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Original Article

IN-SITU FILM-FORMING SOLUTION FOR TOPICAL APPLICATION OF TERBINAFINE HCL: BIOPHARMACEUTICAL EVALUATION AND *IN VIVO* ANTIFUNGAL PERFORMANCE USING ANIMAL MODEL

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ABSTRACT

Objective: The main purpose of this study is to develop a film-forming solution with optimum physical-mechanical characteristics and excellent antifungal activity to enhance deposition and penetration into the stratum corneum (SC).

Methods: The film-forming solutions of terbinafine HCl were formulated using methacrylate copolymers, polyethylene glycol 400, and ethanol as diluent. The selected formulations were subjected to test of physical-mechanical properties, drug release, drug permeation across the stratum corneum and drug deposition study. The best formulation was further evaluated for *in vivo* antifungal efficacy.

Results: The selected formulations exhibited superior pharmaceutical characteristics, including rapid drying, non-stickiness, and being transparency on the skin. Formulation A (FA) had significantly lower tensile strength (4.78 N/m^2, p<0.05) and higher percentage elongation at break (33.61%, p<0.05), which reduced the firmness of the film, allowing it to be super-flexible in following the movement of the skin and preventing loss of film through abrasion. FA showed significantly (p<0.05) rapid drug permeation (1510.51 µg/cm^2) across the stratum corneum (SC) at 24 h when compared with the other formulations and the positive control proprietary drug (PD), Terbex® cream formulation (475.8 µg/cm^2).

Conclusion: Having superior physical-mechanical and drug permeation characteristics, FA can be considered as an efficient, reproducible, and efficacious antifungal formulation for topical application.

Keywords: Terbinafine HCL, *In situ* film-forming solution, Topical application, Dermatophytes, Drug permeation, Stratum corneum, Antifungal

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INTRODUCTION

Dermatophytosis infects more than 20%of the world population [1] and is the most frequent source of infection, especially in tropical countries [2, 3]. These infections are not life-threatening but are responsible for the largest global burden of years lived with disability (YLDs) which are in fact, higher (36.4 million) than diabetes mellitus (29.5 million) and migraine (28.9 million) [4]. Dermatophytosis can cause morbidity, especially amongst immunocompromised patients and those suffering from chronic diseases and amongst the elderly [5].

Topical drugs continue to be the mainstay in treating cutaneous infections [6]. Local effects attained in a topical drug delivery system is superior over oral treatment. The success of topical antimicrobial therapy, however, depends on the ability of a drug to enter the SC and follicular keratin and it must be retained at the infection site for an effective period of time [7]. The skin, in particular the SC, poses a formidable barrier to drug permeation [6, 8]. Recent studies reported that antifungals generally exhibit excellent activity *in vitro*, but have a low capacity for penetrating the SC in skin models [8]. Compromised ability to retain a drug at the site of infection may lead to reduced efficacy and development of drug resistance [5, 9]. This is evident from incidences of resistance to common drugs for treating dermatophytoses such as terbinafine, fluconazole, and griseofulvin [10-12]. Resistance arises as a consequence of non-adherence to prescribed treatment regimens, repetitive use of antifungal drugs more than once in a patient's life, the incidence of reabsorption or washout of drug from the skin and failure of the drug to reach the SC [10, 11]. Higher treatment failures (40–60%) and relapse incidences (22.2% after 3 y) give rise to questions about the efficacy of current anti-microbial preparations with consequent implications on morbidity and mortality [13]. Current topical anti-infective preparations are available in the form of ointments, creams, gels, lotions, and shampoo. These preparations have certain limitations like poor persistent contact with site of treatment, poor drug permeability, and compromised patient compliance due to them being messy, sticky, possessing an unattractive appearance, and interference with daily activities [14].

The use of film-forming dermal and/or transdermal delivery of drugs [15] has therefore been explored. Film-forming solutions have a unique attribute, which is being a non-solid dosage form of solution during the storage period that can be converted into the form of a film *in-situ* upon application. They induce the supersaturation of the drug upon solvent evaporation and the formed film is occlusive and increases skin hydration which leads to a high flux of drug across the SC. Besides, the film is not removed from the site by clothing, gauze, etc. A film-forming system hybridised with econazole loaded nanostructured lipid carrier showed markedly higher drug permeation (1.5 fold) across SC and deposition (3 fold) in the skin layer compared to a conventional marketed product (6).

