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Research Article

AMPHOTERICIN B TOPICAL EXTEMPORANEOUS PREPARATIONS FOR THE TREATMENT OF NON-DERMATOPHYTIC ONYCHOMYCOSIS

KOMESMUNEEBORIRAK PHOJANA[1], WERAWATGANONE PORNPEN[2], MUANGSIRI WALAISIRI[2]*

[1]Department of Siriraj Hospital Pharmacy, Faculty of Medicine Siriraj Hospital, Mahidol University, Salaya, Thailand. [2]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. Email: walaisiri@yahoo.com,

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ABSTRACT

Objective: At present, the nail preparation to cure onychomycosis, caused by non-dermatophyte molds, is not commercially available in Thailand. The physical and chemical stability of amphotericin B (AmB) extemporaneous preparations in the presence of 30% dimethyl sulfoxide (DMSO) and their *in vitro* nail permeation was evaluated.

Methods: AmB extemporaneous preparations in the presence of 30% DMSO were prepared from a commercial sterile injection product, and cream or hydrophilic ointment. Physical stability was tested at 30°C for 2 months, or using 6 heating-cooling cycles. The chemical stability and *in vitro* nail permeation of AmB content were analyzed using high-performance liquid chromatography (HPLC). *In vitro* nail permeation was performed by applying 3.5 mg/mm^2 of the tested formulation on nail clippings for 5 consecutive days.

Results: The AmB cream and ointment extemporaneous preparations containing 30% DMSO, a permeation enhancer, were homogeneous and pale yellow to yellow cream or ointment. The AmB ointment was stable for up to 60 days. The ointment preparation allows *in vitro* penetration through nails up to 14.17 μg/cm^2. The ointment preparation allows significantly better penetration through than the cream preparation due to the presence of DMSO, sodium lauryl sulfate (SLS), and water in the ointment preparation.

Conclusion: The AmB extemporaneous ointment was successfully compounded from a commercial sterile injection product with a beyond-use date of 60 days. The ointment preparation is currently under further investigation for *in vivo* efficacy.

Keywords: Amphotericin B, Onychomycosis, Non-dermatophytes, Beyond-use date, Degradation, Nail preparation.

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INTRODUCTION

Onychomycosis is a fungal infection caused by dermatophytes, yeasts, and/or non-dermatophyte molds [1]. The nail becomes thickened and discolored, resulting in onycholysis, along with separation of the nail plate from the nail bed [2,3]. The dermatophytes, *Trichophyton rubrum* and *Trichophyton mentagrophytes,* cause 60% and 20%, respectively, of onychomycosis worldwide. Terbinafine, Ciclopirox 8% solution, and amorolfine 5% lacquer are commercially available topical preparations for the treatment of onychomycosis caused by dermatophyte molds [4,5]. In contrast, the majority of onychomycosis in Thailand is caused by non-dermatophyte molds, such as *Fusarium* spp. and *Scytalidium* spp. [6,7]. At present, no effective topical antifungal drugs are commercially available to treat it.

Amphotericin B (AmB) has been shown to effectively cure onychomycosis caused by *Scytalidium* spp. and *Fusarium* spp., non-dermatophyte molds [8-10]. About 0.2% topical AmB solution in DMSO:2-propanolol (50:50) is reported to have effectively cured onychomycosis from non-dermatophyte in eight patients resistant to multiple conventional topical and systemic treatments [10]. The solution could not adhere to the nail surface for a long enough period of time such as high treatment failure is due to a low concentration of AmB at infection sites, leading to poor patient compliance.

Our objective was to develop AmB extemporaneous preparations with high amounts of DMSO as a nail permeation enhancer for the treatment of onychomycosis, caused by non-dermatophyte in Thailand – which is in climatic zone IVb. Stable formulations were investigated for *in vitro* permeation in the human nail plate. The obtained extemporaneous preparation would be the first topical preparation in the treatment of non-dermatophyte onychomycosis.

