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2 Study of Fluconazole Release From O/W Cream and 3 Water Soluble Ointment Bases

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9 **ABSTRACT**

10 **Aims:** Study the release of fluconazole from different O/W creams and PEG ointments.

11 **Study design:** In this study, different formulations were prepared with changing one of the added excipients
12 and study the effect of this change on the drug release and then the selected formulations were subjected to
13 antifungal activity study.

14 **Place and Duration of Study:** Faculty of Pharmacy, Department of Pharmaceutics, Assiut University,
15 between December 2011 and March 2012.

16 **Methodology:** O/W creams were prepared with changing either fatty alcohol type or the concentration of the
17 added emulsifying agent. Also, the PEG ointments were prepared with changing the type of the liquid PEG
18 (low molecular weight). Then, the viscosity and the fluconazole release from the prepared formulations were
19 studied.

20 **Results:** changing the fatty alcohol type from stearyl to cetostearyl and cetyl alcohol in the O/W creams
21 caused an increase in the viscosity and a decrease in the drug release. Also, changing the liquid PEG from
22 PEG 400 to PEG 600 resulted in an increase in the formulation viscosity and subsequent decrease in the
23 drug release. Both F1 and F6 showed a good inhibition to the fungal growth against *Candida albicans* and
24 *Trichophyton mentagrophyte* using cup plate method, also PEG base showed a slight fungal growth
25 inhibition.

26 **Conclusion:** Results obtained showed that the PEG ointment formulations showed higher fluconazole
27 release after three hours over the O/W cream formulations. Also, the nature of the PEG base may be
28 adjunctive to the efficacy of the antifungal agent.

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31 **Keywords:** Fluconazole; O/W creams; Fatty alcohol; Poly Ethylene Glycols; in vitro release; kinetics;
32 antifungal activity.

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38 **1. INTRODUCTION**

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

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

48 Petrolatum jelly (White soft paraffin) is used as a base material in formulating ointment and creams. It is a
49 mixture of solid and liquid hydrocarbons and is solid-like at room temperature (6). Some solid aliphatic fatty
50 alcohols like stearyl alcohol, cetyl alcohol and cetostearyl alcohol are reported to be used in oil-in-water
51 emulsions to form a viscoelastic continuous phase in combination with the aqueous emulsifier solution that
52 impart semisolid properties to the emulsion and prevent droplet coalescence and hence increase its stability.
53 Stearyl alcohol is 1-octadecanol (C18), cetyl alcohol is 1-hexadecanol (C16) and cetostearyl alcohol consists
54 mainly of a mixture of them in which stearyl alcohol consists about 50-70 % and cetyl alcohol consists about
55 20-35 % (7). Variations in these base materials lead to variability in cream formulation in an attempt to
56 achieve the optimum high quality topical drug. Different investigations on cream formulations on different
57 drugs are carried out in this concern (3, 5, 8, 9).

58 Different formulations of azole antifungal ointment are postulated by different authors (3-5). It is a semisolid
59 dosage form that contains < 20% water and volatiles and > 50 % hydrocarbons, waxes or polyethylene
60 glycols as the vehicle for external application to the skin. They are opaque or translucent, viscous, greasy;
61 they don't tend to evaporate or be absorbed when rubbed onto the skin. Hydrocarbon bases (oleaginous
62 ointment bases), absorption bases and water soluble bases (greaseless ointment bases) are different types
63 of ointment base. This variability of base materials facilitates the production of optimum formulation. In water
64 soluble bases, polyethylene glycol ointment is the only pharmacopeial preparation. Polyethylene glycols
65 (PEGs) which are known also as macrogols are widely used in topical pharmaceutical formulations since
66 these chemicals are stable, hydrophilic substances that are essentially nonirritant to the skin and easily
67 removed from the skin by washing. In research, authors are trying to obtain optimum release of topically

68 applied drug to increase the bioavailability and obtain a better therapeutic effect with maintaining aesthetically
 69 acceptable formulations for patient and be easily used and adhere to the treated area in the required time
 70 with good physical and chemical stability.

