-bilosomal hydrogel was further evaluated for skin irritation test and antifungal activity. The optimized KET-bilosome loaded hydrogel (FKC934-1%) showed good viscosity, highest spreadability (11.5 ± 0.5 g.cm/sec), and acceptable pH (5.9 ± 0.1) and drug content (98.4 ± 0.5%). Ketoconazole-bilosomes loaded hydrogel was found to be safe and non-irritating when tested on a rat skin. It also exhibited the sustained release profile (82.16% at 8 hours) with a Korsmeyer–Peppas kinetic release model (R^2 = 0.9967). FTIR study shows no important interaction between ketoconazole and the polymers used. The antifungal study revealed a significant ($p<0.05$) enhancement of antifungal activity against *C. albicans* compared to marketed ketoconazole cream.

Keywords: ketoconazole, bilosome, hydrogel, antifungal activity, spreadability

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INTRODUCTION

Ketoconazole (KET) is a broad-spectrum antifungal medication effective against various fungi and yeasts[1]. KET is classified as a Class II biopharmaceutical, which means it has poor aqueous solubility with log P value equal 4.74 which considered that it has good permeability[2]. The creation of a solubilized KET form is difficult, and hence it is required to overcome this limitation to prevent a formulation issue, minimize side effects, and improve the drug's antifungal activity[3].

Antifungal medications treat and prevent the progression of cutaneous fungal disorders. These medications found over the years have little effect on drug resistance, inability to reach target areas, limited residence duration, low bioavailability, lack of penetration, and other issues[4]. One of strategies to overcome these constraints in topical application is the preparation of drug-loaded lipid nanovesicular systems[5]. One of the novel nanocarriers that have been first emerged by Conacher in 2001 is bilosomes, which are defined as bile salt-loaded bilayer vesicles, structurally similar to liposomes and niosomes and differ in composition, stability and storage conditions. Bilosomes are usually made from phospholipid or non-ionic surfactant, cholesterol, bile acid, or salts. They are more stable and flexible compared to conventional liposomes. Bile salts have become widely utilized because of their stability, safety and biological compatibility[6,7]. Also, the bile salts in nanovesicles play an important role as edge activators to impart vesicular elasticity and as charge inducers to supply vesicular stability against aggregation. Due to its biological membrane fluidizing impact, sodium deoxycholate is the most typically used bile salt. It leads to enhanced drug permeation and its ability to enhance the dissolution of insoluble drugs[8,9]. Furthermore, bilosomes have been used as permeation enhancers in topical dosage forms such as buccal, ocular, nasal, and transdermal routes of administration, as well as for increasing the hydrophilicity of water-insoluble active pharmaceutical ingredients[10].

Topical drug delivery is the most basic and easy technique of delivering localized medications to any portion of the body via routes such as ophthalmic, rectal, vaginal, and skin[11].Topical ketoconazole formulations that have been granted approval by the FDA include 2% shampoo, 1% shampoo, 2% cream, 2% gel, and 2% aerosol foam[12]. The bulk of topical dermatological formulations such as creams and ointments, have the drawbacks of having a lower spreading coefficient, being sticky, and requiring rubbing during application. These restrictions can be overcome by using a hydrogel composition[13]. Gels, particularly hydrogels, have attracted much interest due to their appealing look and

nice cold feeling. They are simple to apply and remove[14]. They also provide regulated release characteristics for a variety of drugs[15]. Carbopol vesicular gel with miconazole improved drug permeability, residency time, and medicinal efficacy. A carbopol-gelling substance was additionally identified to extend the liberation time of the drug-loaded nanovesicle system[16]. Since up-to-date there is no research on ketoconazole-loaded bilosomes prepared by ultrasonication and then incorporated into carbopol gel base for topical application. So the goal of this study was the preparation of ketoconazole bilosomal-loaded hydrogel to enhance its solubility, antifungal activity and patient compliance.