METHODS

AmB from *Streptomyces* sp. was purchased from Sigma-Aldrich (St. Louis, MO, USA), while AmB for injection U.S.P was manufactured by Asence Pharma Private Ltd. (390020 Gujarat, India). Acetonitrile, dimethyl sulfoxide, and methanol HPLC grade were purchased from RCI Labscan Ltd., Bangkok, Thailand (as well as analytical grade chemicals, such as hexane); ethylenediaminetetraacetic acid calcium disodium salt was purchased from Farmitalia Carlo Erba SPA, Milan, Italy, hydrochloric acid from Loba Chemie PVT. Ltd., Mumbai, India, Tris-hydroxymethyl-methylamine from Ajax Finechem Pty Ltd., 2229 Australia, and poloxamer 407 and glyceryl monostearate SE from BASF, Bangkok, Thailand. Propylene glycol, sodium lauryl sulfate, Span 60, stearyl alcohol, and Tween 60 (non-ionic detergent) were analytical grade from S. Tong Chemicals, Bangkok, Thailand. Cetyl alcohol, mineral oil, and white petrolatum were chemical grade from S. Tong Chemicals as well. All products were used as received.

HPLC analysis method for AmB

The general HPLC method was modified for quantitative analysis of AmB in extemporaneous preparations [11]. The analysis used a Shimadzu LC-20 AD, equipped with a photodiode array UV detector. The column oven and analytical wavelength were set to 30°C and 405 nm, respectively. The separation was done on a Phenomenex C18 (5 μ, 250 × 4.6 mm), with a corresponding guard column. The mobile phase included methanol:acetonitrile:0.0025 M EDTA (50:35:20 v/v/v). Flow rate and injection volume were set to 1 mL/min and 20 μL, respectively.

The mobile phase was freshly prepared, filtered through a 0.45 μm membrane filter, and degassed by a sonicator before use.

The analytical method for the drug substance could be validated for selectivity, the limit of detection (LOD), the limit of quantification (LOQ), linearity, accuracy, and precision. The analytical method for AmB in the formulations was verified for selectivity, accuracy, and precision. All analytical methods assessed the concentration range of 1–30 μg/mL.

For cream or ointment preparation, the samples (0.5 g) were dissolved in 5 ml of hexane, after which 10 mL of DMSO was added. The mixture was shaken well, centrifuged at 10,000 rpm for 10 min and the AmB was allowed to partition into the lower DMSO layer. Then, 1 mL of the lower layer was pipetted and transferred to a 10 mL volumetric flask and adjusted for volume with the mobile phase. Finally, the amount of AmB in the preparation was analyzed using the HPLC method, described above.

Formulation of extemporaneous AmB topical preparations

In this study, the extemporaneous AmB topical preparations were prepared by dissolving AmB for injection in DMSO. This mixture was added to the base formulation; that is, o/w cream or hydrophilic ointment formulations. The base formulations (Tables 1 and 2) were shown to possess physical stability in the presence of a high DMSO concentration [12]. First, the base formulations were prepared as briefly described below. We then added 5 mL of DMSO to the vial of AmB powder for injection. Finally, 3 mL of the mixture were withdrawn, mixed well, and adjusted to the final weight of 10 g with the base formulations. These extemporaneous AmB topical preparations were kept in amber glass vials, sealed with PTFE-coated stoppers and aluminum seals. Three batches of each formulation were prepared for physical and chemical stability studies.

The o/w cream base was prepared with the beaker method. The oil phase consisted of glyceryl monostearate SE, cetyl alcohol, stearyl alcohol, mineral oil, and Span 60, heated to 75°C. The water phase consisted of tris buffer, and Tween 60, heated to 80°C. Oil was added to the water and gently stirred until congealing.

The hydrophilic ointment base was prepared by melting stearyl alcohol and white petrolatum at 75°C. The aqueous phase consisted of sodium lauryl sulfate, propylene glycol, and tris buffer, heated to 75°C. The oil phase was added to the aqueous phase, with the hydrophilic ointment base stirred gently until congealing.

