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Formulation and *In-vitro* Evaluation of Nanovesicles Bilosomes Loaded with Ketoconazole

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Abstract

Ketoconazole (KET), an imidazole derivative with well-known antifungal properties. It has poor aqueous solubility; therefore, its topical clinical use has practical disadvantages**.** Bilosome plays an important role in topical drug delivery as they can reduce toxicity and modify dermal drug targeting by acting as a drug reservoir that can adjust the drug release rate. This research aimed to formulate and optimize KET bilosomes to enhance its dissolution and topical antifungal activity. Ten 0.25% w/v of KET bilosomes formulas were prepared by probe sonication method using two types of non-ionic surfactants (Span 40 or Span 60) along with cholesterol and sodium deoxycholate (SDC) as lipid strengthen and bile salt stabilizer, respectively. The formulations were evaluated for vesicles size, poly dispersity index (PDI), entrapment efficiency and *in- vitro* drug release. The optimum formula was investigated for its morphological property, zeta-potential, FTIR spectrum and antifungal activity. All prepared bilosomes showed nanosize vesicles of (189.27 ± 2.75 - 251.87 ± 1.74 nm) and entrapment efficiency was found in the range of 32.07 ± 0.67 and 92.33± 0.76 %. The optimum formula that contained span 60: cholesterol: SDC at weight (350:60:15 mg) had spherical shape appearance without aggregation and its zeta potential was – 54.45 mV. The optimum formula was found to provide significant antifungal activity. Its % cumulative KET released was 97% at 8 h. According to obtained result, it was concluded that bilosomes loaded with KET can be developed successfully to improve dissolution and the antifungal activity.

Keyword: Antifungal activity, Bilosoms, Ketoconazole, Span 60, Probe sonication.

Introduction

Ketoconazole (KET) is antifungal phenylpiperazine derivative medication. It is chemically 1-acetyl-4-(4-{[2-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1, 3-(dioxolan-4-yl] methoxy} phenyl piperazine ethenone. It has a high molecular weight of 531.4 Da [(1)]. It is widely used to treat yeast or fungus infections such candidiasis, blastomycosis, coccidioidomycosis, histoplasmosis, and chromoblastomycosis. This medication eliminates the yeast or fungus or stops its growth [(2)]. KET has broad-spectrum antifungal properties and blocks the action of cytochrome P450 14-demethylase to prevent the formation of the fungus ergosterol. Furthermore, KET has anti-inflammatory action by inhibition of lipoxygenase synthesis and thus block leukotriene B4 production in skin, so KET is effective in alleviating symptoms of seborrheic dermatitis and dandruff [(3)].Ketoconazole belongs to a Class II according to Biopharmaceutical Classification System (BCS). Its properties include low water solubility and high lipid solubility with log P equals 4.74, as well as high permeability [(4)]. KET has two weak basic groups with pKa values of 6.5 (imidazole) and 2.9 (piperazine) [(5)]. The challenge is to generate a solubilized KET form in order to bypass a formulation issue, enhance the drug's antifungal effectiveness and lessen its side effects associated with conventional topical preparation like moderate burning at the place of application, serious allergic reactions, blisters, itchiness, discomfort, or a reddish [(6, 7)]. In spite of approximately more than half billons around worldwide people have suffered from cutaneous fungal infections, there are several restrictions of appropriate delivery of antifungal medication across skin to deeper layers by conventional topical pharmaceutical delivery systems [(8)]. Therefore, topical nano-carrier drug delivery systems have been developed to overcome solubility, stratum corneum permeation and depositions issues as well as lessen undesirable systemic side effects [(9, 10)]. One of the novel nanocarriers that had been firstly emerged by Conacher in 2001 was bilosomes which are defined

as bilayer membrane vesicles containing bile salt, structurally similar to liposomes and niosomes and differ in composition, stability and storage conditions. Bilosomes usually made up from phospholipid or non-ionic surfactant and cholesterol in addition to bile acid or its salts. They are more stable and flexible compared to conventional liposomes. Today, bile salts become widely used because of their stability, safety and biological compatibility [11, 12]. Furthermore, the bile salts in nanovesicles play an important role as edge activator to impart vesicular elasticity and as charge inducer to provide vesicular stability against aggregation. Sodium deoxycholate is the most commonly used bile salts due to its biological membrane fluidizing effect leading to enhance drug permeation and also its ability to enhance dissolution of insoluble drug [13, 14]. The dissolution of butenafine HCl was enhanced when prepared as bilosomes and showed the maximum drug release of 81.09 ± 4.01% in 24 h compared to the dissolution of pure drug dispersion which was found to be 17.54 ± 5.37%. Furthermore, the enhancement ratio of permeation flux of butenafine HCl bilosomes was 2.34-fold compared to pure drug [15]. Among different methods, such as ether injection, reverse phase evaporation and thin film hydration techniques that used for bilosomes preparation, ultrasonication by probe sonicator is considered as the simplest, more fast, eco-friendly with no need to utilize organic solvent and more cost effectiveness [16]. However, this research aimed to formulate and optimize sodium deoxycholate bilosomes loaded with KET in order to enhance drug dissolution and topical antifungal activity.

