Formulation of Econazole Nitrate as an Ophthalmic Ointment

Dr. Heba Antwan Fatohy

ABSTRACT

Background and Objectives: Keratomycosis is an important cause of ocular morbidity and can lead to blindness due to lack of ocular antifungal agents. The goal of this study is to formulate a stable effective formula of econazole nitrate ophthalmic ointment.Econazole nitrate has an advantage over other antifungal agents in treatment of mixed infection since it is active against both dermatophytes and candida, and also it is effective in the treatment offilamentous fungi.

Methods: Econazole nitrate ophthalmic ointment of different strength were formulated and compared for their in vitro activity.The effect of different factors (light and type of container) and calculating the expiration date of the selected formula were studied.

Results: Formula A (1 gm Econazole nitrate in 100 gm ophthalmic ointment base) and formula B (2 gm Econazole nitrate in 100 gm ophthalmic ointment base) had good in vitro antifungal activity because both formula A and B inhibit the growth of the fungi used, but formula A was selected because it has the lowest concentration and the same activity. Also the results indicated that the selected formula A was affected by light and its % remaining was (96.7%), while there was no effect of the type of container used to store Econazole nitrate ophthalmic ointment.The expiration date of formula A was calculated to be (2.19) years.

Conclusions: 1% Econazole nitrate ophthalmic ointment appears to be effective in vitro and should be stored in dark container . .

Keywords: Keratomycosis,Econazole nitrate, shelf life.

INTRODUCTION:

Filamentous fungi such as (species of Fusarium, Aspergillus) and yeast like fungi such as Candida, considered as the causative factors for keratomycosis[1,2]. Fungal keratitis is more frequent in rural areas of developing countries [3]. Trauma is the most important cause of keratomycosis especially encountered in farmers after trauma with palm tree leaf, thorn, kernel or other plant objects [4]. In recent decades, the incidence of keratomycosis has been increased,while inadequate and inappropriate treatment has led to poor visual outcomes [5]. Patients having fungal keratitis complain of discomfort, photophobia and mild discharge [6], if these eyes are to be saved, antifungal agent is required, otherwise fungus-infected eyes eventually become blind due to the lack of ocular antifungal agents [5]. Imidazole compounds are useful in the treatment of fungal keratitis, the available imidazoles are clotrimazole, miconazole, ketoconazole & econazole [6]. Econazole nitrate (EN) which is:1-[2,4-dichloro-β-(p-chlorobenzyloxy) phenethyl]-imidazole nitrate [7,8]. It is antifungal agent with broad spectrum activity against yeast, mold, dermatophytes and it is quite active against some bacteria of the actinomycets, and highly active against some gram positive cocci and bacilli [9]. Econazole nitrate is used topically in the treatment of superficial

* Assistant lecturer MSc. Pharmaceutics/College of pharmacy/Hawler medicalUniversity

candidiasis , dermatophytosis & pityriasis versicolor infections, it is also used in the treatment of vaginal candidiasis, and has also been used as eye or ear drops10. Econazole nitrate is very slightly soluble in water, soluble in methanol [7-10], it's melting point is 162 and 166oC 8,11. Pharmaceutical preparations are applied topically to the eye to treat surface or intraocular conditions including bacterial, fungal and viral infections of the eye or eyelids[12]. Ophthalmic ointment is an ophthalmic product used for the application to the conjunctival sac or to the eyelid margin [12,13].Ophthalmic ointments have extended contact time on the surface of the eye compared with ophthalmic solutions, increasing the duration of their surface effect and bioavailability for absorption into the ocular tissues [12].

MATERIALS AND METHODS:

Materials**:** Econazole nitrate powder (Al-Saffa pharmaceutical industry), methanol (BDH limited pool England), phenyl mercuric acetate (Al-Shaffa pharmaceutical industry), liquid paraffin (E,Merk,Darmstadt,Germany), wool fat & soft paraffin (Riedel-De-Haen AG,Seelze-Hannover,Germany), nutrient agar & Candida fungi (Nanakaly Hospital).

Methods**: Formulation of ointment base :** Ophthalmic ointments contain liquid paraffin, wool fat and soft paraffin as the base ingredients that can be varied in proportions to adjust consistency [11,13].

