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PREPARATION OF CLOTRIMAZOLE NANOEMULGEL AND ITS ANTI FUNGAL STUDIES

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ABSTRACT

Clotrimazole is a synthetic derivative of imidazole, a medication with a broad-spectrum antifungal agent that is used to treat localized vaginal, skin, and nail infections. The aim of the study was to develop an innovative technique to improve the permeability and efficacy of topical Clotrimazole. A nanoemulgel of Clotrimazole was formulated by the incorporation of a nanoemulsion and a hydrogel. The nanoemulsion was first optimized using a self-emulsifying technique, and the drug was then loaded into the optimum formulation and evaluated prior to mixing with the hydrogel. Clotrimazole nanoemulgel formulations were evaluated for their physical characteristics and antifungal activity. Based on the results, the formulation with 0.4 % Carbopol showed the highest release profile after 2 h; thus, it was chosen as the optimum formulation. A cell diffusion test was performed to examine the ability of the Clotrimazole nanoemulgel to penetrate the skin and reach the bloodstream. Percentage cumulative drug releases of 29.81 % and 23.60 % after 6 h were achieved for the CNZ nanoemulgel and the commercial cream. The antifungal activity of the novel CNZ nanoemulgel formulation was tested against Candida albicans and compared to marketed cream and almond oil; the results were: 40.8 ± 2.1 mm, 25.6 ± 1.8 mm and 18 ± 2.0 mm, respectively. In conclusion, a novel CNZ nanoemulgel showing superior antifungal activity compared to that of the commercial product has been developed. This nanotechnology technique is a step toward making pharmaceutical dosage forms that has a lot of promise.

Keywords: Clotrimazole, Antifungal, Nanoemulgel, SNEDDs, Almond oil.

INTRODUCTION

Topical nanoemulgel delivery systems have been demonstrated to improve the systemic delivery, pharmacokinetics, pharmacodynamics, and therapeutic profile of lipophilic drugs. In recent years, the use of nanoemulgels has increased due to the higher compliance of patients. This can be attributed to the advantages of nanoemulgels, as this drug delivery method is noninvasive, avoids gastrointestinal side effects, has an excellent therapeutic, and safety profile and is easy to apply (1-2). Topical therapy reduces the risk of systemic side effects, making it the most favorable route of therapy for diseases affecting the skin (3). The compartmentalization of nanostructured drug delivery systems is restricted to specific environments; consequently, the

drug is concentrated at its site of action. Topical nanoparticle drug delivery has emerged as one of the most promising strategies for site-specific drug delivery (4).

In prior studies, nanoemulsion has been used as a delivery system for the transdermal delivery of Clotrimazole is a hydrophobic broad-spectrum antifungal agent that is used for the treatment of dermal infections caused by various species of pathogenic dermatophytes, yeasts, and Malassezia furfur. The primary action of clotrimazole is against dividing and growing organisms. Clotrimazole is effective in preventing the growth of the pseudomycelia and mycelia of Candida albicans. Clotrimazole [1-[(2-(chlorophenyl) diphenyl methyl]-1-H imidazole] is an odourless, white to pale yellow, crystalline powder and is practically insoluble in water, freely soluble in polyethylene glycol 400.It inhibits ergosterol synthesis and promotion of the plasma membrane of fungi leaky. In this study CNZ nanoemulgel was prepared and characterized to provide an agent with good permeability for topical use. An advantage of nanoemulgel delivery systems is their stable formulation, which could improve patient compliance. The aim of the present study was to formulate a novel CNZ nanoemulgel to improve the applicability and permeability of CNZ through the skin. This study will focus on the preparation of a novel nanoemulgel delivery system for CNZ nitrate with enhanced solubility, permeability, spreadability, efficacy, and safety.

MATERIALS AND METHODS

Materials

Clotrimazole marketed currently available on the market were kindly gifted to the researchers by Jerusalem Pharmaceuticals Co. ltd., Palestine. Almond oil was gifted by professional-super pharm company, Israel. Tween 80, Span 80, glycerol, propylene glycol 400, ethanol, and carboxyvinyl polymer (Carbopol 940) were purchased from CBC Co., ltd., Japan. Crystal oil, olive oil, castor oil, and paraffin oil were obtained from the Al-Shams company, Palestine.