Physical stability was evaluated using a heating-cooling method. In each heating-cooling cycle, the samples were stressed at 4°C for 48 h followed by stress at 45°C for 48 h. The samples were exposed to 6 heating-cooling cycles. Organoleptic characteristics such as color, viscosity, and appearance of the formulations were evaluated by visual observation. Moreover, samples were investigated under a microscope for crystal formation of AmB or other excipients in the formulations.

Table 1: The formulation of cream base

Ingredients	Functions	Gram
Glyceryl monostearate SE	Emollient, emulsifying agent	5
Cetyl alcohol	Stiffening agent	5
Stearyl alcohol	Stiffening agent	2.5
Mineral oil	Emollient	9
Tween 60	Emulsifying agent	2
Span 60	Emulsifying agent	0.5
Tris buffer qs to	Vehicle, buffer	q.s. to 70

Table 2: The formulation of hydrophilic ointment base

Ingredients	Functions	Gram
Sodium lauryl sulfate	Emulsifying agent	1
Stearyl alcohol	Stiffening agent	25
White petrolatum	Emollient	25
Tris buffer qs to	Vehicle, buffer	q.s. to 70

The extemporaneous AmB preparations in amber glass vials were sealed with PTEF-coated stoppers, and the aluminum seals. The preparations were investigated for the active drug by the HPLC method. The preparations were stored in the dark, at 30±2°C, for up to 60 days [13]. Sample preparation procedures before analysis by HPLC are described above.

***In vitro* permeation of extemporaneous AmB topical preparations in human nail clippings**

Protocol number 0.79.1/57 was approved by the Ethics Review Committee for Research Involving Human Subjects, Health Science Group, Chulalongkorn University (on August 15, 2014).

Fifteen healthy volunteers, ranging from 18 to 60 years, participated in this study. The volunteers could drop out of study at any time. Inclusion criteria: Healthy volunteers from 18 to 60 years with permission to participate in the study. Exclusion criteria: Volunteers with a history of fungal nail disease or those with a current manicure or nail varnish. The nail plate clippings in this study could be at least 3 mm in length. The subjects cut the nail plate from each finger as a whole piece.

The nail clippings from each volunteer were cleaned with gentle soap and dried overnight on a watch glass at room temperature. Each clipping was again cut into squares, yielding a surface area of about 20 mm^2. Before the experiment, the weight and surface area of each piece were recorded. Ten finger nail clippings from a single subject were used in each iteration of the experiment. The total surface area of nail clippings in each experiment was around 200 mm^2. The experiment started by applying 3.5 mg/mm^2 of the tested formulation on nail clippings, with each set of nail clippings kept in an incubator (KBF 720, Binder) at 32±2°C and 75±5% RH for 8 h. After this, they were washed with 5 mL of hexane. The cream or ointment was similarly applied for 5 consecutive days. After testing, each sample set was pooled and analyzed for non-permeated and permeated AmB content. Each formulation was tested in triplicate.

To determine non-permeated drug content, the nail clippings were cleaned twice with cotton and about 2.5 mL hexane. The cotton and hexane were pooled and transferred to a 15 mL centrifuge tube, while 5 mL of DMSO was added to dissolve AmB. The mixture was centrifuged at 10,000 rpm for 10 min. Then, 2 mL of the DMSO layer was transferred to a 5 mL volumetric flask and adjusted to volume using mobile phase before analysis, to determine the amount of AmB, with the HPLC method.

The permeated drug was now able to be determined. After the cleaning process, nail clippings were cut into small pieces and transferred to an Eppendorf. In addition, 1 mL of DMSO was added to dissolve AmB in the nail clippings. Finally, the amount of AmB was investigated with the HPLC method, as described above.

RESULTS

HPLC method for analysis of AmB

HPLC chromatogram of AmB standard solution showed a major peak of AmB reference standard at a retention time of around 5 min (Fig. 1a). The analytical method's selectivity was performed by exposure of AmB to 0.1 N hydrochloric acid, 0.01 N sodium hydroxide, and 0.5% hydrogen peroxide or water at 45°C. In all cases, peak purity indices of the main peak were more than 0.9999. The limit of detection and quantification of the HPLC method were calculated at 0.50 and 0.75 μg/mL, respectively. The plot between the concentration of AmB solutions and their corresponding peak areas showed a linear correlation in the range of 1–30 μg/mL, with a coefficient of determination (R^2) greater than 0.999. The determination of accuracy at 1.5, 15, and 25 μg/mL was in the range of 98.12–102.49%. Within-run precision and between-run precision were in the range of 0.41–1.60% to 0.86–1.50%, respectively.