Materials and Methods

Materials

Ketoconazole, sodium deoxycholate, cholesterol, sorbitan monostearate (span 60) and span 40 (Baoji Guokang bio-technology Co., China). Methanol (Thomas Baker, India). Phosphate buffer 5.5 (Himedia laboratories, India). Dialysis membrane, M.wt 8000-14000 Da, (Special products laboratory, USA).

Methods

Preparation of ketoconazole bilosomes

The bilosomes performance and properties, such as EE%, uniform stable nanosized with acceptable safety profile were largely affected by the chemistry nature and the amount of the selected lipid bilayer builder and bile salts additives. So, span®60 and span® 40 were 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 [17]. Ultra-sonication method was carried out for the preparation of formulas ketoconazole bilosomes [16]. Ten formulas (BF1-BF10) were prepared by mixing a fixed amount (350 mg) of span 60 or span 40 and KET with different amounts of cholesterol (membrane stabilizer), and SDC (bile salt) as shown in Table 1. Then, 20 ml of distilled water was added to the prepared mixture. The resultant dispersion was homogenized by a homogenizer (Homogenizer HG-150, Witeg Labortechink, Germany) operating 5 min at 5000 rpm. After that, the resultant dispersion was subjected to probe sonication (QSONICA Sonicator, Qsonica, USA) for 5 min (50 seconds on and 10 seconds off with 30% amplitude). Finally, the resultant milky dispersion was stored at the refrigerator overnight to allow vesicles to mature and remained there until further physicochemical evaluation of the formulas. The amount of stabilizer (cholesterol), amount of SDC and the type of bilayer forming component (span) were varied to optimize bilosomes formulation.

Table1. Composition of different KET-loaded bilosomes formulas

Formulas code	Ketoconazole (mg)	Span 60 (mg)	Span 40 (mg)	Cholesterol (mg)	SDC (mg)	Total weight (mg)	Distilled water (ml)
BF1	50	350	----	15	10	425	20
BF2	50	350	----	30	10	440	20
BF3	50	350	----	60	10	470	20
BF4	50	350	----	90	10	500	20
BF5	50	350	----	60	15	475	20
BF6	50	٣٥٠	----	60	5	465	20
BF7	50	350	----	90	15	505	20
BF8	50		----	90	5	495	20
BF9	50	----	350	60	15	475	20
BF10	50	----	350	90	15	505	20

In-vitro characterizations of KEC bilosomes

Determination of bilosomes size and their polydispersity index (PDI)

For bilosomes size and PDI measurements using Zetasizer Ultra analyzer (Malvern analytical,

UK), one mL of each prepared formula was diluted with 10 ml of distilled water to reach faint opalescence. The molecules' ability to scatter light at angle of 90º was recorded as scattering intensity versus vesicles diameter at a constant temperature, 25 º C. Measurement of each formula were done in triplicate [18].

Determination of drug content and entrapment efficiency

A direct method was applied for determining KET entrapment efficiency % (EE %). A volume (1 ml) of each bilosome formula was subjected to centrifugation using cooling centrifuge operating at 9000 rpm at temperature 4 ºC for 90 min, the supernatant was discarded and the precipitates were dissolved in methanol to obtain 10 ml, with the aid of sonication using bath sonicator operated at 56 ºC for 5 min. The entrapped KET was determined spectrophotometrically at KET λmax 243 nm 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 entrapment efficiency of each formulation was calculated using the equation below [19]:

Entrapment efficiency % = (Entrapped drug /Total drug content) × 100… Equation 1[19]