Wavelength screening of a Clotrimazole using UV spectrophotometry

A sample of the medication, which consisted of 0.02 g of the active ingredient CNZ, was dissolved in 10 ml of methanol so that the optimum wavelength could be identified. The solution was mixed with a vortex mixer to ensure a homogenized solution, then the absorbance was measured using a UV spectrophotometer (7315; Jenway, United Kingdom) within a wavelength range of 200–600 nm (5).

Calibration curve for Clotrimazole

A standard stock solution was prepared according to the Indian Pharmacopoeia. The stock solution was prepared by dissolving 10 mg of CNZ in 100 ml of methanol (100µg/ml). From this stock solution, 0.5–3 ml was diluted with up to 10 ml of methanol (5–30µg/ml) and examined using a UV spectrophotometer. To generate the calibration curve, the absorbance results were plotted against the prepared concentrations (5).

Solubility of Clotrimazole in different surfactants and oils

The solubility of CNZ in different oils and surfactants was determined in order to select the most suitable oil and surfactant as the drug vehicle, which would then be used to prepare the nanoemulsion. By dissolving the active ingredient CNZ at a concentration of 2 % in different oils (castor oil, paraffin oil, olive oil, crystal oil, almond oil, and pine oil) and surfactants (propylene, Span 80, Tween 80, Tween 20, and glycerol), the solubility could be determined. The mixtures

Section: Research Paper
ISSN 2063-5346

were prepared and centrifuged for 5 min at 6000 rpm, and then the supernatants were collected to measure the absorption using a UV spectrophotometer (6-7).

Preparation of nanoemulsion

To optimize the nanoemulsion formulation, the drug vehicles (surfactants and oils) were selected based on the results of the CNZ solubility test. The nanoemulsion was chosen for the preparation, optimize the nanoemulsion formulation, different compositions of olive oil, almond oil, Span 80, and Tween 80 were tested. The different formulations were weighed and vortexed for 1 min with delicate agitation to self-emulsify the formulations in distilled water.

Index analysis, polydispersity, and droplet size analysis of the almond and olive oil nanoemulsions

The size distribution and droplet size of the almond and olive oils and the surfactant emulsion were measured using a sampler and a laser diffraction particle size analyzer (SALD-MS23 and SALD-2300; Shimadzu Corp., Japan), which permitted the measurement of the diameter of the droplets and the polydispersity index (8).

Clotrimazole loading in nanoemulgel

Based on the droplet size results, the optimal nanoemulgel formulation was chosen, and CNZ was loaded into it. The loading process was performed by dissolving CNZ in Tween 80, Span 20, and almond oil.

Nanoemulgel preparation

Hydrogel was prepared by adding water to Carbopol 940, and then the mixture was homogenized to achieve uniform dispersion. The pH of the hydrogel was adjusted using a few drops of 2 M sodium hydroxide (NaOH), which were added under constant stirring. Then the mixture was constantly stirred for 24 h to complete the gelation.

Preparation of the Clotrimazole nanoemulgel

The optimized CNZ-loaded nanoemulsion formulation was incorporated into the Carbopol 940 hydrogel at several concentrations (0.4 %, 0.6 %, and 0.8 % Carbopol). Polydispersity index, particle size, and zeta potential analyses were performed for the attained nanoemulgel formulations.

Measurement of the zeta potential of the Clotrimazole nanoemulgel formulations

The Omni (Brookhaven Instruments Corporation, New York, USA) was used to measure the zeta potential of the formulations. Measurements were performed in triplicate, and the average was calculated. The zeta potential was determined for each sample, and then the zeta potential was graphed against the Carbopol concentration.

Measurement of the rheological behavior of the Clotrimazole nanoemulgel

There were several differences in the behavior of nanoemulgel formulations with different concentrations of Carbopol (the thickening agent). The temperature was measured using a rotational viscometer (DVI; Brookfield, USA) at the same value of 25 °C. The viscosity shear rate values were between 0 and 100 rpm.

