

Formulation, *In-vitro* and *In-vivo* Evaluation of Nystatin Topical Gel

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Abstract: The aim of this study was to enhance solubility and dissolution of antifungal drug, nystatin, with two different techniques, nanoemulsion and solid dispersion systems and then incorporate them into gel base. Several nanoemulsion formulations were prepared and evaluated as mentioned in the previous study. One nanoemulsion formulation was optimized and incorporated into gel base (F₁ NE). Several solid dispersion formulations at 1:1 and 1:2 drug: carrier ratios were prepared. These solid dispersion formulations were evaluated for solubility, drug content and *in-vitro* drug release. The solid dispersions were then incorporated into gel base and tested for pH, drug content, viscosity, *in-vitro* drug release, IR studies and DSC studies. A comparison between the nanoemulsion based gel formulation (F₁ NE), solid dispersion based gel and a commercially available product, Nystatin[®] cream, was carried out to judge their efficacy. (F₁ NE) showed the highest drug release percent (49.13%) followed by F₁ solid dispersion (F₁ SD) (36.37%) in contrast, the marketed formulation released (22.88%) of the drug in 24 hrs. The *in-vitro* nystatin release data were fitted to Korsmeyer peppas's release model. The optimized F₁ NE was chosen for further clinical trial. **Background:** Cutaneous candidiasis treatment usually requires topical application of an antifungal agent for 2 to 4 weeks. **Methods:** Patients (n = 40) were randomly selected to apply nystatin, F₁ NE or Nystatin[®] cream twice daily for 30 days and were periodically assessed until day 42. **Results:** F₁ NE recipients had significantly higher rates of mycological cure beginning at day 14 (50% vs 35%) with continued improvements through day 30 (95% vs 50%). **Conclusions and Relevance:** With the outcome mycological cure at the end of treatment, there was significant difference between F₁ NE and Nystatin[®] cream. The F₁ NE was found to be more efficient in cutaneous candidiasis management.

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Keywords: Nanoemulsion, Solid dispersion, Nystatin, *Candida albicans*, Nanoemulsion based gel, Topical delivery.

1.Introduction

Candidal infections of the skin/nails and vagina are very common worldwide. Cutaneous candidiasis is a superficial mycotic infection of the skin usually caused by the yeast *Candida albicans*. The intertriginous skin folds are most frequently affected and patients who are obese or who have diabetes are at particular risk⁽¹⁾. Cutaneous candidiasis, usually caused by *Candida albicans*, may colonize occluded areas or folds of the skin, producing infection in areas such as the groin, axillae, and interdigital spaces. Clinical manifestations include erythema, scaling, maceration, vesicles, and pustules⁽²⁾.

The outcome of treatment depends on the susceptibility of the pathogenic fungi to the antifungal agent. Little information is available, comparing the antifungal activity of commonly used agents against yeasts⁽³⁾.

Nystatin is a polyene antifungal characterized by a potent broad-spectrum antifungal action including a wide range of pathogenic and nonpathogenic yeasts and fungi. The Nystatin is active against a variety of

fungal pathogens including: *Candida*, *Aspergillus*, *Histoplasma*, and *Coccidioides* and has been used for years to treat *Candida* at the skin and those for the mouth. Nystatin exerts its antifungal activity by binding to sterols in the fungal cell membrane. As a result of this binding, the membrane is no longer able to function as a selective barrier, and potassium and other cellular constituents are lost⁽⁴⁾. This information, combined with the facts that the incidence of disseminated fungal infections has risen over the past decade, and that *Candida* is now the fourth most commonly encountered nosocomial bloodstream pathogen, shows that it is increasingly important to make available new products to fight these alarming trends⁽⁵⁾.

The aim of this study is improving nystatin solubility, dissolution rate and subcutaneous absorption to increase its efficacy for topical application. This achieved by formulating nystatin in two different techniques, nanoemulsion and solid dispersion systems, then incorporating them into a gel base. Gel bases have gained more and more

importance. This is because the gel bases are better percutaneous absorbed than cream and ointment⁽⁶⁾.

The nanoemulsion systems are comparatively thermodynamically stable systems because they contain surfactant, cosurfactant, and oil. Nanoemulsion-based drug delivery systems have gained wide acceptance because of their enhanced drug solubilization, thermodynamic stability, and ease of manufacture. Delivery of drugs using these nanoemulsions through skin increases the local/systemic delivery of the drug by different mechanisms that make them suitable vehicles for the delivery of Antifungals⁽⁷⁻¹¹⁾. Solid dispersion is another effective technique which can easily enhance the solubility and dissolution rate of drugs. The term 'solid dispersion' has been utilized to describe a family of dosage forms whereby the drug is dispersed in a biologically inert matrix. It may be defined as the dispersion of one or more active ingredients in an inert carrier matrix at solid-state prepared by the melting (fusion) or solvent method⁽¹²⁻¹⁴⁾. A comparison between the nanoemulsion based gel formulation (F₁ NE), solid dispersion based gel and a commercially available product, Nystatin[®] cream, was carried out to judge their efficacy. The optimized formula was chosen for further clinical study. The antifungal activity of the optimized prepared nystatin gel was evaluated and compared with Nystatin[®] cream in patients with skin candidiasis.