Formulation of Econazole nitrate as an ophthalmic ointment :

After grinding of the particles of EN pure powder and obtaining microfine particles, two different concentrations of EN ophthalmic ointment 1% and 2% (w/w) were prepared by mixing the microfine particles of EN powder with small quantity of ointment base, and then incorporated with the remainder of the base, as shown in (Table 1).

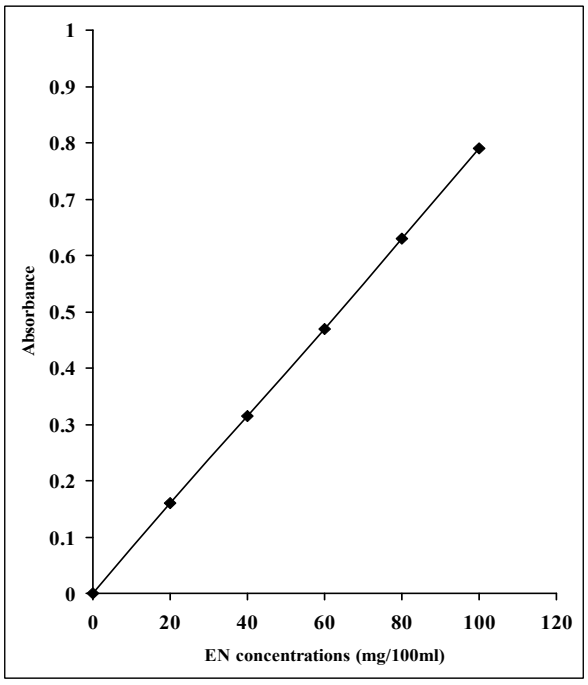
(Table 1): Different formulas of EN as an ophthalmic ointment dosage form.

Formula	EN* (gm)	Phenyl mercuric acetate** (gm)	Ointment base (gm)
A	1	0.002	100
B	2	0.002	100

*Econazole nitrate, ** preservative.

All materials used in the preparation of eye ointment sterilized by heating in oven 13.

Assay of Econazole nitrate ophthalmic ointment:A calibration curve of EN in methanol was plotted as shown in (Figure 1).



(Figure 1): Calibration curve of Econazole nitrate in methanol

A simple procedure for the determination of EN based on the UV spectrophotometry was applied as follows [14].

Test sample: 5 gm of EN ophthalmic ointment contains 50 mg of EN was dissolved in 50 ml of methanol then the volume was completed to 100 ml with methanol.

Reference sample: 50 mg of pure EN was weighted and transferred into 100 ml volumetric flask. 50 ml of methanol was added, shacked well until it was dissolved, completing the volume to 100 ml with methanol.

The UV absorbance was then recorded for the reference and the test samples against pure methanol. The quantity of EN in ophthalmic ointment was calculated using the following equation:

%Econazole nitrate=[(A sample x mg standard) / (A standard x mg sample)] x100

Preliminary in vitro study of antifungal activity of Econazole nitrate ophthalmic ointment: This study was done using nutrient agar inoculated with Candida fungus species, EN ophthalmic ointment formula (A & B) was spreaded on the agar to follow its effect based on the inhibition of the growth.

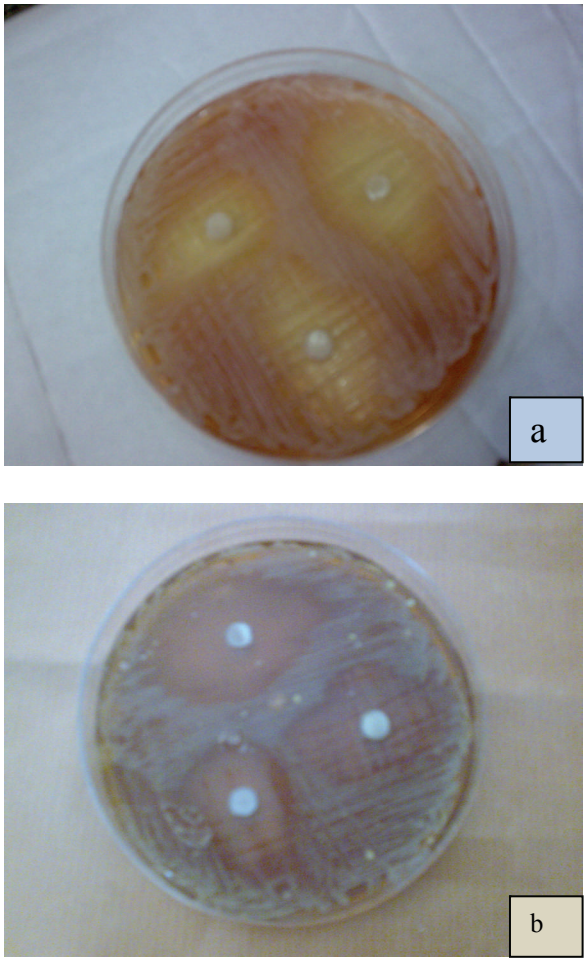
Effect of light:This factor was studied by placing formula (A) in two glass containers (clear and dark), at room temperature for two months. Samples were analyzed for their drug content every two weeks.