In vitro drug release of the selected bilosomes formulations

In- vitro drug release was performed for the selected bilosomal formulations that showed high EE% and low nanosize bilosome distrbution. One milliliter of selected bilosomes dispersion formulas which contained equivalent amount of 2.5 mg KET was taken and dispersed in 5ml of dissolution medium before placing in dialysis bag. Then the dialysis bag was placed in type II dissolution apparatus (paddle type). The dissolution medium was 500 ml of 30% ethanolic phosphate buffer (pH 5.5) solution to achieve sink condition. The apparatus temperature was maintained at 37 ± 0.5 ºC and the paddles rotation speed was 100 rpm. At predetermined time (1, 2, 3, 4, 5, 6, 7 and 8 h), 5ml samples were withdrawn and replaced by fresh ethanolic phosphate buffer solution [20]. The withdrawal samples were tested for KET concentration spectrophotometrically by reading the absorbance at 235 nm after an 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). The dissolution test was repeated twice of each selected formula.

Release kinetic modeling study

The mechanism and kinetic of KET release from the selected bilosomes formulas were ascertained by fitting the obtained *in-vitro* release data to various mathematical equations of (zero order, first order, Higuchi and Korsmeyer Peppas) release kinetic model using the DDsolver software. Model with the highest correlation coefficient was selected to be the best fitted model [21].

Optimization of KET bilosomes

Depending on the data of the prepared bilosomes formulations, the formula that exhibited the highest amount of KET released after 8 h with acceptable EE% and nanosize as well as good PDI was chosen as the best formula to be further studied.

Surface morphology determination

To check the shape of prepared vesicles, an aliquot of optimum bilosome formula dispersion was observed under the optical microscope to ensure vesicles formation. Also, the surface morphology was investigated by transmission electron microscope (TEM) at accelerating voltage of 100KV. Before examination, one drop of optimum formula was diluted and employed on carbon coated copper grid and left till dry to form thin film. Then, the sample was viewed at suitable magnification power [22].

Zeta potential measurement

The zeta potential serves as a significant predictor of the *in-vivo* behavior and storage stability of colloidal nanosystems. In general, reducing the aggregation of colloidal particles depends on the electrostatic repulsion that high zeta potential values between particles impart. The diluted bilosomes dispersion was placed in a disposable cuvette of Zetasizer analyzer (Malvern instruments, UK) with in-built software to measure electrophoretic mobility and then converted to zeta potential [23].

Fourier-transform infrared spectroscopy (FTIR) analysis

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.

Antifungal activity study

The antifungal activities of the optimum KET bilosomes formula, placebo bilosome, as a control, which had the same composition of the optimum formula except not contain KET, and marketed cream (kenazole ®cream 2% w/w) were evaluated on *C Candida albicans ATCC 10231*, fungal strains, using the agar diffusion method. The autoclaved aqueous solution of the required quantity of Muller-hinton agar with 2% glucose for supports fungal growth were prepared and poured into sterilized petriplates. Each plate was then planted with fungi and 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 º C for 48 h [24]. The zones of inhibition (ZOI) were measured to compare the results.

Statistical analysis

All evaluation tests were done in triplicate and results expressed as mean ± SD. One-way ANOVA was employed for the statistical analysis by Microsoft Excel2010. The P values of 0.05 or less were regarded as statistically significant, whereas values more than 0.05 was regarded as statistically insignificant.

Results and Discussion

Preparation of ketoconazole bilosomes

The appearance of the ten prepared formulas was a homogenous milky white liquid dispersion. The impacts of studied variables include; different amount of cholesterol, SDC and span type on the characteristics of bilosomal vesiceles were investigated and the results were reported in Table 2. It was found that bilosomes had nanosize that ranged from 189.27 ± 2.75 to 251.87 ± 1.74 nm, and the PDI varied between 0.281 ± 0.035 and 0.47± 0.049 (Table 2), all the PDI value were less than 0.5 that indicated an acceptable vesicular size distribution of the prepared bilosomal dispersion [8]. On the other hand, data for the EE % were between 32.07 ± 0.67 and 92.33± 0.76%.

Table2. The bilosomal vesicular size, PDI and Entrapment Efficiency % of the prepared formulas

