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Original Article

New Topical Pharmaceutical Formulations of Econazole& their Evaluation

Sabati A. M, and Othman A. M.

Department of Pharmaceutics, Faculty of Pharmacy, Sana'a University, Yemen.

Abstract

Econazole is an antifungal drug present in the Yemeni market as a 1% cream only, in this study a new topical formulations of econazole were prepared such as emulgels, gels, ointments (PEG, oleaginous), and o/w cream.
In-vitro release studies of the above mentioned formulations were done in comparison with market's cream by using permeable membrane "tea bags", cellophane membrane & and rabbit skin in diffusion cell , the release of econazole from the above mentioned bases were arranged in the following order:
Emulgel > gel > polyethylene glycol ointment > o/w cream > market's cream > oleaginous ointment with tea bag and cellophane membrane, while in case of using rabbit skin as a membrane there is no penetration for the drug through rabbit skin.
The correlation coefficient (r), the order of the drug release, and the half-life (t1/2) for each base were determined kinetically.
The above mentioned results were confirmed by the study of antimicrobial activity against some strains of fungi such as (Candida Albicans , Aspergillus Niger , Trichophyton Memtography ,Trichophyton Rubrum , Microsporum canis , and Epidermophyton Floccosum) and against some strains of gram positive bacteria such as (Staphylococcus Aureus , and Streptococcus Pyogens)
The results (inhibition zone in mm) were found to be identical to that results obtained in case of in-vitro release study.

Introduction:

Econazole nitrate is an antifungal drug, it's a member of azole class, it is interfering with fungal oxidative enzyme to cause lethal accumulation of hydrogen peroxides, also reduce the formation of ergosterol by interact with C-14 alpha-demethylase (cytochrome P450 enzyme) to block demethylation of lanosterol to ergosterol, the inhibition will disrupt membrane function & increase permeability to intra cellular constituents I -

Econazole nitrate is effective against candida spp, blastomyces dermatitidis coccidioides immitis, cryptococcus neformans, histoplasma capsulatum, paracoccidioids brasiliensis, Malessezia furfur, Aspergillus spp, sporothrix schenckii, most dermatophytes including epidermophyton floccosum, microsporum canis trichophyton mentography, trichophyton rubrum and some gram-positive bacteria including staphylococcus, streptococcus and nocardia minutissima.4,8

Econazole weakly absorbed orally, that the mean peak plasma concentration was 2.6µg/ml 2.5hours after oral administration of 250mg of econazole, about 40% of the oral dose administered is exerted in the urine and 30% was eliminated in the faeces.

Econazole is widely distributed to body tissues, particularly the joints, skin and eyes but don't penetrate CSF, absorption of econazole nitrate is not significant when applied topically (10% is absorbed after topical application to the skin and 5% after vaginal application.3,8

Econazole nitrate is applied topically as a 1% cream, lotion, powder or solution in the treatment of fungal infections such as candidiasis, tinea and pityriasis versicolor. Econazole nitrate has also been administered

Corresponding Author: Sabati A. M, and Othman A. M. Department of Pharmaceutics, Faculty of Pharmacy, Sana'a University,P.o.Box 13078 Sana'a Yemen.

as eye-drops, and has been given by intravenous infusion.3,5,7

This investigation is an attempted to prepare econazole in different new pharmaceutical formulations of 1% econazole nitrate for improvement of econazole release and getting better effect than the 1% econazole cream formulation found in the market .

Materials and equipments

Materials

-Econazole nitrate (A moon company, Egypt)

-Glycerin(pharmacare, int MfG .CO ,Yemen.

-Liquid paraffin (shiba pharmaceuticals& chemicals MfG .CO.Yemen)

-Span 80 and Tween 80 (Merck company, Germany).

The following chemicals are of pharmaceutical grade: Polyethylene glycol 4000, ethanol, methyl cellulose, avicil, cetyl-alcohol, poly-ethylene glycol 400), triethanolamine, and propanol.

Media for microbiological activity test such as:

-Sabouraud's dextrose agar (65gm/L) + chloramphenicol (0.05gm/L) for Candida Albicans and Aspergillus Niger.

-Sabouraud's dextrose agar (65gm/L) + chloramphenicol (0.05gm/L) + cyclohexamide (0.5gm/L) for dermatophytes.