METHODOLOGY

Materials

Ketoconazole, sodium deoxycholate, cholesterol, span 60 and span 40 were purchased from Baoji Guokang Bio-technology Co., China. Methanol was supplied from Thomas Baker, India. Phosphate buffer 5.5 from Himedia laboratories, Carbopol 934, Carbopol 940 (Hi Media lab., Ltd, Mumbai, India), glycerin and triethanolamine (Thomas baker/ India).

Preparation of ketoconazole bilosomes

The ultra-sonication method was carried out for the preparation of different ketoconazole bilosomes[17]. Briefly, this method involves mixing 350 mg of span 60 and 50 mg of KET with 60 mg of cholesterol and 15 mg of sodium deoxycholate. Then, 20 mL of distilled water was added to the prepared mixture and the resultant dispersion was homogenized with a homogenizer (Homogenizer HG-150, Witeg Labortechink, Germany) operating for 5 minutes at 5000 rpm. After that, the resultant dispersion was subjected to probe sonication (QSONICA Sonicator, Qsonica, USA) for 5 minutes (50 seconds on and 10 seconds off with 30% amplitude). Finally, the resultant milky dispersion was stored in the refrigerator overnight to allow vesicles to mature and remained there until further evaluation of the bilosomal formula's as previously prescribed[2,18].

Preparation of ketoconazole bilosomal hydrogel / ketoconazole plain gel

Bilosomal hydrogels containing KET-loaded biolosomes were formulated with varying percentages (1% and 2% w/w) of Carbopol 934 and Carbopol 940. The calculated amounts of Carbopol 934 and Carbopol 940 were soaked in distilled water overnight. A defined quantity of KET bilosomal suspension underwent centrifugation at 4 °C and 30,000 rpm for 90 minutes in a cooling centrifuge. The resulting semisolid bilosomal mass, equivalent to 1% w/w, was separated

from the supernatant and incorporated into the Carbopol 934 and Carbopol 940 hydrogel base using an electric homogenizer (1000 rpm) for 30 minutes. Triethanolamine (TEA) was added dropwise for neutralization until the desired pH range of 5.5-7 was achieved. The plain hydrogel was prepared by following above mention method but replacing bilosomal dispersion by pure KET levigated with propylene glycol.

The prepared hydrogels were then stored in the refrigerator for subsequent hydrogel evaluation[19]. Table 1 illustrated the composition of the prepared KET-bilosomal hydrogel and plain ketoconazole hydrogel.

Table 1. Formulas composition of ketoconazole plain hydrogel and ketoconazole bilosomal hydrogel

Formula Code	Carbopol 934 (% w/w)	Carbopol 940 (% w/w)	Ketoconazole bilosomes (% w/w)	TEA	Distilled water (g)
FKC934 1%	1%		1%	q.s	Up to 100
FKC934 2%	2%		1%	q.s	Up to 100
FKC940 1%		1%	1%	q.s	Up to 100
FKC940 2%		2%	1%	q.s	Up to 100

Ketoconazole plain hydrogel (1% w/w) was made by dissolving a measured quantity of Carbopol 934 1% in distilled water to form an aqueous dispersion. A known amount of ketoconazole (equivalent to 1% w/w) was levigated with 5% w/w propylene glycol and added to the hydrogel base, where it was thoroughly dispersed with continuous homogenization until a homogeneous hydrogel was formed. Triethanolamine drops were added until the desired pH was achieved.

Evaluation of ketoconazole bilosomal hydrogel

Physical appearance

All preparation formulas were visually evaluated for color, uniformity, homogeneity, aggregation, presence of grittiness, and separation. Once the hydrogels had been set in their receptacles[20].

pH measurement

The pH of KET-bilosomal hydrogels were measured utilizing a digital pH meter. The pH of topical formulations is meaningful for their association with the skin pH to avert any irritation[21].

Viscosity measurement

A digital viscometer with spindle number R7 (Myr Rotational Viscometer, Spain) was used for investigating the rheological properties of prepared KET-bilosomal hydrogel. Viscosity measurements were taken at room temperature. The spindle was immersed in the tube, and the speed was rotating at 6, 10, 12, 20, 30, 50, 60, 100 and 200 rpm. The observed viscosity values had been expressed in centipoise[22].