Section: Research Paper
ISSN 2063-5346

Assessment of the release of Clotrimazole from nanoemulgel systems containing different concentrations of Carbopol

The dialysis test was used to study the release of the CNZ from the nanoemulgel system. Five grams of CNZ were added to each sample, consisting of three different concentrations of Carbopol (0.4 %, 0.6 %, and 0.8 %). One liter of phosphate-buffered saline (PBS) was prepared by dissolving 0.19, 2.38, and 8 g of potassium dihydrogen phosphate (PDP), disodium hydrogen phosphate (DHP), and sodium chloride (NaCl), respectively, in distilled water, then making it up to 1 L. The pH of the PBS stock was adjusted to 7.4 pH with 1 M HCl.

The next step was to add the sample to a dialysis bag and place it in an isothermal shaker containing 40 ml of PBS, maintaining the temperature at 37 ± 1.0 °C. To determine the amount of the drug released from the nanoemulgel, samples from the buffer solution were taken at 10, 20, 30, 40, 50, 60, 90, and 120 min At a wavelength of 230 nm, UV spectrophotometry was used to measure the CNZ absorbance. The release test was also performed on the market product (CNZ cream). Lastly, the results of the formulated CNZ nanoemulgel were compared to those of the market CNZ cream (9).

Antifungal test

The antifungal activity was assessed by the agar-well diffusion method using Candida albicans. A plate containing Muller–Hinton agar was used for the inoculation of a standard inoculum of fungal culture. Two wells (A and B) with a diameter of 6 mm were punched into the agar: A was filled with the market CNZ cream, and B was filled with the formulated CNZ nanoemulgel. The plates were incubated for 48 h at 37 °C. The diameter of the zone of inhibition was measured to evaluate the antifungal activity (10).

Skin penetration study using the Franz cell diffusion test

A cell diffusion test was performed to examine the ability of the CNZ nanoemulgel to penetrate the skin and reach the blood stream. This is essential to determine whether the drug is suitable for topical use. Mice (10–12 weeks old) were sacrificed with carbon dioxide when the full thickness of their skin was reached. The media for the Franz cell diffusion test was PBS (pH 7.4). The diffusion cell was kept at 37 ± 1 °C during the test by heating re-circulated water with oscillating (electromagnetic) stirring. Samples (1 ml) were taken from the receptor compartment at specified time intervals (0.5, 1, 2, 3, 4, 6, and 24 h) and replaced with fresh medium. The CNZ absorbance was assessed by ultraviolet (UV) spectrophotometry at a wavelength of 230 nm. The concentration was then calculated using the calibration curve. The release profile was determined by plotting the cumulative amount of CNZ released (mg/ml of the acceptor media) versus time (h) (8).

Statistical assessment

Each of the experiments was performed in triplicate, and the values were expressed as mean ± standard deviation (SD). Statistical significance was considered when the p-value was ≤ 0.005.

RESULTS AND DISCUSSION

Screening for the Clotrimazole wavelength

To find the optimum wavelength for CNZ, screening was carried out by UV spectrophotometry. The optimum absorbance was achieved at 230 nm.

Section: Research Paper
ISSN 2063-5346

Calibration curve for Clotrimazole

To determine the CNZ nitrate concentration in an unknown sample, a calibration curve was prepared with standards of different concentrations. The unknown samples were compared to a set of known values. Fig. 1 shows the results of the CNZ calibration curve.

