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FORMULATION AND EVALUATION OF CLOTRIMAZOLE OINTMENT

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ABSTRACT

The in vitro release of clotrimazole from different ointment bases was studied. The bases tested were oleagenous, absorption, water-soluble, an O/W emulsion and a W/O emulsion ointment base. The release profile agreed with the first order mechanism. Among the tested bases, the water soluble base provided the best release rate.

Clotrimazole exhibited antimycotic effect against C. albicans. The data had confirmed the in-vitro release results and proved the superiority of the water soluble bases.

The clinical evaluation was performed on patients complaining of T. circinata. Three ointment formulations of different release pattern viz; water soluble , O/W emulsion and an oleagenous ointment each incorporating 1% clotrimazole, were selected to conduct the clinical test. Good efficacy was obtained with 1% clotrimazole in water soluble base composed of. PEG 4000 and PEG 400 (4:6).

INTRODUCTION

Superficial fungus infection caused by dermatophytes or yeast are common dermatological problems. The humid atmosphere, warm temperature, dust, socioeconomic standards and abuse of antibiotics may contribute to the high incidence of this type of infection [1]. Relatively, a few advances were reported in the field of therapy of superficial fungus infections. In 1970, imidazole antifungal compounds were made available [2]. Of these, clotrimazole enjoys a broad range of activity against almost all dermatophytes and Candida of clinical interest [3,4]. It has less irritant effect, potent and well to be tolerated by the skin [3,5]. Clinically, clotrimazole is selected as the drug of choice for the topical treatment of Tinea capitis in Egypt, together with systemic griseofulvin therapy.

The efficacy and safety of clotrimazole was conducted by Spiekerman and Young [6] who found that 1% clotrimazole solution or cream was more effective for the treatment of Tinea, as compared with its respective vehicle. Also, 1% clotrimazole solution was more effective clinically than 1% haloprogin solution [7]. Kusunoki and Harada [8] have studied the antifungal activity of clotrimazole and other antifungal agents against clinical isolates of dermatophytes. They found that the rank order of activity against the dermatophytes was: clotrimazole econazole nitrate > miconazole nitrate > exalamide. Duhm *et al.* [9] compared the topical absorption of 1% clotrimazole from cream, solution and 100 mg vaginal tablet. Their results revealed that cream formulation was more effective than solution or tablets.

The *in vitro* drug release from various ointment bases may be difficult to correlate with *in vivo* results. But, such studies may be useful for detecting interaction between drugs and bases which influence drug penetration.

The objective of this present work is to formulate clotrimazole in an ointment dosage form. Moreover, the work aimed to evaluate the best ointment formula clinically on infected human skin. Comparative studies with two locally manufactured brands namely Canesten® and Candistan cream are also intended.

EXPERIMENTAL

Materials:

Clotrimazole, (kindly supplied by the Arab Drug Co., Cairo, Egypt), Span 65 (Roth, G.F.R.), PEG 400, 4000 (Fluka AG, Switzerland), Tween 80 (Merck, G.F.R.), Wool alchol, Soft paraffin, Hard paraffin, Cetyl alcohol, Stearyl alcohol, Glycerine, Anhydrous lanoline, and all other chemicals were of official grade samples and used as received.

Equipment:

Double-beam spectrophotometer (U.V. 150-62, Shimadzu, Japan), magnetic stirrer regulator hot plate (Gallenkamp, G.F.R.), and semipermeable Fischer cellophane 30/32 membrane (Fisher Scientific Co., London, U.K.).

Methods

Preparation of Ointments:

6 different ointment bases were used for formulation (Table 1), and were prepared by the fusion method. The drug was incorporated in the melted base and vigorously triturated until congealing.

In the emulsion bases, the drug was suspended in the aqueous phase. The aqueous phase was then warmed and incorporated in the melted oleagenous phase. Stirring was continued until congealing.

The drug was incorporated into each of the tested bases at 1, 2. and 3% w/w.