These delivery systems are, however, not without disadvantages. A voriconazole-loaded film-forming transdermal spray required a long time for film formation (up to 4 min) and to completely treat the infection site (up to 14 d) on male Wrister rats [16]. Gel formulation of rotigotine with hydroxypropyl cellulose and carbomer 934 also required about 3–5 min for film formation and had only 22.6% absolute bioavailability [17]. A tolterodine film-forming hydrogel precipitated within 3 min to form whitish films [18]. Yang *et al.* reported that terbinafine HCl and urea-loaded film-forming system for onychomycosis took 2 to 3 min to form a clear flexible film but had poor correlation coefficient of drug release profile [15]. The commercial preparation Lamisil ONCE® film-forming solution that consists of terbinafine HCl (1% (w/w), forms a rough film with particles upon application that causes poor appearance and patient compliance [15]. Lamisil ONCE® shows poor drug permeation and retention due to the crystallisation of terbinafine HCl resulting in failure of drug penetration across the SC which leads to treatment failure and high relapse incidences [19]. All these findings emphasise the need to take into account the physical and chemical characteristics of topical products when designing film-forming

solutions. Besides satisfying consumer needs, formulation concerns i.e., drying time, formation of a transparent and flexible film, ease of application, high drug permeation across the SC, and effectiveness of formulation, must be considered. This study, therefore, is aimed at developing a topical dosage form using a film-forming solution to overcome the drawbacks of the existing conventional topical formulations. We hypothesise that the film-forming terbinafine HCl formulation in the present study will form a thin, transparent, non-sticky and flexible film which dries in less than 1 min and has good drug permeation across the SC to improve dermatologic outcomes for the treatment of cutaneous fungal infections.

MATERIALS AND METHODS

Materials

The film-forming solutions were prepared using methacrylate copolymer (Evonik Röhm GmbH, Darmstadt, Germany) of Eudragit L100, S100, and L100-55; hydroxypropyl methylcellulose (HPMC, Merck Milipore, USA); polyethylene glycol 400 (PEG400, Merck Milipore, USA), ethanol (Thermo Fischer Scientific, USA) and terbinafine HCl (USP Convention, USA). Deionised water was obtained using a Milli-Q system from Millipore. The proprietary drug Terbex® (1% terbinafine HCl cream, Bemxico Pharma, Bangladesh) was used for positive control. Acetonitrile and tetrahydrofuran HPLC grade (Thermo Fischer Scientific, USA) were used for HPLC assays. *Trichophyton rubrum* (ATCC-10218), sabouraud dextrose agar (SDA, Life Technologies, USA), RPMI 1640 with glutamine (Life Technologies, USA), and cocktails of ketamine, xylazine, tiletamine and zolazepam (Virbac, France) were used for an *in vivo* animal study.

Formulation

The film-forming solution was formulated by dissolving 10–20% (w/v) methacrylate copolymers alone or in combination with hydroxypropyl methylcellulose (HPMC) in 70% (v/v) ethanol that was left to stir overnight. To this solution, 3–5% (v/v) PEG400 and 1% (w/v) terbinafine HCl were finally added. The volume of ethanol was made up to 100% and left to stir overnight. The prepared formulations were screened and selected for desired quality attributes i.e., low viscosity, fast drying (less than 1 min), forms a non-sticky, transparent, and detachable film as described by Zurdo Schroeder *et al.* but with slight modifications [20]. Five formulations which fulfilled all specified criteria were subjected to tests of physical and mechanical properties. The prepared formulations were stored in an amber bottle at room temperature until further use.

Determination of physical-mechanical properties

a) Uniformity of thickness and weight of forming films

Film thickness was measured at six different points by a digimatic thickness gauge (Mitutoyo, Japan) and each dried film was weighed individually. The mean values for thickness and weight of six films were recorded as specified in USP 34,<905>Uniformity of Dosage Unit [21].

b) Measurement of pH

The pH of various formulations was determined using a digital pH meter [14]. The pH of each formulation was measured in triplicates and mean values were calculated.

c) Water content

The films were immersed in 5 ml of PBS pH 5.5 for 24 h after which they were placed on filter papers to remove excess water. Swollen films were weighed and left to dry at room temperature until constant weight (Ws). The films were dried again in a vacuum oven (Labwit ZRD–5055, Malaysia) for 6 h at 60 °C to determine if the films were able to stand without changing its physical and chemical characteristics [14]. The dried films were weighed (Wd), and the percentage (%) of water content was calculated as follows:

$$\text{Water content (\%)} = \frac{(\text{Ws} - \text{Wd})}{\text{Ws}} \times 100 \text{ Eq. (1)}$$

d) Percentage of moisture uptake capacity

The weighed film was kept in a desiccator at room temperature for 24 h and was exposed to 84% relative humidity until a constant weight was obtained. The percentage of moisture uptake was calculated as the difference between the final and initial weights with respect to initial weight [22].

e) Water vapor permeability (WVP)

A 10 ml glass vial was filled with 10 ml of phosphate buffer solution (PBS) pH 5.5 at 32±2 °C and covered with the dried films and sealed tightly with aluminium foil which was kept in place by a silicone ring [14]. The vials (n = 6 per formulation) were left on a heater (32±0.5 °C) and the relative humidity (RH) was monitored. The samples were individually weighed at 0 and 24 h. WVP was calculated from the weight loss of the vials W (g) in relation to time t (24 h) and surface permeability area A (1.54 cm^2).