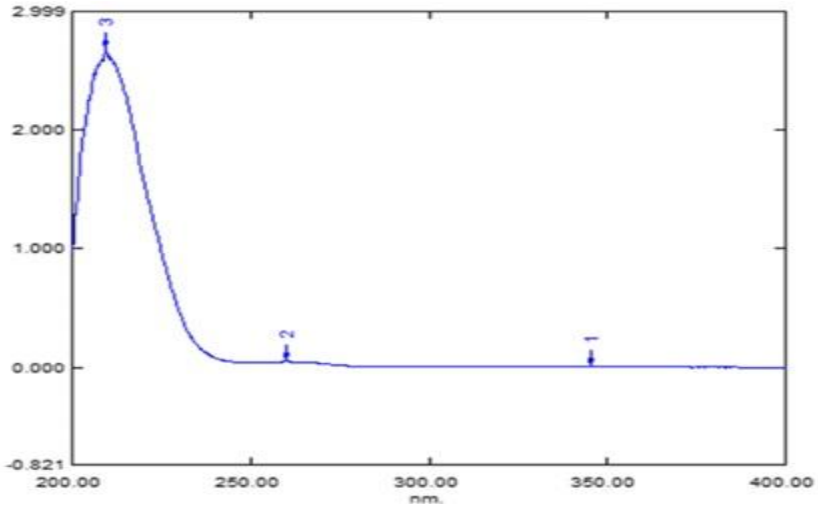

Figure 1: Calibration curve of Clotrimazole

The calibration curve will be used in the research to calculate concentrations from the UV-spectrophotometer absorbance results using the equation that correlates absorbance and concentration, $y = 0.042x + 0.018$.

3.3. Screening of Clotrimazole solubility in several oils and surfactants

By dissolving CNZ in several oils and surfactants, its solubility was determined, and the absorption was measured using a UV spectrophotometer. The results achieved are shown in Table 1 below

Table 1: The solubility results of Clotrimazole in different oils and surfactants.

Oil/surfactant	**Concentration (mg/ml)**
Almond oil	51.624
Span 80	51.449
Tween 80	52.148
Olive oil	52.378
Crystal oil	2.610
Paraffin oil	1.440
Tween 20	34.240

Section: Research Paper
ISSN 2063-5346

Pine oil	27.000
Propylene	31.040
Glycerol	0.1640
Castor oil	11.314

Based on the results presented in Table 1, the best oil for dissolving CNZ were olive oil and almond oil, with concentrations of 52.378 and 51.624mg/ml, respectively. Moreover, Tween 80 and Span 80 showed the highest solubilizing capability for CNZ amongst the surfactants, with concentrations of 52.148 and 51.449mg/ml, respectively. Hence, they were chosen as the surfactants and co-surfactants, respectively. These oils and surfactants were used as the drug vehicle for the production of CNZ nanoparticles using the self-emulsifying technique.

Optimization of olive and almond oil nanoemulsion formulations

Olive and almond oil nanoemulsion formulations were optimized using the self-emulsifying technique. A ternary phase diagram was constructed to determine the optimum nanoemulsion formulations using several concentrations of oils (olive and almond oils), Tween 80, and Span 80. The green area represents those compositions that produced nanoemulsion formulations with droplets smaller than 1 µm in size, whereas the red area represents the compositions that were able to produce macroemulsions with droplets between 1 and 20 µm in size. The optimum nanoemulsion formulations were chosen according to the droplet size and polydispersity index (PDI) of the two oil formulations. Those formulations with droplets smaller than 200 nm were chosen.

Table 2: The selected nanoemulsion formulations for both oils nanoemulsion

Tween 80	**Span 80**	**Olive oil**	**Particle size (nm)**	**PDI**
64 %	16 %	20 %	190 ± 3.7	0.27 ± 0.03
Tween 80	Span 80	Olive oil	Particle size (nm)	PDI
72 %	8 %	20 %	175 ± 2.2	0.182 ± 0.06

The best formulations were those that obtained the smallest particles and were loaded with CNZ. The obtained formulations were measured for their particle size and polydispersity index in triplicate.

Particle size and polydispersity index of the Clotrimazole nanoemulsion

The results showed no significant change after loading the CNZ into the selected nanoemulsion formulations, as shown in Table 3.

Table 3: The particles size and polydispersity index of Clotrimazole nanoparticle

	Tween 80	Span 80	Oil	CNZ	Particle size (nm)	PDI

Almond	72 %	8 %	20 %	0.02 g	170 ± 3.1	0.193 ± 0.06
Olive	64 %	16 %	20 %	0.02 g	201 ± 4.2	0.300 ± 0.04

By comparing the formulations, it can be seen that the formulation loaded in almond oil presented the smallest particle size (170 nm) and polydispersity index (0.193). Thus, the formulation with almond oil was chosen for further experiments.

Clotrimazole nanoemulgel particle size, polydispersity index, and zeta potential

Nanoemulgel formulations of CNZ were prepared after preparing the drug nanoparticles using the self-emulsification technique and incorporating them into Carbopol hydrogel (Carbopol concentrations of 0.4 %, 0.6 %, or 0.8 %). The results for CNZ particle size and polydispersity index are presented in Fig. 3, whereas the zeta potential results for CNZ can be seen in Fig. 2.