To study the effect of particle size on the *in vitro* release, clotrimazole of different size fractions viz; > 315, 315-200 and 90-63 um was incorporated into some of the tested bases at 3% concentration. For each medicated base, placebo preparation was similarly prepared.

Release of Clotrimazole from Ointments:

1 gm sample of the ointment under investigation was placed over an area of 2 cm^2 of the cellophane membrane which was previously soaked in water over night and then dried. The loaded membrane was firmly stretched over one end of a glass tube 2 cm in diameter. The tube was suspended in a 250 ml beaker containing preheated 100 ml of the release medium (25% dimethyl formamide in 0.02 N HCl) and maintained at 37±1°C. Agitation was affected by magnetic stirring at 70 rpm. The amount of clotrimazole released at time intervals was determined spectrophotometrically at 261 nm [10] against blank similarly treated.

Mycological Study:

Agar-cup diffusion method was adopted [11], and Candida albicans was used. The test was carried out as follows: 15 ml of nutrient media seeded with 24 hours-subculture C. albicans was distributed in each petri-dish (10 cm-diameter). On solidification, 8 mm holes were made and filled with an accurately weighed 0.25 gm ointment. In each plate, 2 holes for the medicated ointment base and another two for the placebo. The petri-dishes were left for two hours, then incubated at 37°C for 48 hours. The extent of release (Zone of inhibition) was measured by taking the mean of 4 readings. Each two were taken from one hole.

The prepared formulations under test as well as the marketed ones

each incorporating 1% clotrimazole were used.

Clinical Study:

Eighty four patients were selected from out-patient clinic of Dermatology Department, Assiut University Hospital. All patients were complaining from superficial Tinea circinata. Male or female patients were randomly chosen in different ages (3-50 years), but they were mainly of ages ranged from 3-15 years old. All cases have no history of previous antifungal therapy whether locally or systemically. The ointments were applied to the skin lessions twice daily for a period of 21 days. The cases were clinically followed up.

Some of the patients did not terminate the hole course of treatment. These cases were fifteen patients and were excluded from the test. Accordingly the actual cases studied were 69 patients, nearly 12 case for each medicated formula while 3 patients for each control base. The drug was incorporated into each of tested formulations at 1% concentration.

RESULTS AND DISCUSSION

In Vitro Results:

The *in vitro* release of clotrimazole from the tested ointment bases, through cellophane membrane was investigated. The release data were analysed according to zero [12], first-order [13] and diffusion controlled release mechanism [13]. The highest correlation coefficient values, obtained with linear regression analysis of the logarithm of the amount retained versus time attest to first kinetics Figure 1 and 2. Further confirmation according to the equation: log G = Log K + ½ log t [14], excludes the applicability of diffusion model, as the slope was found to be not equal to half as required by the linear equation.

From the data given in (Table and illustrated by Figure 1 and it is clear that water soluble bas (I and II) provided the best relea profile followed by o/w emulsio absorption, w/o emulsion and oleag nous ointment base. The obtain results could be explained on t basis of composition and consisten of the ointment bases used.

The higher release of clotrim zole from the water soluble bases and II) is attributed in one hand the solubility enhancing effect base components on the drug [21] a on the other hand, to the high te dency of such base to attract wat molecules inside its strucure [15-2] However, the release from the wat soluble base II (K = 1.24) was fou to be lower than that from base I = 2.04). This result may be due the increased viscosity of base provided by cetyl alcohol.

The observed decrease in dr release rate from the oleageno base (K = 0.33) may be explained the hydrophobic nature of the dr which favours solubility in the ba rather than passing into the aqueo medium [15,18]. Moreover, the compos tion and consistency of the ba which hinders the penetration of t release medium and consequently r tarding the drug release [20].