Spreadability test

The spreadability of the prepared formulations was studied. Briefly, 0.5 g of hydrogel were placed in the center of a glass slide (14x14 cm) after that, another glass slide had to be placed on top of the first one and then 500 grams weight was placed on the upper glass slide[13]. Following the removal of the weight, the final diameter of the spread hydrogel was measured.

Drug content determination

The drug content in the hydrogel was determined by utilizing a UV spectrophotometer. One gram of the hydrogel was first dissolved in 50 milliliters of methanol. Following that, the solution was sonicated to guarantee full drug solubility in the methanol. Approximately 1 milliliter of this solution was extracted and diluted to a final volume of 10 milliliters. The absorbance was then measured at 243 nanometers after appropriate dilution depending on previous constructed calibration curve which gave straight line equation ($y= 0.029x-0.0007$) and revealed high correlation coefficient ($R^2 = 0.9993$). Finally ,the content of the drug was calculated by using a linear regression of the drug in methanol[23].

Extrudability

The typical method for determining the force required to push material out of a tube is to conduct an extrudability test. In this test, 30 grams of hydrogel were put within a closed collapsible tube, and the plunger was adjusted to keep the tubes in place. A load of 1 kg was used for 30 seconds, and the poured hydrogel was precisely weighed. This procedure was done three times throughout the tube at regular intervals[13].

In-vitro drug release from a hydrogel loaded with ketoconazole bilosomes

In-vitro drug release was performed for the KET-bilosomal hydrogel formulations utilizing a Paddle type II dissolution apparatus[24]. A 0.5 gram hydrogel containing 5 mg of ketoconazole was uniformly put over a dialysis membrane

that soaked overnight in the dissolution medium, about 150 mL of 30% ethanolic phosphate buffer solution is used as dissolution medium to achieve sink condition. With the aid of a rubber band, the dialysis membrane was affixed to the circular open end of a tube. The tubes were then inverted and secured to the lower part of a paddle with rubber bands, ensuring that the gel's lower portion was just submerged beneath the surface of a 150 mL solution of phosphate buffer (pH 5.5) as the receiving medium. The apparatus temperature was maintained at 37 ± 0.5 °C, and the paddle rotation speed was 100 rpm. At predetermined time (1, 2, 3, 4, 5, 6, 7 and 8 hours), five milliliters' samples were withdrawn and replaced by fresh ethanolic phosphate buffer solution. The withdrawal samples were tested for KET amount spectrophotometrically by measuring the absorbance at the maximum wavelength (λ_{max}) of KET in ethanolic phosphate buffer at 235 nm, after appropriate dilution depending on previous constructed calibration curve which gave straight line equation (y= 0.0327x-0.0006) and revealed high correlation coefficient (R^2 = 0.9994).

Kinetic model study

The release mechanism of KET from the prepared formulas was investigated by fitting the release data into the Zero, First, Higuchi, and Korsmeyer Peppas equations. Using a DDSolver Excel Microsoft Add-in application, k and R^2 values were calculated for each equation, as well as the n value for the Korsmeyer Peppas equation at 60% release[25].

FTIR spectrum for the optimum KET bilosomes loaded-hydrogel

Fourier-transform infrared spectroscopy (FTIR) analysis was used to ensure purity, compatibility and the absence of drug-excipient interaction; it was performed for the pure KET, physical mixture of KET with utilized formulation excipients and the optimum bilosome formula[26].

Skin irritation test

This test was carried out by applying sufficient hydrogel to a small region of rat skin under supervision (at the time of application, after one hour and 24 hours). The skin was observed for any visible change and checked for skin hypersensitivity (redness, irritation and edema) or any visible necrosis that could be happened[27].

Antifungal activity of optimum KET bilosome-loaded hydrogel

The antifungal activities of the optimum KET-bilosomal hydrogel formula, plain hydrogel as a control and local marketed cream were evaluated using the agar diffusion method on *C. albicans* ATCC 10231, fungal strains. The auto-

claved aqueous solution of the required quantity of Muller-Hinton agar with 2% glucose for support fungal growth was prepared and poured into sterilized Petri plates. Each plate was then planted with fungi, and the holes were made in the agricultural media after solidification. The samples to be examined were added into the holes, and then plates were incubated at 28°C for 48 h[28]. The zones of inhibition (ZOI) were measured to compare the results.