$$\text{WVP (g cm}^{-2}\ 24\text{h}^{-1}) = \frac{\text{W}}{\text{A} * \text{t}} \text{ Eq. (2)}$$

f) Mechanical properties

The dried films were subjected to a tensile tester (TA. XT plus Texture analyser, USA) with a mounting load of 30 kg according to Ng and Tan but with slight modifications [22]. Six samples (2 cm x 2 cm) of each formulation were placed between two vertical grips and tested with an extension speed of 5 mm/min until the film ruptured. From the recorded load time profiles, tensile strength (σ) and elongation at break were calculated as follows:

$$\sigma\ (\text{N m}^{-2}) = \frac{\text{Fmax}}{\text{Ai}} \text{ Eq. (3)}$$

$$\varepsilon\ (\%) = \frac{\text{Lr}}{\text{Lo}} \times 100 \text{ Eq.(4)}$$

Where F*max* is the maximum force and A*i* is the initial cross-sectional area of the sample, L_r (m) is the extension of the sample at the moment of rupture, and L_o (m) is the original sample length. An average of six readings was recorded.

Quantification of terbinafine HCl using reverse phase-HPLC

The HPLC assay was adopted from terbinafine HCl standard drug monograph, USP (2009) Terbinafine HCl was analysed using RP-HPLC that consisted of a pump (Agilent 1200), UV detector and reversed-phase HPLC column (Agilent Zorbax SB-C18 HPLC (250 mm x 4.6 mm, 5 µm). The mobile phase was acetonitrile: water: tetrahydrofuran (70:25:5 (v/v)). The analysis time was about 35 min with a flow rate of 0.7 ml/min. UV detection was performed at 254.16 nm at a retention time (Rt) of 13.5 min, which resulted in a good resolution and separation efficiency. The method was found to be accurate and precise with 95.01 to 101.63% of recovery and 0.34 to 1.34% for R. SD, respectively. HPLC assay showed good linearity (R^2=0.9997) with limit of detection and limit of quantitation for terbinafine at 0.1 µg/ml and 0.5 µg/ml, respectively.

Drug content analysis

Each prepared film-forming solution or Terbex®cream was weighed (1 g) and diluted with 100 ml of pure ethanol in a 250 ml volumetric flask. From this solution, 1 ml was transferred to a 10 ml volumetric flask and the volume topped up to 10 ml. Two ml of the solution was filtered through a 0.45 µm nylon filter and subjected to RP-HPLC for quantitative assay. The experiment was performed in six replicates for each batch and the average value was calculated.

In vitro drug release analysis

Release of terbinafine HCl from *in-situ* film-forming solutions (FA-FE) and Terbex® cream was performed using a Franz-type diffusion cell with a nylon membrane (0.45 µm) placed in between the donor and receptor compartments. Five ml PBS (pH 5.5) was employed as the receptor phase and the temperature was maintained at 32±0.5 °C using a circulating water jacket and agitated by a magnetic stir bar. The receptor fluid was removed at 8-time points; post 0.25 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h and immediately replaced with an equal volume of fresh diluents. All samples were filtered through a 0.45 µm nylon filter and analysed by HPLC. The assay was performed in triplicates and at three different sites of donor skin samples. The data were fitted to four kinetic models [23, 24] i.e.

zero-order kinetic, first-order kinetic, Higuchi classical kinetic, and Korsmeyer-peppas kinetic to investigate the kinetic mechanisms of terbinafine HCL release from formulations.

Animals

The present study was approved by the Committee on Animal Research and Ethics (CARE) of UiTM (UiTM Care: No 196/2017). Male Sprague Dawley (SD) rats (n=30) weighing 150–300 g were obtained from LAFAM, Faculty of Pharmacy, UiTM. All the animals have housed in individually ventilated cages [IVC] (Modular Animal Caging Systems, Alternative Design, Manufacturing and Supply, Inc., Siloam Springs, Arkansas, USA) at 10% negative pressure with respect to a room that had a natural light-dark cycle (12 h each). Corn cobs (Bed-O' Corbs ¼) were used as bedding material and changed weekly or as and when required. All rats had access to food (Gold Coin Feed Mills, Penang, Malaysia) and water *ad libitum*.

Drug permeation and drug retention analysis

Permeation analysis was conducted by using the hairless abdominal skin (25 pieces) of SD rats [25]. The animals were sacrificed by overdose inhalation of diethyl ether. Hair on the dorsal side of the animal was removed with a 0.1 mm animal hair clipper, in the direction of tail to head. The dermis part of the skin was wiped 3 to 4 times with a wet cotton swab soaked in isopropanol to remove any adhering fat and the skin was then examined thoroughly for any surface skin abnormalities such as fissures or tiny hole. The skin was mounted on the receptor chamber of the Franz Diffusion Cell (PermeGear, USA). The surface area exposed to the test compound had a cross-sectional area of 3.14 cm^2 (2 cm in diameter). PBS (pH 5.5, 5 ml) was employed as the receptor phase and the temperature was maintained at 32±0.5 °C using a circulating water jacket and agitated by a magnetic stir bar. The receptor fluid was removed at 6-time points; post 1 h, 2 h, 4 h, 6 h, 12 h, and 24 h. Fluid removed from the receptor compartment was immediately replaced with an equal volume of fresh diluents. All samples were filtered through a 0.45 µm nylon filter and analysed by HPLC. The assay was performed in triplicates and at three different sites on the skin samples.