Regarding the emulsion base the release rate from the o/w emu sion ointment is nearly twice th of the w/o type (K = 0.95, 0.38 r spectively). In the case of o emulsion type, the emulsion extern phase is miscible with the relea medium. In addition, the presen of Tween 80 in the base increas wetting and facilitated better flu penetration into the base as a r sult of interfacial tension loweri effect. This effect of surfacta brings closer contact between t external diffusion medium and t drug itself [22].

Figure 1 and 2 showed the effect of drug concentration in the ointment on the release rate. It is clear that, increasing drug concentration from 1-3% w/w resulted in an increased drug release from the ointment bases.

Drug particle size also plays a role in the drug release from the base. The effect of particle size was studied from three bases viz; water soluble, an o/w emulsion and an oleagenous ointment base. It is obvious from the results illustrated in Figure 3 that, reduction of particle size was accompanied by a slight increase of drug release from the water soluble and oleagenous bases. This result could be attributed to the solubility and distribution of the drug in the base and hence, the transport of drug particles.

In the case of o/w emulsion base the effect was more pronounced. Fine fractions (90-63 mu) showed an increase in the release rate of the drug, as compared with the fraction size > 315. The possible explanation of this is that, large proportion of the drug being suspended in the aqueous phase, hence, the reduction of particle size will be accompanied by an enhanced release rate [23].

The same evaluating procedure was carried out on two commercial brands of clotrimazole. These brands are available as cream (1%) in the Egyptian market under the trade name Canesten and Candistan. Three batches from each brand were used. It is clear from (Table 3) that differences in drug release can exist between the two brands, and also between batches of the same brand. For Canesten, the inter-batch release variability was more detected than for Candistan. Moreover, the release rates of the different batches of Canesten were higher than that of Candistan. Variation in the above results could be referred to base composition used and to some variables during manufacturing processing. It is obvious also, that higher release of the drug was observed from Canesten cream as compared to other tested formulations.

In Vitro Antimycotic Study:

The effect of base composition, as well as drug concentration on the antimycotic activity of clotrimazole against C. albicans using the agar-cup diffusion method was investigated.

The primary results revealed that the factor of concentration difference seemed to have undetectable effect on the activity of the drug against C. albicans; thus, the drug was used in 1% concentration to test the effect of base composition (Table 4). From the data presented in (Table 4), the bases can be arranged according to their antimycotic activity of the drug as follows: water soluble base I > water soluble base II > w/o emulsion > o/w emulsion > absorption > oleagenous base. This sequence of arrangement appears to be in agreement with that based on the *in vitro* release through cellophane membrane except with emulsion systems. In this respect Joune and Bayomi [24] studied the release of sulphonamides from different ointment bases into agar gel diffusion medium. They found that the release was superior from the water soluble base followed in the order by the o/w emulsion and oleagenous base. On trying the release from ointment bases into the agar medium, affected by the solubility of the drug in the ointment base, its solubility in the gel base (agar) and the inter-molecular forces of attraction between the drug and diffusion medium. In addition, the diffusion of drugs through the agar media can be described in

two steps [25] partition at the boundary between the diffusion medium and the ointment, producing mobile and diffusible molecules and then diffusion process in which the molecules transported into medium by virtue of their random molecular motion, from higher concentration regions to lower concentration regions.

Consequently, the PEG base being a water soluble base in which clotrimazole in completely soluble by its components [21]. The base containing the drug absorbs water from the diffusion medium then diffuses rapidly through the pores within the hygrogel and hence inhibits the growth of candida. The reverse was true for fatty bases where the delayed diffusion obtained could be attributed to differences in partitioning the drug at the boundary between the diffusion medium and the ointment. With respect to emulsion systems, the w/o emulsion base containing Span 65 showed better antimycotic activity of clotrimazole than the o/w emulsion type that contain Tween 80. This behaviour of the emulsion systems could be explained by Iwata and Yamaguchi [26] who reported that the activity of clotrimazole against C. albicans was enhanced *in vitro* by anionic surfactants that did not contain ethylene oxide groups. Surfactants containing these groups diminished this activity irrespective of their ionic or nonionic nature. Levenson *et al.* [27], reported that Tween 80 induced aggregation and hence promote the growth of micro-organism. On the other hand, the organism hydrolysed the surfactant with the release of oleic acid necessary as carbon source for the organism [28].