Statistical analysis

All evaluation tests were carried out in triplicate, and the findings were presented as mean ± SD. Microsoft Excel2010 utilized one-way ANOVA for statistical analysis. P values of 0.05 or less were considered statistically significant, whereas values of 0.05 or higher were considered statistically insignificant.

RESULTS and DISCUSSION

Preparation of ketoconazole bilosomes

Span60, sorbitan monoester, was selected as membrane forming nonionic surfactant own to its lipophilic saturated alkyl chain, high transition temperature and optimum HLB value (4-8) that can create stable single and/or multilamellar nanovesicles layers. The appearance of the prepared bilosomal formula was a homogenous milky white liquid dispersion. Furthermore, it had optimum vesicles nanosize (229.63 ± 6.22 nm) and PDI about 0.376 ± 0.029 that indicate homogeneity and approximately can be considered monodispersion as well as good % EE which was 83.13 ± 1.21%. similar result were attained by previous study[29].

Evaluation of ketoconazole bilosomal hydrogel

Physical appearance

All prepared KET-bilosomal hydrogel formulas had an off-white appearance and were smooth in texture with no grittiness or evidence of phase separation. This result agrees with the previous study, which stated that all prepared gelling systems were evaluated for visual appearance, clarity, and pH. The pH of the formulation decreases as the carbopol concentration increases. The pH of the formulation becomes acidic due to the polymer's acidic nature[30].

pH measurement

The developed KET-bilosomal hydrogel formulations had pH values ranging from 5.9 ± 0.1 to 5.5 ± 0.1 as shown in Table 2, which in agreement with the regulations required for topical treatment to avoid any skin irritation[31].

Table 2. The pH, spreadability, extrudability and % drug content evaluation of the different KET-bilosomal hydrogel

Formula	pH	Spreadability (cm)	% Drug content	Extrudability
FK C934 1%	5.9 ± 0.1	11.5 ± 0.5	98.4 ± 0.5	Excellent 93%
FK C934 2%	5.8 ± 0.1	9 ± 1	98.3 ± 1.05	Good 85%
FK C940 1%	5.6 ± 0.05	7 ± 0.5	98.7 ± 0.6	Fair 76%
FK C940 2%	5.5 ± 0.1	5 ± 0.5	98.76 ± 0.25	Fair 66%

Spreadability

Spreadability is an important issue in topical drug delivery systems since the efficacy of therapy is dependent on patients evenly applying the drug formulation to give a standardized dosage. It was revealed that increasing the polymeric content has a significant impact on spreadability since increasing the viscosity of the gel diminishes the formulation's spreadability. It is clear from the Table 2 that the spreadability of hydrogel formulas of Carbopol 940 is less than Carbopol 934. This is because the spreadability of any semisolid preparation decreases as the viscosity of the polymer increases. Similar results were attained by previous study which stated that the ejected gel was gathered and measured. The results were explained by difference in the concentration and nature of crosslinking of polymers[32].

Extrudability

The amount of extruded gel was measured and collected. The extrudability of the product exhibited decreasing as the gelling polymer percent raised[13], and the result shown in Table 2 that Carbopol 934 1% has excellent outcome. Similar results were gained by prior study[33].

Drug content

The ketoconazole content in the hydrogel formulations was ranged from 98.3 ± 1.05 to 98.76 ± 0.25% which indicated uniform distribution of KET in all hydrogel formulations and to be in good agreement with the theoretical drug content.

