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Preparation, in Vitro and in Vivo Evaluation of Liposomal/Niosomal Gel Delivery Systems for Clotrimazole

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ABSTRACT Clotrimazole, which is an imidazole derivative antifungal agent, was widely used for the treatment of mycotic infections of the genitourinary tract. To develop alternative formulation for the vaginal administration of clotrimazole to provide sustained and controlled release of appropriate drug for local vaginal therapy, liposomes/niosomes were evaluated as delivery vehicles. To optimize the preparation of liposomes/niosomes with regard to size and entrapment efficiency, multilamellar liposomes/niosomes containing drug were prepared by lipid hydration method. The prepared liposomes/niosomes were incorporated into 2% carbopol gel, and the systems were evaluated for drug stability in phosphate-buffered saline (pH 7.4) and simulated vaginal fluid at 37±1°C. Further, the vesicle gel system was evaluated by antifungal activity and tolerability on tissue level in rat.

KEYWORDS Clotrimazole, Liposomes, Niosomes, Vaginal drug delivery

INTRODUCTION

Vulvovaginal candidiasis is the infection with Candida albicans (Lanchares & Hernandez, 2000). Approximately 75% of women have a vaginal infection with a candida strain during their life, about 40 to 50% of them suffer a second one, and a small percentage show a chronic course (Ferrer, 2000). Clotrimazole, which is an imidazole derivative, is widely and effectively used for the treatment of vulvovaginal candidiasis (Ceschel et al. 2001). Unfortunately, oral use of clotrimazole is unacceptable due to the severe side effects. The plasma half-life of clotrimazole is 3–6 hr, suggesting that frequent dosing is needed. Thus, topical administration of clotrimazole is recommended. However, it is limited by its very low water solubility, requiring it to be incorporated into a suitable vehicle. Commercial conventional clotrimazole vaginal delivery systems, such as creams, foams, and gels, are considered to reside for a relatively short period of time at the targeted site and have higher systemic absorption of the drug, resulting in systemic side effects. The entrapment of drug in vesicles may help in the localized delivery of the drug, and an improved solubility and availability

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of the drug at the site may reduce the dose and systemic side effect.

Liposomes are being widely investigated in topical applications for skin (Kim et al., 1997; Schmid & Korting, 1994; Valenta et al., 2000), oral (Farshi et al., 1996), and vaginal diseases (Pavelié et al., 1999, 2001). Liposomes have been shown to enhance the penetration of vesicle-bound drugs into the skin after topical application and act as a "drug localizers," with low systemic absorption of the drugs, as compared with other galenical formulation, resulting in less drug side effect and sustained drug releasing (Kim et al., 1997). Analogous to liposomes, niosomes are formed from the self-assembly of nonionic amphiphiles in aqueous media, resulting in closed bilayer structures. They need some forms of energy to form the vesicles and offer several advantages over liposomes such as higher chemical stability, intrinsic skin penetration-enhancing properties, and lower costs (Yoshika et al., 1994). Therefore, niosomes have also been widely studied as drug carriers for controlled and targeted delivery (Uchegbu et al., 1995; Varshosaz et al., 2003). Preliminary studies indicate that niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug to alter its organ distribution and metabolic stability, or prolonging the contact time of drug with the applied tissues in topical application (Fang et al., 2001; Manconi et al., 2001; Perini et al., 1996; Shahiwala & Misra, 2002; Vora et al., 1998), which demonstrated that niosomes could improve drug skin penetration and increase its accumulation in the superficial skin strata. However, little work has been carried out on the application of drug-loaded niosomes in vaginal therapy investigation.