Formula Code (span type (Cholesterol: SDC amount))	Bilosomes size (nm)*	PDI*	Entrapment Efficiency %*
FB1 (Span 60 (15:10))	216.53 ± 2.99	0.311 ±0.011	32.07 ± 0.67
FB2 (Span 60 (30:10))	217.43 ± 4.31	0.281 ± 0.035	59.10 ± 0.75
FB3 (Span 60 (60:10))	228.73 ± 3.37	0.379 ± 0.023	82.00± 1.00
FB4 (Span 60 (90:10))	241.87 ± 5.31	0.414± 0.061	88.03±1.05
FB5 (Span 60 (60:15))	229.63 ± 6.22	0.376± 0.029	83.13 ±1.21
FB6 (Span 60 (60:5))	222.93 ± 2.96	0.333 ± 0.04	70.4 ± 2.07
FB7 (Span 60 (90:15))	251.87 ± 1.74	0.463±0.098	92.33± 0.76
FB8 (Span 60 (90:5))	230.66 ± 4.4	0.47 ± 0.049	84.17 ± 2.75
FB9 (Span 40 (60:10))	189.8 ± 3.44	0.295± 0.02	43.93± 1.65
FB10 (Span 40 (90:10))	198.27 ± 2.75	0.284±0.019	58.20± 1.3

*The results were expressed as mean ± SD.

The impact of formulation variables on bilosomal size and percent of entrapment efficiency

Among the critical factors that control drug release, bilosomal stability, penetration and deposition into deeper skin layers is vesicle's size [14]. In this study, it was found that increasing cholesterol amount (from 15 mg in FB1 to 90 mg in FB4) led to larger vesicles obtained ($p<0.05$) with increasing in % EE, which may due to the bilosmoal's surfactant bilayer lipophillicity, packing tightness and stability were enhanced with increasing cholesterol amount leading to higher aqueous phase inside bilosomes and also more lipophilic drug loaded in membrane bilayer, both reasons may contributed in the increasing of bilosomes size and %EE, by decreasing the drug leakage and vesicles permeability. The aforementioned results were in agreement with that obtained by Al-mahallawi et al who reported that there was direct correlation between particle size and the amount of drug entrapped and included in the hydrophobic space between bilayers of the bilosome [25]. On another hand, replacing of span 60 by span 40 as in formulas (BF9 and BF10 compared to corresponding formulas BF3 and BF4, respectively) caused significant ($P<0.05$) decrease in bilosomal size and EE%. Similar results were achieved by Zafar et al. [26] who attributed such results to the longer alkyl chain of span 60 (C18), higher transition temperature (Tc ~53 ^{o}C) with lower HLB value 4.7 in comparison with span 40 which had alkyl chain of C16, its Tc ~ 42 ^{o}C with HLB value 6.7. Even both span 60 and span 40 have same polar head group but higher lipophilic nature of span 60 leading to higher amount of KET was entrapped. Similar finding was reported by Salem et al. [27]. By contrary, span 40 gave significant ($P < 0.05$) tiny bilosomes vesicles due to its higher HLB with more reduction of interfacial energy and properly mixed micelles formation. Furthermore, it was found when increasing the amount of SDC from 5 mg (formulas BF6 and BF8) to 10 mg (BF3 and BF4) caused significant ($P< 0.05$) increasing in vesicles size and EE% which may be attributed to promotion of KET solubility and bilayer flexibility by SDC as it has surface active property and anionic nature [15]. Meanwhile, increase SDC amount from 10 mg (BF3 and BF4) to 15 mg (BF5 and BF7) did not have significant effect ($P > 0.05$) on neither vesicles size nor EE%. Similar results were achieved by Al-mahallawiet et al. [25].

***In-vitro* drug release of the selected bilosomes formulations**

The bilosomes that revealed high EE% (more than 80%) were evaluated for their *in-vitro* % of cumulative KET released. The release profile of selected five (BF3, BF4, BF5, BF7and BF8) formulas and pure drug suspension was shown by Figure 1. The % cumulative of KET released from bilosomes at 8 h was ranged from 59.8 to 97.0 % compared to 17.5% of pure KET with significant enhancement ($p< 0.05$) in their dissolution profiles.

Similar results attained by Zafaar A. et al. [15] who explained that poor water solubility of pure drug exhibited slow-release pattern. Meanwhile the drug entrapped in nanobilosome showed higher release due to nanosize of vesicles and the presence of surfactant and SDC would help in reduction of interfacial tension and increase drug solubility in medium and then showed slow diffusion from bilosomal matrix. Both the amount of cholesterol and SDC showed statistically significant ($P < 0.05$) effect on % cumulative KET released. It was found that increasing cholesterol caused decreasing % KET released due to the bilosomal wall became more compact and impair KET elution from vesicles, comparable results was found by the Shirsand et al. [6] who stated the ability of cholesterol to cement the leaking space in the bilayer membranes, which in turn allow enhanced drug level (EE%) in niosomes and more time was taken for maximum drug release. By contrary, increasing amount of SDC led to increase KET release which might attributed to increase vesicles flexibility by intercalation of bile salts into membrane core, but KET release decrease at too high SDC amount [26].