HPLC chromatograms of AmB extemporaneous preparation showed the main peak of AmB at a retention time of ~5 min (Fig. 1b and c). The AmB

peak had purity indices which were greater than 0.999. AmB could be separated from other impurities and excipients in preparations using the HPLC analytical method. The average percentages of analytical recovery in cream and ointment were 98.18 and 93.57%, with a relative standard deviation of 6.86 and 4.09%, respectively. The recovery of AmB in cream and ointment formulations was <100%, and the relative standard deviations were more than 2%; AmB was lost during the sample extraction process.

Formulation of extemporaneous AmB topical preparations

AmB extemporaneous cream and ointment appear as homogeneous opaque pale yellow to yellow cream and ointment, respectively. Physical stability of the extemporaneous AmB topical preparations (i.e., o/w cream and hydrophilic ointment) was evaluated after passing through 6 heating-cooling cycles. The color of the cream and ointment products was not changed after 6 heating-cooling cycles, and phase separation was not observed. Under a microscope, crystallization was not observed.

The chemical stability of each extemporaneous AmB preparation was evaluated at 30°C for 2 months (Tables 3 and 4). Concentration time profiles of AmB extemporaneous ointment are shown in Fig. 2. The pH of AmB cream and ointment was found in a range of 6.9–7.1 through the experiment period.

Table 3: Percentage of the labeled amount (%LA) of AmB in extemporaneous cream

Lot No.	Time (days)							
	0	**3**	**11**	**23**	**28**	**36**	**48**	**59**
C-1	112.3	107.3	107.7	-	-	110.0	-	110.3
	0	**7**	**14**	**23**	**28**	**35**	**48**	**62**
C-2	119.0	119.3	111.6	115.7	118.7	116.3	97.3	82.7
C-3	108.7	108.7	98.0	96.0	89.3	97.3	111.7	119.0

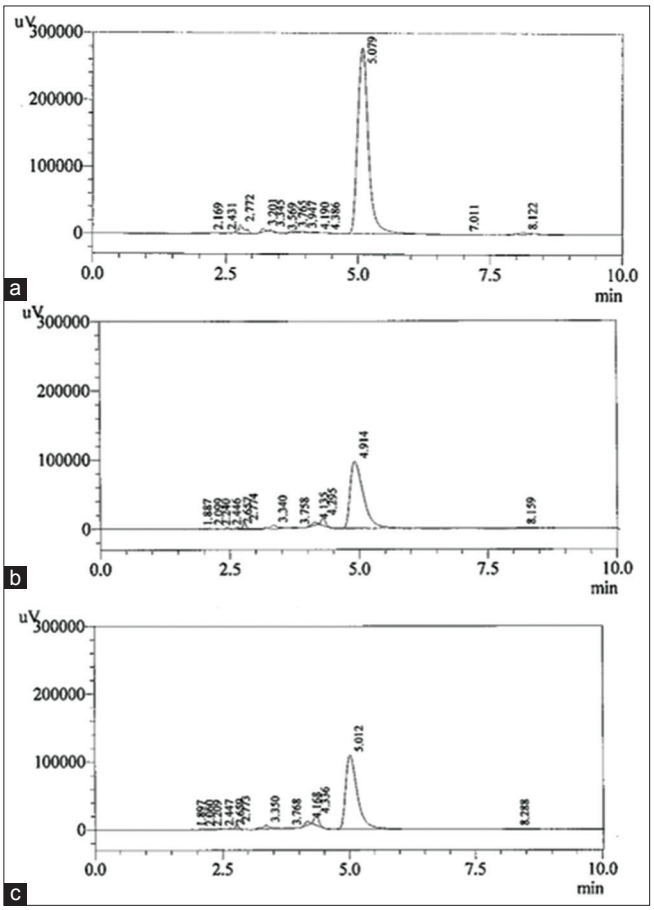

Fig. 1: HPLC chromatogram of AmB reference standard (a), AmB in cream preparation (b), and AmB in ointment preparation (c)

