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Study of Fluconazole Release from O/W Cream and Water Soluble Ointment Bases

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Authors' contributions

This work was carried out in collaboration between all authors. Author AIAAM managed the literature searches, carried out the experimental work, performed the statistical analysis, and wrote the first draft of the manuscript. Authors MF and SES designed the study, wrote the protocol and managed the analyses of the study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Study the release of fluconazole from different O/W creams and PEG ointments.
Study Design: In this study, different formulations were prepared with changing one of the added excipients and study the effect of this change on the drug release and then the selected formulations were subjected to antifungal activity study.
Place and Duration of Study: Faculty of Pharmacy, Department of Pharmaceutics, Assiut University, Assiut, Egypt, between December 2011 and March 2012.
Methodology: O/W creams were prepared with changing either fatty alcohol type or the concentration of the added emulsifying agent. Also, the PEG ointments were prepared with changing the type of the liquid PEG (low molecular weight). Then, the viscosity and the fluconazole release from the prepared formulations were studied.
Results: Changing the fatty alcohol type from stearyl to cetostearyl and cetyl alcohol in the O/W creams caused an increase in the viscosity and a decrease in the drug release. Also, changing the liquid PEG from PEG 400 to PEG 600 resulted in an increase in the formulation viscosity and subsequent decrease in the drug release. Both F1 and F6 showed a good inhibition to the fungal growth against *Candida albicans* and *Trichophyton mentagrophyte* using cup plate method, also PEG base showed a slight fungal growth inhibition.
Conclusion: Results obtained showed that the PEG ointment formulations exhibited

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higher fluconazole release after three hours over the O/W cream formulations. Also, the nature of the PEG base may be adjunctive to the efficacy of the antifungal agent.

Keywords: Fluconazole; O/W creams; Fatty alcohol; Polyethylene glycols; In vitro release; Kinetics; Antifungal activity.

1. INTRODUCTION

Topical products for the treatment of dermatological diseases include a wide choice of vehicles ranging from solids to semisolids and liquid preparations including creams, gels, ointments, pastes, aerosols and solutions.

Cream and ointment are topical formulations that offer better patient compliance and hence become more acceptable to patients [1]. Cream is an emulsion semisolid dosage form that contains > 20% water and volatiles and/or < 50% hydrocarbons, waxes or polyethylene glycols as the vehicle for external application to the skin [2]. There are two types of creams; an oil-in-water cream with the water as the continuous phase and a water-in-oil cream with oil as the continuous phase. Creams are opaque, viscous and non-greasy to mildly greasy, tend to mostly evaporate or be absorbed when rubbed onto the skin. Generally, cream is preferred by many investigators in azole group with different formulations [3-5].

Petrolatum jelly (White soft paraffin) is used as a base material in formulating ointment and creams. It is a mixture of solid and liquid hydrocarbons and is solid-like at room temperature [6]. Some solid aliphatic fatty alcohols like stearyl alcohol, cetyl alcohol and cetostearyl alcohol are reported to be used in oil-in-water emulsions to form a viscoelastic continuous phase in combination with the aqueous emulsifier solution that impart semisolid properties to the emulsion and prevent droplet coalescence and hence increase its stability. Stearyl alcohol is 1-octadecanol (C18), cetyl alcohol is 1-hexadecanol (C16) and cetostearyl alcohol consists mainly of a mixture of them in which stearyl alcohol consists about 50-70% and cetyl alcohol consists about 20-35% [7]. Variations in these base materials lead to variability in the cream formulations in an attempt to achieve the optimum high quality topical dosage form. Different investigations on cream formulations containing different drugs are carried out in this concern [3,5,8,9].

