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Ketoconazole Emulgel Formula Activity Test against *Microsporum gypseum* and *Candida albicans*

Sulistyaningsih[1], Dolih Gozali[2], Rd. M. Bambang[1], Resmi Mustarichie[3*]

[1]*Department of Biological Pharmacy, Faculty of Pharmacy, Universitas , Padjadjaran, Indonesia 45363*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia 45363*
[3]*Department of Pharmaceutical Analysis and Medicinal Chemistry, Universitas , Padjadjaran, Indonesia 45363*

Abstract

This research aims to create a formula emulgel ketoconazole with good physical stability as well as comparing the antifungal activity of ketoconazole emulgel formula with ketoconazole cream products against gypseum Microsporum and Candida albicans. Formulating ketoconazole emulgel followed by an evaluation of the result of the storage stability, pH, and anti-fungus effect against Trichopyton metagrophytes and Candida albicans. The test results emulgel formula antifungal activity using agar diffusion method, showed that the formula emulgel ketoconazole have better activity compared to ketoconazole cream products against Candida albicans. The results of the evaluation of stability during 35 days of storage which includes observation of the organoleptic and centrifugation test of formula emulgel said to be stable, while the pH and viscosity decreased, but still met the requirements set. It is concluded that emulgel formula antifungal activity using agar diffusion method, showed that the formula emulgel ketoconazole has better activity compared to commercially ketoconazole cream products.

Keywords: Formulation, Emulgel, ketoconazole, *Microsporum gypseum, Candida albicans*

INTRODUCTION

The geographical condition of Indonesia which is a tropical region with high temperatures and high humidity will facilitate the growth of mold so that a fungal infection in Indonesia, in general, are found [1]. Fungi are not photosynthetic protists that grow as a mass of very fine filaments once and branches (hyphae) that consist of a series of cells which then form a network known as mycelium. About 100 species of fungi that cause disease in humans and animals, it is often transmitted from one person to another only species Candida and demartofita groups such as Trichophyton mentagrophytes [2].

Candida albicans is one example of an opportunistic fungus, the fungus that can cause disease only in people with impaired body's defense mechanism (Pelczar, 1998). It is the main cause of candidiasis of the mucous membranes, the female genitals, skin, nails, lungs, kidneys, also in the eye.Trichophyton mentagrophytes is one class of dermatophyte fungi that invade only superficial keratinized tissue, causing infections dermatophytosis fungus (Tinea disease). A group of dermatophyte fungi can cause some forms of typical clinical, depending on anatomic location [2].

Ketoconazole is a synthetic imidazole derivative antimicotica which have antimycotic activity against dermatophytes and yeasts, such as Microsporum sp, Tricophyton sp, Epidermophyton sp and Candida sp. Ketoconazole works by interfering with the synthesis of ergosterol which is a vital component of fungal cell membranes by inhibiting the fungal cytochrome P450 [3].

Emulgel is a mixture of emulsion gel. The emulsion is a dispersion in which the dispersed phase consists of small dots dispersed throughout a liquid carrier which is not mixed [4]. Gels are a semisolid system consisting of a suspension made of small inorganic particles or large organic molecules that are penetrated by a fluid. Emulgel is a preparation which has a high acceptance for emulgel has various advantages same as in form of an emulsion or gel [5].

This study designed different formula to other ketoconazole emulgel studies by other researchers [6-8].

MATERIAL AND METHOD

Collection of Materials

Raw materials research in the form of Ketoconazole obtained from PT. Meprofarm, Bandung) as well as the test fungus Candida albicans and Trichophyton mentagrophytes obtained from PT. Biofarma, Bandung.

Raw Material Inspection

The examination was conducted on the examination of the active substance (Ketoconazole) according to the Indonesian Pharmacopoeia [9,10], and an examination of the gel base Aqupec HV 505 according to the Handbook of Pharmaceutical Excipients [11].

Emulgel base formulation

Emulgel was prepared with the guidance of British Pharmacopea [12] and Rowe et.al [11].

Making Ketoconazole Emulgel

The steps in the creation of ketoconazole emulgel formula based on modified Mohamed et.al method [5]. Ketoconazole gel emulsion mixed into Aqupec HV-505 with a ratio of 1: 1 while still stirring using a turbo mixer for 10 minutes to form a homogeneous emulgel.