***In vitro* permeation of extemporaneous AmB topical preparations in human nail clippings**

The permeation study of each AmB extemporaneous preparation was evaluated by analyzing the amount of non-permeated and permeated drug. In each set of experiments, the percentage of recovery of non-permeated and permeated drug fell in a range of 106.8–107.8%. For the ointment preparation, 29.26±5.03 µg of AmB permeated through 205.64±12.40 mm^2 of the nail clippings (n=3), as shown in Table 5.

DISCUSSION

At present, there is no AmB nail product commercially available in Thailand. In addition, the AmB drug substance is expensive in comparison to the AmB for injection product. Therefore, in this study, the extemporaneous preparations were made from the manufactured product. The selected formulations were oil in water (o/w) emulsions and hydrophilic ointments, since the nail plates are composed of more water than lipid structure [14-16]. Water content in nails, responsible for nail elasticity and flexibility, is approximately 10–30% and is associated with relative humidity (RH). Total lipid content of the nail plate is between 0.1 and 1% [17]. The topical nail formulations must be viscous and able to adhere to the nail plate for a reasonable period of time so that the permeation enhancer can facilitate drug permeation in the nail plate. In this study, the cream base formulations were modified from those in the Handbook of Pharmaceutical Manufacturing Formulations: Semisolid Products, and the hydrophilic ointment base formulation followed that of hydrophilic ointment in USP41 [18,19].

DMSO is an organic solvent, known as a permeation enhancer, and is safe for humans [20-23]. Development of a physically stable formulation with a high concentration of DMSO was a challenge. The formula containing DMSO has been shown to increase the permeation of econazole lotion through the deeper human nail layer [20,24]. DMSO forms hydrogen bonds with the OH or NH groups of the keratin side chain [25]. Therefore, it can act as a permeation enhancer through the nail plate. Although DMSO can cause skin irritation, it is well tolerated with apparent low systemic toxicity [26]. The US FDA has approved its use as an inactive ingredient in topical formulations up to 45.5 % w/w [22]. DMSO can cause formulation problems, being an organic solvent by nature, which can increase solubility of the oil phase of o/w emulsion and increase solubility of the oil component in the hydrophilic ointment. As such, the development of formulations containing a high concentration of DMSO is quite a challenge. However, both of the

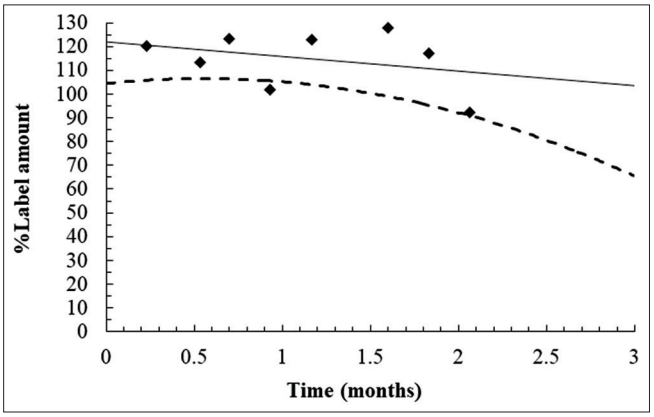

Fig. 2: Concentration time profile of AmB ointment (♦). The solid line and the dash line represent a linear regression line and the lower one-sided 95% confidence limit of the mean around the linear regression line, respectively

Table 4: %LA of AmB in extemporaneous ointment

Lot No.	Time (days)								
	0	**7**	**16**	**21**	**28**	**35**	**48**	**55**	**62**
0-1	120.3	113.3	123.3	102.0	123.0	128.0	117.3	92.0	109.7
0-2	121.0	110.7	121.0	123.7	116.7	118.7	110.7	110.0	104.0
0-3	110.7	117.0	118.3	106.3	118.3	121.3	122.7	108.0	96.3

Table 5: The amount of AmB permeated in the nail µg/cm^2 clippings after 5 consecutive days of application

Set	Amount of AmB in nail (µg/cm^2)	
	Cream	**Ointment**
1	6.57	12.65
2	3.86	15.30
3	7.48	14.55
Mean ± SD	5.97±1.88	14.17±1.36

selected base formulations in this study contained up to 30% DMSO with good physical stability after passing through 6 heating-cooling cycles [12].