The current study investigates the feasibility of liposomes and niosomes to formulate the vaginal administration of model drug clotrimazole. Multilamellar vesicles (MLVs) formed using conventional lipid film evaporation method Formulations composed of egg lecthin and nonionic surfactant compositions have been characterized by particle morphology and encapsulation efficiency. To enhance the stability and increase the viscosity of this system, a convenient self-administration dosage form-carbopol gel was prepared. The prepared liposomes/niosomes and vesicle gel systems were evaluated for drug stability in phosphate-buffered saline (PBS) (pH 7.4) and simulated vaginal fluid at 37±1°C. Further, we examined in vivo antifungal activity testing and tissue tolerability of the most promising liposomes/niosome gel preparations in Sprague-Dawley (SD) rat. We report here that liposomal/niosomal gel offered prolonged antifungal activity over several days against C. albicans vaginitis.

MATERIALS

Clotrimazole and egg phospholipids (EPs) (>98%) were the generous gifts by Drs. Fu and Cheng (Xi'an Libang Liposomes Pharmaceutical Company). Sorbitan monoesters (Span40, HLB = 6.7), dicetylphosphate (DCP), and cholesterol (CH) were brought from Sigma. Cellulose nitrate membrane filters (0.22 μm, Whatman, Majdstone, UK). Buffer PBS (pH 7.4) was made of 8 g NaCl, 0.2 g KCl, 0.025 g $Na_2HPO_4 \cdot 2H_2O$, and 0.050 g $NaH_2PO_4 \cdot 2H_2O$ per 1 L. All other reagents used in the study were of analytical grade.

METHODS

Preparation of Liposomes/Niosomes

To study the effect of composition of the vesicles containing clotrimazole, a series of formulations containing different compositions with EP, sorbitan ester (Span™), and cholesterol were designed (CPL, clotrimazole, phospholipids, liposomes; CSN, clotrimazole span 40 niosomes). Conventional MLV were prepared by thin lipid evaporation method. The formulations containing phospholipid or nonionic surfactants, cholesterol and DCP were resolved in ethanol, and the desired volumes were added to a 100 mL round-bottom flask. The flask was attached to a rotary evaporator (Büchi Rotavapor R 110, Flawil, Switzerland) and lowered into a 30°C water bath (BÜCHI 461 water bath, Switzerland), and the organic solvents were evaporated under reduced pressure at 150 rpm to form a thin, dry film on the wall of the flask. Any excess organic solvents were removed by leaving the flask in a desiccator under vacuum for 12 hr. The dried lipid film was hydrated when required with buffer PBS, pH 7.4, followed by vigorous shaking in an incubator at 30°C (for liposomes) or 60°C (for niosomes) for about 60 min to form large multilamellar blank liposomes/niosomes. Conventional, drug-containing liposomes/niosomes were prepared by adding drug (clotrimazole was

dissolved in ethanol previously) to the surfactant mixture prior to evaporating the organic solvent.

Purification of the Resultant Liposomes/Niosomes

The unencapsulated materials and residual organic solvent were removed by dialysis against PBS (pH 7.4) solution overnight, using cellulose membrane tubing with molecular weight cut-off at 8000–12,000, which was previously stored in PBS (pH 7.4) before use. The system was maintained at 25°C. The dialyzing solution was continuously stirring with magnetic bar.

Morphology and Particle Size of Liposome/Niosome Particles

Multilamellar vesicles after dilution with 5% mannitol were viewed under optical microscope with photograph auto-selecting system (Axiokop 40, ZEISS, Welwyn Garden City, Hertfordshire, UK) to observe the shape and lamellar nature of vesicles. Some of photomicrographs as shown in Fig. 1 were prepared by photograph auto-selecting system in 10×100 magnifications. The size distribution of the resultant dispersion was characterized using a laser particle size analyzer based on laser diffraction using the Beckman LS Particle Size Analyzer (Beckman Coulter, Fullerton, CA, USA).