71 2. MATERIALS AND METHODS

72 2.1. Materials

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

81 2.2. Preparation of fluconazole gel formulations

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

84 **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

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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 (if present) 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 70°C. 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 25°C using 20g 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

110 glass tube of 2.5 cm internal diameter and made watertight by rubber band. The tube was then immersed in a
111 beaker containing 25 ml of phosphate buffer pH 7.4 which is the release medium (receptor compartment).
112 The system was maintained for 3 hours at $37 \pm 0.5^\circ\text{C}$ in a thermostatic shaking water bath at 50 rpm.
113 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
114 sample was replaced by the same volume of fresh buffer (kept at the same temperature) to maintain constant
115 volume. Samples were analyzed for fluconazole content spectrophotometrically at λ_{max} 261 nm against blank
116 similarly treated.

117 **2.3.3. Analysis of the release data.**

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

121 Zero order kinetics

$$122 \quad Q = k_0 t \quad (10) \quad (1)$$

123 Where Q is the % of drug released at time t, k_0 is the zero order release constant and t is the time in hours.

124 First order kinetics

$$125 \quad \ln(100-Q) = \ln 100 - k_1 t \quad (10) \quad (2)$$

126 Where K_1 is the first order release constant.

127 Higuchi kinetics

$$128 \quad Q = k_H t^{1/2} \quad (11) \quad (3)$$

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

$$130 \quad K_H = 2C_0 (D/\pi)^{1/2} \quad (4)$$

131 Where C_0 is the initial drug concentration & D is the diffusion coefficient.

132 Korsmeyer peppas equation

$$133 \quad M_t/M_\infty = kt^n \quad (12) \quad (5)$$

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

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

140 **2.3.4. Statistical analysis..**

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

146 **2.3.5. In vitro antifungal activity**

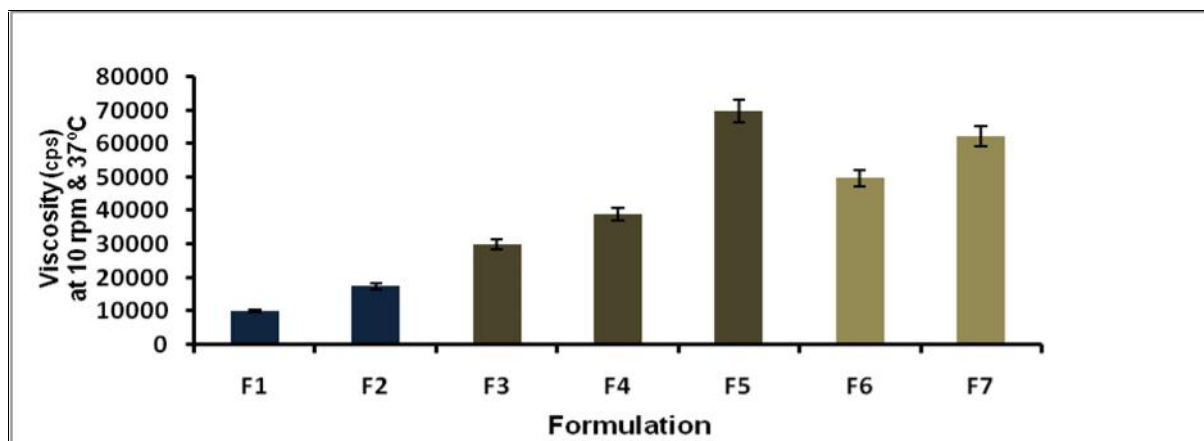
147 Agar cup-plate method was adopted for this study. The *in vitro* antifungal activity of the selected fluconazole
148 formulations; O/W cream (F1) and PEG ointment (F6) against **two isolates of *Candida albicans*** (as a
149 representative Yeast fungus) and **two isolates of *Trichophyton Mentagrophyte***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

159 **3. RESULT AND DISCUSSION**

160 **3.1. Evaluation of the prepared fluconazole gel formulations.**

161 **3.1.1. Viscosity.**

162 The viscosity of the prepared formulations is illustrated in figure 1. The viscosity differed according to the
163 change in type of fatty alcohol and concentration of added Tween 80 (for O/W creams) and the molecular
164 weight of the liquid polyethylene glycol (for PEG ointments).