On comparing the antimycotic activity of clotrimazole in the tested ointments with that in the marketed preparations, the data revealed that, the activity of clotrimazole ointments exceeded that of Canesten and Candistan (Table 4). This result not agreed with the *in vitro* release results through cellophane membrane.

Clinical Evaluation:

Three ointment formulations with different *in vitro* release pattern viz; the water soluble I (of high release rate), o/w emulsion (intermediate release rate) and an oleagenous formula (low release rate) were selected and were compared with Canesten and Candistan cream in the treatment of superficial T. circinata. The patients were examined every week for 21 days (Table 5).

It is clear from the data that, application of the water soluble formula proved its high efficiency as appeared from the disappearance of lesions during the course of treatment. The group treated by the application of oleagenous formula showed the best results after one week. This unexpected results may be attributed to the increase in skin hydration achieved by the oleagenous base, leading to accumulation of sweat [29]. The group receiving the o/w emulsion formula showed moderate response.

Regarding the marketed preparations, Canesten provided good results after one week treatment and a 100% response was obtained after two weeks; although it exhibited the minimum antimycotic effect. However, Candistan exhibited no effect during the first week but after application for 21 days the response was 91.6%.

In conclusion 1% clotrimazole, in the water soluble ointment base composed of PEG 4000 and PEG 400 (4:6), or in oleagenous base composed of white soft paraffin, can be recommended for optimal treatment of superficial T. circinata.

Table 1: Composition of Ointment Bases Used.

Base type	Composition	% W/W
Oleagenous	White soft paraffin	100
Water soluble		
I	PEG 4000	40
	PEG 400	60
II	PEG 4000	47.5
	PEG 400	47.5
	Cetyl alcohol	5
Absorption		
	Hard paraffin	24
	Wool alcohol	6
	Soft paraffin	10
	Liquid paraffin	60
Emulsion		
O/W	Stearyl alcohol	25
	White petrolatum	25
	Glycerine	12
	Tween 80	5
	Distilled water	33
W/O	White soft paraffin	64
	Span 65	6
	Distilled water	30

Table 2: Release Characteristics of Clotrimazole (1% W/W) from the different Ointment Bases.

Base type	Zero-order		First-order			Diffusion model			
						O/A vss	$t_{½}$	Loga vss	log t
	r	k	r	$Kx10^{-1}$	$t_{½}$	r	$DK\ 10^{3}$	r	k
Oleagenous	0.998	0.267	0.999	0.332	20.90	0.487	0.401	0.984	0.467
Water soluble									
I	0.983	1.041	0.990	2.04	3.40	0.986	7.69	0.975	0.569
II	0.994	0.712	0.987	1.24	5.60	0.984	3.51	0.984	0.358
Absorption	0.994	0.401	0.994	0.530	13.08	0.989	1.130	0.976	0.337
Emulsion									
O/W	0.981	0.633	0.990	0.944	7.34	0.997	2.80	0.996	0.416
W/O	0.991	0.314	0.994	0.340	18.02	0.977	0.707	0.994	0.374

r = Correlation Coefficient.

K = Rate Constant min^{-1}.

D = Diffusion Coefficient.

Table 3: *In Vitro* Release Characteristics of Clotrimazole (1% W/W) from the Commercial Preparations.

Formula	Batch	r	K $(hr^{-1})x10^{-1}$	$t_{½}$ (hr)
Candistan Cream*				
	I	0.962	0.783	8.85
	II	0.979	0.716	9.7
	III	0.975	0.730	9.49
Canesten Cream**				
	I	0.990	1.92	3.6
	II	0.997	2.06	3.36
	III	0.994	2.95	2.35

* The Arab Drug Comp., Cairo, Egypt.