Drug loading in these topical preparations was limited to 3 mg/mL or 0.3% AmB based on the solubility of the AmB commercial product in DMSO. The AmB for injection, a manufactured product commercially available in Thailand, was supplied in vials as a sterile lyophilized powder containing 50 mg of AmB in 41 mg sodium desoxycholate and 25.2 mg sodium phosphate. Although the solubility of AmB (as a drug substance) in DMSO is 30–40 mg/ml, to obtain a clear solution, the highest concentration of AmB for injection in DMSO was about 10 mg of AmB per mL [27]. The incomplete dissolution was due to the solubility limit of sodium desoxycholate (10 mg/mL) in DMSO, resulting in an unsuitable suspension for topical preparations. The o/w emulsion and hydrophilic ointment base preparations were developed to contain up to 30% DMSO with reasonable physical stability [12]. Thus, 0.3% AmB is the highest concentration loaded in base preparations.

After 6 heating-cooling cycles, the physical stability of AmB extemporaneous preparations was evaluated. The appearance of cream and ointment preparations was unchanged.

The physical appearance of the extemporaneous cream and ointment preparations was determined at 30°C for 2 months in the climatic zone IVb [28]. The humidity effect was not assessed, since the primary packaging material for both cream and ointment formulations was amber glass vials with PTFE-coated stoppers and aluminum seals, which were impermeable to gases and moistures. After storage at 30°C for 2 months, the color of the extemporaneous cream and ointment was unchanged. pH values of the cream and ointment preparations were in the range of 6.9–7.1 throughout the experiment period.

Beyond-use dates of AmB extemporaneous ointment at 30°C based on chemical stability were estimated to be about 60 days. USP41 states that AmB ointment contains the active ingredient in a range of 90–125% of the labeled amount (%LA) [19]. The concentration time profiles of AmB in the extemporaneous AmB cream or ointment after storage at 30°C for 2 months is shown in Fig. 2. The beyond-use dates were estimated by identifying the 1st time the lower one-sided 95% confidence limit of the mean around the linear regression curve intersected the proposed acceptance criterion. Although a concentration of AmB in the extemporaneous cream was between 90 and 125% LA, the beyond-use date of the AmB extemporaneous cream could not be assessed, due to high variability of data. However, another set of stability studies should be performed at 25°C to obtain correct beyond-use dates for both preparations in climatic zone II (25±2°C, 60±5%RH).

The permeation of AmB through the healthy human nail clippings for each preparation was also evaluated and compared. McAuley *et al*. found that the permeation of hydrophobic drugs through healthy and fungal nail plates is not significantly different [29]. This implies that permeation of AmB, a hydrophobic drug, through diseased nails is the same as through healthy nails. After an application of the testing formulation on the nail clippings, they were kept in an incubator at 32±2°C and 75±5% RH for 8 h. These experimental conditions simulated patient prescription instructions, in which patients would be directed to apply the formulation on the infected nails and then cover the area with a bandage (occlusive dressings) once daily at bedtime. The incubator was controlled at 32±2°C and 75±5% RH to simulate normal conditions, given that skin surface temperature is around 32°C, and % RH of the climatic zone IVb is around 75%, respectively.

The *in vitro* nail permeation study was done in triplicate, using nail clippings from three different volunteers. Nail clippings from the volunteers were separately exposed to the AmB extemporaneous ointment. Hexane was employed to dissolve non-permeated wax, oil, and/or AmB on the nail clippings. The AmB extemporaneous ointment was able to permeate nail clippings (Table 5). However, the amount of AmB from the AmB extemporaneous ointment that did permeate the nail clippings was higher than the MIC_{90} of *Scytalidium* spp. and *Fusarium* spp. [8,9]. The authors note that the MIC values in nails are likely to be much higher than reported MIC values; therefore, a clinical study will be conducted to corroborate the efficacy of the extemporaneous preparation.