2. Material, Patients and Methods

A. Nanoemulsion and solid dispersion systems

Material

Nystatin was kindly donated as a gift from Delta Pharm Company. Labrafil M 1944 (polyoxyethylated kernel oil), was a gift sample from GlaxoSmithkline Pharmaceutical Company, (Egypt). Tween[®] 80 (polyoxyethylene 20 sorbitan monooleate) and Polyvinylpyrrolidone K30 (PVP K30) were gift samples from ADCO, (Egypt). Ethanol, urea and cetyl alcohol were purchased from ADWIC, (Egypt). Methyl cellulose was purchased from Fluka. Nystatin[®] skin cream (Nystatin 1% w/w, manufactured by Pharaonia Pharmaceuticals) was purchased from a local pharmacy store. All other materials were of analytical grade.

Methods

Preparation of nystatin nanoemulsion formulation

The nanoemulsion formulation (F₁ NE) was selected because it showed proper stability, small droplet size and highest extent of drug release as mentioned before in the previous study⁽¹⁵⁾. It prepared by spontaneous emulsification method as follow. Appropriate quantities of oil Labrafil M 1944, surfactant Tween[®] 80 and co-surfactant ethanol were weighed and mixed well. The drug was accurately weighted to represent 1% of the total weight of the

formulation and added to the previous mixture and stirred with a magnetic bar on magnetic stirrer, at room temperature until the drug completely dissolved. The weighed amount of water then added drop wise with continuous mixing.

Preparation of nystatin solid dispersion formulations

The required amount of nystatin and carrier in 1:1 and 1:2 ratio were dissolved in sufficient volume of suitable solvent with continuous stirring. The solvent from the solution was removed using a rotary evaporator. Afterwards the formed solid dispersion is stored in a dessicator to remove the residual solvent. The dried mass was pulverized passed through 250 µm sieve and stored in desiccator until used for further studies.

Characterization of solid dispersions

Solid dispersions obtained from the above method were evaluated for their solubility, drug content and *in-vitro* release studies.

Solubility studies:

An excess amount of nystatin solid dispersions was added to 25 ml of citrate-phosphate buffer (pH 5.5) in screw capped bottles. Samples were shaken for the 24 hours at room temperature. Subsequently, the suspensions were filtered. Filtered solution diluted properly and measured spectrophotometrically (Jenway Ltd, Model 6105 UV/V, United Kingdom) at 305 λ max⁽¹⁶⁾.

Drug content:

Precisely weighed amounts of solid dispersions equivalent to 20 mg of nystatin was taken in 100 mL volumetric flask containing 15 mL methanol and stirred for 30 min. The volume was made up to 100 mL with citrate-phosphate buffer (pH 5.5) and appropriate dilutions were made. The resultant solution was filtered. The absorbance of the solution was measured spectrophotometrically at 305 nm.

In-vitro release studies:

In-vitro release studies were performed in 900 mL citrate-phosphate buffer (pH 5.5) at 37 ± 0.5°C; using USP dissolution apparatus (paddle method, 75 rpm). Twenty miligrams of pure nystatin drug and equivalent amount of solid dispersions were spread over the surface of the dissolution medium. At fixed time intervals (30, 60, 90, 120, 150, 180, 210 and 240 min) aliquots of 5 mL samples were withdrawn and simultaneously replenished with fresh 5 mL of citrate-phosphate buffer (pH 5.5) solution maintained at same temperature to maintain sink conditions. Samples, so withdrawn were immediately filtered through Whatman filter paper and assayed spectrophotometrically for drug content at 305 nm⁽¹⁶⁾.

Release kinetic studies of solid dispersion

To study the drug release mechanism of each formulation, the release data were fitted to the general

exponential function: $M_t / M_0 = kt^n$; where M_t / M_0 represents the fractional uptake of solvent (or release of a solute) normalized with respect to the equilibrium conditions; n is a diffusion exponent characteristics of the release mechanism, and k denotes properties of the polymer and the drug. When n is ≤ 0.5 , the drug is released from the polymer with a Fickian diffusion mechanism. If $0.5 < n < 1$ this indicates anomalous or non-Fickian release. While if $n = 1$ this indicates Case II transport. Lastly, when n is > 1.0 , Super Case II transport is apparent. Kinetic studies were performed by adjusting the release profiles to Higuchi and Zero order equations⁽¹⁷⁾.

Preparation of nanoemulsion and solid dispersion based gel

The nanoemulsion and solid dispersion based gel were prepared by sprinkling the weighed amounts of the methyl cellulose powder (5%) gently in sufficient quantity of warm distilled water, and magnetically stirred at high speed. Stirring was continued until a thin hazy dispersion, without lumps, was formed. For complete gel dispersion it was necessary to leave samples overnight in the refrigerator⁽¹⁸⁾. Then the nystatin loaded nanoemulsion was slowly added to the viscous solution of methyl cellulose under magnetic stirring⁽¹¹⁾. While the nystatin solid dispersions was dissolved in methanol and then slowly added to the viscous solution of methyl cellulose under magnetic stirring⁽¹⁹⁾.