** The Alexandria Pharm. Comp., Alex., Egypt.

Table 4: *In Vitro* Antimycotic Activity of 1% Clotrimazole in Different Formulations Using Agar-Cup Method and C. albicans as Test Organism.

Formula	inhibition Zone Diameter (mm)
Oleagenous	37.00
Water-Soluble	
I	54.2
II	50.7
Absorption	38.5
Emulsion	
O/W	40.8
W/O	44.3
Canesten	34.8
Candistan	37.5

Table 5: Clinical Efficiency of Clotrimazole (1%) in its Tested Formulations on T. circinata.

Tested formula	Clinical response (%) after (days) 7	14	21
Oleagenous	58.3	58.33	100
Water soluble	50	83.3	100
O/W emulsion	25	50	70
Canesten	41.7	100	
Candisten	No response	33.3	91.6

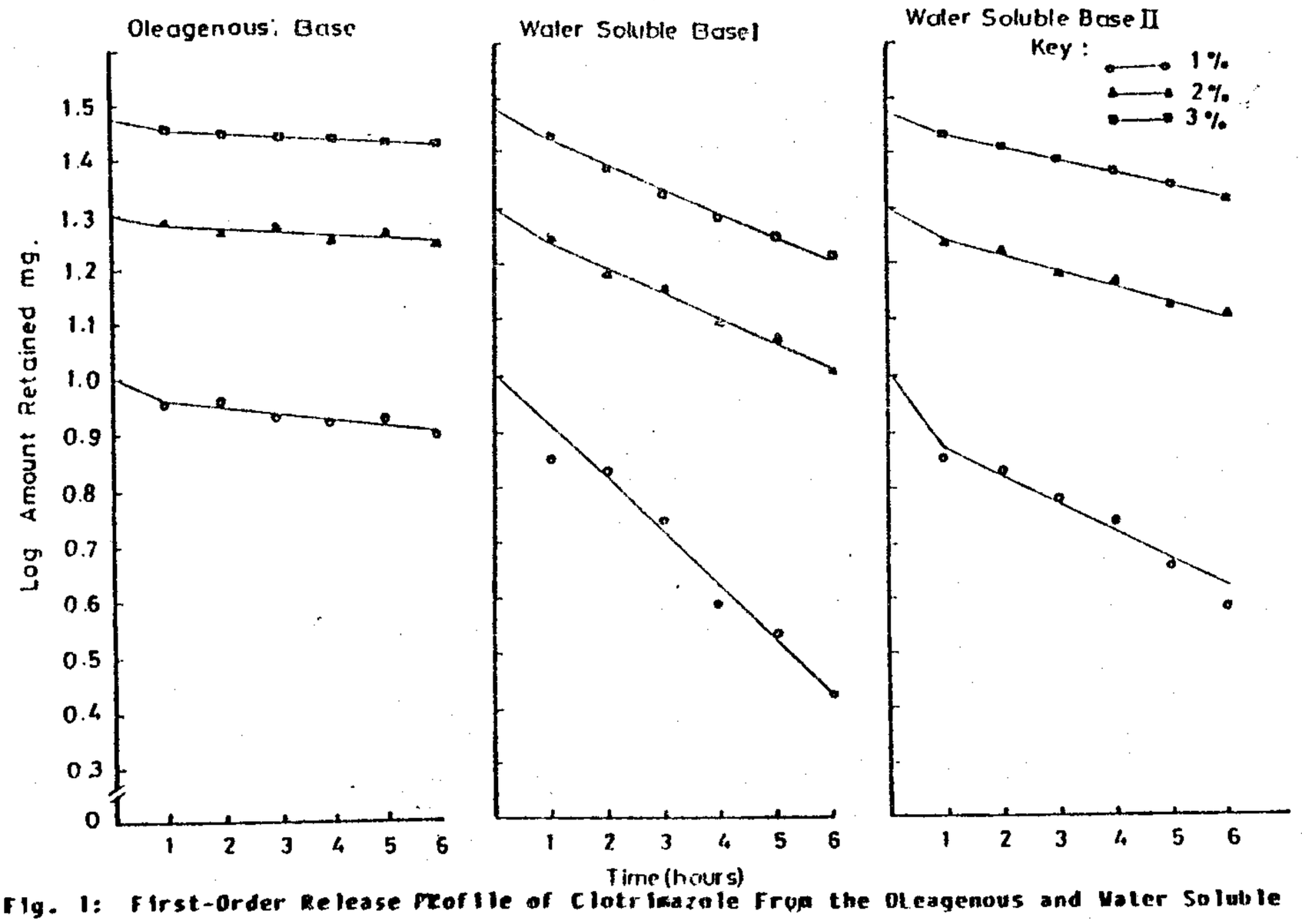

Fig. 1: First-Order Release Profile of Clotrimazole From the Oleagenous and Water Soluble Bases Containing Different Drug Concentrations.