Viscosity

Viscosity plays a meaningful role in determining drug content and release from prepared KET-bilosomal hydrogel formulations. The viscosity experiment was carried out to determine the impact of Carbopol base type and concentration

on hydrogel viscosity. Hydrogel viscosity was measured at various shear rates was shown in Figure 1. It was revealed that as the shear rate raised, the viscosity of the hydrogel decreased, Likewise, it was found that increasing in the viscosity of the prepared hydrogel is caused by an increase in the amount of polymer. The results agreed with the earlier study, which stated that viscosity greatly affected the drug's content and release. The viscosity had done to evaluate the base's type and concentration impact on gel viscosity. The study showed that the gel's viscosity a decrease as the shear rate increases[34].

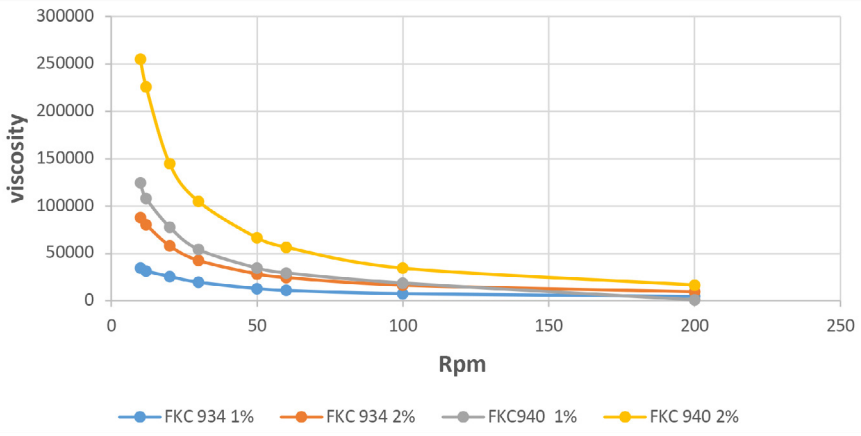

Figure 1. Viscosity versus share rate of the KET-bilosomal hydrogel

In-vitro drug release from ketoconazole –bilosome-loaded-hydrogel

The study of ketoconazole's *in-vitro* release patterns from several bilosome hydrogel compositions was performed and Figure 2 showed the results which revealed that the formulations containing 1% Carbopol 934 exhibited the highest drug release, whereas those containing 2% Carbopol 934, 1% Carbopol 940, and 2% Carbopol 940 exhibited slower drug release rates. This indicated the importance of Carbopol grade and concentration as gelling agent on drug release kinetics[35].

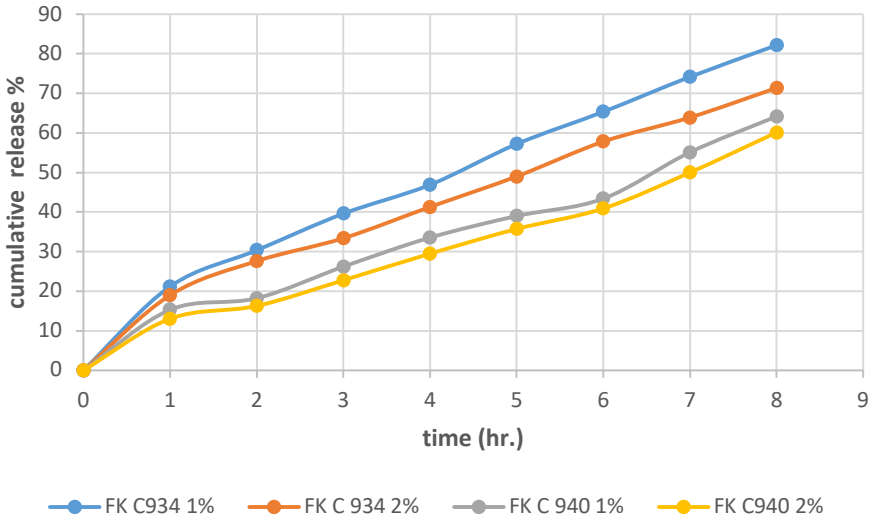

Figure 2. *In-vitro* drug release from ketoconazole -bilosome-loaded-hydrogel

Kinetics of release

The values of the release rate constant and regression coefficient (R^2) of the KET released profile obtained by applying different mathematical models are listed in Table 3. KET releasing profile for all tested bilosomal formulas best fit the Korsmeyer-Peppas model since it exhibited the highest R^2. The release exponent (n value) was less than 0.85 and larger than 0.43, which indicated that non-fickian diffusion (anomalous) is a drug transport mechanism[29].