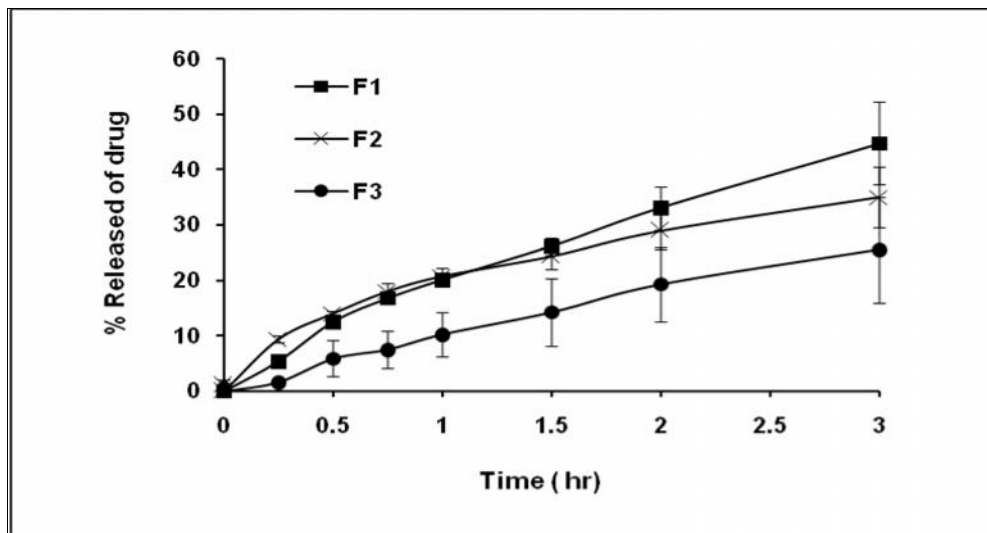
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Fig.1. Viscosity of different ointment and cream formulations at 10 rpm at 37°C

168 As shown, formulations F3 (that contained cetyl alcohol) exhibited higher viscosity over F2 (that contained
169 cetostearyl alcohol) and F1 (that contained stearyl alcohol). The effect of increasing the added Tween 80
170 percent from 2 % to 6 % w/w on the viscosity of the prepared O/W cream containing 20 % w/w stearyl alcohol
171 (F1) was studied. It is obviously clear that increasing the Tween 80 concentration resulted in a large increase
172 in the viscosity of the formulations. Therefore, the viscosity of F5 containing 6 % w/w T80 was much higher
173 than F4 containing 4 % w/w T80 and F1 containing 2 % w/w T80. Similar results were obtained by Patel et al.
174 (14) who found that increasing the concentration of the emulsifying agent in the psoralen cream formulation led
175 to increased viscosity of the formulation. In case of PEG ointment formulations, the viscosity was increased
176 with increasing the molecular weight of the liquid PEG used. So, F7 containing PEG 600 exhibited higher
177 viscosity over F6 containing PEG 400.

178 **3.1.2. In vitro release studies.**

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

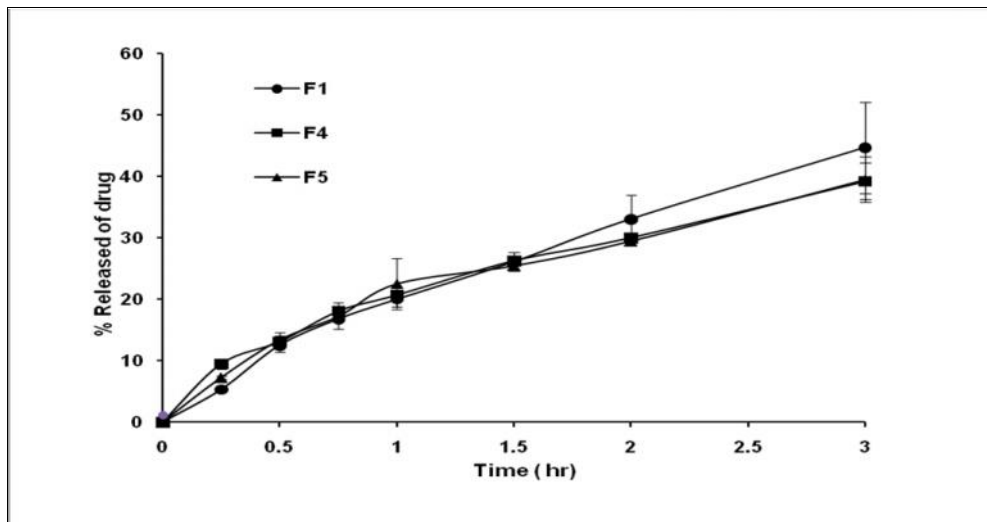


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Fig.2. Effect of different fatty alcohols on the fluconazole release from O/W creams