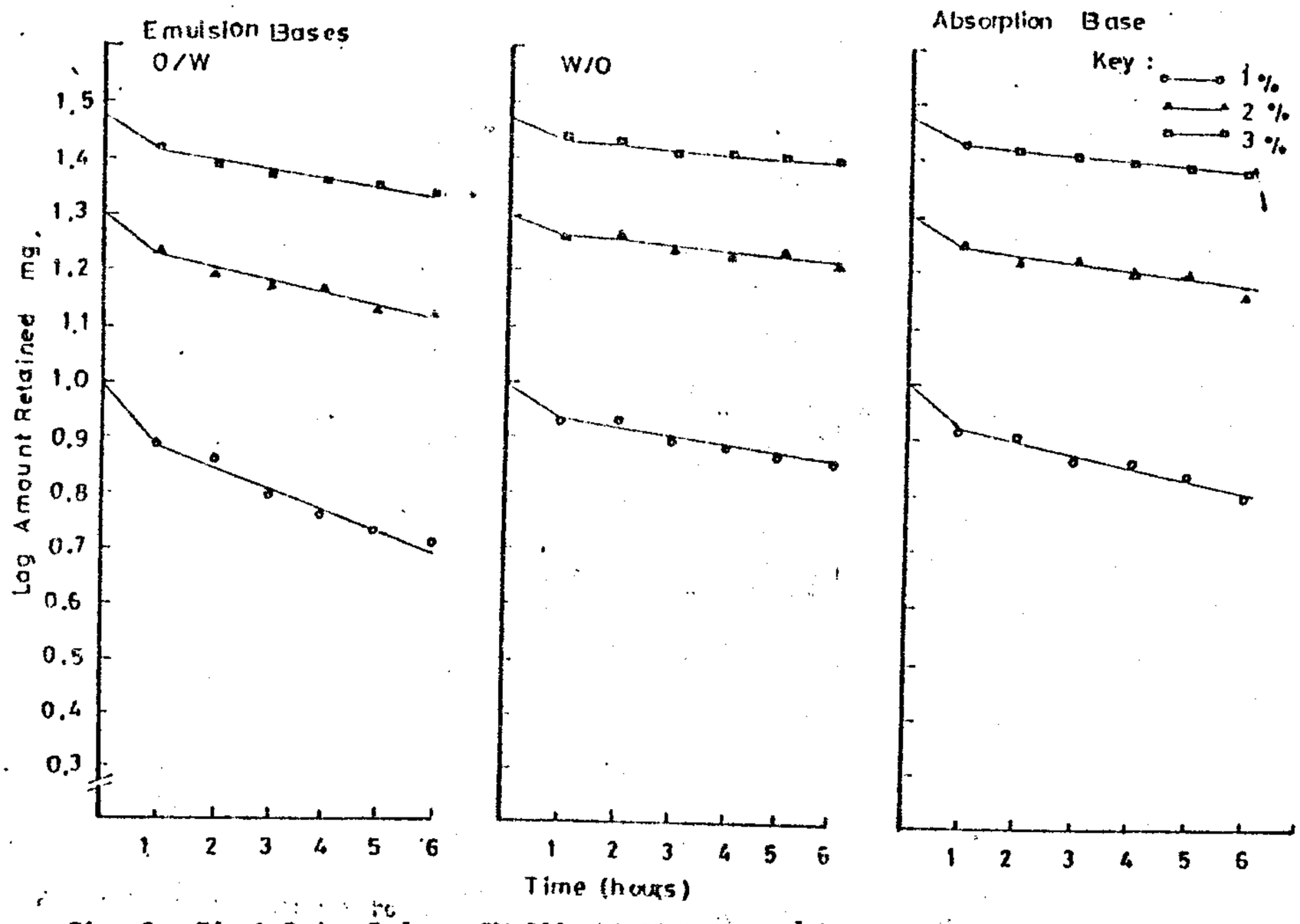

Fig. 2: First-Order Release Profile of Clotrimazole From the Emulsion and Absorption Ointment Bases Containing Different Drug Concentrations.