At the end of the experiment, the skin was wiped twice with cotton gauze to remove the residual formulation. Tape disk was applied and removed with pincers, using gentle pressure with the blunt end after application to ensure good skin contact. The application of tape disk at the respective sites was repeated 13 times. To avoid potential contamination by drug residue, the first tape strip was discarded, and remaining tapes were extracted in groups of three by shaking overnight with 5.0 ml of PBS. The mixture was filtered and quantified using RP-HPLC. The drug permeation and drug retention were determined using the Area under Curve (AUC) method and compared with calibration plots that were generated with pure compounds.

The drug permeation profile of terbinafine HCl in each tested formulation was determined over 24 h and plotted based upon the cumulative amount of drug permeated against time. The flux (µg cm^{-2} h^{-1}) of terbinafine HCl was calculated from the slope of the obtained plot. The steady state permeability coefficient (*Kp*) of the drug through the SC was deduced by using the Fick's law equation:

$$\mathrm{Kp} = \frac{\mathrm{J}}{\mathrm{C}} \quad \text{Eq. (5)}$$

Where J is the flux and C is the concentration of terbinafine HCl in the formulations.

Cutaneous infection and treatment

Cutaneous infection studies were carried out according to Mori *et al.* with slight modifications [16]. Male SD Rats (150–300 g) were anesthetised intraperitoneally with a cocktail of ketamine 100 mg/ml, xylazine 20 mg/ml, tiletamine 50 mg/ml, and zolazepam 50 mg/ml at a dose of 0.1 ml/100 g. Electric clippers and depilatory cream were used to remove hairs from rat flanks. An area of skin (20 mm in diameter) on each flank was scarified with coarse sandpaper to irritate the SC of the rats. *T. rubrum* (200 µl; $1x10^6$ conidia) were inoculated at the respective areas and covered by a sterile adhesive bandage with extra adherent tape. The bandage was held in place for 72 h before initiating treatment. On the third day, infected animals were randomly assigned into four groups comprising of six animals per group, including negative control (without any treatments), placebo (FA without drug), Terbex®, and FA. Each treatment (40 µl) was applied once daily for seven consecutive days. The efficacy of the treatments was examined using morphological and mycological evaluations.

Morphological evaluation

The mean areas of crust formation and skin lesions were examined visually daily. The area of crust formation, skin lesions, and papules formation (fig. 1) of each animal was measured using transparent graph paper. The mean area is the sum of the area of crust, skin lesion, and papules formed of all test animals in each group divided by the number of animals in that group.

Mycological evaluation

The work of Thapa *et al.* was adopted with a slight modification for mycological evaluation of cutaneous infections [19]. The animals were exposed to diethyl ether at the end of the experiment. The infected skins were excised and cut into 10 small blocks of 2 mm x 2 mm each and implanted on SDA plates containing 50 µg/ml of chloramphenicol and 50 µg/ml of gentamycin. The inoculated plates were incubated at 28 °C for 7 d and examined for evidence of visible growth. The fungal growth of any skin block was considered fungus-positive skin. Intensity of infection was assessed with scores ranging from 0 to 10, based on the number of culture-positive tissue blocks from the 10 tissue blocks/skin studied.

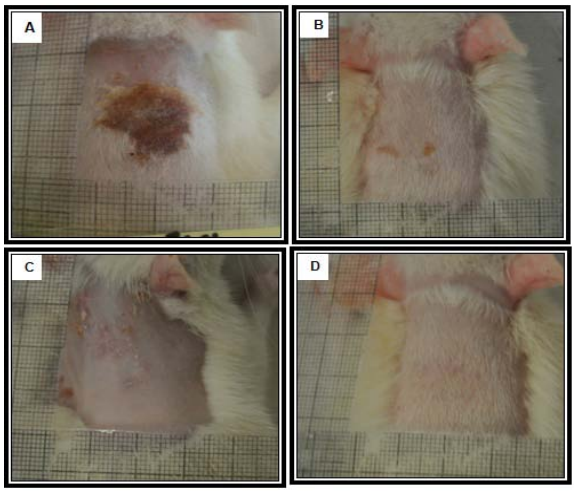

Fig. 1: Clinical scores. (A) Presence of crust, (B) Small erythematous (redness of skin) and lesion score normal, (C) Small erythematous and presence of small papules, and (D) Completely healed and growth of hair

Statistical analysis

The results were presented as mean±standard deviation of the mean (SD). Analysis of variance (ANOVA) test with Duncan's post-hoc test were used to analyse and compare between the group data, with $p<0.05$ as the limit of significance.