Entrapment Efficiency of Drug in the Liposomes/Niosomes

One milliliter of clotrimazole liposomal/niosomal suspension each of which before and after dialysis was diluted and adjusted to volume with methanol in a 10-mL volumetric flask, and the amount of drug was determined by high-performance liquid chromatography (HPLC). The system was maintained at 25°C (150 rpm) by means of shaker. The percentage entrapment efficiency of drug was calculated by:

$$\% \text{ Entrapment efficiency} = [\text{Content of clotrimazole in postdialyzed vesicles}/(\text{Content of clotrimazole in predialyzed vesicles})] \times 100$$

The HPLC system consisted of Gold Nouveau software workstation, a Beckman 126 NM solvent delivery system, Beckman 508 autosampler with a 100-μL loop, and Beckman 168 NM PDA detector. The

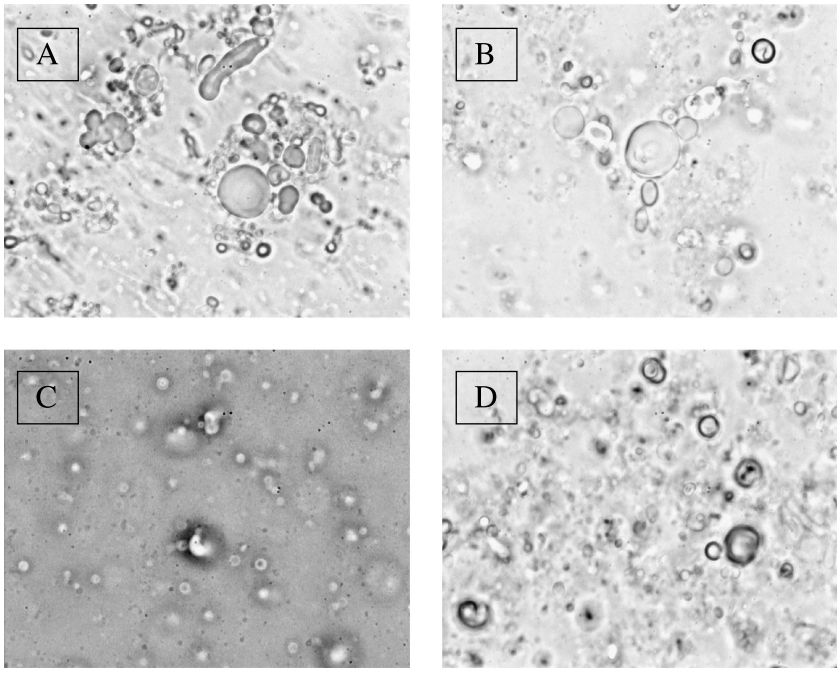

FIGURE 1 Optical Micrographs (×1000) of Clotrimazole-loaded Vesicles Composed of Phospholipid and Sorbitan Esters (Span™) Prepared by Classic Film Method: (A) CPL6 Liposomes, (B) CSN6 Niosomes, (C) CPL6 Liposomal Carbopol Gel, (D) CSN6 Niosomal Carbopol Gel. CPL, Clotrimazole, Phospholipid, and Liposomes; CSN, Clotrimazole, Surfactant, and Niosomes.

column used was Beckman C18 dp 5 μm, 4.6 mm × 25 cm (Beckman, Fullerton, CA, USA). The mobile phase consisted of methanol and H_2O (pH 3.0) (95:5, v/v). The flow rate was 0.8 mL/min. The chromatogram was monitored at a wavelength of 220 nm.

This method was validated in terms of specificity, linearity, and reproducibility. The limit of quantification was 1 μg/mL. The exact amounts of clotrimazole were using a calibration curve.

Preparation of Carbopol Gels and Incorporation of Liposomes/Niosomes into 2% Carbopol Gel

The gel (2%) was prepared by the following as described. Briefly, Carbopol 934 (1 g) was dispersed in distilled water (44 g) in which glycerol (5 g) was previously added. The mixture was stirred until thickening occurred, and then neutralized by adding triethanolamine (pH 5.5) until a transparent gel appeared (the plain drug gel as control in this experiment).