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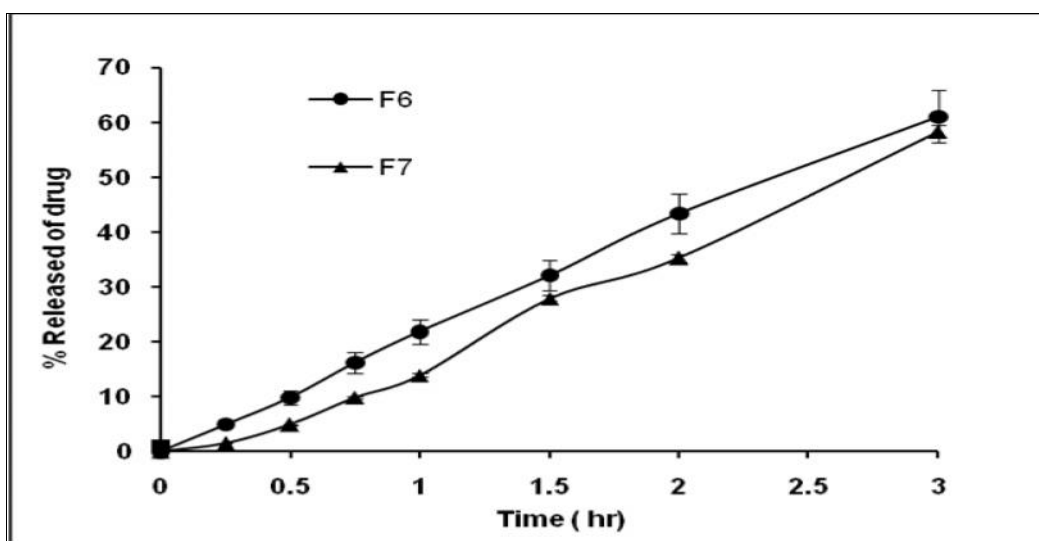
Fig.3. Effect of Tween 80 concentration on the fluconazole release from O/W creams

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

197 also attributed to the higher viscosity of formulations F3 containing cetyl alcohol over formulations F2 and F3
198 containing cetostearyl and stearyl alcohols, respectively. Figure 3 shows the effect of increasing the concentration
199 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
200 % w/w liquid paraffin. It was found that the release of the drug from F5 containing 2 % w/w Tween 80 was
201 insignificantly ($p > 0.05$) higher than F10 & F11 containing 4 % and 6 % w/w Tween 80, respectively. This might
202 be attributed to the higher viscosity of the formulations upon increasing the Tween 80 concentration.

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



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211 **Fig.4. Effect of the liquid PEG molecular weight on the fluconazole release**
212 **from PEG ointment formulations.**

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

217 **3.1.3. Analysis of the release data**

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

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

Polymer	Zero Order		First Order			Higuchi Diffusion		Best fitted model
						Q/A vs. $T^{1/2}$		
	R^2	K_0 (% 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

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227 R^2 : Coefficient of determination, K_0 : Zero order release constant, K_1 : First order release constant,

228 $T_{0.5}$: Half-life of first-order reaction, D: Diffusion coefficient.

229 **3.1.4. In vitro antifungal activity.**

230 **The antifungal activity** of the selected medicated formulations; F1 and F6 are described in table 3 and the
 231 growth inhibition zones of **two fungal isolates of them** are shown photographically in figures 4 & 5.

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235 Table.3. *In vitro* antifungal activity of the selected medicated and plain formulations using agar-
 236 diffusion method.

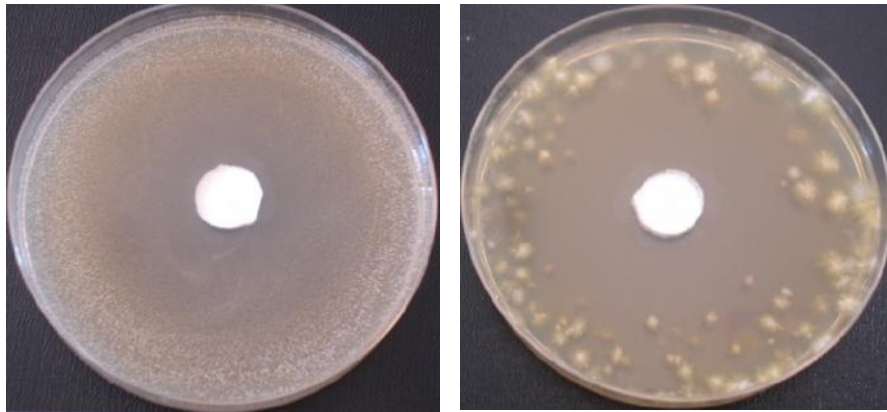
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Type of formula	Type of fungi and isolate number			
	<i>Candida albicans</i> *		<i>Trichophyton mentagrophyte</i> **	
	No:11	No:17	No:5500	No:5508
Average diameter of growth inhibition zone (mm) ± SD				
Medicated cream (F1)	45.0 ± 5.00	46.0 ± 1.73	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*.