Evaluation of nanoemulsion and solid dispersion based gel

Determination of pH

The pH of nanoemulsion and solid dispersion based gel was measured on digital pH meter standardized using pH 4.0 and 7.0 standard buffers before use⁽²⁰⁾.

Drug content studies

The nanoemulsion and solid dispersion based gel equivalent to 20mg of nystatin was taken in 100 mL volumetric flask containing 15 mL methanol and stirred for 30 min. The volume was made up to 100 mL with phosphate citrate buffer (pH 5.5) and appropriate dilutions were made. The resultant solution was filtered through 0.45 μm membrane filter. The absorbance of the solution was measured spectrophotometrically at 305 nm.

Viscosity measurement

A Cole parmer viscometer was used to measure the viscosity of the prepared gel bases. The spindle was rotated at 10 rpm. Samples of the bases were allowed to settle over 30 min at room temperature before the measurements were taken⁽¹⁸⁾.

In-vitro release studies

The *in-vitro* release studies of nystatin nanoemulsion and solid dispersion based gel were investigated through Semipermeable membrane

obtained from Sigma which has molecular wt cut off 12,000 Daltons, was used in this study using a modified USP 17 dissolution apparatus I. A glass cylindrical tube (2.5 cm in diameter and 6 cm in length) was attached instead of the basket and was tightly covered with the semipermeable membrane. Nystatin nanoemulsion and solid dispersion incorporated gel bases were placed in the cylindrical tube at the semipermeable membrane surface. The cylindrical tube was dipped in 100 ml methanolic citrate-phosphate buffer (30%:70%) at pH 5.5 to allow the establishment of the sink conditions and to sustain permanent solubilization. The release study was carried out for 24 hrs. at 32 °C. the stirring shaft was rotated at speed of 100 r.p.m⁽¹⁵⁾.

At predetermined time intervals (1, 2, 4, 6, 8, 12, 20, 24 hrs.) aliquots of one milliliter of the release medium were withdrawn and diluted then filtered for analysis and replaced with equal volume of the buffer solution to maintain a constant volume. The absorbance of the collected samples was measured by UV at λ_{max} of 305 nm.

Release kinetic studies of nanoemulsion and solid dispersion based gel

To study the drug release mechanism of each formulation, the release data were fitted to the general exponential function: $M_t / M_0 = kt^n$; where M_t / M_0 represents the fractional uptake of solvent (or release of a solute) normalized with respect to the equilibrium conditions; n is a diffusion exponent characteristics of the release mechanism, and k denotes properties of the polymer and the drug. When n is ≤ 0.5 , the drug is released from the polymer with a Fickian diffusion mechanism. If $0.5 < n < 1$ this indicates anomalous or non-Fickian release. While if $n = 1$ this indicates Case II transport. Lastly, when n is > 1.0 , Super Case II transport is apparent. Kinetic studies were performed by adjusting the release profiles to Higuchi and Zero order equations⁽¹⁷⁾.

Fourier Transform Infrared Spectroscopy (FTIR) studies

Instrument used was Shimadzu FTIR-84005 spectrophotometer, Japan. In this study, potassium bromide disc method was employed. Pure drug, pure polymer and selected solid dispersion studied by IR. The powdered sample was intimately mixed with dry powdered potassium bromide. The mixture was then compressed into transparent disc under high pressure using special dies. The disc was placed in IR spectrophotometer using sample holder and spectrum was recorded⁽²¹⁾.

Differential Scanning calorimetry (DSC) studies

The thermal characteristics of the pure materials and the selected solid dispersion were determined by differential scanning calorimetry (DSC-60, Shimadzu, Japan). The scanning rate was 10°C/min, and the

scanning temperature range was between 30°C and 300°C. Samples of about 5 mg were sealed into aluminum pans⁽²¹⁾.

B. Clinical trial

Patients and clinical specimens

This trial was conducted in Dermatology department, Faculty of medicine, Tanta University over a period of 6 months. Forty patients with diagnosis of cutaneous candidiasis (20 patients with candidal intertrigo, 20 patients with erosiointerdigitalis) were enrolled in this randomized prospective controlled trial and identified by coded numbers to maintain their privacy. Patients unlikely to receive a therapy and with an ability to give a written consent were eligible to be enrolled in this work while pregnant or lactating women, those had an evidence of human immunodeficiency virus or other life threatening infection, unable to give a written consent, having history of hypersensitivity to nystatin, or included in any other clinical trial within the previous month were excluded from this study.

The patients were randomized by closed envelope into one of two groups.