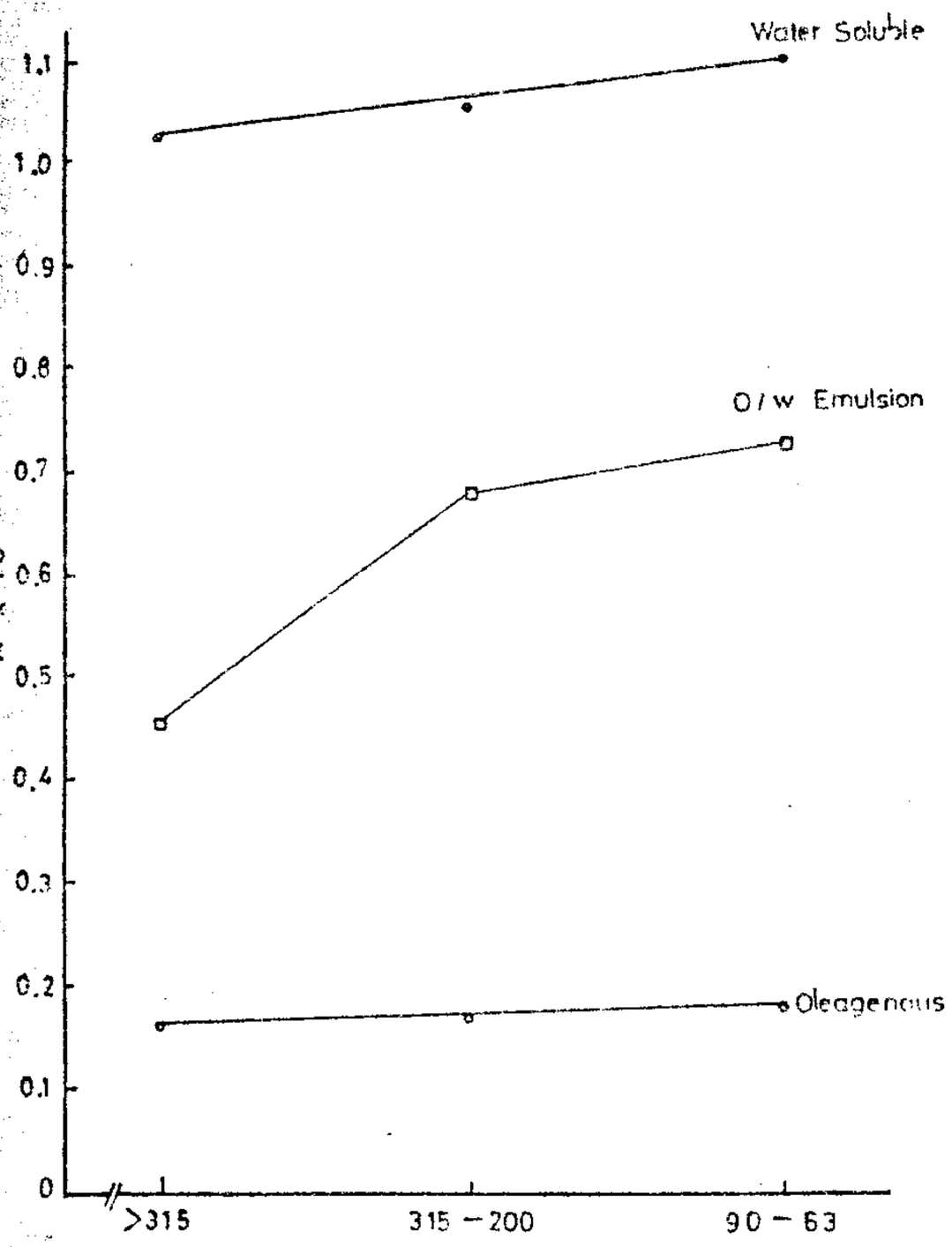

Fig. 3: Effect of Particle Size of Clotrimazole(3%)on its Release Rate Using Different Ointment Bases.

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صياغة وتقويم مرهم الكلوتريمازول

السيد على ابراهيم ـ احسان حافظ ابراهيم ـ سهير مصطفى الشـــــــــنوانى ـ

ابراهـيم الجبالـــــى

قسم الصيدلانيات ـ كلية الصيدلة ـ جامعة اســـيوط

اختص هذا البحث بدراسة تأثير نوع القاعدة المرهمية وتركيز العقار وحجم حبيباته على معدل انطلاق الكلوتريمازول وكذلك فعاليته المضادة للفطريات كانت القواعد المستخدمة فى تحضير المرهم هى : قاعدتين تذوبين فى الماء (١،٢)، قاعدة ممتصة ، قاعدة دهنية. قاعدتين مستحلبتين ماء فى زيت وزيت فى ماء .

واستخدم العقار فى كل من هذه القواعد المستخدمة بتركيز ١، ٢، ٣ فى المائة . وكما استخدمت ايضا احجام مختلفة من حبيباته هى اكبر مـــــــن ٣١٥ ، ٣١٥ ـ ٢٠٠ ، ٢٠٠ ـ ٩٠ ميكرومتر مع بعض من هذه القواعد .