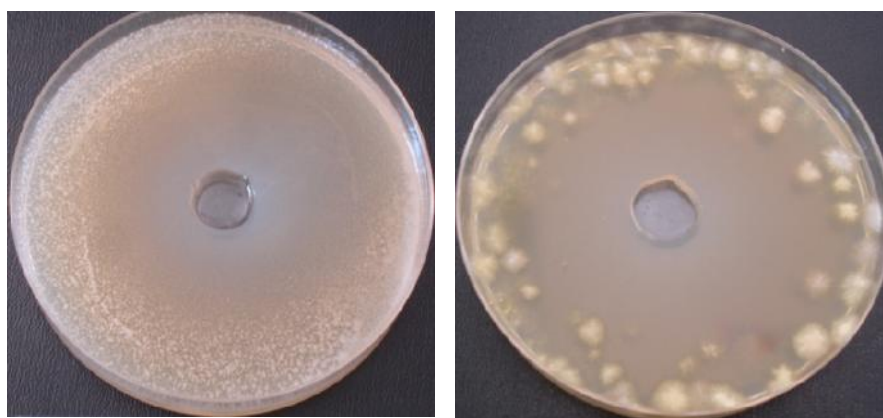
***Trichophyton mentagrophyte*; No: 5500 was isolated from patient with *Tinea pedis* and No: 5508 was isolated from patient with *Tinea capitis*.

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240 Fig.5. The growth inhibition zone of the medicated cream (F1) against (a) *Candida albicans* (No: 11) &
 241 (b) *Trichophyton mentagrophyte* (No: 5500).



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243 **Fig.6. The growth inhibition zone of the medicated PEG ointment (F6) against (a) *Candida albicans***
244 **(No: 11) & (b) *Trichophyton mentagrophyte* (No: 5500).**

245 As illustrated in table 3 and figures 4 & 5, the tested formulations exhibited a good growth inhibition zone for
246 **all** the tested fungal isolates. It was found that the plain formulation of F1 have showed a normal fungal
247 growth in the agar plates. So, excipients used in **the preparation of the O/W cream had** no growth inhibitory
248 effect on the tested fungi. In contrast, the plain polyethylene glycol (F6) ointment showed some growth
249 inhibition to the tested fungi. This might be due to the PEG effect on the water activity in the culture medium.
250 Similar results were obtained by Inch and Trinci (16) who found that PEG 200 is inhibitory to *Paecilomyces*
251 *farinosus* because of its effect on water activity. They mentioned also that there was a linear relationship
252 between the decrease in the water activity of the medium and the decrease in the growth yield. The inhibitory
253 effect was more pronounced in the *Trichophyton* isolates than the *Candida* isolates. Klipp (17) mentioned
254 that the pathogen *Candida albicans* can adapt to different environmental conditions such as osmotic
255 changes. This ability plays an important role in the fungus virulence. The osmoadaptive response is not
256 identical in different fungi and the fungus ability to survive depends on its capability to alter the morphogenic
257 programs(18). Gleason et al. (19) predicted different fungal growth response according to the different water
258 potential. With increasing the water potential, the fungus may not be affected until the response mechanisms
259 are overwhelmed and growth ceases or the growth of the fungus may slow or the fungus may be adapted to
260 this high water potential and the growth will increase until the response mechanisms are overwhelmed.

261 **4. Conclusion.**

262 Results obtained showed that the PEG ointment formulations showed better fluconazole release over the
263 O/W cream formulations. For PEG ointments, the nature of the base itself may be adjunctive to the efficacy
264 of the antifungal agent. So, PEG **ointments** could be a promising topical antifungal agent.

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