Different formulations of azole antifungal ointment are postulated by different authors [3-5]. It is a semisolid dosage form that contains < 20% water and volatiles and > 50% hydrocarbons, waxes or polyethylene glycols as the vehicle for external application to the skin. They are opaque or translucent, viscous, greasy; they don't tend to evaporate or be absorbed when rubbed onto the skin. Hydrocarbon bases (oleaginous ointment bases), absorption bases and water soluble bases (greaseless ointment bases) are different types of ointment base. This variability of base materials facilitates the production of optimum formulation. In water soluble bases, polyethylene glycol ointment is the only pharmacopeial preparation. Polyethylene glycols (PEGs) which are known also as macrogols are widely used in topical pharmaceutical formulations since these chemicals are stable, hydrophilic substances that are essentially nonirritant to the skin and easily removed from the skin by washing. In research, authors are trying to obtain optimum release of topically applied drug to increase the bioavailability and obtain a better therapeutic effect with maintaining aesthetically acceptable formulations for patient and be easily used and adhere to the treated area in the required time with good physical and chemical stability.

2. MATERIALS AND METHODS

2.1 Materials

Fluconazole (FLZ.) was kindly provided by CIDCO, Cairo, Egypt. The Spectra/Por® dialysis membrane 12000 to 14000 molecular weight cut off (Spectrum Laboratories Inc., USA). propylene glycol (PG), white soft paraffin, stearyl alcohol, Tween 80, polyethylene glycol 4000 (PEG 4000), polyethylene glycol 600 (PEG 600) (Adwic, EL-Nasr Pharmaceutical Chemicals Co., Egypt). Polyethylene glycol 400 (PEG 400) (LOBA CHEMIE PVT. LTD. Mumbai, India). Liquid paraffin, cetyl alcohol and cetostearyl alcohol (ISO-CHEM, Egypt). Organisms: *Candida albicans* No. 11 & 17 and *Trichophyton mentagrophyte* No. 5500 & 5508 (supplied from Mycological center, Faculty of Science, Assiut University, Egypt).

2.2 Preparation of Fluconazole Gel Formulations

The composition of the prepared ointment and cream formulation bases containing 1% w/w fluconazole is shown in Table 1.

Table 1. Composition of the prepared ointment and cream formulations containing 1% fluconazole

Composition (%)	O/W emulsified bases (O/W creams)					PEG ointment bases	
	F1	F2	F3	F4	F5	F6	F7
White soft paraffin	10	10	10	10	10		
Liquid paraffin	10	10	10	10	10		
Propylene glycol	20	20	20	20	20	20	20
Stearyl alcohol	**20**			20	20		
Cetostearyl alcohol		**20**					
Cetyl alcohol			**20**				
Tween 80	2	2	**2**	**4**	**6**		
Water	38	38	38	36	34		
Polyethylene glycol 4000						20	20
Polyethylene glycol 400						60	
Polyethylene glycol 600							60

2.2.1 Preparation of O/W emulsion ointments (O/W creams)

White soft paraffin and the fatty alcohol used (stearyl alcohol, cetostearyl alcohol or cetyl alcohol) were melted in a porcelain dish over a boiling water bath. Liquid paraffin was heated to approximately the same temperature and added to the melted base. Fluconazole (1% w/w) dissolved in 20% propylene glycol and the specified concentration of tween 80 were added to the calculated amount of water. Both the aqueous and the oily phases were heated to 70ºC. The oily phase then was added gradually to the aqueous phase with continuous stirring until the O/W cream was formed.

2.2.2 Preparation of water soluble ointments

The specified concentration of polyethylene glycol (PEG) 4000 was melted in a porcelain dish over a boiling water bath. PEG 400 or PEG 600 was heated to approximately the same

temperature and added to the melted PEG 4000. The mixture was then removed from heat and stirred. Then, fluconazole (1% w/w) dissolved in 20% propylene glycol (which is slightly heated) was added to the PEGs mixture and stirred until congealing.

2.3 Evaluation of the Prepared Fluconazole Gel Formulations

2.3.1 Viscosity

The viscosity of the prepared gel formulations was determined using BrookField DV-III ULTRA programmable rheometer model RV, helipath spindle set (Brookfield Engineering laboratories, USA) using T-bar spindle. The viscosity was measured in centipoises (cps) at 10 rpm for 1 minute and temperature 25ºC using 20 gram sample. This experiment was performed for both the plain and the medicated formulations.