RESULTS AND DISCUSSION

Physical-mechanical properties

The physical-mechanical properties of each experimental batch are crucial to ensure that the laboratory data can be translated or scaled up for bulk manufacturing. In the formulation process, ratios of excipients (polymer, plasticiser, and diluents) are crucial. Different ratios of plasticiser and polymer can alter the physical and mechanical properties of prepared formulations.

The findings of this study (table 1) showed that the weight and thickness of all the tested formulations passed the requirement for uniformity (acceptance value or L within 15% variation) except the uniformity of thickness of formulation C (FC). The film formation of formulation A (FA, 10.57±0.03 mg, 0.24±0.01 mm) and formulation D (FD, 10.74±0.01 mg, 0.25±0.02 mm) were significantly ($p<0.05$) lighter and thinner than those of formulation B (FB, 12.35±0.09 mg, 0.39±0.02 mm), FC (12.03±0.09 mg, 0.37±0.07 mm) and formulation E (FE, 15.73±0.07 mg, 0.45±0.03 mm) (table 1). The findings of this study showed that film thickness and weight were significantly affected by the incorporation of HPMC and PEG400 in the formulations. Films which contained HPMC (FE) were thicker and weighed more than those prepared using a single polymer (Eudragit L100) due to the higher molecular weight of HPMC compared to the methacrylate copolymer. Previous studies also reported that an increase in the concentration of plasticiser (FB) affected film thickness and weight [26]. The uniformity of film thickness and weight are of utmost importance as they are directly related to (1) uniformity and accuracy of drug content, (2) bio adhesion of formed film and (3) reproducibility of the employed formulations.

Table 1: Details on the composition and physical, mechanical properties of the formulations that passed all five criteria

	Composition % (w/w)			Physical-mechanical properties								
	Eudragit L 100	HPMC	PEG 400	Weight (mg)	Thickness (mm)	pH	Moisture uptake (%, w/w)	Moisture content (%, w/w)	WVP (g/cm².24h)	Tensile Strength (N/m²)	Percent Elongation at Break (%)	Film flexibility
FA	10	-	3	10.57^{a}±.03	.24^{a}±.01	5.4	10.90^{a}	39.98^{a}	.0014^{a}±.001	4.78^{a}±0.14	33.61^{b}±2.229	Soft and tough
FB	5	5	5	12.35^{b}±.09	.39^{b}±.02	5.1	14.33^{a}	51.41^{b}	.0010^{a}±.001	18.33^{c}±3.10	5.49^{a}±1.394	Hard and brittle
FC	5	5	3	12.03^{b}±.09	.37^{b}±.07	5.3	13.08^{a}	47.01^{b}	.0014^{a}±.002	23.30^{c}±5.93	3.81^{a}±0.806	Hard and brittle
FD	10	-	5	10.74^{a}±.01	.25^{a}±.02	5.3	12.57^{a}	38.83^{a}	.0013^{a}±.002	4.60^{a}±0.44	43.11^{c}±3.199	Soft and tough
FE	10	5	3	15.73^{c}±.07	.45^{c}±.03	5.7	15.07^{a}	47.50^{b}	.0014^{a}±.001	10.77^{b}±3.30	5.76^{a}±1.740	Soft and weak

Mean±SD (n=6). $^{a-c}$Mean values within a column with no common superscript differ significantly ($p<0.05$, ANOVA and Duncan's test).

The pH of the film-forming solution is important to ensure that the film that is formed does not cause damage to skin or membrane leading to patient discomfort. Chemical properties of the active ingredient and its excipients determine whether the formulation is acidic or basic. In this study, the pH of all formulations (pH 5.1–5.7, table 1) was in the normal pH range of human skin (pH 4–6) indicating that the formed films would be a non-irritant to skin upon application [27].

The findings revealed that the dried film formulations which contained PEG400 and HPMC had significantly higher ($p<0.05$) water content (FB, FC, and FE, 47.5%–51.4%) than those without HPMC (FA and FD, 38.8%–40%) due to hydrophilic properties since water molecules would be attracted to enter the formed films. No significant differences in moisture uptake of the formed films (10.9–15.07%) were observed. With regard to water vapour permeability (WVP), all five films showed low water permeation with WVP less than 0.0014±0.002 g/cm².24h indicating a blockage in trans-epidermal water loss to the external environment causing saturation in the skin layer due to the hydrophobic properties of the Eudragit L100. This may lead to keratinocyte swelling and softening of skin tissue and thus accelerating drug permeability across the SC [19]. A film that has a high water absorption capacity and swelling capacity would be expected to show (1) better bio adhesion and (2) ease the release of drug principally by diffusion and/or erosion mechanism [28].