Group I: 20 candida patients (9 patients with candida intertrigo and 11 patients with erosio interdigitalis) who applied commercially available product, Nystatin[®] cream (Pharonic Company) topically on the affected areas twice daily for 4 weeks. The mean age of this group was 45 years (range, 19-82), 13 females and 7 males. 6 patients were diabetic, 7 were hepatic and 3 were on steroid therapy

Group II: 20 candida patients (12 patients with candida intertrigo and 8 patients with erosio interdigitalis) who applied nanoemulsion formulation (F₁ NE) of nystatin topically on the affected areas twice daily for 4 weeks. The mean age in of this group was 38 years (range, 16-60), 12 females and 8 males. 5 patients were diabetic, 6 were hepatic and 1 was on steroid therapy.

An informed consent was taken from all participants and the research ethical committee of Tanta Faculty of Medicine approved this trial (Code: 2284).

Table 1: Dermographic data of patients

	Group I	Group II	p- Value
Age (Mean) ± SD	45 ± 15.2	38 ± 15.8	0.16NS*
Sex			
Female	13	12	0.74 NS
Male	7	8	
Type			
<i>Candida intertrigo</i>	9	12	0.52 NS
<i>Erosio interdigitalis</i>	11	8	
Precipitating factors			
Diabetes mellitus	6	5	0.72 NS
Hepatic disease	7	6	0.7 NS
Steroid therapy	3	1	0.59 NS

NS= Non Significant P- value =0.05, using Qui Square test.

* using student t-test.

2. Material and Methods

All Microbiological raw materials were supplied by Oxoid, UK and were prepared in Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University according the manufacturer's instructions. Cases of candidiasis enrolled in this study were identified using standard microbiological techniques including microscopic examination, culture on Sabouraud dextrose agar media, germ tube test (Figs. 1a, b and c, respectively) and biochemical reactions.⁽²²⁾ The efficacy of the nanoemulsion gel were evaluated by the clinical presentation and the microbiological tests results for each patient before and after two weeks of topical application of the therapy and comparing the results between group I and group II.

3. Results and Discussion

A. Nanoemulsion and solid dispersion systems

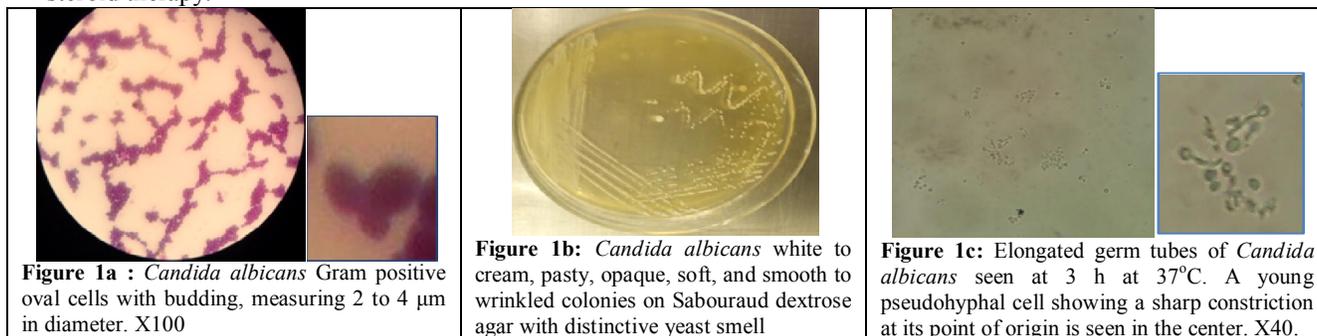


Figure 1a : *Candida albicans* Gram positive oval cells with budding, measuring 2 to 4 μ m in diameter. X100

Figure 1b: *Candida albicans* white to cream, pasty, opaque, soft, and smooth to wrinkled colonies on Sabouraud dextrose agar with distinctive yeast smell

Figure 1c: Elongated germ tubes of *Candida albicans* seen at 3 h at 37°C. A young pseudohyphal cell showing a sharp constriction at its point of origin is seen in the center. X40.

Evaluation of solid dispersion of nystatin

Solubility studies:

Figure 2 displays the amount of nystatin solubilized from solid dispersion systems with different carriers at different drug: carrier ratios (1:1

and 1:2) prepared with solvent evaporation method. Urea and PVP K₃₀ in the drug: carrier (1:2) ratio showed the highest solubilizing effect. These formulations increased the solubility almost 2 fold compared to that of pure drug.

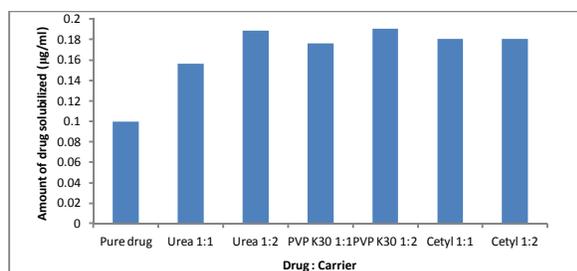


Figure 2. Solubility studies of nystatin solid dispersions

Drug content studies

Drug content of all solid dispersions was in the range (95.34 - 104.1 %).