2.3.2 *In vitro* release studies

The *in vitro* release of fluconazole from the prepared formulations was studied using dialysis method. A one gram sample of each formulation was accurately weighed and placed on a semi permeable cellophane membrane (previously immersed in phosphate buffer pH 7.4 for 24 hours) to occupy a circle of 2.5 cm diameter. The loaded membrane (donor compartment) was firmly stretched over the lower open end of a glass tube of 2.5 cm internal diameter and made watertight by rubber band. The tube was then immersed in a beaker containing 25 ml of phosphate buffer pH 7.4 which is the release medium (receptor compartment). The system was maintained for 3 hours at 37 ± 0.5ºC in a thermostatic shaking water bath at 50 rpm. Samples of 5 ml were withdrawn at intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, and 3 hours. The volume of each sample was replaced by the same volume of fresh buffer (kept at the same temperature) to maintain constant volume. Samples were analyzed for fluconazole content spectrophotometrically at λ_{max} 261 nm against blank similarly treated.

2.3.3 Analysis of the release data

The release mechanisms of fluconazole from the semisolid formulations were elucidated by fitting the release data to four kinetic models. Regression analysis was adopted to compute the constants and correlation of data (r^2).

Zero order kinetics

$$Q = k_o t \ [10] \quad (1)$$

Where Q is the % of drug released at time t, k_o is the zero order release constant and t is the time in hours.

First order kinetics

$$\ln (100-Q) = \ln 100 - k_1 t \ [10] \quad (2)$$

Where K_1 is the first order release constant.

Higuchi kinetics

$$Q = k_H t^{1/2} \ [11] \quad (3)$$

Where Q is the amount of drug released at time t per unit area & K_H is the Higuchi release rate constant.

$$K_H = 2C_o (D/\pi)^{1/2} \quad (4)$$

Where C_o is the initial drug concentration & D is the diffusion coefficient.

Korsmeyer peppas equation

$$M_t/M_\infty = kt^n \text{ [12]} \quad (5)$$

Where M_t/M_∞ is the fraction of released drug at time t & n is the release exponent.

n value is indicative for the drug release mechanism, If $n \leq 0.5$ it is a fickian diffusion mechanism, $0.5 < n < 1$ it is a non-fickian mechanism (anomalous diffusion) and if $n = 1$, so release mechanism from the formulation follows a zero order mechanism (case-2 relaxation). In case of $n > 1$, it indicates a super case-2 transport. Anomalous diffusion or non-fickian diffusion refers to combination of both diffusion and erosion controlled release rate while case-2 relaxation and super case-2 transport refer to erosion of the polymeric chain.

2.3.4 Statistical analysis

All studies were performed in triplicate and the values were expressed as mean ± S.D. The data were analyzed by one way ANOVA and Post Hoc Turkey-Test at a significance level of .05, homogeneity of variance was evident by Levene's test in most cases and assumed in few others since no transformations were valid. Student T-test was also considered in some cases at a significance level of .05. SPSS statistical package [13] was used in these analyses.

2.3.5 *In vitro* antifungal activity

Agar cup-plate method was adopted for this study. The *in vitro* antifungal activity of the selected fluconazole formulations; O/W cream (F1) and PEG ointment (F6) against two isolates of *Candida albicans* (as a representative Yeast fungus) and two isolates of *Trichophyton Mentagrophyte* (as a representative Dermatophyte fungus) was studied. A single isolate of each fungus was picked from the agar slab culture to prepare spores suspensions in sterile water and was adjusted to be 1×10^6 spores/ml. One ml of the spores' suspension was mixed with Sabouraud agar (15-20 ml) in sterile Petri dish (9 cm in diameter) and the agar plates were allowed to solidify. After solidification, a single well was made in each agar plate using a porer of size 1 cm and filled with an accurately weighed 0.5 gm of each formula (either medicated or plain).The plates were incubated at 25± 1ºC for 3 days (for Candida isolates) and 8 days (for Trichophyton isolates) and then they were examined for the inhibition zone diameter which is an indicator for the antifungal activity. Plain formulations (without drug) were also tested as a positive growth control result. The mean value of the inhibition zone diameter from three plates was calculated.