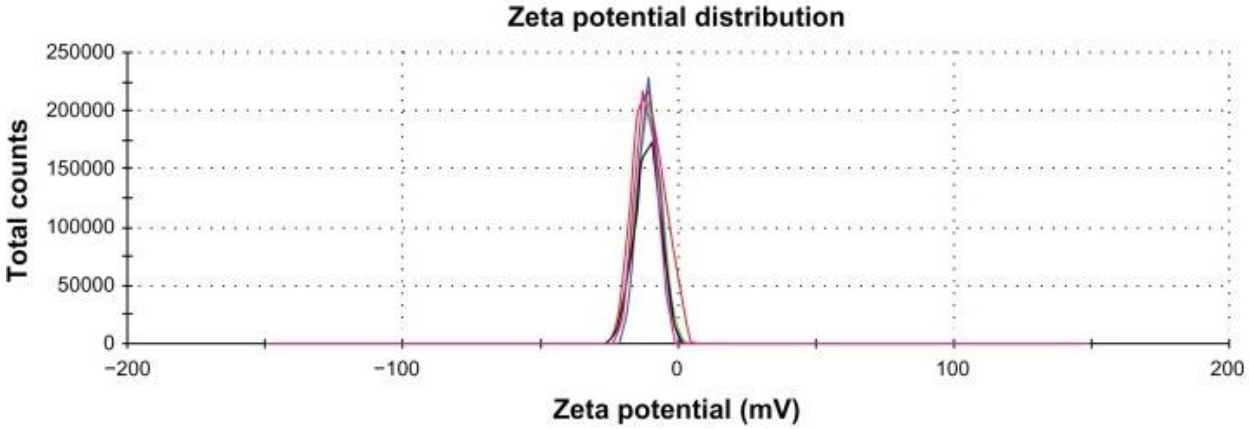

Figure 2: Zeta potential of Clotrimazole nanoemulgel formulations.

The drug particle size and PDI results did not significantly differ between the CNZ nanoemulsion form and when it was converted to a nanoemulgel. The results for the three different concentrations of Carbopol were in the range of 170–180 nm. A slight increase was observed at higher concentrations of Carbopol, but generally the behavior of the three tested concentrations was similar.

The zeta potential results for the nanoemulgel formulations were below −30 mV for both drugs. This fact suggests that the formulations adequately prevented the agglomeration of particles, and, therefore, presented appropriate stability.

Rheological properties of the Clotrimazole nanoemulgel and cream

Evaluation of the rheological properties is of great importance for semisolid forms, like that of our formulated drug, as they indicate the efficacy and quality of the formulations.

The behavior of the nanoemulgel formulations was similar for all three Carbopol concentrations tested; however, the viscosity increased as the Carbopol concentration increased. Furthermore, the viscosity decreased with an increase in the shear rate, which indicates that the drug nanoemulgel formulations presented pseudoplastic behavior.

Section: Research Paper
ISSN 2063-5346

Release of Clotrimazole (CNZ) from the nanoemulgel formulation

To study the release of CNZ from the nanoemulgel formulations, release tests were performed. This study was also important for selecting the optimum Carbopol concentration for use as a thickening agent for the preparation of the hydrogel used in the nanoemulgel formulations. The release of the drug from the nanoemulgel formulations was tested using the dialysis method and compared to the market product. The release profile of Clotrimazole nanoemulgels contains different Carbopol concentrations compared to the market product.

The release profiles of the different formulations are presented in Fig. 3. It is notable that there was an inverse relationship between the Carbopol concentration and the release profile, where the formulation with the lowest concentration of Carbopol (0.4 %) presented the highest release profile.