In-vitro release studies:

The solid dispersions of nystatin with carriers urea, PVP K₃₀ showed an increase in the dissolution rate in pH 5.5 citrate-phosphate buffer (Figure 3). Dissolution of the nystatin increased with increasing proportions of carriers. These observations indicate the enhanced dissolution of SDs with increase in the concentration of carriers possibly due to the increased wettability of the drug by the carrier, drug particle size reduction in the course of the solid dispersions preparation and converting drug crystals to amorphous state⁽²³⁾. In case of cetyl alcohol carrier, the dissolution rate of drug decreased. It is slower than the dissolution rate of pure drug. That is due to that the cetyl alcohol is slightly water soluble carrier so it sustains the drug release from the matrix⁽²⁴⁾.

The dissolution rate of drug from cetyl alcohol decreased as the proportion of the carrier increased.

The in-vitro dissolution data were fitted to Korsmeyer peppas's release model and interpretation of release exponent values (n) enlightens us in understanding the release mechanism from the solid dispersion formulations (Table 2). The release exponent values of the formulations obtained were from 0.0179 to 0.3388. Based on these values we can say that the formulations exhibited Fickian release. These results are in agreement with a result by Krishnamoorthy *et al*⁽²⁵⁾. All formulations showed higher (r) values drug release followed Higuchi model kinetics except cetyl alcohol 1:1 formula showed higher (r) value for zero order plots indicating that drug release followed zero order kinetics.

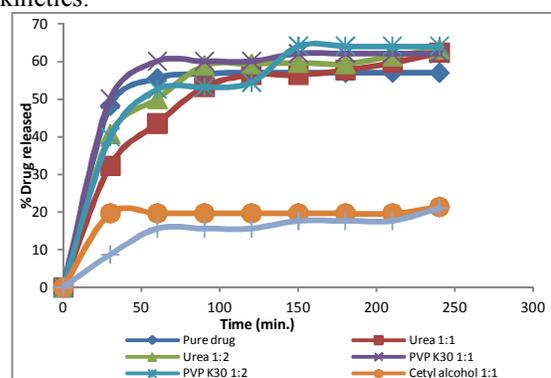


Figure 3. In-vitro dissolution profiles of pure drug and its solid dispersions

Table 2. Kinetics of in-vitro release from different formulations of nystatin solid dispersion (using regression coefficient (r) and 'n' value)

Code	(n) value	r	Zero-order kinetic		Higuchi model		Possible mechanism Of drug release
			r	k	r	k	
Pure drug	0.0689	0.8326	0.6617	0.0275	0.7500	0.6666	Higuchi model, Fickian
Urea 1:1	0.2995	0.9630	0.8902	0.1207	0.9406	2.7231	Higuchi model, Fickian
Urea 1:2	0.1965	0.9403	0.8424	0.0860	0.9014	1.9643	Higuchi model, Fickian
PVP 1:1	0.0901	0.8680	0.7335	0.0407	0.8068	0.9550	Higuchi model, Fickian
PVP 1:2	0.2266	0.9565	0.8985	0.1068	0.9367	2.377	Higuchi model, Fickian
Cetyl alcohol 1:1	0.0179	0.4331	0.5774	0.0047	0.5081	0.0881	Zero-order, Fickian
Cetyl alcohol 1:2	0.3388	0.9094	0.8644	0.0416	0.8980	0.9228	Higuchi model, Fickian

Evaluation of nanoemulsion and solid dispersion based gel

Determination of pH

The pH of nanoemulsion and solid dispersion based gel was found to be in the range of 5.88 to 6.16 (Table 3) which was within the acceptable limits for topical application.

Drug content studies

Drug content of all formulations was in the range (97.4-103.6%) indicating uniform dispersion of nystatin (Table 3).

Viscosity measurement

The viscosity of the nanoemulsion and solid dispersion based gel could be observed from Table 3. The most viscous formulation was formulation F₆SD which has 2680 Ps. followed by formulation F₅ which has 1980 Ps. F₁NE has the lowest viscosity compared to other formulations which was 348.1 Ps.

In-vitro release studies

Dissolution profiles of the commercial product, Nystatin[®] cream, solid dispersion and nanoemulsion based gel formulations are represented in Figure 4. As it is apparent, F₁ NE, F₁ SD and F₂ SD improved the

dissolution rate of nystatin. F₁ NE showed the highest drug release percent (49.13%) followed by F₁ SD (36.37%) and F₂ SD (34.5%) which were significantly (ANOVA-one way, $p < 0.05$ level) higher than that of the commercial formulation (22.88 %) of the drug in 24 hrs due to low aqueous solubility. In case of the nanoemulsion technique, reduction in the droplet size leads to higher surface area and higher dissolution of nystatin in oily phase of nanoemulsion based gel

formulation eventually permitted drug release at faster rate from nanoemulsion based gel formulation showing the significance of the nanosizing of the oils globules⁽²⁶⁾. While in case of the solid dispersion, the enhancement can be attributed to the great hydrophilic character of the system due to the presence of the carrier, which can reduce interfacial tension between a poorly water-soluble drug and dissolution medium⁽²⁷⁾.