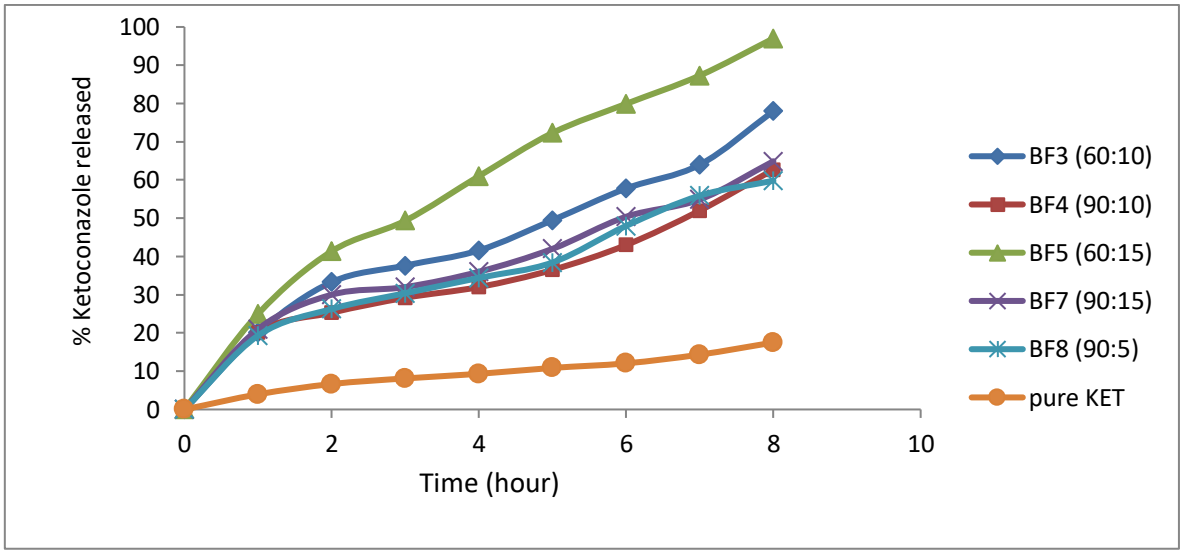

Figure 1. The *in-vitro* % ketoconazole released versus time profiles for the prepared bilosomes at different (cholesterol: SDC) weight amount compared to pure KET suspension.

Release kinetic modeling

The values of release rate constant and regression coefficient (R^2) of KET released profile that obtained by applying different mathematical models were listed in Table 3. KET releasing profile for all tested bilosomal formulas were best fit with 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 non-fickian diffusion (anomalous) was drug transport mechanism [12].

Table 3. Mathematical Model Data of *in-vitro* Ketoconazole Releasing from Bilosomes

formula	Zero order		First order		Huguchi model		Korsmeyer Peppas model		
	k_0	R^2	k_1	R^2	k_H	R^2	k_{KP}	n	R^2
BF3	9.962	0.9128	0.155	0.9594	23.827	0.9566	18.173	0.663	**0.9790**
BF4	7.806	0.8855	0.107	0.9190	18.676	0.9325	14.482	0.653	**0.9510**
BF5	13.371	0.9163	0.264	0.9818	32.046	0.9794	25.234	0.644	**0.9987**
BF7	8.514	0.8658	0.123	0.9343	20.473	0.9662	17.593	0.592	**0.9741**
BF8	8.091	0.8934	0.114	0.9490	19.401	0.9639	15.690	0.628	**0.9785**

Optimization of KET bilosomes

Regarding the results finding, formula BF5 was selected for further investigation. Since, it had optimum nanosize (229.63 ± 6.22 nm) and PDI about 0.376± 0.029 that indicated homogeneity and approximately can be considered monodispersion. Furthermore, it had good EE% which was 83.13 ±1.21% and showed the highest % KET release about 97% at 8 h.

Surface morphology of KET bilosomes

Both light microscope and TEM photos illustrated the formation of spherical vesicles with slight thick dark wall surrounded the aqueous core. The pictures depicted acceptable dispersion of

bilosomal vesicles without any aggregation; they are pointed by arrow (Figure 2).