Table 3. Mathematical model data of in-vitro ketoconazole bilosome releasing from hydrogel

Formula	Zero order		First order		Higuchi model		Korsmeyer Peppas model		
	k_0	R^2	k_1	R^2	k_H	R^2	k_{KP}	n	R^2
FKC934 1%	10.993	0.9514	0.181	0.9836	26.194	0.9599	19.269	0.671	0.9967
FKC934 2%	9.556	0.9453	0.145	0.9854	22.792	0.9619	17.064	0.662	0.9939
FKC940 1%	7.936	0.9732	0.108	0.9689	18.731	0.9067	12.809	0.682	0.9904
FKC940 2%	7.288	0.9828	0.096	0.9706	17.135	0.8905	10.486	0.753	0.9912

So, 1% Carbopol 934 (FKC934 1% formula) was selected as the optimum formula for KET-bilosomal hydrogel with good and acceptable physical properties include its in-vitro release, viscosity, spreadability and extrudability.

FTIR spectrum for the optimum KET bilosomes loaded-hydrogel

Any interaction or incompatibility of KET with the hydrogel formulation excipient mixture was investigated by comparing their FTIR spectra, as illustrated in Figure 3. The primary ketoconazole characteristic bands have been identified at 1645.28, 1583.58, 1510.26, 1285, and 814 cm^{-1}, which correspond to C=O stretching, C=C aromatic symmetrical stretching, C=C aromatic asymmetrical stretching, tertiary amine, and -C-Cl stretching, respectively. The FTIR spectrum of the physical combination revealed all of the principal KET peaks with no alterations in position. That suggests no interaction exists between ketoconazole and the components employed in crucial hydrogel production[36].

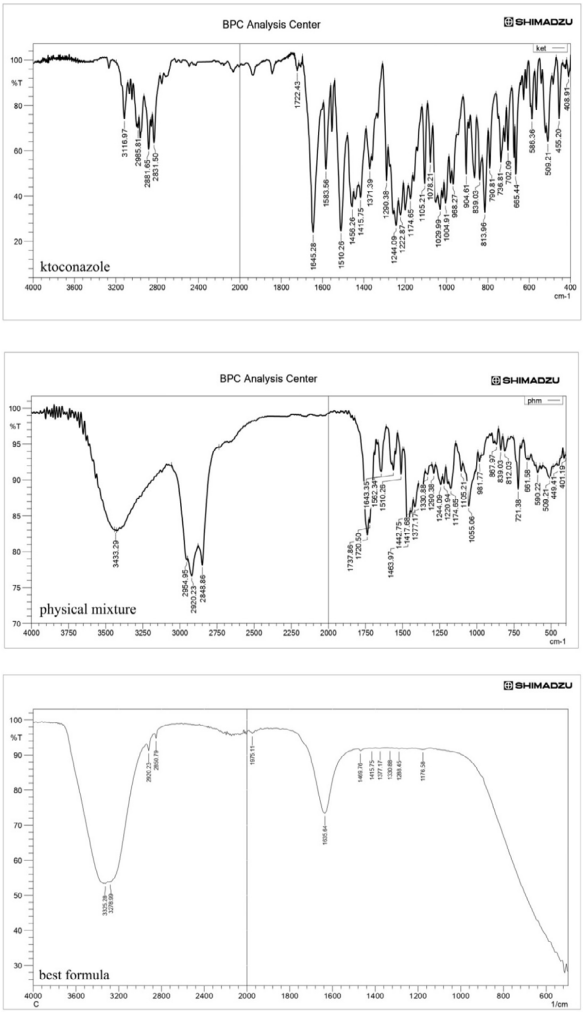

Figure 3. The FTIR spectra of ketoconazole, physical mixture and the best selected formula

Skin irritation test

To achieve the safety of the optimal hydrogel formula (FKC934 1%), a skin irritation test was done after topical hydrogel application on skin rats. Application of the hydrogel on the skin was found to have no irritation and no redness as shown in Figure 4, and this result agrees with a previous study[37].