Table 3. Characterization of Nystatin solid dispersion and nanoemulsion based ge

Code	Drug:Carrier ratio	% w/w of Components in Nanoemulsion formulation			pH	% Drug Content	Viscosity (Ps)
		Oil	Smix	Water			
F ₁ SD	Drug:Urea 1:1	-	-	-	6	100.7±0.88	1859±11.4
F ₂ SD	Drug:Urea 1:2	-	-	-	6	97.4±2.6	870±5.7
F ₃ SD	Drug:PVP K ₃₀ 1:1	-	-	-	6.12	101.5±0.74	1880±8.9
F ₄ SD	Drug:PVP K ₃₀ 1:2	-	-	-	5.96	101.9±1.56	860±5.3
F ₅ SD	Drug:Cetyl alcohol 1:1	-	-	-	5.89	103.6±1.33	1980±10.7
F ₆ SD	Drug:Cetyl alcohol 1:2	-	-	-	5.88	99.2±0.92	2680±7.4
F ₁ NE	-	Oil 8.6	Smix 51.5	Water 40	6.16	99.25±1.2	348.1±10.2

The *in-vitro* dissolution data were fitted to Korsmeyer peppas's release model and interpretation of release exponent values (n) enlightens us in understanding the release mechanism from the commercial product, solid dispersion and nanoemulsion based gel formulations (Table 4). The release exponent values of F₃ SD, F₄ SD, F₅ SD and F₆ SD obtained were from 0.3052 to 0.4972. Based on these values we can say that these formulations exhibited Fickian release. While the release exponent values of commercial product, F₁ SD, F₂ SD and F₁ NE obtained were from 0.6385 to 0.8882 so they exhibited Non-fickian release. These results are in agreement with a result by Gaudin *et al.* where the calculated *in-vitro* release exponent (n) value suggested that artesunate is released from the gel by non-Fickian transport⁽²⁸⁾. All formulations showed higher (r) values for zero order plots indicating that drug release followed zero order kinetics except commercial product showed higher (r) value for Higuchi model plots indicating that drug release followed Higuchi model kinetics.

FTIR studies

FTIR spectroscopic studies were conducted to determine possible drug:carrier interactions. Based on the previous results FTIR studies of pure drug nystatin, urea and its solid dispersion 1:1 (F₁ SD) were selected and shown in Figure 5. The typical FTIR absorbance bands in the spectrum of pure nystatin displays broad intense absorption bands with maxima at 3415 cm⁻¹ due to the stretching vibration of bonded hydrogen; at 2927 cm⁻¹ due to the asymmetric and

symmetric stretching vibration of CH₂ group, 1705 cm⁻¹ due to the stretching vibration of carbonyl from ester and carboxylic acids, 1400 cm⁻¹ due to the bonding vibration ν (C-H) and at 1068 cm⁻¹ due to the hydroxyl groups from nystatin. It was also found to be comparable with the previous recorded FTIR spectra of nystatin⁽⁵⁾. All the characteristic peaks of nystatin and urea were present in the solid dispersion, thus indicating no significant evidence of chemical interaction between drug and carrier. This confirms the stability of drug with its solid dispersion.

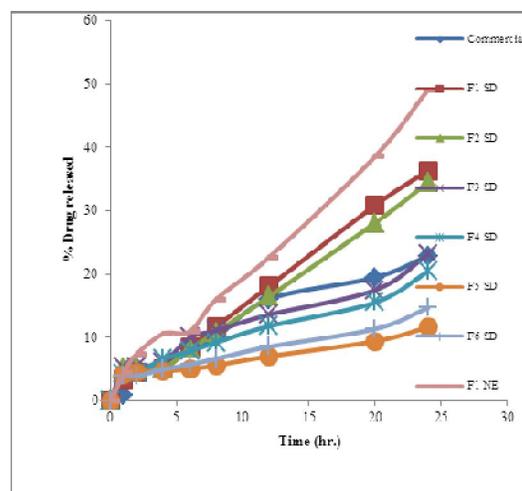


Figure 4. *In-vitro* dissolution profiles of nystatin solid dispersions and nanoemulsion based gel.

Table 4. Kinetics of *in-vitro* release from different formulations of nystatin solid dispersion (using regression coefficient (r) and 'n' value)

Code	(n) value	r	Zero-order kinetic		Higuchi model		Possible mechanism Of drug release
			r	k	r	k	
Commercial	0.8882	0.9504	0.9777	0.0147	0.9892	0.7016	Higuchi model, Non-fickian
F ₁ SD	0.7871	0.9685	0.9969	0.0248	0.9683	1.1342	Zero order, Non-fickian
F ₂ SD	0.6385	0.9219	0.9917	0.0222	0.9519	1.0003	Zero order, Non-fickian
F ₃ SD	0.4827	0.9572	0.9861	0.0124	0.9735	0.5746	Zero order, Fickian
F ₄ SD	0.4972	0.9786	0.9930	0.0111	0.9784	0.5121	Zero order, Fickian
F ₅ SD	0.3052	0.9055	0.9889	0.0052	0.9484	0.2363	Zero-order, Fickian
F ₆ SD	0.4171	0.9528	0.9937	0.0075	0.9680	0.3446	Zero order, Fickian
F ₁ NE	0.7233	0.9803	0.9946	0.0313	0.9634	1.4261	Zero-order, Non-fickian