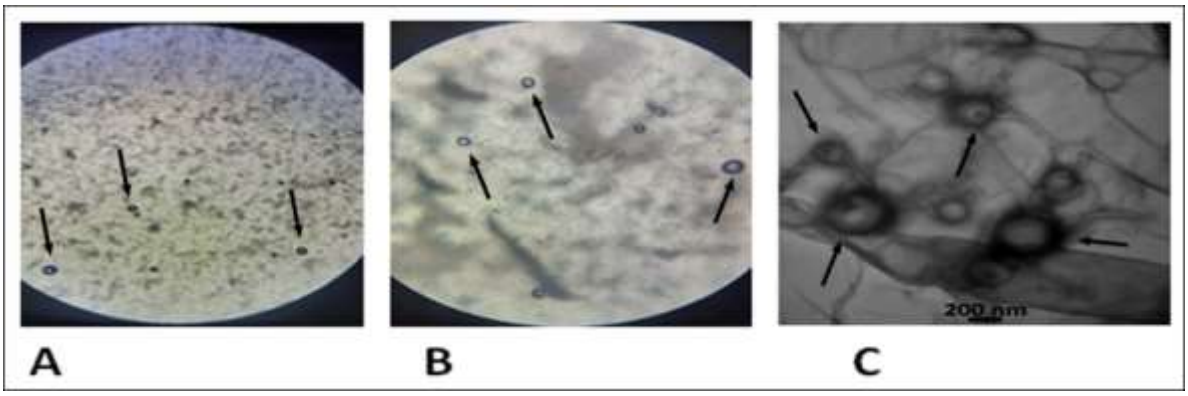

Figure 2. Surface morphological photos of KET bilosomes (Formula BF5) taken by; A and B: optical microscope, and C: transmission electron microscope.

Zeta potential of KET bilosomes dispersion

Bilosomal total surface charges magnitude determined by measuring zeta potential which is one of the confirming indicators for vesicles stability. In general, a high absolute value (± 30 mV) of the zeta potential of the delivery system is suggested to be more physically stable by maintaining electrical repulsion among them and hinder particles aggregation [28]. The optimum KET-loaded bilosomes formula (BF5) revealed negative zeta potential value of -54.45 mV as shown in Figure 3. This charge was due to anionic nature of the used bile salt, SDC [29].

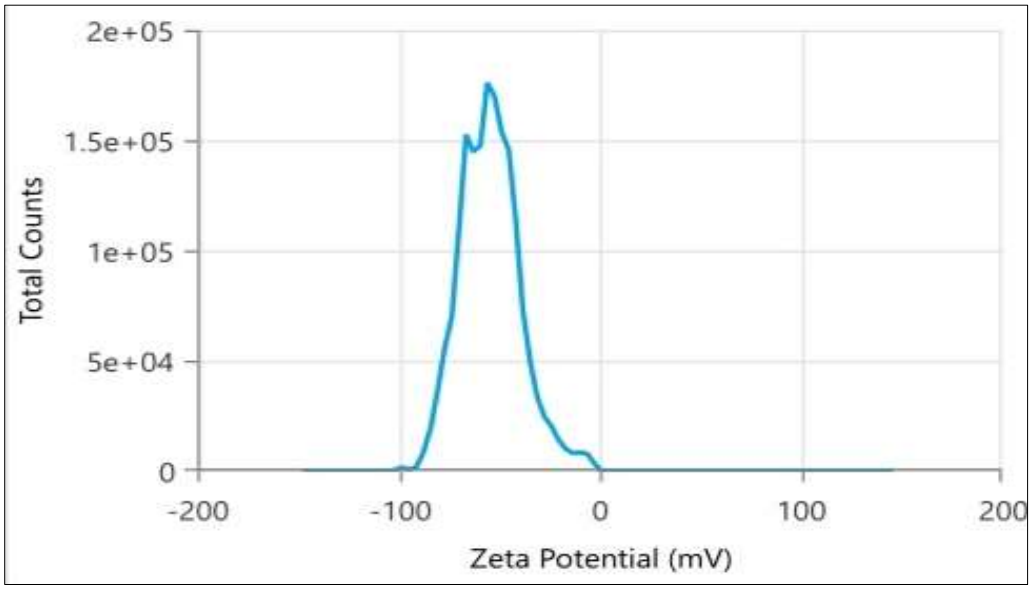

Figure 3. Zeta potential graph of KET-loaded bilosome formula (BF5).