Evaluation of Emulgel made

A. Examination of the organoleptic
   Ketoconazole emulgel organoleptic checked by observing changes in consistency, color, smell and homogeneity during 35 days of storage. Homogeneity

emulgel was checked by applying a number of emulgel lightly on the slide and saw whether or not its homogeneous. Organoleptic examination is commonly used in observation of research products [13].

B. Measurement of pH

Emulgel declared stable if there was no change significant pH during storage time.

C. Viscosity Measurement

The viscosity measurements done using Rion Viscotester VT-04, by entering the spindle appropriate (spindle No. 2) into the container containing the preparation up to the mark. Safety valve was released and the rotor was turned until the figure was stable (± 2 min) indicated by the pointer. The viscosity measurements carried out during the storage period on days 1, 3, 7, and subsequently during 35 days of storage.

D. Test centrifugation

Centrifugation method performed at room temperature, emulgel weighed as much as 1 g and put in a centrifuge tube and then centrifuged at room temperature at 25 ° C with a speed of 2500, 3000 and 3750 revolutions per minute (rpm), for five hours. Stable emulsion system showed no occurrence of phase separation by centrifugation at 2000 to 3000 rpm. No occurrence of phase separation for five hours at a speed of 3750 rpm indicating that the preparation is stable for one year at room temperature [14].

Antifungal Activity of Ketoconazole emulgel made

The assay was intended to determine the antifungal activity of prepared ketoconazole emulgel against ketoconazole in other dosage forms that were on the market. Testing the antifungal activity through several stages, including:

a. Setup Media Sabourade Dextrosa Agar

Media test was made by weighing 6.5 grams Sabourade Dextrose Agar (SDA) and dissolved in about 100 ml of distilled water, then heated over a water bath until dissolved (clear), then sterilized using an autoclave at a temperature of 121^{o}C.

b. Setup Test mushrooms

Mushrooms test made by breed fungus Candida albicans and Trichophyton mentagrophytes in Dextrose Sabourade media and incubated at 25 ° C for ± 3 days, after which the cultures were suspended in sterile physiological NaCl solution.

c. Testing Antifungal Activity

Testing the antifungal activity of ketoconazole emulgel formula done by entering the fungal suspension into a sterile Petri dish 0.5 ml using a micro-pipette, then pour as much as ± 20 ml media SDA thawed into a sterile petri dish containing a suspension of the fungus. The mixture was rotated until homogenous and become solid, then made three holes using the perforator. After that, the formula emulgel and product comparison which would be tested was added to each of the holes that exist in a petri dish aseptically, then incubated at 25 ° C for ± 3 days. After incubation diameter measuring inhibitory marked by a clear zone using a caliper.

RESULTS AND DISCUSSION

Emulgel base formulation with varying concentrations of surfactants and propylene glycol are made as shown in Table 1.

Tabel 1. Emulgel base formula

Compounds	Formula (%)			
	FB0	FB1	FB2	FB3
Ketoconazole	-	-	-	-
Aqupec HV 505	1	1	1	1
Triethanolamine	1,4	1,4	1,4	1,4
Oleum olivarum	5	5	5	5
Span 20	0,9	1,5	0,9	1,5
Tween 20	0,6	1	0,6	1
Propylene-glycol	10	10	15	15
Glycerine	2,5	2,5	2,5	2,5
Acnibio	0.25	0.25	0.25	0.25
Aquadest ad	100	100	100	100

Notes :
FB0: Emulgel base with 1.5% surfactant and propylene glycol 10%.
FB1: Emulgel base with 2.5% surfactant and propylene glycol 10%.
FB2: Emulgel base with 1.5% surfactant and propylene glycol 15%.
FB3: Emulgel base with 2.5% surfactant and propylene glycol 15%.

Other researcher such as Yenti et.al [15] and Dewi et. al [16] mentioned of using other combinations for making of emulgel base. Actually, the use emulgel has been published in many journals [17-20].