-Mueller Hinton agar + blood 5% for Streptococcus pyogenes

-Mueller Hinton agar for Staphylococcus aureus

2.2.Equipments:

-Dissolution Testor (Erweca GMBH D-63150 Heusenstamm – Germany serial No 1105416).

-Spectrophotometer (UV-visible spectrophotometer-shimadzu corporation assembled in Australia CAT No-206-67501-93,serial No- A10773680120sm.

-Heater(ingenic urburo CAT, M Zippers GMbH-Germany-serial NO.525017).

-Electronic digital balance (sartorius AG Gottin Gen-Germany)

-Water bath.

-Incubator at 37°C (Ecnomy size3 gallenkamp)

-Incubator at 25°c(memmert co)

-Autoclave(serial No 980295355-manfactured by Hirayama-japan).

- Millimetre measurement roller.

- Plastic petridish (90mm in diameter and 4mm in depth)

-Micropipette of 20µl volume.

Methods

Pharmaceutical part

3.1.1.Preparation of different semisolid formulations:

The following topical formulations were prepared in which 1% w/w econazole was incorporated:

3.1.a. Ethanolic gel (9)

Ethanol: H2O : Glycerin: Methylcellulose

40 gm : 40 gm : 18.8 gm: 0.2 gm

The calculated amount of econazole (1gm) was dissolved in ethanol / H2O /glycerin system, then 0.2gm of methyl cellulose as a gelling agent was added, then left aside for 24hours for complete swelling.

3.1.b. O/W Cream (10)

- Cetyl alcohol 10 gm
- Liquid paraffin 20 gm
- Tween 80 4 gm
- Span 80 6 gm
- Glycerin 7gm
- Water 52 gm

The aqueous phase (Tween 80, glycerin, water), and the oil phase(Span 80,

cetyl alcohol, liquid paraffin) placed in separate containers and heated to 70°C.

Econazole (1gm)was dissolved in the oil phase, then the aqueous phase was added to the oil phase at the same temperature with continuous stirring until cold and congealed.

3.1.c.Polyethylene glycol base(USP,XXII)

- PEG 4000 40 gm
- PEG 400 60 gm

PEG 4000 was melted at 60°C on water bath then PEG 400 containing the drug was added with continuous stirring until congealed.

3.1.d. Emulgel

- Liquid paraffin 20 gm
- Tween 80 1 gm
- Water 75 gm
- Methyl cellulose 2 gm
- Avicil 1 gm

The liquid paraffin was mixed with Tween 80, then econazole(1gm) was added to the mixture and mix quickly until the drug was dissolved, water was added, the mixture was stirred until primary emulsion was formed, then the avicil and methyl cellulose were added and left aside for 24 hours for complete formation of emulgel.

3.1.e.Oleaginous base(USP,XXII)

99gm of white soft paraffin was melted, then 1gm of econazole was dissolved with continuous stirring until congealed.

3.1.2.In vitro release studies of econazole11-13:

The extent of econazole released from different topical formulations were determined by using a dissolution tester as a diffusion cell.

Three different kinds of membranes were used as the following:

- Rabbit skin membrane14 (prepared as mentioned by El-Nabarawi,1996)
- Tea-bags membrane (to prevent turbidity).
- Cellophane membrane.

The membrane was fixed to the vertical basket with a rubber band, two grams of the tested formulation contains 20 mg of econazole was accurately weighed in the basket, then basket was fixed to the apparatus which operating at 37±5°C and rotate at 50 rpm., The basket was hanged into the center of 1000 glass vessels containing 50 ml of the receptor media (mixture of 50% propanol, 50% distilled water, and 1% triethanolamine) to avoid evaporation. The vessels were kept covered during the experiment and the sampling was done through a small orifice.

Three 3 ml samples were withdrawn at specified times intervals and replaced with an equal volume of receptor medium at 37±5°C to keep the volume constant during the experiment study.

The amount of drug released to the receptor phase was assayed spectrophotometrically at 272 nm using a receptor medium as a blank.

Each experiment was done in triplicate and their average was calculated.

3.1.3. Regression analysis of the data15,16

The amount of drug released across each membrane from each base was determined according to the following kinetics equations.

Zero order kinetic:

$A = K0.t + A0$ Where

$A0$ = the amount of drug at $t = 0$

$K0$ = zero order release rate constant.