3. RESULTS AND DISCUSSION

3.1 Evaluation of the Prepared Fluconazole Gel Formulations

3.1.1 Viscosity

Viscosity of the plain and the medicated formulations didn't differ. The viscosity is illustrated in Figure 1. Viscosity differed according to the change in type of fatty alcohol and concentration of added Tween 80 (for O/W creams) and the molecular weight of the liquid polyethylene glycol (for PEG ointments).

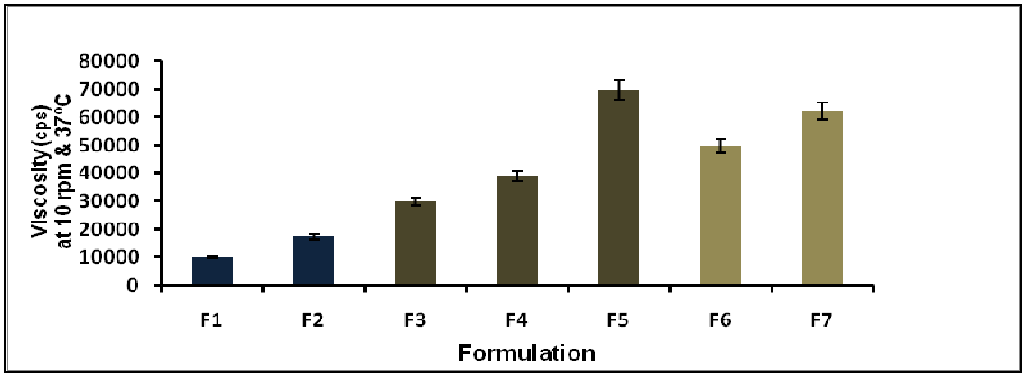

Figure 1. Viscosity of different ointment and cream formulations at 37ºC and 10 rpm

As shown, formulations F3 (that contained cetyl alcohol) exhibited higher viscosity over F2 (that contained cetostearyl alcohol) and F1 (that contained stearyl alcohol). The effect of increasing the added Tween 80 percent from 2% to 6% w/w on the viscosity of the prepared O/W cream containing 20% w/w stearyl alcohol (F1) was studied. It is obviously clear that increasing the Tween 80 concentration resulted in a large increase in the viscosity of the formulations. Therefore, the viscosity of F5 containing 6% w/w T_{80} was much higher than F4 containing 4% w/w T_{80} and F1 containing 2% w/w T_{80}. Similar results were obtained by Patel et al. [14] who found that increasing the concentration of the emulsifying agent in the psoralen cream formulation led to increased viscosity of the formulation. In case of PEG ointment formulations, the viscosity was increased with increasing the molecular weight of the liquid PEG used. So, F7 containing PEG 600 exhibited higher viscosity over F6 containing PEG 400.

3.1.2 *In vitro* release studies

The percent of fluconazole that was released over a period of three hours from the prepared ointment and cream formulations containing 1% w/w fluconazole is shown in Figures 2 - 4. Figures 2 & 3 showed the release data of FLZ from the prepared O/W cream formulations where the type of fatty alcohol and the percentage of the emulsifying agent (Tween 80) were varied.