The mechanical properties of the formed film are important to ensure that the film has sufficient flexibility and elasticity. A deficiency in mechanical properties would result in cracks and fissures in the film when the patient moves. Sakellariou and Rowe classified polymeric films based on two parameters, tensile strength and elongation at break [29]. The ideal film for a topical or transdermal delivery system is one that is both soft and tough [30]. A low value for tensile strength (soft) indicates that the film is flexible enough to follow the movements of the skin, while a high percentage of elongation at break (tough) may prevent abrasion of the film. These factors would ensure patients feel more comfortable whilst using topical film-forming solutions as there would be little interference with their daily activities. In the present study (table 1), films from FA and FD showed significant ($p<0.05$) preferred mechanical properties of the low tensile strength (4.78 N/m² and 4.60 N/m², respectively) and high elongation at break (33.61% and 43.11%, respectively). The tensile strength of FE was about double at 10.77±3.3 N/m², while those of films FB and FC were about five times the tensile strengths of films FA and FD at 18.33±3.10 and 23.30±5.93 N/m², respectively. Films FA and FD were devoid of HPMC. Incorporation of HPMC into the formulations (FB, FC and FE) caused a significant ($p<0.05$) increase in the tensile strength and lowered the percentage of elongation at break (table 1), allowing the films formed by these formulations to be hard and tough [31]. Increase of PEG400 concentrations in the formulation (FA versus FD) showed a significantly ($p<0.05$) lower percentage of elongation at break (table 1). PEG400 is a hydrophilic polymer acting as a plasticizer that ensures the flexibility of the formed film. It has multiple hydroxyl groups and is capable of reducing the viscosity of the film-forming solution and inducing film flexibility and extendibility by reducing the glass transition temperature of the polymer [14]. Its incorporation with Eudragit L100, an anionic hydrophobic polymer, is advantageous due to the formation of hydrogen bonds with the latter [32]. This leads to a reduction in intermolecular interactions and the creation of enlarged spaces between the polymeric chains leading to an enhanced free volume which would reduce the firmness of the film and facilitate the mobility of the polymer chains [14].

Drug content analysis

Drug content uniformity remains a major challenge in formulating dosage forms. Uniformity of drug distribution is crucial in scaled-up

product manufacturing and its validation. The drug content of each formulation (97.21–101.12%) showed that the data obtained (table 2) were acceptable according to US Pharmacopoeia specifications (USP34).

Table 2: Summary of drug content, *in vitro* drug release, *ex vivo* skin permeation, flux, permeability coefficient and drug deposition in SC of terbinafine HCl in tested formulations

	Drug content (%)	Cummulative drug release (%)	Cummulative amount permeated (µg/cm²)	Flux, Jss (µg/cm². *h*)	Permeability coefficient, Kp (cm/*h*)	Amount of terbinafine HCl retained in SC (µg/cm²)
FA	98.74±3.565	47.43^{c}	1510.51^{e}±47.58	1.1577	5.789E^{-4}	6.86±0.47
FB	99.35±4.278	30.57^{b}	973.57^{c}±63.22	0.9823	4.912E^{-4}	6.35±0.98
FC	100.08±5.102	33.71^{b}	1073.57^{d}±75.55	0.9836	4.918E^{-4}	6.18±1.05
FD	97.21±2.114	35.71^{b}	1137.26^{d}±69.23	1.0628	4.814E^{-4}	5.38±0.79
FE	101.12±5.211	7.76^{a}	247.13^{a}±27.33	0.2299	1.150E^{-4}	6.15±1.28
Terbex®	103.34±6.273	14.94^{a}	475.8^{b}±5.60	0.444	2.220E^{-4}	6.51±0.58

Cumulative amount is shown as mean±SD (n=6). $^{a-e}$Mean values within a column with no common superscript differ significantly (p<0.05, ANOVA and Duncan's test).

Drug release kinetics

Terbinafine HCl that is present in a formulation has to be released from the polymer matrix network prior to crossing the skin barrier, the mechanism of which is driven by (1) drug dissolution, (2) drug diffusion and (3) swelling or erosion of the polymer [33]. The release kinetics of film-forming solutions were evaluated by employing data related to the kinetics of release that were fitted to several kinetic models. The results showed that most of the formulations were best fitted to the Higuichi Kinetic Model (table 3) and the coefficient of determination (R^2) ranged from 0.9527 to 0.9997, indicating the terbinafine HCl release from the optimised formulations is based on Fick's law diffusion process [34]. The present tested formulations also followed the Korsmeyer-Peppas equation ($0.918<R^2$ value<0.995) with exponent n in the range of $0.38<n<0.86$ (table 3), which represents the anomalous release mechanism [14, 15] in which the release is controlled both by drug diffusion, chain relaxation and erosion mechanisms [35]. This is attributed to the swelling of the matrix polymer chain and causes structural disentanglement and relaxation, which leads to diffusion of the terbinafine HCl through the swollen matrix [14]. All findings suggest that the drug release followed a diffusion-controlled mechanism by the formation of film through solvent evaporation leading to skin hydration and supersaturation of drug, which caused an initial rapid dissolution phase followed by a second slower release phase caused by swelling of the polymer and diffusion of drug through the swollen matrix.