The FTIR spectrum for the optimum KET bilosomes formula

Any interaction or incompatibility of KET with bilosomal excipients mixture was investigated by comparing their FTIR spectra as illustrated by Figure 4. The main characteristics bands of KET were observed 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, teriary amine and -C-Cl stretching, respectively [30, 31]. The FTIR spectrum of physical mixture demonstrated all main KET peaks without shifting in their position. This indicated that there was no interaction between ketoconazole and the materials used for physical mixture bilosome preparation [17]. Formula BF5 exhibited fewer characteristic peaks of the drug at (1605 and 1285.4 cm^{-1}) with overlapping in the fingerprint region, which indicated a greater degree of trapping of KET inside the bilosomes components which reflected KET solubilization, and hence enhance its dissolution. The obtained spectrum was consistent in shape with the reported one [6].

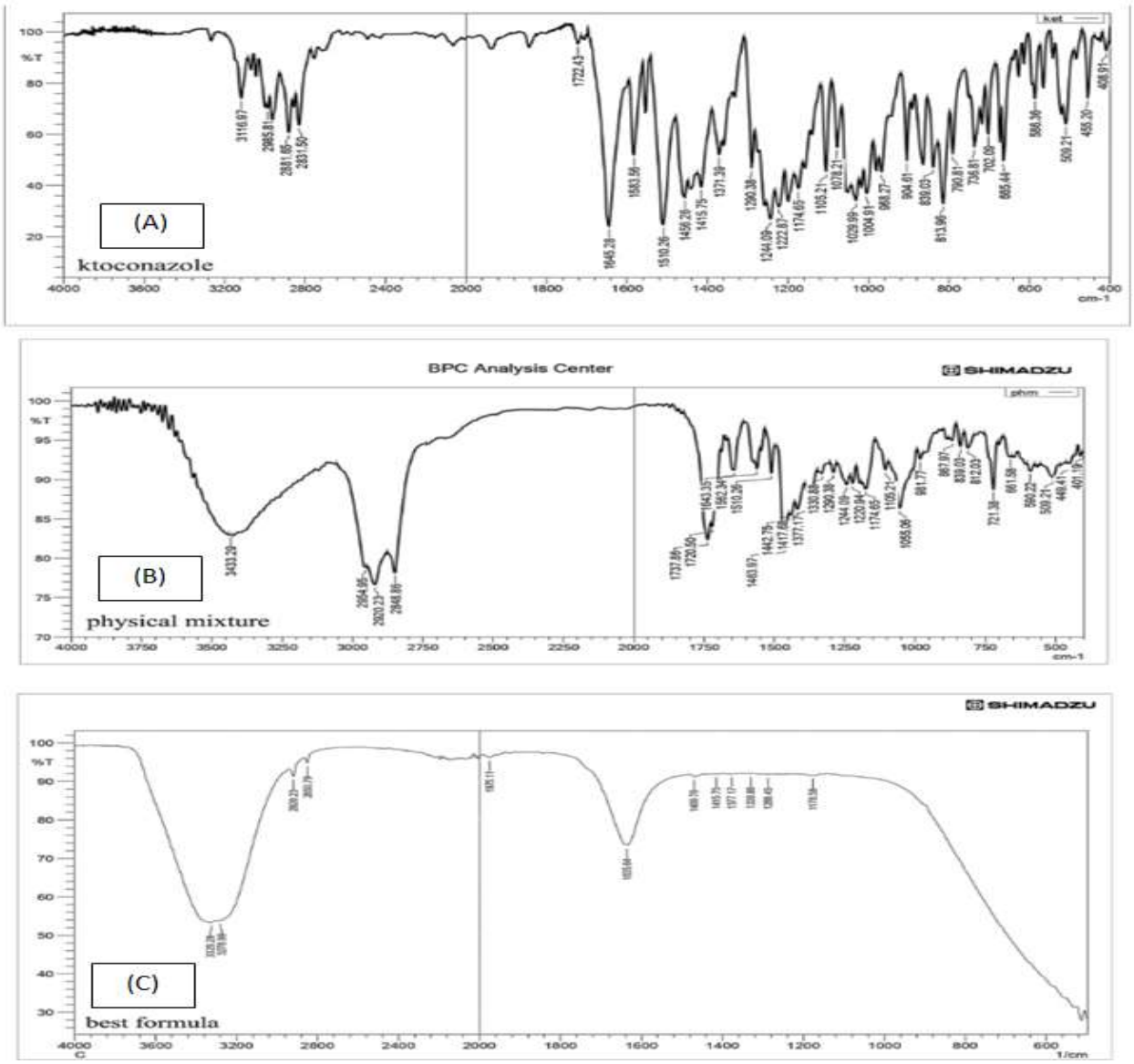

Figure 4. The FTIR spectra of (A) ketoconazole, (B) physical mixture and (C) the best selected formula (BF5) of KET-loaded bilosome.