Liposome/niosome gels were prepared using the same formula. For this purpose, equivalent amount optimized liposome/noisome preparations [CPL6= clotrimazole: egg phospholipid: cholesterol (2:7:3, molar ratio) and CSN6=clotrimazole: span 40: cholesterol (1:8:2, molar ratio)] were centrifuged (7000 rpm, and 4°C for 30 min), and the pellets obtained were incorporated into the previous Carbopol preparation by an electric mixer (25 rpm, 5 min) into liposomal/niosomal gel.

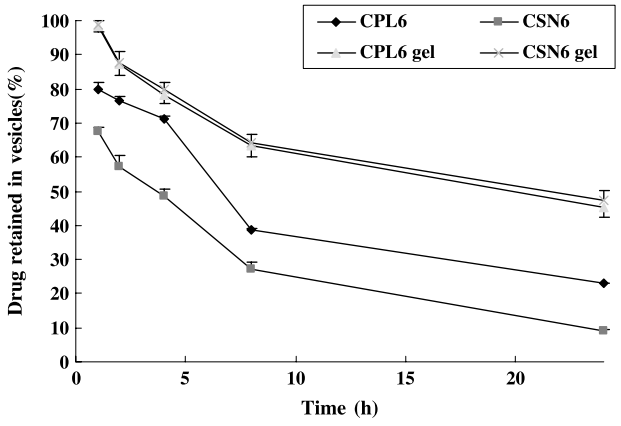

FIGURE 2 **Stability of Liposomes/niosomes Containing Clotrimazole Expressed as Percentage of Originally Entrapped Drug Still Present in Liposomes/niosomes After at Simulated Vaginal Fluid (pH 4.2) at 37±1°C (x±SD, *n*=8). CPL6=Clotrimazole:Egg Phospholipid:Cholesterol (2:7:3 Molar Ratio); CSN6=Clotrimazole:Span 40:Cholesterol (1:8:2, Molar Ratio).**

Stability of Vesicles and Gel at Storage Condition

The CPL6 and CSN6 were stored in glass vials after purging with nitrogen and kept in a refrigerator (4±1°C), room temperature (RT, 25±2°C), and 37±1°C for 3 mo. The samples from liposomes/niosomes were withdrawn at definite time intervals; the residual amount of drug in vesicles after dialysis was determined as described previously.

In Vitro Release Studies at Simulated Physiological Condition

To investigate the formulation's stability in simulated physiological condition, the drug amount retained in vesicles change of liposomes/niosomes and corresponding vesicles gel were tested in simulated vaginal fluid (SVF) (pH 4.2) at 37±1°C for 24 hr. The samples from liposomes/niosomes were withdrawn at definite time intervals; the residual amount of drug in vesicles gel was abstracted by chloroform: methanol (1:3) solution; the residual amount of drug in vesicles after dialysis was determined as described previously. The drug percent retained in vesicles and gel results is shown in Fig. 2.

SVF (Owen and Katz, 1999) for 1 L given as compound and weight (g) is as follows: NaCl, 3.51 g; KOH, 1.40; $Ca(OH)_2$, 0.222; bovine serum albumin, 0.018; lactic acid, 2.00; acetic acid, 1.00; glycerol, 0.16; urea, 0.4; and glucose, 5.0. Once these compounds are combined, the mixture is adjusted to pH of 4.2 using HCl.

Antifungicidal Efficacy Studies

Because cyclic changes in the thickness and histology of rodent species vaginal epithelium have marked effects on the vaginal absorption of drugs, oophorectomized female SD rats, 200±10 g (body weight), receiving subcutaneous administration of estradiol benzoate (25 mg/kg) every 2 days during the experiment, were selected and housed in individual cages and received food and water ad libitum. The animals were infected by intravaginal inoculation of *C. albicans* [CMCC(B) 98 001] suspended in sterile saline containing 10^8 c.f.u./mL. A vaginal smear was taken 2 days after the challenge to confirm the establishment of infection.