Skin penetration study using the Franz cell diffusion test

An in vitro Franz cell diffusion test was performed to determine the percentage cumulative drug release from the CNZ nanoemulgel and from the conventional Marketed cream. The results presented in Fig. 3 show the results of the diffusion cell test for freshly prepared CNZ nanoemulgel formulation and market CNZ cream through the skin of a mouse**.**

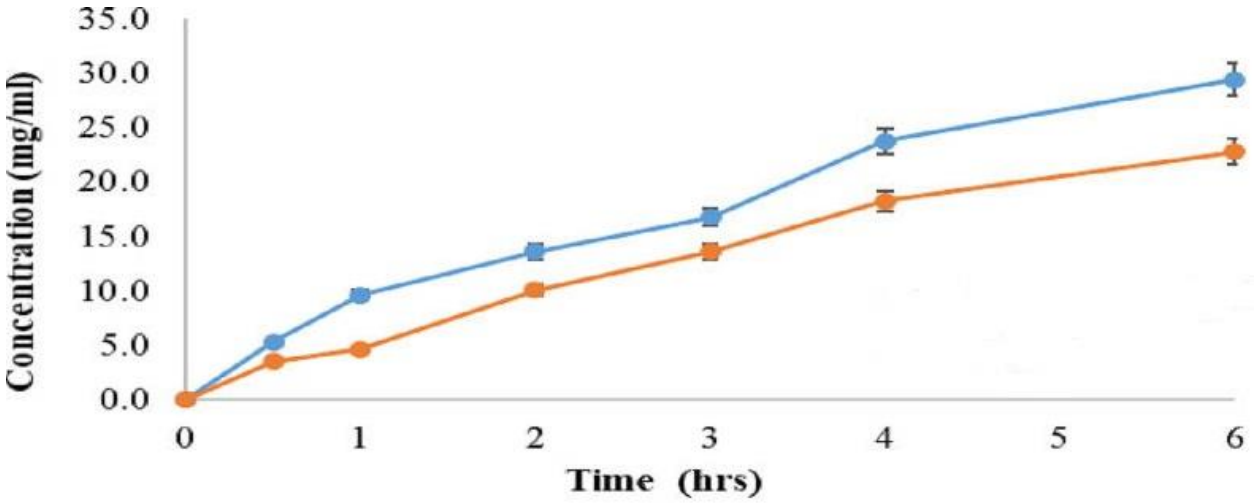

Figure 3: In vitro Franz diffusion profile of the Clotrimazole nanoemulgel compared to the market product.

A percentage of cumulative drug release of 29.81% and 23.60% after 6 h was acheived for the nanoemulgel CNZ and the conventional Marketed cream, respectively.

Evaluation of the antifungal effect of the Clotrimazole nanoemulgel

An antifungal test was performed on C. albicans grown in agar media on Petri dishes to assess the antifungal activity of the CNZ nanoemulgel and compare it to the market product. This was achieved by measuring the inhibition zone. The antifungal activity results, with the CNZ nanoemulgel showing the highest activity (40.8± 2.1 mm), are presented in Table 4

Section: Research Paper
ISSN 2063-5346

Table 4: Antifungal activity of clotrimazole nanoemulgel, compared to marketed product and almond oil

Marketed product	CNZ nanoemulgel (mm)mean ± SD	Almond oil (mm)mean ± SD
25.4 ± 2.7	40.9 ± 2.3	18 ± 1.9

DISCUSSION

In this study, we investigated a novel nanoemulgel formulation for the topical delivery of CNZ nitrate, with the aim to improve its solubility, therapeutic activity, thermodynamic stability, and penetration, and consequently improve patient compliance. To accomplish this aim, the self-nanoemulsifying technique was used to prepare a CNZ nanoemulgel, which was later integrated into a Carbopol hydrogel. Tests of the drug release profile and antifungal activity were performed for the novel CNZ nanoemulgel in comparison to Marketed, the market product.

Clotrimazole is a lipophilic imidazole antifungal drug (1). The skin penetration of CNZ is limited, presenting a challenge for the topical application of CNZ for the treatment of cutaneous fungal diseases. To provide efficient treatment, the concentration of the drug that is delivered to the site of infection must be sufficient (11). One of the modern solutions for improving the therapeutic profile and systemic delivery of hydrophobic drugs is the use of a nanoemulgel drug delivery system. This delivery method has been shown to substantially improve the pharmacodynamic and pharmacokinetic profiles of lipophilic drugs, in addition to their skin permeability. Nanoemulgels are a drug-containing nanoemulsion in a gel base (12).