Antifungal activity of optimum KET bilosome formula

The antifungual activity assessment of KET marketed cream and bilosomal dispersion was carried out against *Candida albicans* the most common human pathogenic fungus. Placebo bilosome showed no growth inhibition and it's represented a negative control. Meanwhile KET bilosome formula (BF5) gave zone of inhibition diameter about 15.0 ± 0.28 mm compared to marketed cream (kenazole ®cream 2% w/w) which exhibited 1.2 ± 0.35 mm diameter of inhibition zone. Such results might attribute to faster KET diffusion through agar media after releasing from formula to exert its antifungal activity [8] and this in agreement with the previous study [32].

Conclusions

In the current study, KET bilosomes was successfully prepared by probe sonication method. KET bilosomes dispersion was introduced as promising drug carrier to be applied topically or incorporated into topical dosage form. KET bilosomes entrapment efficiency% and vesicles size increased with increase amount of cholesterol added but resulting in decreasing the dissolution rate of KET. The % KET released from bilosomes can be improved by the increase amount of SDC. The optimized KET formula (BF5) showed a nanosized range, an acceptable zeta potential, high entrapment efficiency and 97% of drug release over 8 h with good growth suppression of *C. albicans*.

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Conflicts of Interest

We declare that there are no conflicts of interest.

Funding

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Ethics Statements

The research did not include any study on animal or human

Author Contribution

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

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صياغة وتقييم في المختبر لحويصلات البايلوزم النانوية المحملة بالكيتوكونازول

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الخلاصة

كيتوكونازول (KET)، هو أحد مشتقات الإيميدازول ذو خصائص مضادة للفطريات معروفة، وذو قابلية ذوبان مائية ضعيفة، وبالتالي فإن استخدامه الطبي كعلاج موضعي يعتبر احد اهم المشاكل العملية. وحاليا تلعب الحويصلات البايلوزومية دورًا مهمًا في توصيل الأدوية الموضعية حيث يمكنها تقليل السمية وتحسين استهداف الأدوية الجلدية من خلال عملها كمستودع للأدوية يمكنه ضبط معدل إطلاق الدواء.

يهدف هذا البحث إلى صياغة وتحسين جسيمات KET البايلوزومية لتعزيز انحلالها ونشاطها المضاد للفطريات عند استخدامها موضعيا على الجلد.

تم تحضير عشرة صيغ من بايلوزومات KET بطريقة الموجات فوق الصوتنة باستخدام نوعين من المواد الخافضة للتوتر السطحي غير الأيونية (سبان40 أو سبان60) إلى جانب الكولسترول وديوكسيكولات الصوديوم (SDC) ,احد الاملاح الصفراوية, كمقوي للدهون ومثبت ، على التوالي.

تم تقييم المستحضرات في المختبر من حيث حجم الحويصلات و مؤشر التشتت المتعدد وكفاءة الحصر وإطلاق الدواء. والصيغة التي اظهرت خصائص المثلى تم فحص الشكل السطحي لها باستخدام المجهر، اضافة الى تعيين جهد زيتا،و طيف الاشعة تحت الحمراء ونشاطها المضاد للفطريات.

أظهرت جميع البايلوزومات المحضرة احجام نانوية وتراوحت بين (١٨٩,٢٧ ± ٢,٧٥ - ٢٥١,٨٧ ± ١,٧٤ نانومتر) ووجدت كفاءة االحصر في حدود ٣٢,٠٧ ± ٠,٦٧ و ٩٢,٣٣ ± ٠,٧٦. وان الصيغة المثلى التي تحتوي على سبان ٦٠: الكولسترول: SDC بوزن (١٥:٦٠:٣٥٠) كانت ذا مظهر كروي الشكل بدون تجمعات وجهد زيتا حوالي – ٥٤,٤٥ ملي فولت .وكان للصيغة المثلى نشاط مضاد للفطريات كبير.و بلغت نسبة KET التراكمية المتحررة منها حوالي ٩٧٪ في ٨ ساعات.

ووفقا للنتائج التي تم الحصول عليها فانه يمكن تحضير الحويصلات البايلوزمية المحملة بـ KET بنجاح لتحسين ذوبانيه ونشاطه المضاد للفطريات.

الكلمات المفتاحية:فعالية مضادة للفطريات، بايلوزوم، كيتوكونازول، مسمار الموجات فوق الصوتية، سبان ٦٠.

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