Before intravaginal treatment, animals were anesthetized with intraperitoneal injection of 100 mg/kg ketamine hydrochloride. Animals were treated intravaginally with blank control 1 and 2 (liposomal/niosomal gel without drug, as negative control); various formulations were applied into the vagina of rats at a CT dose of 25 mg/kg, using a stomach sondle needle once per day for 3 consecutive days starting 24 hr after challenge (day 0). For the vaginal colony counts, an analysis of variance was done on the log10 colony counts for each day of vaginal culture and each drug formulation from days 4 to 7. Vaginal lavage samples were collected with 100 μL saline by washing the fluid three times up and down in the vagina. The fluid was then plated onto sabouraud dextrose agar and incubated for 72 hr at 37±1°C and c.f.u. values were recorded.

Morphology Study of Vaginal Tissues After Application of Liposome/Niosome Gels

Oophorectomized female SD rats weighing 200±10 g were used after a recovery period of at least 7 days. Liposomal/niosomal gels were administered into the vagina of the rats at a CT dose of 25 mg/kg, the vaginal tissues of the blank gel-treated rats (A), liposomal gel-treated rats (B), and niosomal gel-treated rats (C) were isolated, fixed in 10% neutral carbonated-buffered formaldehyde, embedded in paraffin, and cut into slices. After hemotoxylin-eosin staining, the slices were observed under a light microscope.

Data Analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) using Microsoft Excel 2000 and by the Student's *t*-test (level of significance for $p < .05$).

RESULTS AND DISCUSSION

Particle Size and Size Distribution of Liposomes/Niosomes

The micrographs in Fig. 1 confirm the formation of multilamellar structures from phospholipid and sorbitan ester nonionic surfactants by classic film method. From the microscopic observation, it is evident that large and small multilamellar vesicles were formed. In addition, the mean diameter was 4.111±1.921 μm for liposomes (CPL6) and 3.432±2.226 μm (CSN6) for niosomes by dynamic laser light-scattering measurement. There was no significant difference between the sizes of the liposomes (CPL6) and niosomes (CSN6) ($p > .05$). The gel formed did not influence the vesicles, and made the vesicle more dispersive to prevent the vesicle aggregating and fusing. As for the niosomes, a previous document reports HLB numbers between 4 and 8 were found to be compatible with vesicle formation with the sorbitan monostearate surfactants (Yoshika et al., 1994). In this study, we chose the span 40 whose corresponding HLB is 6.7 to form the lipophilic drug clotrimazole-loaded niosomes. In addition, there was no significant difference between the sizes of optimized vesicles: the liposomes CPL6 and niosomes CSN6 ($p > .05$). The size distribution of vesicles trends to be fairly wide, although this can be modified by altering the hydration time and degree of shaking.

Determination of Content and Entrapment Efficiency

The regression equation for clotrimazole content (μg/mL) in methanol ranging from 10 to 500 μg/mL was $C = 18013A - 28.537$, ($R^2 = 0.9995$), where C (μg/mL) and A represented the concentration and peak area of clotrimazole, respectively. The mean recovery was 98.50±1.73 ($n = 3$). The precision assay showed that relative standard deviations within 1 day and on every other day were all less than 10%. This method was validated in terms of specificity, linearity, and reproducibility. The limit of quantification was 0.1 μg/mL. The exact amounts of clotrimazole were using a calibration curve.

In this study, the dialysis method was applied to determine entrapment efficiency. Recovery of drug was determined for all samples and was between 94.5% of the amount taken into preparation.

The contents of clotrimazole-liposomes/niosomes were determined by the HPLC method. For liposomes, an increasing of the chol level (molar ratio of lipid: chol from 1:9 to 6:4) decreases the entrapment of drug from 93.72±0.91% to 79.87±1.50%. For niosomes, similar to the result of liposomes, with an increasing of the chol, the entrapment efficiency increases from 60.19±3.85% to 88.18±0.72% with

increasing of the chol : lipid ratio from 5:5 to 2:8. According to the results of the entrapment efficiency and drug : lipid ratio, formulations CPL6 (clotrimazole : phospholipid : cholesterol=2 : 7 : 3) and CSN6 (clotrimazole : span 40 : cholesterol=1 : 8 : 2) was chosen to do further experiments.