A self-emulsification technique was used to formulate a novel nanoemulsion with suitable physiochemical properties. The ingredients of the system, whether inactive or active, needed to be carefully selected to ensure the optimum combination of oil, surfactant, and co-surfactant. Firstly, the solubility of CNZ in various oils, surfactants, and co-surfactants was evaluated to determine the optimum components of the self-emulsification system to achieve the desired CNZ solubility.

To achieve this goal, we tested this technique with different oils, surfactants, and co-surfactants (13-14). To enhance and improve the penetration and absorption of CNZ, as indicated by an increase in the amount of drug transported, we needed to identify the oil with the best solubilization of the lipophilic drug CNZ. The selection of the oil phase is the most important parameter when attempting to achieve a stabilized nanoemulsion with the maximum amount of solubilized drug. In general, the oil (Almond oil) with the best solubilization potential (53.125 mg/ml) for the selected drug candidate is selected as the oily phase for the nanoemulsion formulation. This helps to achieve the highest drug load in the nanoemulsion.

Almond oil was found to show considerable antifungal activity (15). Accordingly, these findings support the high antifungal activity of the novel formula, as illustrated by the marked inhibition zone for this oil in the antifungal assay.

The hydrophilic–lipophilic balance (HLB) value is another significant criterion for the selection of a surfactant. Hydrophilic surfactants are considered to give priority to the interface and reduce the energy required to form the nanoemulsion, thereby improving its stability. Nonionic surfactants are usually chosen because they have been found to be least affected by changes in

ionic strength and pH, and they are also known to be safe and biocompatible. Based on toxicological concerns, ionic surfactants were excluded (11).

The hydrophilic Tween 80 surfactant was chosen as the nonionic surfactant for this formulation, as it had an elevated emulsifying activity with a HLB value of 15 (16). This helps to lower the surface tension at the water–oil interface and makes the droplet size, causing reduced dispersion of the self-nanoemulsifying drug delivery system (SNEDDS) (17). Span 80 was chosen as a co-surfactant as the HLB value was 4.3, indicating enhanced drug absorption and dispensability (18-20).

The self-emulsifying technique was used to prepare the nanoemulsion. In order to find the optimum nanoemulsion components, pseudoternary phase diagrams were constructed for almond oil and olive oil with different surfactants and co-surfactants. Two ternary phase diagrams were constructed: ternary phase diagram A was composed of almond oil, Tween 80, and Span 80; whereas ternary phase diagram B consisted of olive oil, Tween 20, and Span 80. Plotting these diagrams allowed us to determine which formulation obtained the desired droplet size (smaller than 200 nm), representing the optimum formulation.

The measurement of the zeta potential is important because it is related to the physical stability and surface charge of the nanoformulation. Ordinarily, the opportunity for aggregation is reduced as soon as the zeta potential increases above 30 mV, either positively or negatively, due to electrostatic repulsion within the particles. The nanoemulgel formulated in the current study presented an adequate negative value (below −35 mV), indicating a stable nanoemulgel (21).

The rheological behavior is an important criterion for topical products, as it is related to the release of the drug from the formulated nanoemulgel. The spreadability, flowability, and rheology are all important to ensure consumer acceptance of the product. The flow behavior of the formulation in the current study presented a nonlinear relationship between the shear rate and viscosity; hence, the behavior of the nanoemulgel is considered pseudoplastic. This result was expected on the basis of the positive correlation observed between Carbopol concentration and Jadhao and his research team on the viscosity. Similar findings were previously obtained by formulation of Clotrimazole hydrogel (22).

Carbopol, as a rheological modifier, achieves excellent results in enhancing the physical appearance and stability of nanoformulations. To assess the speed of drug release from the novel formulation, a drug release test was performed. The results of this test showed a remarkably higher release of CNZ compared to the commercial product. The reduced droplet size of the formulation enhanced the drug release rate, as evidenced by increased permeation of the active ingredient through the membrane, indicating higher bioavailability compared to the commercial product. Moreover, the amount of drug released from the nanoemulgel decreased as the concentration of Carbopol was increased. Accordingly, the best formulation in terms of drug release was the formulation containing 0.4 % Carbopol. Similar results were presented (23), who developed sodium fusidate and fusidic acid nanoemulgels. For both of these nanoemulgels, the highest release and pseudoplastic behavior were observed for formulations containing 0.4 % Carbopol.