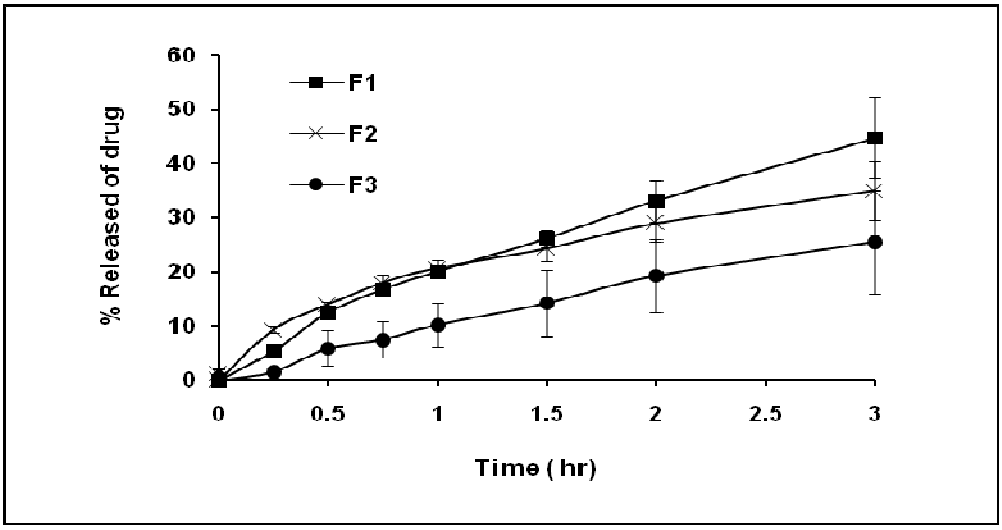

Figure 2. Effect of different fatty alcohols on fluconazole release from O/W creams

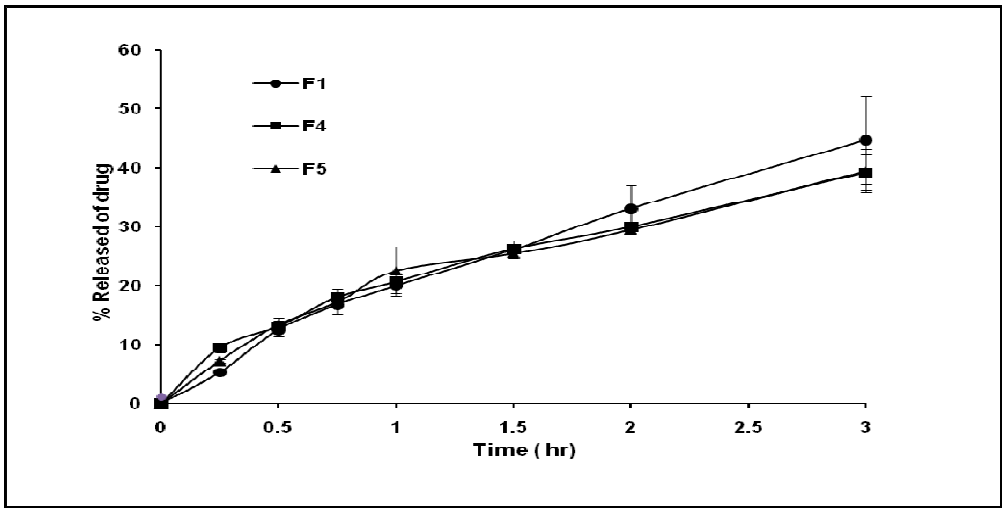

Figure 3. Effect of different Tween 80 concentrations on fluconazole release from O/W creams

As noticed from Figure 2, F1 (that contained stearyl alcohol) exhibited significantly ($p<0.05$) higher release of FLZ over F2 (that contained cetostearyl alcohol) and F3 (that contained cetyl alcohol). Halpern and Zope [15] studied the hydrophilic properties of the ointment base constituents. They reported that stearyl alcohol caused the greatest potentiating effect on water number of petrolatum over cetyl alcohol and other studied fatty alcohols. Accordingly, the presence of stearyl alcohol increased the hydrophilic properties of these formulations over those containing cetostearyl alcohol and cetyl alcohol. This increased the affinity of the base to absorb water from the release medium and subsequently increased the drug diffusion and release. Cetostearyl alcohol exhibited higher release over cetyl alcohol as stearyl alcohol represents about 70 % w/w of its constituents. These results were also attributed to the higher