Effect of container: Samples of EN ophthalmic ointment formula (A) were stored in plastic and metal containers for 120 days. (Eye ointment can be packed in collapsible metal or plastic containers 15).

Stability study :Stability of EN ophthalmic ointment in accelerated conditions was carried out by incubating samples of formula (A) in ovens at 40, 50 and 60 oC for 120 days. Samples were taken and assayed for their drug content at suitable time intervals 16.

RESULT:

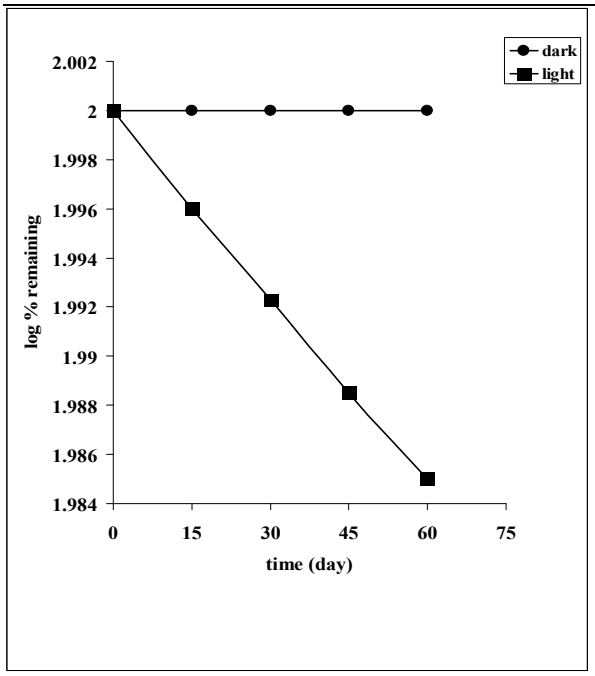
The growth of the fungi was inhibited in both test samples, (formula A & B), so formula (A) selected to complete the study because it have the lower concentration and it is effective as formula (B). As shown in (Figure 2).



(Figure 2): a) In vitro activity of Econazole nitrate formula (A)

b) In vitro activity of Econazole nitrate formula (B).

There was linear relation for logarithmic plot of the % remaining of EN versus time, when the drug was stored under light. As shown in (Figure 3).



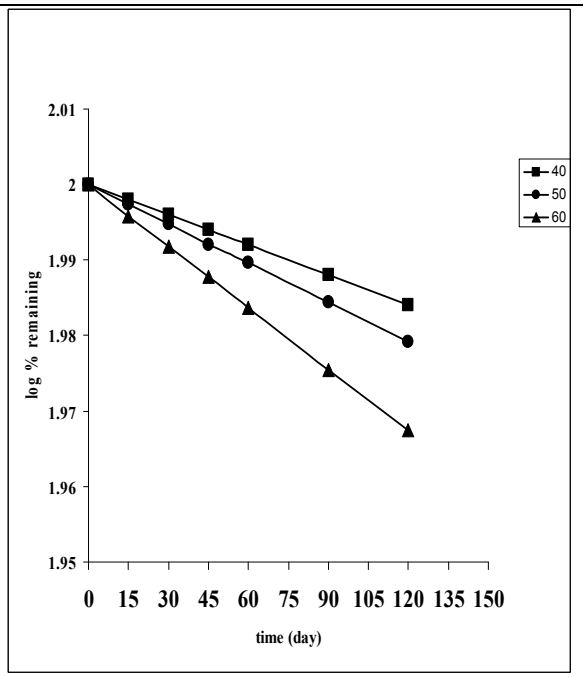
(Figure 3): Effect of light on the rate of hydrolysis of Econazole nitrate ophthalmic ointment formula (A) at room temperature.