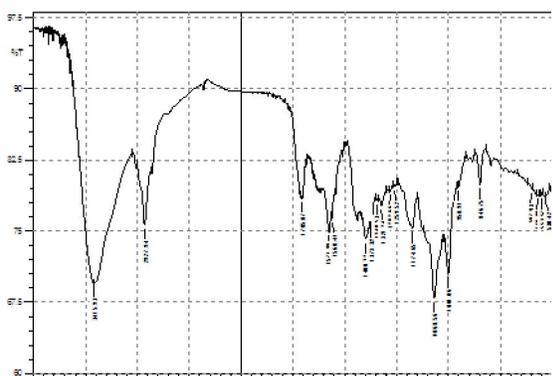
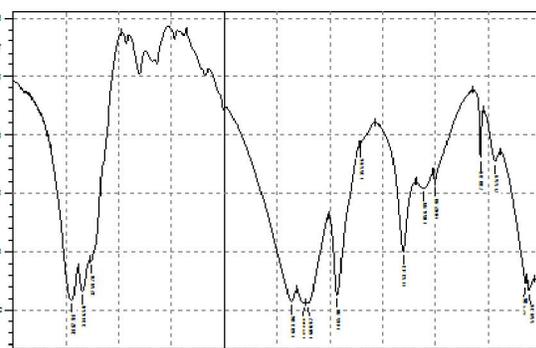
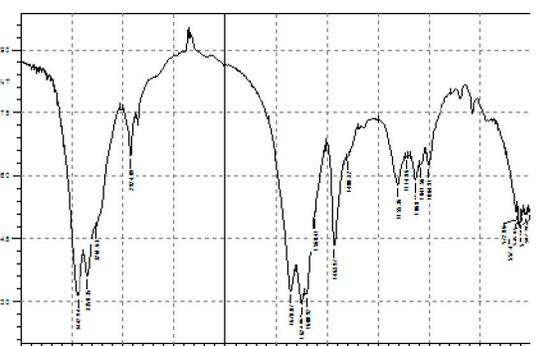
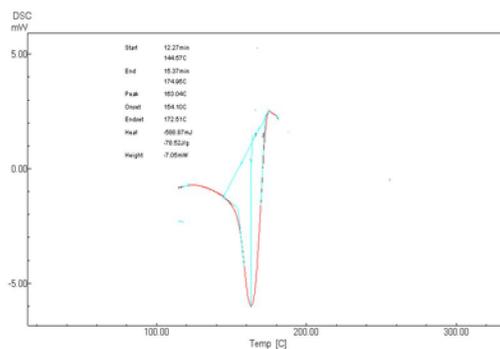
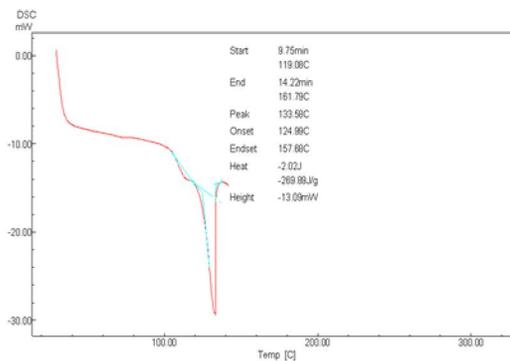
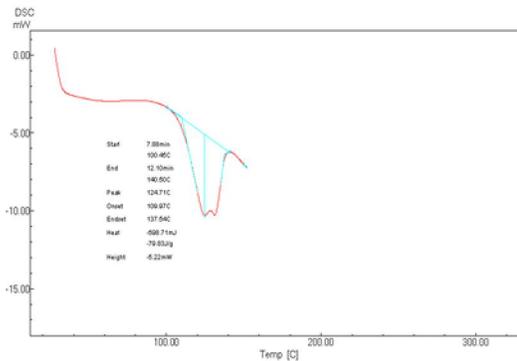
**A****B****C****Figure 5. FTIR spectra of A) pure nystatin, B) pure urea and C) solid dispersion with urea in the ratio (1:1).****A****B****C****Figure 6. DSC thermograms of A) pure nystatin, B) pure urea and C) solid dispersion with urea in the ratio (1:1).**



Figure 7. The effect of nystatin nanoemulsion based gel and commercially available product, Nystatin® cream on cutaneous candidiasis after 4 weeks of treatment.