Table 3: Release kinetics of the terbinafine HCl film-forming solutions

Formulation code	Zero-order (r value)	First-order (r value)	Higuchi (r value)	Korsmeyyer-peppas		Best fit model
				(r value)	(n value)	
FA	0.7849	0.8626	0.9554	0.9288	0.4631	Higuichi
FB	0.9793	0.8987	0.9814	0.9945	0.8635	Korsmeyyer
FC	0.9902	0.8921	0.9527	0.9697	0.6829	Korsmeyyer
FD	0.8863	0.9032	0.9536	0.9180	0.3758	Higuichi
FE	0.9560	0.9607	0.9959	0.9913	0.6863	Higuichi
Terbex®	0.9858	0.9903	0.9997	0.9907	0.7089	Higuichi

Drug permeation and retention across SC

The most challenging task in cutaneous drug delivery is to overcome the strong permeation barrier of the SC, which limits drug transport. Drug penetration across the SC into viable epidermis at effective concentrations is the main goal in determining the efficiency of a topical antifungal medication. FA showed significantly (p<0.05*)* the highest cumulative drug release and permeation across the SC at 24 h (47.43% and 1510.51 µg/cm², respectively) when compared to the rest of the formulations (table 2) and three-fold higher than the proprietary drug, Terbex® (14.9% and 475.8 µg/cm², respectively). Eudragit L100 exhibits ionic interactions and hydrogen bonding with PEG400; this is of importance in stabilising the amorphous state of drugs to prevent crystallization. Drugs in an amorphous state are thermodynamically metastable relative to the crystalline state, which leads to an enhanced drug release and permeation profile [36]. Increasing Eudragit L100 concentration from 10% (FA) to 15% (FE) decreased the cumulative amount of drug permeation across the SC after 24 h (247.13±27.33 µg/cm²) due to concentration-dependent matrix formation that may retard drug release from the film-forming solution. The combination polymer Eudragit L100 and HPMC in formulations (FB and FC), resulted in a significant (p<0.05) decrease in skin permeation (973.57±63.22 µg/cm² and 1073.57±75.55 µg/cm², respectively) of the drug across the SC. Previously, the use of a methacrylate copolymer alone in a formulation resulted in a significantly higher drug permeation across the SC than when it was incorporated with HPMC [37].

A low volatility of solvent upon application to the skin causes drug recrystallization [38]. In the present study, 70% (v/v) ethanol was used, and it evaporated rapidly (1 min) as compared to the previous study done by Reid *et al.* where solvent evaporation took 30 min leading to re-crystallization [38]. Anwar *et al.* and Ahad *et al.* reported that the use of ethanol in formulations significantly enhanced the flux of drugs across the SC compared with other solvents [25, 39]. The supersaturation of the drug prior to application, or known as transiently supersaturated preparation, is also associated with high thermodynamic activity. This would enhance cutaneous drug delivery as there will be a strong thermodynamic drive for the drug to leave the formulation leading to an increase in flux. FA was superior (K_p of 5.789E^{-4} cm/h) than the other formulations (FB–FE, 1.15E^{-4}–4.92E^{-4} cm/h) or Terbex®(K_p of 2.220E^{-4} cm/h). Improvement in permeability was seen in all formulations except in FE (1.15E^{-4} cm/h) when compared to Terbex®. Ethanol also acts as a penetration enhancer by disrupting the intercellular lipid packing and causes diffusivity of drug across the SC [19]. The combination of ethanol and PEG400 increased the solubility of terbinafine in the formulation and caused a higher permeability coefficient [25].

The highest amount of terbinafine HCl deposited after 24 h was found in the SC of FA (6.86 µg/cm²) followed by Terbex® (6.51 µg/cm²), FB (6.35 µg/cm²), FC (6.18 µg/cm²), FE (6.15 µg/cm²), and FD (5.38 µg/cm²). However, the differences were not significant. Since ethanol alters the solvency and barrier properties of the SC, it

would be expected to be partially responsible for the deposition of terbinafine HCl [38].

Rapid permeation and drug depletion of tested formulations compared to Terbex® could be due to the dynamic interactions of alcohol as a chemical enhancer (in the first 1 minute of application), supersaturation of drugs and excipients (due to the evaporation of the diluents), and hydration of skin layer. Ethanol may act as a penetration enhancer whilst causing supersaturation of drugs which is the driving force for diffusion of terbinafine HCl across the SC. The formation of thin-film was observed after 1 min of application. The film may prevent water loss from the skin layer and hence cause skin hydration. This is supported by the low water vapor permeability study, which indicated a blockage of trans-epidermal water loss (less than 0.0014 g/cm^2.24h) to the external environment causing saturation of the skin layer. Saturation of water in the skin may lead to swelling and softening of skin tissue and a marked increase in its permeability. In combination, these effects produced by the film-forming solution led to synergy in terbinafine HCl permeation across the SC.