Process variables, hydration medium, hydration time, and speed of flask rotation were optimized to prepare lipid vesicles of clotrimazole. The rotational speed of the flask demonstrated discernible influence on the thickness and uniformity of the lipid film. The speed of 100 rpm yielded a uniform thin, lipid film yielding vesicular preparation of desired characteristics on hydration, although lower and higher rates of rotation resulted in preparations with noticeable agglomerate on the wall of flask. Multilamellar vesicles are prepared by constant vortexing for 15 min on a vortex mixer until no residual lipid film remains on the wall glass of flask: the hydrating temperatures used to make liposomes/niosomes were above the gel-to-liquid phase transition temperature of the system. During the preparation of niosomes, although the surfactant film was thin, it has finite thickness, and hydration initially occurs at the surface of the glass. As a result, full hydration of the surfactant film is difficult, and the preparation of liposomes with lower molar ratio of cholesterol did not produce such phenomena. The hydration of phospholipid film was relative easy.

The presence of ionic surfactants in the formulation is generally used to stabilize niosomes by means of an increase of their zeta potential and optimized ion–dipole interaction. DCP is a charge inducer, and dicetylphosphate was added to all the formulations to increase the vesicles stability in this study.

Carbopol was chosen in this study, which is due to its hydrophilic nature and bioadhesive properties, which may result in an increased residence time of a drug at the site of absorption by interacting with the mucosa and proved to be compatible with liposomes. In addition, it could achieve an adequate pH value corresponding to physiological conditions and desirable viscosity (Pavelié et al., 2001).

Physical Stability at Storage Condition

Table 1 shows liposomes/niosomes were relative stable at 4±1°C storage condition. The drug leakage percent amounts of original entrapped in liposomes/niosomes are very small (<5%) and have no significant difference after 1 mo compared with immediately after preparation ($p > .05$). The results of drug retention studies show higher drug leakage at higher temperature. This may be due to the higher fluidity of lipid brilayers at higher temperature, resulting in higher drug leakage. Below the testing temperature of 25°C, the niosome formulation is less stable than liposomes. This result might be due to the fact that the multiniosomes forming the nonionic surfactant were less stable than multiliposomes. When the testing temperature is above 25°C, the results are contrary to the previous, which might result from the fact that the span 40 niosome transition temperature is higher than liposomes.

In Vitro Release Studies at Simulated Physiological Condition

It is known that the pH value of the healthy human vagina ranges between 4.0 and 5.0, in our study; the SVF was chosen to be release medium in vitro. Figure 2 shows liposomes/niosomes and the corresponding gel release profile. The release of

TABLE 1 **Stability of Liposomes/Niosomes Containing Clotrimazole Expressed as Percentage of Originally Entrapped Drug Still Present in Liposomes/Niosomes After at Storage Conditions at 4±1°C; RT (room temperature about 25±2°C), 37±1°C (*n*=3±SD)**

	Formulations	1 mo	2 mo	3 mo
4±1°C	CPL6	97.37±2.55	86.53±1.37	69.36±2.32
	CSN6	98.22±1.54	77.87±3.15	67.27±2.19
25±2°C	CPL6	86.33±1.55	73.24±3.17	55.28±2.79
	CSN6	90.37±1.55	71.83±4.11	50.24±2.20
37°C	CPL6	76.33±1.75	53.24±3.29	25.28±2.15
	CSN6	80.37±1.52	64.83±3.18	45.24±1.10