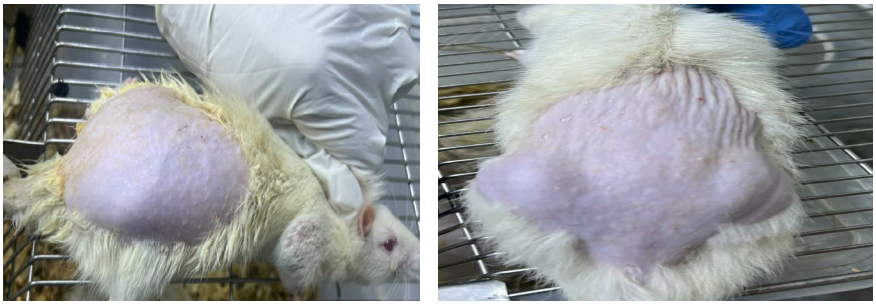

Figure 4. Rats' skin at time of application (left) and after one hour (right) after application KET bilosomal hydrogel

Antifungal activity of optimum hydrogel loaded with ketoconazole bilosomes

The antifungal activity assessment of KET-bilosomal hydrogel, plain hydrogel, ethanolic ketoconazole solution, and marketed ketoconazole cream was carried out against *Candida albicans,* the most common human pathogenic fungus. As shown in Figure 5, the plain hydrogel showed no growth inhibition. Meanwhile, KET-bilosomal hydrogel (FKC934 1%) gave a zone of inhibition diameter of about 18.5 ± 0.5 mm, ethanolic KET-solution 1% (as positive control used) showed growth inhibition 19.16 ± 0.28 mm and marketed ketoconazole cream zone inhibition 1.06 ± 0.11 mm. The highest inhibition zone of ketoconazole compared to plain hydrogel could be due to the highest release of the ketoconazole from hydrogel bilosomal formulation. This result confirmed with the earlier study[38].

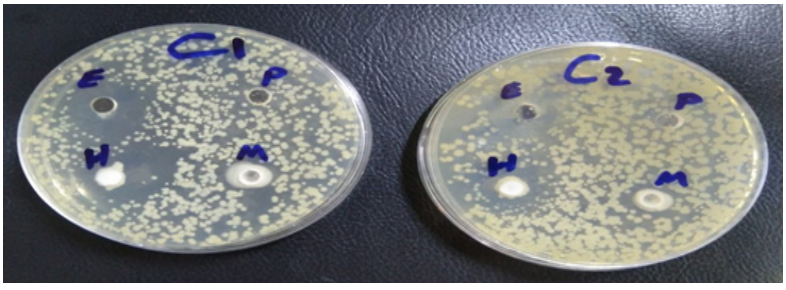

Figure 5. Photo illustrated; H: KET-bilosomal hydrogel, E: ethanolic ketoconazole solution, P: plain hydrogel, M: marketed ketoconazole cream

The characterization approaches and *in-vitro* release studies of the prepared KET-bilosomal hydrogel using Carbopol exhibited acceptable pH, good viscosity, spreadability, excellent extrudability, and good release profile. The optimum formula that containing KET bilosomes loaded in 1% Carbopol 934 showed 18- and 17-fold enhancement in antifungal activity against *C. albicaes* compared to plain gel and local marketed cream, respectively which provide a promising result for further studies.

STATEMENT OF ETHICS

All the necessary ethical rules were followed while performing research. Research ethical approval form number RECAUBcD151020BA have assigned by Research Ethics Committee at College of Pharmacy/University of Baghdad.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the study as follows: study conception and design: Lubna Abdalkarim Sabri (L.A.S.); methodology and data collection: Amer Sajjed (A.S.); analysis and interpretation of results: L.A.S and A.S.; draft manuscript preparation: A.S.; reviewed the results and approved the final version of the manuscript: L.A.S.

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