The 95% confidence interval calculated from linear regression (R^2 values) of the kinetic profile along with the physical-mechanical properties and drug permeation study indicates strong evidence that FA was superior to the other formulations (table 1and3). Thus, FA was further examined for *in vivo* efficacy study.

Morphological evaluation of *in vivo* antifungal potency

All experimental rats were successfully infected with 1x10^6–6x10^{6}cfu/unit of *T. rubrum* after 3 d of inoculation as skin lesions were observed. On Day 4 of treatment (fig. 2), both FA and Terbex® elicited a significant (p<0.05) reduction in the size of crust formation, redness, scales, and papules of 2.25±0.11 cm^{2}and 2.88±0.13 cm^2, respectively, compared to the negative control, untreated (4±0.08 cm^2) or placebo (4±0.09 cm^2) groups and this trend continued until complete healing on Day 7 of treatment. The placebo group was not significantly different from the baseline, indicating that the vehicle was without effect.

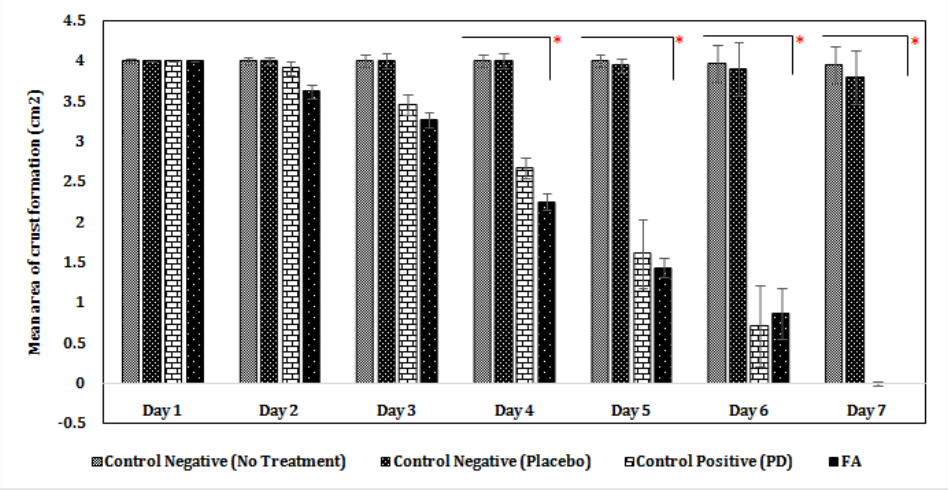

Fig. 2: Mean area of crust formation, skin lesion and papules (cm^2±SD), Note: '*' Significant difference in mean areas of crust when compared to the negative control

Mycological evaluation

The mycological study (table 4) showed a significant difference (p<0.05) between treatment groups and negative control groups (placebo-treated and untreated groups). The results showed that the treatment groups (FA and Terbex®) eradicated the dermatophyte infection almost completely (number of fungus-positive skin were 3 and 6, respectively). The average intensity of infection score of the FA (0.5±0.84) was not significantly different from that of the proprietary drug, Terbex® (1.0±0.89). This showed that terbinafine HCl in FA and Terbex® were fungicidal, which is preferred in anti-dermatophytosis compared to a fungistatic effect. Fungicidal drugs help prevent the recurrence of dermatophyte infections, while fungistatic drugs only render the infective organism unable to grow or divide.

Table 4: Therapeutic efficacy of different terbinafine formulations in the SD rats' model of dermatophytosis

	Number of fungus-positive skin* (n = 60)	Average intensity of infection score
Negative control (Untreated)	56^a	9.33±0.82^a
Negative control (Placebo)	53^a	8.83±1.17^a
PD	6^b	1.00±0.89^b
FA	3^b	0.50±0.84^b

*Viable growth/total number of specimens, n = 60 per group. $^{a\text{-}b}$Values within a column with no common superscript differ significantly (p<0.05, ANOVA and Duncan's test)

CONCLUSION

The 1% (w/w) terbinafine HCl film-forming solution showed advantages i.e., ease of use, convenience, and formed a thin, transparent, non-sticky film which dried in less than 1 minute. The film had high flexibility; thus it would be supple on the skin and would not be easily removed by abrasion or contact with clothing and other surfaces. All these properties would improve the compliance of patients to topical antifungal treatment. Terbinafine in both FA and Terbex® penetrated the SC and was deposited in the viable epidermis to provide a continuous drug supply over a prolonged period of time. The new formulation, FA showed a significantly higher drug flux than Terbex®. In addition, the amount of terbinafine HCl which permeated across the SC was higher following the application of the developed formulation when compared with the control.

Furthermore, FA had high drug content uniformity, which complied with US Pharmacopoeia specifications making it a suitable candidate for further research prior to commercialization.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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