viscosity of formulations F3 containing cetyl alcohol over formulations F2 and F3 containing cetostearyl and stearyl alcohols, respectively. Fig. 3 shows the effect of increasing the concentration of Tween 80 from 2% to 6% w/w on the FLZ release from O/W cream containing 20% w/w stearyl alcohol and 10% w/w liquid paraffin. It was found that the release of the drug from F5 containing 2% w/w Tween 80 was insignificantly ($p>0.05$) higher than F10 & F11 containing 4 % and 6% w/w Tween 80, respectively. This might be attributed to the higher viscosity of the formulations upon increasing the Tween 80 concentration.

Release profile of fluconazole from water soluble ointment bases is illustrated in Figure 4. It showed that FLZ release from PEG ointments was higher than that from the O/W creams (O/W emulsified ointments). This finding was due to the high solubility of the drug in PEG base. De Muynck and Remon [3] also reported that polyethylene glycol ointment has shown the highest release rate of metronidazole compared to O/W emulsion. The formulation F6 (containing low molecular weight; PEG 400) exhibited a higher drug release over F7 (containing higher molecular weight; PEG 600). These results could be explained by the reduced viscosity of the formulation upon using lower molecular weight PEGs.

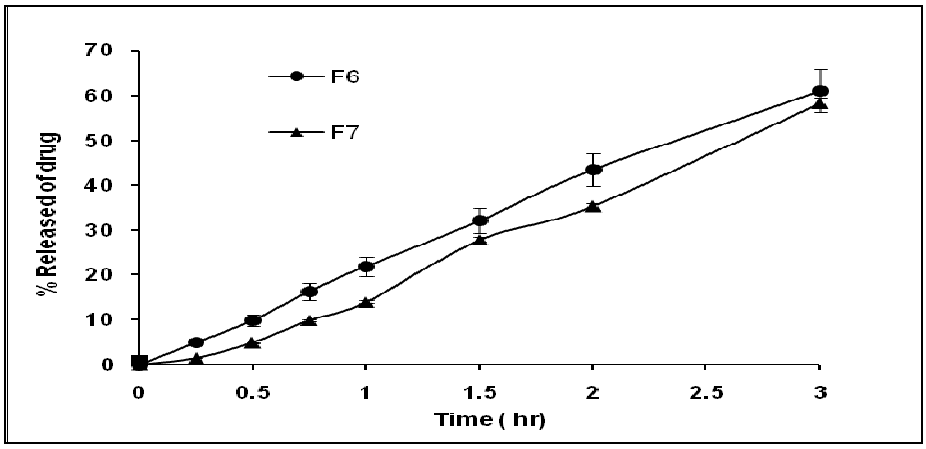

Figure 4. Effect of liquid PEG molecular weight on fluconazole release from PEG ointment formulations

In conclusion, the diffusion of any drug through the different bases depends on the nature and the composition of the bases. So, the release rate can be altered by changing the nature and the composition of the bases.

3.1.3 Analysis of the release data

The kinetic analysis of the in vitro release data of FLZ from all the prepared formulations is presented in Table 2. The preference between the release mechanisms was dependent on the coefficient of determination (R^2; squared correlation coefficient) and the release exponent (n) of korsmeyer-peppas equation. As shown in the table, R^2 and n values ($0.5 < n < 1$) indicated that the release of FLZ from O/W emulsified formulations followed first order kinetics and was based on non-fickian diffusion. While the drug release from water soluble ointment bases followed zero order kinetics with n values = 1 indicating a case-2 relaxational release for F6 and $n > 1$ indicating super case -2 transport for F7. In both cases, this referred to erosion of the polymeric chain.