CPL6=clotrimazole : egg phospholipid : cholesterol (2 : 7 : 3, molar ratio); CSN6=clotrimazole : span 40 : cholesterol (1 : 8 : 2, molar ratio); CPL, clotrimazole, phospholipid, and liposomes; CSN, clotrimazole, surfactant, and niosomes.

clotrimazole from vesicle gel in SVF is much slower than from clotrimazole vesicles. Clotrimazole from vesicles showed release of about 70% within 8 hr. After 24 hr, the drug percent of original entrapped retained in liposomes and niosomes are 22.86% and 9.25%, respectively, and had significant difference between two vesicles ($p < .05$). In addition, there is a significant difference between the vesicles and their corresponding gel ($p < .05$), the drug retained percents of the corresponding gels were 45.25% and 47.27%, respectively. However, the two gels had no significant difference ($p > .05$). As can be seen from Fig. 2, after 24 hr the leakage was approximately 50% of original entrapped clotrimazole, which suggested liposomal/niosomal gel delivery system might provide controlled and prolonged release of an adequated drug in vaginal local treatment. The results obtained from Fig. 3 were that niosomes containing clotrimazole were less stable than liposomes, especially after 8 hr.

Antifungicidal Efficacy Studies

Experimentally induced vaginal candidiasis in this model is hormonal dependent, making treatment with estradiol valerated essential. Positive/negative cultures taken immediately before challenge were all negative, indicating no prechallenge *C. albicans* vaginal colonization. *C. albicans* challenge inocula were verified by serial plating on sabouraud dextrose agar to be 5.0×10^5 blastospores for study. This provided a well-established infection in all challenged animals (Table 2) with a range of 4.89 to 5.05 $\log_{10}$ c.f.u./mL on day 0 prior to treatment. All animals had well-established infections on day 0 with very little variability. The blank 1 and 2 (without drug liposomal/niosomal gel-treated controls) maintained an excellent level of infection throughout the studies (day 7). This was an indication and an appropriate challenge inoculum. In addition, drug dosages and clinical dosing regimens vary greatly when compared with commercially available vaginal preparations. These differences make direct comparisons between compounds difficult. One way to compare various formulations is to subject them to the same dosing regimen and allow the concentration of drug to be the variable.

In study, formulations including the control and standard ointment (Table 2) reduced the number of yeasts in the vaginas on days 4, 5, and 6 ($p < .05$) compared with day 0. However, there are differences between niosomal/liposomal gel and plain drug gel or commercial ointment on observed days 5, 6, and 7. On day 4, the ointment group decreased greatest in all formulations, which resulted from drug from ointment more rapidly than others. However, there was no significant difference between commercial product and liposomes/niosomes. In addition, it did not significantly reduce the number of yeasts in the vagina on day 7 ($p > .05$) when compared with day 0. However, the liposomal/niosomal gel shows different results compared with the ointment on day 7, which suggested a longer-term reduction of yeasts in the vagina with liposomal/niosomal gel. Because Table 2 also showed no significant differences, the liposomal/niosomal formulations were detected on days 4 and 5. However, there was a significant difference between niosomal and liposomal gel on days 6 and 7. The findings of in vivo studies suggested that the prolonged and enhanced antifungal activity might be due to the higher drug vaginal mucosa retention, which may be due to creation of reservoir effect for drug in mucosa due to deposition of components of liposomes/niosomes with drug into the mucosa, thereby increasing the drug retention capacity into the mucosa.