Table 5: Clinical and mycological cure after two weeks of treatment with nystatin nanoemulsion based gel or commercially available nystatin cream

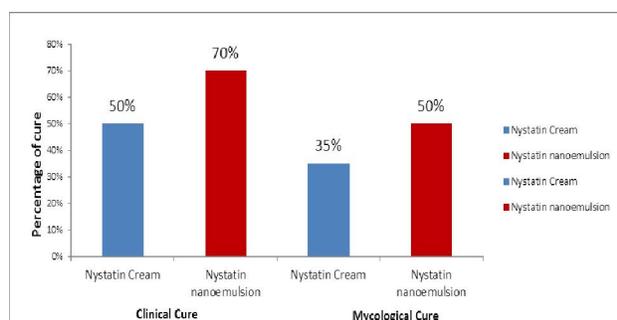
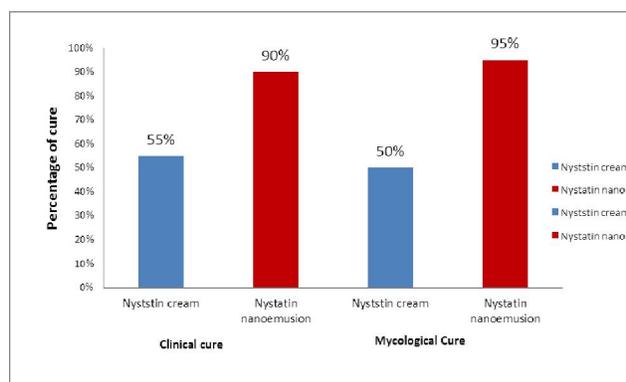
	Clinical cure				Mycological cure			
	Group I N=20	Cure %	Group II N=20	Cure %	Group I N=20	Cure %	Group II N=20	Cure %
Cure number	10	50%	14	70%	7	35%	10	50%
p-value	0.33 NS				0.5 NS			

NS= Non Significant using Qui square test.

Table 6: Clinical and mycological cure after four weeks of treatment with nystatin nanoemulsion based gel or commercially available nystatin cream

	Clinical cure				Mycological cure			
	Group I N=20	Cure %	Group II N=20	Cure %	Group I N=20	Cure %	Group II N=20	Cure %
Cure number	11	55%	18	90%	10	50%	19	95%
*p-value	0.03 *				0.004*			

*= Significant using Qui square test

**Figure 8. Clinical & Mycological Cure after 2 weeks of treatment with nystatin nanoemulsion based gel or commercially available nystatin cream****Figure 9. Clinical & Mycological Cure after 2 weeks of treatment with nystatin nanoemulsion based gel or commercially available nystatin cream**

DSC studies

The DSC thermograms of nystatin, urea and the corresponding solid dispersion (F₁ SD) are shown in Figure 6. The DSC thermogram of crystalline nystatin alone was characterized by a sharp endothermic peak at 163 °C corresponding to its

melting point while sharp endothermic peak at 133 °C was observed for urea. This is in the good agreement with the previous findings on the thermal analysis of nystatin⁽²⁹⁾. The DSC thermogram of the solid dispersion containing urea shows that nystatin lost its shape and distinctive appearance and shifted to a lower melting point at 124.7 °C. It was suggested that a very small crystalline portion of nystatin existed in the solid dispersion melted at a lower melting point than of intact nystatin. The previous results were concluded when studying the DSC of allopurinol-urea solid dispersions⁽²⁷⁾. The increase in the dissolution rate was thus attributed to an increase in the available surface area of the drug due to improve wettability provided by the carrier used.

Based on the previous results F₁ NE selected as optimum formulation. As it showed higher release than F₁ SD and F₂ SD (ANOVA-one way, p < 0.05 level).

B. Clinical trial

The age, type of fungal infection (Candida intertrigo or Erosio interdigitalis) and precipitating factors (diabetes mellitus, hepatic diseases and steroid therapy) were did not differ significantly between the studied groups at the beginning of the study (p>0.05) as shown in Table 1. After two weeks of treatment there was no significant difference between the studied groups either by clinical evaluation or mycological results (p>0.05, Table 5& Figure 8). While after four weeks of treatment the clinical evaluation was shown significant increase in cure rate in group II compared to group I (p<0.05). Also there was very significant increase in mycological cure in group II compared to group I (Table 6& Figure 9).

The results show consistent evidence of the superiority of nanoemulsion formulation in the

treatment of cutaneous candidiasis. Only 2 patients suffered contact dermatitis with nanoemulsion formulation.

Conclusion

Nanoemulsion recipients had significantly higher rates of mycological cure beginning at day 14 (50% vs 35%) with continued improvements through day 30 (95% vs 50%). They also had higher rates of effective treatment.

This study reveals that the nanoemulsion formulation enhance nystatin solubility, dissolution rate and subsequently improve the subcutaneous absorption so it increased its efficacy for topical application. The clinical trial showed that a nanoemulsion based gel formulation (F₁ NE) of nystatin is more efficacious and better tolerated than commercially available product, Nystatin[®] cream for the treatment of cutaneous candidiasis. Further studies are warranted to confirm the therapeutic benefits of this nanoemulsion formulation.

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