Table 2. Kinetic analysis of the release data of fluconazole from prepared formulations

Polymer	Zero Order		First Order			Higuchi Diffusion model		Best fitted model
						Q/A vs. $T^{1/2}$		
	R^2	K_o (% h^{-1})	R^2	K_1 (h^{-1})	$T_{0.5}$ (h)	R^2	D(cm^2/hr)	
F1	0.973	14.454	**0.994**	0.192	3.604	0.836	1.33E-03	first order
F2	0.882	10.546	**0.927**	0.133	5.229	0.857	1.33E-03	first order
F3	0.981	8.747	**0.990**	0.101	6.887	0.803	4.75E-04	first order
F4	0.927	11.964	**0.965**	0.154	4.488	0.854	1.50E-03	first order
F5	0.921	12.188	**0.958**	0.157	4.418	0.851	1.48E-03	first order
F6	**0.997**	20.743	0.989	0.315	2.198	0.796	2.07E-03	zero order
F7	**0.987**	20.116	0.955	0.292	2.377	0.725	1.70E-03	zero order

R^2: Coefficient of determination, K_o: Zero order release constant, K_1: First order release constant, $T_{0.5}$: Half-life of first-order reaction, D: Diffusion coefficient.

3.1.4 *In vitro* antifungal activity

The antifungal activity of the selected medicated formulations; F1 and F6 are described in Table 3.

Table 3. *In vitro* antifungal activity of the selected medicated and plain formulations using agar cup-plate method

Type of formula	Type of fungi and isolate number			
	*Candida albicans**		*Trichophyton mentagrophyte***	
	No:11	No:17	No:5500	No:5508
	Average diameter of growth inhibition zone (mm) ± SD			
Medicated cream (F1)	45.0 ± 5.00	46.0 ± 1.73	50.0 ± 5.00	43.3 ± 2.89
Medicated ointment (F6)	48.3 ± 2.89	47.3 ± 2.08	51.7 ± 2.89	50.0 ± 0.00
Plain cream	0.00	0.00	0.00	0.00
Plain ointment	Not well marked	Not well marked	Not well marked	Not well marked

**Candida albicans; No: 11 was isolated from patient with Tinea capitis and No: 17 was isolated from patient with Onychomycosis.*

***Trichophyton mentagrophyte; No: 5500 was isolated from patient with Tinea pedis and No: 5508 was isolated from patient with Tinea capitis.*

As illustrated in Table 3, the tested formulations exhibited a good growth inhibition zone for all the tested fungal isolates. It was found that the plain formulation of F1 have showed a normal fungal growth in the agar plates. So, excipients used in the preparation of the O/W cream had no growth inhibitory effect on the tested fungi. In contrast, the plain polyethylene glycol (F6) ointment showed some growth inhibition to the tested fungi. This might be due to the PEG effect on the water activity in the culture medium. Similar results were obtained by Inch and Trinci [16] who found that PEG 200 is inhibitory to *Paecilornyces farinosus* because of its effect on water activity. They mentioned also that there was a linear relationship

between the decrease in the water activity of the medium and the decrease in the growth yield. It was also observed that the inhibitory effect was more pronounced in the Trichophyton isolates than the Candida isolates. Klipp [17] mentioned that the pathogen *Candida albicans* can adapt to different environmental conditions such as osmotic changes. This ability plays an important role in the fungus virulence. The osmoadaptative response is not identical in different fungi and the fungus ability to survive depends on its capability to alter the morphogenic programs [18]. Gleason et al. [19] predicted different fungal growth response according to the different water potential. With increasing the water potential, the fungus may not be affected until the response mechanisms are overwhelmed and growth ceases or the growth of the fungus may slow or the fungus may be adapted to this high water potential and the growth will increase until the response mechanisms are overwhelmed.

4. CONCLUSION

The obtained results showed that the PEG ointment formulations exhibited better fluconazole release over the O/W cream formulations. For PEG ointments, the nature of the base itself may be adjunctive to the efficacy of the used antifungal agent. So, PEG ointments could be a promising topical antifungal drug.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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