By plotting of A (amount of drug released in mg) versus time (t) would yield a straight line with correlation coefficient (r) and intercept (y) equal to A0 and the slope of the line would be equal to K0 .

First order kinetics:

$\text{Log } A = -K.t/2.3 + \text{Log } A0$

Where A = amount of drug at time (t)

T = time interval.

K = the first order constant.

By plotting of log A (amount of drug released in mg) versus time (t) would yield a straight line ,with correlation coefficient (r) and the intercept(y) equal to log A0 , the slope equals to -K/2.3, and half life equal to 0.693/k.

Higuchi-diffusion model:

Fick's second law of diffusion states that drug molecules diffuse from a region of higher concentration to a region of lower concentration.

The equation for the release rate of drugs from an ointment base derived by T. Higuchi and subsequently W. Higuchi simplified an equation to: (17)Where

Q = the amount of drug released to the membrane at time (t) per unit area contact (µg/cm2)

C0 = the initial concentration of drug in the vehicle.

D = the diffusion coefficient of drug in the vehicle.

This equation describe drug release as being linear with the square root of time or .

3.2. Microbiological part18.

In order to confirm the results obtained from release study of econazole nitrate from different formulations, a microbiological studies were done against some dermatophytes human pathogens such as Candida Albicans, Aspergillus Niger, Microsporum Canis, Epidermophyton Floccosu, Trichophyton Rubrum, Trichophyton Mentography, and against some gram positive bacteria including Staphylococcus Aureus, and Streptococcus Pyogens, according to the following method:

To sterile Sabouraud's agar plates, specific amount (2 ml) of each species of dermatophytes suspension was poured and distributed equally and then wells or pores of 5 mm in diameter was made, to these wells 20 µl of the tested formulation (PEG ointment, gel, emulgel, and o/w cream) containing 200µg of econazole was added, growth control plates of each organism without drug were made, the plates were incubated aerobically at 25°C for 21 days, the radius inhibition zone was measured by millimeter roller .About 2-5 colonies of bacteria (Staphylococcus aureus, Streptococcus pyrogens) were incubated into 5ml of sterile normal saline and turbidity was adjusted to match an opacity tube containing 0.5ml of 1% of barium chloride in 1% sulphuric acid, then by the used of a sterile cotton-wool swab, the test culture was streaked evenly over the Mueller Hinton plate.

And wells of about 5mm were made and to these wells a 20µl of different formulations containing 200µg of econazole was added, the plates were incubated aerobically at 37°C for 24 hours, the inhibition zone measured by the millimeter roller.

Results

Fig.1: Amoumts of econazole released from different bases using tea membrane

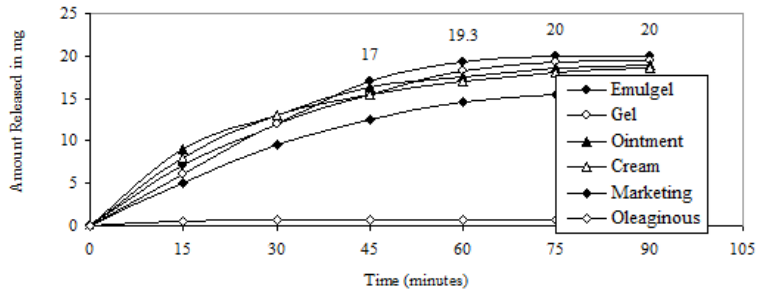

Fig.2: Amounts of econazole released from different bases using cellophane membrane

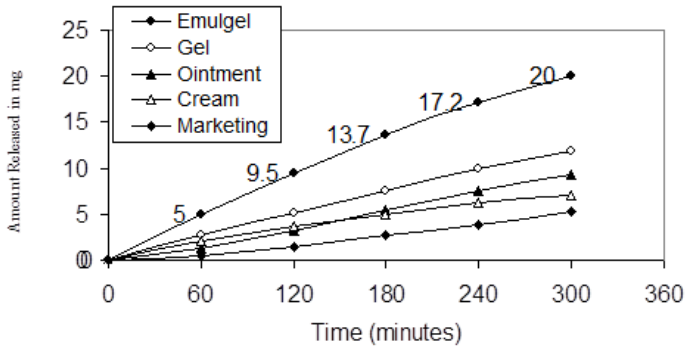

Table (1): The Kinetic data of 1% econazole released from different formulations through permeable membrane (tea bag)