Franz diffusion vertical cells (FDVC) provide a reproducible and reliable means of in vitro drug release (IVDRT) testing for different dosage forms (24). The cumulative percentage of CNZ released from the nanoemulgel was 29.67 %, which was significantly higher than the amount

released from the conventional Marketed cream (23.79 %, $p < 0.05$). The improved permeation of MNZ in the nanoemulgel can be related to the decreased particle size, as smaller particles can easily penetrate the skin and overcome the barrier by squeezing between the intracellular lipids of the stratum corneum. Comparable results were previously presented, who prepared transfersomes of CNZ to overcome the skin barrier function (8).

The antifungal activity against Candida albicans of Marketed, the formulated nanoemulgel, and almond oil was investigated and compared. The zone of inhibition of the formulated CNZ nanoemulgel was significantly higher (40.9 ± 2.3 mm) than that of the commercial product (25.4 ± 2.7 mm) and almond oil (18 ± 1.9 mm). This improvement could be attributed to the decreased size of the particles (nanoscale), which increases the surface area and consequently increases the penetration of the drug through the C. albicans cell membrane, where it inhibits ergosterol synthesis. The same findings were reported in a study [1], in which CNZ-loaded solid lipid nanoparticles were developed and evaluated (6). These authors confirmed that the smaller the particle size, the better the antifungal activity. Similar results were presented by Shinde (2013) in a study that investigated the nanoemulsion formulation potential for vaginal CNZ delivery (Shinde 2013). The antifungal activity of almond oil was supported by the findings of Kumar et al. (2012), as we got an 18 mm zone of inhibition when testing the pure almond oil. This also supports the improvement in the zone of inhibition observed for the novelly formulated nanoemulgel (15).

The space between the cells of the skin is 70 nm. Conventional semisolid products, such as creams, penetrate the skin slower than nanoemulgels, which rapidly penetrate the skin and can deliver the active substances quicker and deeper (18-20). Moreover, the nanoemulgel delivery system is associated with improved solubility of lipohilic drugs, such as CNZ, which improves drug loading and increases the bioavailability of the drug. The nanoemulgel drug delivery system has a longer residence time and time of contact with cells (25). The use of methylene blue carbon nanotubes enhanced the antimicrobial activity against gram negative and positive bacteria as a result of the accumulation of the photosensitizer in the cell membrane, which causes critical damage to the bacteria (26-29). Another research study was conducted (30-34), which highlighted that the loading capacity and entrapment efficiency of carbon nanotubes are two important parameters in antimicrobial photodynamic inactivation (35-39).

These findings support the hypothesis that the antifungal activity of the CNZ nanoemugel formulation was improved when compared to the conventional CNZ cream, as seen in the inhibition zone test, as well as an improvement in all the outcomes reported in the study

Conclusion

Based on the outcomes of the present study, we can conclude that the stable CNZ nanoemulgel was superior to the marketed cream in the studies performed. The nanoemulgel was prepared by incorporating a nanoemulsion and hydrogel base, with the inclusion of Carbopol as a thickening agent. Using the self-nanoemulsifying technique, we obtained the optimum CNZ formulation for the nanoemulsion, composed of almond oil, Tween 80, and Span 80. This formulation presented the dissolving power and improved nanoemulsion properties, including nanoscale droplet size, elevated negative zeta potential a narrow PDI, and improved permeation through the skin of mice, indicating better cumulative drug release. Moreover, we observed excellent antifungal activity against Candida albicans when compared to the marketed marketed cream. In conclusion, the preparation of CNZ as a nanoemulgel has the potential to overcome the challenge

posed by the poor solubility of CNZ. Hence, this formulation will be able to overcome the skin barrier and increase the antifungal activity, leading to a shorter healing time and the maximum activity of the drug with the minimum frequency and dose, which will improve patient compliance.

The nanoemulgel drug delivery system has many advantages over conventional cream. The preparation of CNZ nitrate in such a dosage form enhanced CNZ solubility, skin penetration, drug loading, and bioavailability. It also facilitated CNZ application, associated with improved patient compliance, together with an increase in the efficacy of the drug and a decrease in side effects. This dosage form will play a major role in formulating more effective novel dosage forms for pharmaceutical companies.

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Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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