Emulgel formulationl

Ketoconazole emulgel formula was designed as shown in Table 2**.**

Table 2. Ketoconazole emulgel formula

Compounds	Formula (%)				
	F0	F1	F2	F3	F4
Ketoconazole	-	1	1,5	2	2,5
Aqupec HV 505	1	1	1	1	1
Triethanolamine	1,4	1,4	1,4	1,4	1,4
Oleum olivarum	5	5	5	5	5
Span 20	0,9	0,9	0,9	0,9	0,9
Tween 20	0,6	0,6	0,6	0,6	0,6
Propylene glycol	15	15	15	15	15
Glycerine	2,5	2,5	2,5	2,5	2,5
Acnibio	0.25	0.25	0.25	0.25	0.25
Aquadest ad	100	100	100	100	100

Notes:
F0: Emulgel without active ingredient Ketoconazole (blank)
F1: Emulgel with ketoconazole 1%.
F2: Emulgel with ketoconazole 1,5 %.
F3: Emulgel with ketoconazole 2%.
F4: Emulgel with ketoconazole 2,5%.

Formula from Table 2 different formula of Jain *et al* [6] who used carbopol 934 and carbopol 930, liquid paraffin to make their ketoconazole emulgel. Verma et al. [8] also used carbopol for their ketoconazole nanoemulgel. Triethanolamine combined with methyl and propyl paraben dissolved in ethanol and mixed with aqueous phase has been reported by Priya et al.[21]. The use of propylene glycol with carbopol reported by Melani et al. [22].

Inspection organoleptic Ketoconazole

Ketoconazole active substances obtained from PT. Meprofarm with a certificate of analysis accompanying substances. The results of the examination of active substances Ketoconazole based Indonesian Pharmacopoeia [9], acquired the characteristics of ketoconazole has met the requirements.

Inspection Aqupec Gel Base HV-505

Examination gel base Aqupec HV 505 is based on the Handbook of Pharmaceutical Excipients [10], 505 HV Aqupec acquired characteristics were meet the requirements.

Result Emulgel preparations Ketoconazole

Ketoconazole made was shown in Figure 1.

Evaluation of preparations Emulgel Ketoconazole

Based on observation for 35 days, it appeared that the form and the four formulas emulgel ketoconazole could be said to be stable because no change in organoleptic, where the formula emulgel did not show phase separation and remain visible white with the consistency of a thick and homogeneous, and smelling of oil as shown in Table 3**.**

Fig.1 Emulgel. made

Notes :
F0: Emulgel without ketoconazole (blank).
F1: Emulgel with ketoconazole 1%.
F2: Emulgel with ketoconazole 1,5 %.
F3: Emulgel with ketoconazole 2%.
F4: Emulgel with ketoconazole 2,5%.

Table 3 Observations during the organoleptic emulgel ketoconazole 35 days of storage

Formula	Observation	Organoleptic observations on day -						
		1	3	7	14	21	28	35
F0	Consistency	vh	vh	vh	vh	vh	vh	vh
	Colour	white	white	white	white	white	white	white
	Odor	so	so	so	so	so	so	so
F1	Consistency	vh	vh	vh	vh	vh	vh	vh
	Colour	white	white	white	white	white	white	white
	Odor	so	so	so	so	so	so	so
F2	Consistency	vh	vh	vh	vh	vh	vh	vh
	Colour	white	white	white	white	white	white	white
	Odor	so	so	so	so	so	so	so
F3	Consistency	vh	vh	vh	vh	vh	vh	vh
	Colour	white	white	white	white	white	white	white
	Odor	so	so	so	so	so	so	so
F4	Consistency	vh	vh	vh	vh	vh	vh	vh
	Colour	white	white	white	white	white	white	white
	Odor	so	so	so	so	so	so	so

Notes:
F0: Formula emulgel without ketoconazole (blank).
F1: Formula emulgel with ketoconazole 1%.
F2: Formula emulgel with ketoconazole 1,5 %.
F3: Formula emulgel with ketoconazole 2%.
F4: Formula emulgel with ketoconazole 2,5%.
Vh: viscous homogeneous.
Bm: smell of oil

Results of pH measurement of Ketoconazole emulgel

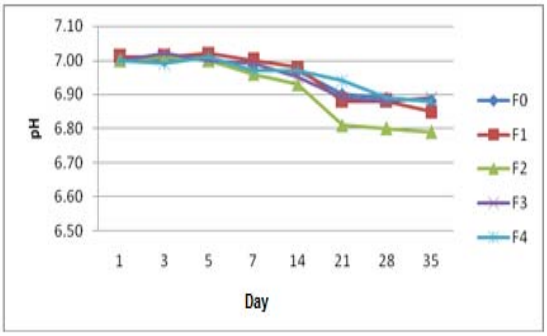

Figure 1 The observation of the average change in pH during 35 days of emulgel storage