Water, a well-known nail plasticizer, swells the nail plates. Since the nail plate is described as hydrogel, swelling results in the separation of keratin fiber and the formation of pores, which molecules can transverse [13]. Gunt *et al*. reported an *in vitro* enhancement of ketoconazole permeation through human nails from an increase of water content in the nails [30]. Formulations with more hydrophilic properties, which increase nail hydration, may improve topical nail permeation. The presence of water (21% w/w) in extemporaneous AmB hydrophilic ointment facilitated AmB permeation through the nail plates.

The permeation of the hydrophilic ointment preparation may also come from the presence of SLS and the viscosity of the preparation. SLS is known as a human skin permeation enhancer. Moreover, the ointment adheres to the nail surface for a longer period of time and allows greater contact time for AmB to permeate the nail plates. The amount of AmB permeating the nail clippings from both preparations was higher than the minimum inhibitory concentrations (MICs) of the investigated fungi [9]. A clinical study is currently being carried out at a hospital in Bangkok, Thailand, to determine the efficacy of the AmB extemporaneous ointment.

CONCLUSION

In this study, a commercially available sterile product was used in the preparation of AmB extemporaneous ointment and cream. The preparations contained 30% DMSO as a permeation enhancer. The AmB extemporaneous ointment was successfully compounded with beyond-use date of 60 days at 30°C. The nail clipping permeation of the ointment preparation was superior to that of the cream preparation. As stated above, a clinical trial is now in process to evaluate the efficacy of the AmB extemporaneous ointment.

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AUTHORS' CONTRIBUTIONS

P Komesmuneeborirak proposed the research topic, prepared the research protocol, and collected the data. P Werawatganone contributed to the development and discussion of the work. W Muangsiri contributed to the development of the work, the statistical analysis of data, the discussion of results, and writing and revision of the article.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest with respect to the research and/or publication of this article.