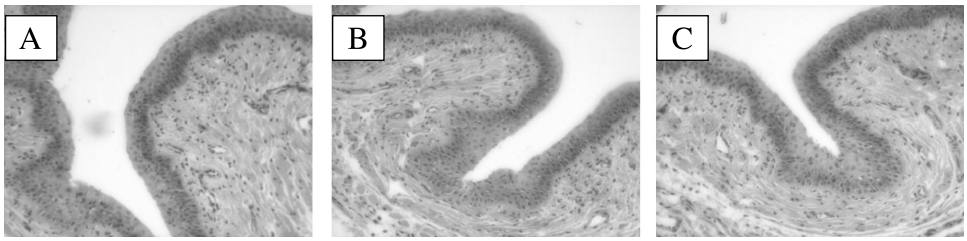

FIGURE 3 **Morphology of Vaginal Tissues After Application of Liposome/niosome Gels. Liposome/niosome Gels were Administered into the Vagina of the Rats at a CT Dose of 25 mg/kg. The Vaginal Tissues of the Blank Gel-Treated Rats (A), Liposomes Gel-Treated Rats (B), and Niosomes Gel-Treated Rats (C) were Isolated, Fixed in 10% Neutral Carbonated-Buffered Formaldehyde, Embedded in Paraffin, and Cut into Slices. After Hemotoxylin-eosin Staining, the Slices were Observed under a Light Microscope (×100).**

TABLE 2 Antifungicidal Efficacy Studies of the Prepared Liposomes/Niosomes Systems Concentrations of *C. albicans* Log c.f.u./mL) ($n=3\pm SD$)

Time (d)	Blank 1	Blank 2	Control	Standard	CPL6 gel	CSN6 gel
0	4.89±0.22	4.99±0.23	5.05±0.33	4.95±0.44	4.98±0.51	4.98±0.42
4	4.90±0.51	4.88±0.41	1.70±0.21[a]	1.02±0.12[a]	1.08±0.01[a]	1.07±0.01[a]
5	4.90±0.53	4.95±0.46	1.85±0.23[a]	1.82±0.24[a]	1.44±0.02[a,b]	1.47±0.13[a,b]
6	4.95±0.44	5.03±0.51	2.21±0.32[a]	2.91±0.29[a,b]	1.53±0.09[a,b,c,d]	2.08±0.12[a,c]
7	4.95±0.25	4.95±0.28	4.23±0.26[a]	4.84±0.25[b]	2.25±0.24[a,b,c,d]	2.85±0.23[a,b,c]

Blank 1, group treated with liposomal gel without drug; blank 2, group treated with niosomal gel without drug; control, group treated with plain drug gel; standard, group treated with marketed clotrimazole ointment; SPL6 = clotrimazole:egg phospholipid:cholesterol (2 : 7 : 3, molar ratio); CSN6 = clotrimazole : span 40 : cholesterol (1 : 8 : 2, molar ratio); CPL6 gel, group treated with liposomal gel; CSN6 gel, group treated with niosomal gel.

[a] $p<.05$, compared with the day 0.

[b] $p<.05$, compared with the control.

[c] $p<.05$, compared with the standard.

[d] $p<.05$, compared with the niosomal gel.

Tolerability of Clotrimazole Liposome/Niosome Gels in Tissue Level

Liposome/niosome gel did not alter the morphology of vaginal tissues. Figure 3 shows the histopathology of the vaginal mucosa after intravaginal application of CT-containing liposomal and niosomal gels. Compared with the control with no treatment, the vesicle gel-treated group showed no visible sign of inflammation or necrosis. Liposomal/niosomal gels did not affect the morphology of vaginal tissues at 24 hr postdose, which indicated such drug delivery systems are safe for vaginal delivery.

CONCLUSION

The goal of this study is to prepare and investigate the liposome/niosome delivery system for local vaginal treatment of clotrimazole. The results show that the optimized multilamellar liposome/niosome prepared in this study have higher drug : lipid ratio (>85 μg/mg with liposome; >75 μg/mg with niosomes) and were stable in 4±1°C storage condition. The vesicle gel systems can provide sustaining release in simulated vaginal fluid at 37±1°C for 24 hr. Our results suggest that CT-containing vaginal liposomal/niosomal gels would be useful for effective and convenient treatment of vaginal candidasis with reduced dosing interval. Moreover, liposomal and niosomal gels did not affect the morphology of vaginal tissues at 24 hr postdose; the morphology results support the safety of vesicle gels for vaginal application.

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