It was found that (Fig. 1) prepared emulgel ketoconazole had a pH value suitable for topical preparations during the 35 days of storage. Moreover, it appeared that the pH value of each of the stocks declined during the storage time. The pH decreased might be due to the acidic nature of its base, ie Aqupec HV 505 (pH 5.5), which reacted with other additives used in the formula emulgel stretcher. The addition of ketoconazole in preparation in small amounts, ie one gram in 100 grams of preparation, might not affect the pH of the preparation. This was seen from the pH value of the stocks of blank forms (F0) during 35 days of storage were almost the same as the pH value of the stocks F4.

Results of observation of viscosity, it was found that the viscosity of the emulgel formula fifth experienced little change during the 35 days of storage time. F2 and F4 showed viscosity values were relatively larger and relatively more stable than F1 and F3. It was likely influenced by differences in the number of VCO used in the system affect the viscosity emulsion in the preparation of the preparation emulgel concluding the greater the number of VCO was used, the greater the viscosity of the resulting value. The addition of ketoconazole in preparation emulgel in small dosage, ie one gram to 100 gram dosage was unlikely to affect the viscosity of the preparation, which shown from the viscosity of the blank (F0) during 35 days of storage equal to the value of the viscosity of dosage F2.
Physical Stability Testing Results Emulgel Ketoconazole with centrifuge method seen directly after centrifugation carried out for 5 hours at a speed of 2500, 3000 and 3750 rpm. It found that after centrifugation with a speed of 2500 rpm, 3000 rpm, and 3750 rpm separation occurs only in formula F1. The use of centrifugation method in view emulsion phase separation was very useful for predicting the shelf life of a preparation. Centrifugation at 3750 rpm in a centrifuge radius of 10 cm for 5 hours equivalent to the effects of gravity for approximately one year. Based on the conversion, it was found that centrifugation at a speed of 2,500 rpm for 5 hours equivalent to the effects of gravity approximately 8 months, while for the 3000 rpm speed is equivalent to the effect of gravity is approximately 10 months. Our data showed the F1 formula phase separation occurs after centrifugation at a speed of 2500 rpm, therefore excluded F1 back on further centrifugation. Based on the above facts, the creamy formula F1 is not resistant to storage at room temperature for less than 8 months.

Antifungal Activity Assay results

Results of testing the activity of ketoconazole 2% emulgel formula using the agar diffusion method (perforation) compared with 2% ketoconazole cream products against Candida albicans and Microsporum gypseum can be seen in Table 4 and 5.

Table 4 Antifungal activity against Candida albicans

Petri dish	Diameter (mm)		
	F_3	FP_1	FP_2
I	25.8	24.2	0
	25.9	24.3	0
	25.9	24.1	0
Mean	25.87	24.2	0
II	25.9	24.2	0
	25.8	24.2	0
	25.9	24.1	0
Mean	25.87	24.17	0

Notes:
F_3: Formula emulgel with ketoconazole 2%
FP_1: Comparative product 1, Cream Ketoconazole 2%
FP_2 : Comparative product 2, Cream Ketoconazole 2%

Tabel 5 Antifungal activity against Microsporum gypseum

Petri dish	Diameter (mm)		
	F_3	FP_1	FP_2
I	0	0	0
	0	0	0
	0	0	0
Mean	0	0	0
II	0	0	0
	0	0	0
	0	0	0
Mean	0	0	0

Notes:
F3: Formula emulgel with ketoconazole 2%
FP1 : Comparative product 1, Cream Ketoconazole 2%
FP2: Comparative product 2, Cream Ketoconazole 2%

Based on Table 4 & 5, it appeared that the formula emulgel had an activity that was as good as ketoconazole cream products, as seen from the diameter of inhibition against Candida albicans. Table 5, however, showed either the formula and comparators did not have activity against Microsporum gypseum characterized by the absence of inhibition diameter.

CONCLUSIONS

From the research formulation and stability evaluation emulgel ketoconazole observed from an examination of the organoleptic, pH, viscosity, and the levels of ketoconazole in preparation for the storage time, it can be concluded that the viscosity of the preparation emulgel F4 during 35 days of storage is relatively more stable compared to the fourth formula emulgel others. Levels of ketoconazole in the preparation of F4 also relatively stable during the 35 days of storage.

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