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REFERENCES

1. André J, Sass U, Theunis A. Diseases of the nails. In: Calonje JE, editor. McKee's Pathology of the Skin. 5th ed. China: Saunder; 2020. p. 1129-55.
2. Welsh O, Vera-Cabrera L, Welsh E. Onychomycosis. Clin Dermatol 2010;28:151-9.
3. Maddy AJ, Tosti A. Hair and nail diseases in the mature patient. Clin Dermatol 2018;36:159-66.
4. Rodgers P, Bassler M. Treating onychomycosis. Am Fam Physician 2001;63:663-73.
5. Tabara K, Szewczyk AE, Bienias W, Wojciechowska A, Pastuszka M, Oszukowska M, *et al*. Amorolfine vs. ciclopirox-lacquers for the treatment of onychomycosis. Postepy Dermatol Alergol 2015;32:40-5.
6. Ungpakorn R. Mycoses in Thailand: Current concerns. Nihon Ishinkin Gakkai Zasshi 2005;46:81-6.
7. Chaowattanapanit S, Pattanaprichakul P, Leeyaphan C, Chaiwanon O, Sitthinamsuwan P, Kobwanthanakun W, *et al*. Coexistence of fungal infections in psoriatic nails and their correlation with severity of nail psoriasis. Indian Dermatol Online J 2018;9:314-7.
8. Alastruey-Izquierdo A, Cuenca-Estrella M, Monzón A, Mellado E, Rodríguez-Tudela JL. Antifungal susceptibility profile of clinical Fusarium spp. isolates identified by molecular methods. J Antimicrob Chemother 2008;61:805-9.
9. Lacroix C, de Chauvin MF. *In vitro* activity of amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin and terbinafine against *Scytalidium dimidiatum* and *Scytalidium hyalinum* clinical isolates. J Antimicrob Chemother 2008;61:835-7.
10. Lurati M, Baudraz-Rosselet F, Vernez M, Spring P, Bontems O, Fratti M, *et al*. Efficacious treatment of non-dermatophyte mould onychomycosis with topical amphotericin B. Dermatology 2011;223:289-92.
11. Wilkinson JM, McDonald C, Parkin JE, Sunderland VB. A high-performance liquid-chromatographic assay for amphotericin B in a hydrophilic colloidal paste base. J Pharm Biomed Anal 1998;17:751-5.
12. Komesmuneeborirak P, Werawatganone P, Muangsiri W. Formulation of topical preparations containing high concentration of permeation enhancer in a treatment of onychomycosis. Thai J Pharm Sci 2013;38:192-4.
13. Harmanization of Standard and Technical Requirements in ASEAN. ASEAN Guideline Onstability Study of Drug Product, R1; 2013. Available from: https://www.asean.org/wp-content/uploads/2012/10/asean-guideline-on-stability-study-of-drug-product-r1-2013.pdf. [Last accessed on 2020 Jun 24].
14. Murdan S. Drug delivery to the nail following topical application. Int J Pharm 2002;236:1-26.
15. Fischer-Levancini C, Sanchez-Regana M, Llambi F, Collgros H, Exposito-Serrano V, Umbert-Millet P. Nail psoriasis: Treatment with tazarotene 0.1% hydrophilic ointment. Actas Dermosifiliogr 2012;103:725-8.
16. Valdes BS, Serro AP, Gordo PM, Silva A, Gonçalves L, Salgado A, *et al*. New polyurethane nail lacquers for the delivery of terbinafine: Formulation and antifungal activity evaluation. J Pharm Sci 2017;106:1570-7.
17. Walters KA, Abdalghafor HM, Lane ME. The human nail-barrier characterisation and permeation enhancement. Int J Pharm 2012;435:10-21.
18. Niazi S. Handbook of Pharmaceutical Manufacturing Formulations: Semisolid Products. New York: CRC Press; 2009. p. 184-327.
19. United States Pharmacopeia Commission. United States Pharmacopeia and National Formulary, USP41-NF36. Washington, DC: United States Pharmacopeia Commission; 2018.
20. Vejnovic I, Simmler L, Betz G. Investigation of different formulations for drug delivery through the nail plate. Int J Pharm 2010;386:185-94.
21. Swati H, Krishan K, Chandra NB, Richa S. Design, formulation and *in vitro* drug release from transdermal patches containing imipramine hydrochloride as model drug. Int J Pharm Pharm Sci 2017;6:220-5.
22. The United States Food and Drug Administration. Inactive Ingredient Search for Approved Drug Products; 2019. Available from: https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm. [Last accessed on 2019 Nov 15].
23. Putri MC, Harmita H, Iskandarsyah I. Improving transdermal drug delivery system for medroxyprogesterone acetate by olive oil and dimethylsulfoxide (DMSO) as penetration enhancers: *In vitro* penetration study. Int J Pharm Pharm Sci 2020;4:12-5.
24. Franz TJ. Absorption of amorolfine through human nail. Dermatology1992;184:18-20.
25. Myoung Y, Choi HK. Permeation of ciclopirox across porcine hoof membrane: Effect of pressure sensitive adhesives and vehicles. Eur J Pharm Sci 2003;20:319-25.
26. Sheskey PJ, Cook WG, Cable CG. Handbook of Pharmaceutical Excipients. 8th ed. London: Pharmaceutical Press; 2017. p. 331-3.
27. Asher IM, Schwartzman G. Amphotericin B. In: Florey K, editor. Analytical Profiles of Drug Substances. Vol. 6. New York: Academic Press; 1997. p. 1-42.
28. World Health Organization. Forty-third Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations. Geneva: World Health Organization; 2009. p. 99.
29. McAuley WJ, Jones SA, Traynor MJ, Guesné S, Murdan S, Brown MB. An investigation of how fungal infection influences drug penetration through onychomycosis patient's nail plates. Eur J Pharm Biopharm 2016;102:178-84.
30. Gunt HB, Kasting GB. Effect of hydration on the permeation of ketoconazole through human nail plate *in vitro*. Eur J Pharm Sci 2007;32:254-60.