(Table 2) shows the % remaining of EN when placed in plastic and metal container at room temperature for four months.

(Table 2): Percentages remaining of Econazole nitrate in different containers after 120 days.

Formula (A)	% Remaining
Plastic	98.8
Metal	98.7

The degradation of EN in formula (A) follows first order kinetics since straight lines were obtained by plotting the logarithm of % remaining of EN versus time as shown in (Figure 4).



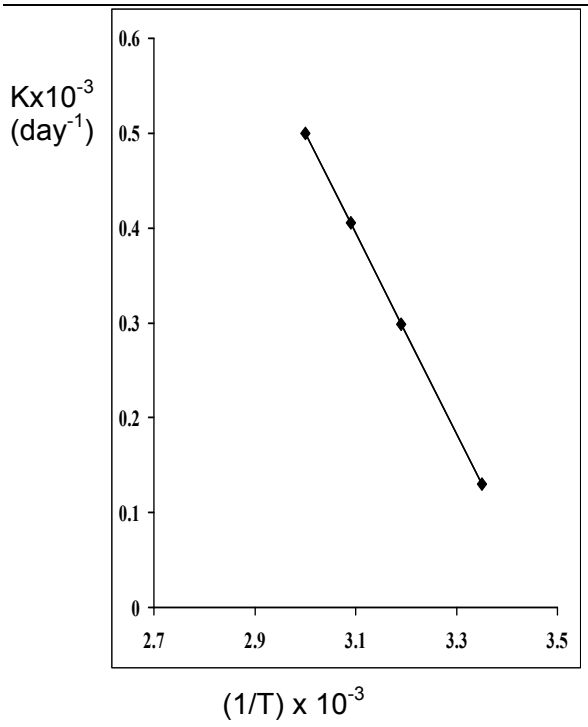
(Figure 4): Degradation curve of Econazole nitrate ophthalmic ointment formula (A) at 40, 50 and 60 ^{o}C.

The degradation rate constant (k) at different temperatures were calculated from the slopes of the lines as illustrated in (Table 3).

(Table 3): Degradation rate constant (k) of Econazole nitrate ophthalmic ointment formula (A) at different temperatures.

Temperature ^{o}C	Kx10^{-3} day^{-1}	1/T x 10^{-3}
40	0.290	3.19
50	0.405	3.09
60	0.500	3.0

To determine the expiration date ($t_{10\%}$) at 25^{o}C, Arrhenius plot was constructed as shown in (Figure 5) to determine the degradation rate constant of EN formula (A) at 25 ^{o}C which was found to be (0.13 x10^{-3} day^{-1}).



(Figure 5): Arrhenius plot for estimation of the expiration date (t_{10}%) of Econazole nitrate ophthalmic ointment formula (A).

Since the degradation of the drug follows first order kinetics therefore the expiration date can be calculated using the following equation [17]: $t_{10\%} = 0.104 / k_{25^oC}$ .The results indicate that formula (A) has $t_{10\%}$ value of about (2.19) years.

DISCUSSION:

Econazole nitrate pure powder was milled to obtain microfine particles which were used to formulate EN ophthalmic ointment. (Microfine particles are necessary to enhance the absorption of the drug and to decrease the irritation of it on the eye [18,19]. The growth of the fungi (Candida species) was inhibited by the two tested samples (1% & 2% (w/w)), and this result indicate that EN has good antifungal activity at low and high concentration as shown in (Figure 2), this was consistent with the reference which state that EN was effective against Candida species and has broad spectrum activity[20]. The % remaining of the drug was (96.7%) when exposed to light at room temperature after two months, this was consistent with the reference which state that EN is known to be affected by light and should be stored in a well closed container protected from light [8], on the other hand the sample which was stored in dark container showed no detectable change in the % remaining when exposed to the same conditions. There was no detectable change in % remaining of EN ophthalmic ointment samples when stored at room temperature in different containers, that's to say metal and plastic containers had no effect on EN ophthalmic ointment as shown in (Table 2). The expiration date of EN ophthalmic ointment formula A was calculated from the results of the stability study which found to be (2.19) years. Inspired from a study in animal models econazole was effective in the treatment of experimental ocular fungal diseases in rabbit 20, also a study which state that focal corneal lesion resection combined with dusting mixed imidazole powder is relatively effective in treatment of filamentous fungal keratitis 21so further study on formula (A) will be done to prove its in vivo effectiveness.

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