وقد اجريت دراسة مقارنة مع مستحضرين للعقار فى السوق المحلى .

وقد امكن ترتيب القواعد المستخدمة حسب معدل انطلاق العقار الى قاعـــــدة تذوب فى الماء رقم ١ ثم رقم ٢ ، قاعدة مستحلب زيت فى الماء قاعدة ممتصـــــة، قاعدة مستحلب ماء فى زيت ثم القاعدة الدهنية . ووجد ان هناك علاقة مباشرة بين معدل انطلاق العقار من القواعد المختارة وبين تأثيره المضاد للفطريات .

وعلى اساس النتائج التى تم الحصول عليها ، اختيرت القاعدة التـــــى تذوب فى الماء رقم (١) وقاعدة مستحلب زيت فى ماء والقاعدة الدهنية بالاضافة الى مستحضرات السوق للدراسة الاكلينكية لعلاج التينيا الحلقية .

وبعد ترتيب المستحضرات حسب كفاءتها الاكلينيكية يمكن التوصية باستخدام ١/ من العقار فى قاعدة تذوب فى الماء وتتكون من جليكول عديد الايثيلين ٤٠٠٠، ٤٠٠ بنسبة ٤ : ٦ او فى قاعدة دهنية تتكون من الفازلين لعلاج الامراض الفطرية السطحية .

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