The formulation	Correlation coefficient (r)			Observed order	slope	$t_{1/2}$
	Zero order	First order	Diffusion model			
Emulgel	0.950	0.830	0.980	D.M	2.27	19.4 min
Gel	0.943	0.833	0.985	D.M	2.26	19.6 min
PEG(ointment)	0.906	0.772	0.988	D.M	2.08	23.1 min
O/w cream	0.912	0.538	0.988	D.M	2.04	24 min
Market's cream	0.949	0.845	0.989	D.M	1.825	30min
Oleaginous base	0.745	0.094	0.907	D.M	0.075	12 days

D.M = Diffusion model

Table (2) : The Kinetic data of 1% econazole released from different formulations through cellophane membrane

The formulation	Correlation coefficient (r)			Observed order	slope	$t_{1/2}$ in (min)
	Zero order	First order	Diffusion model			
Emulgel	0.995	0.908	0.975	Zero	0.067	149
Ethanolic gel	0.998	0.953	0.966	Zero	0.039	256
PEG(ointment)	0.997	0.974	0.934	Zero	0.032	313
O/w cream	0.989	0.956	0.986	Zero	0.023	434
Market's cream	0.989	0.916	0.904	Zero	0.018	556

Table (3) Zone-size-Interpretative chart for different formulations against some human pathogenic fungi-strains

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Type of fungi	Radius inhibition zone in (mm) *												
	Emulgel			Gel			Ointment			O/w cream			Market Cream
	S	B	N.I.Z	S	B	N.I.Z	S	B	N.I.Z	S	B	N.I.Z	N.I.Z
Candida Albicans	32	6	26	20	0	20	20	4	16	16	0	16	14.3
Aspergillus Niger	19	4	15	14	0	14	16	5	11	11	0	11	9.5
Microsporum Canis	33	5	28	27	0	27	32	8	24	23	0	23	22
Epidermophyton Floccosum	31	2	29	16	0	16	28	13	15	12	0	12	12
Trichophyton Rubrum	40	8	32	32	0	32	39	9	30	28	0	28	27
Trichophyton Mentography	36	6	30	32	3	29	36	8	28	21	0	21	21

* Results are the mean of three experiments

S = Sample B = Blank N.I.Z = Net Inhibition Zone

Table (4): Zone-size-Interpretative chart for different formulations against some human pathogenic bacteria

Type of bacteria	Radius inhibition zone in (mm)*												
	Emulgel			Gel			Ointment			O/w cream			Market Cream
	S	B	N.I.Z	S	B	N.I.Z	S	B	N.I.Z	S	B	N.I.Z	N.I.Z
Staphylococcus aureus	13	0	13	11	0	11	11	0	11	10	0	10	7.3
Streptococcus pyrogens	5	0	5	4.5	0.5	4	4	0	4	3	0.5	2.5	2.3

* Results are the mean of three experiments

S = Sample B = Blank N.I.Z = Net Inhibition Zone

Discussion

Econazole nitrate is an antifungal agent; it is sparingly soluble in water.

The release of the drug from different bases depends on the various factors such as, the physicochemical properties of the drug and the nature of the drug-carrier matrix. Therefore, the greater release of drug is expected when there is less affinity of drug for the base.

The amount of the drug released from different bases by using different kinds of membranes were done, the following results were obtained:

- When using rabbit skin no amount of econazole penetrated through rabbit skin from the different prepared formulations, this means it have no systemic effect when used topically.
- When using tea bags it was illustrated by figure (1).
- When using cellophane membrane it was illustrated by figure (2).

The amount of econazole released from different bases can be arranged according to the following descending order:

Emulgel base > gel base >PEG ointment base > o/w cream base > market's cream > oleaginous ointment base.

It is clear that the emulgel base gave highest release, this can be attributed to the presence of certain amount of liquid paraffin in emulgel that increases the solubility of the drug, and the presence of Tween 80 (HLB value15) (8) as a hydrophilic and acts also as solubilizer, it considers as surfactant to mix the oil phase and aqueous phase and as enhancer that increase the release of the drug from the base, either methyl cellulose and avicil act as emulsifying agent and consider hydrophilic derivatives that decrease affinity of the drug to the base and increase its release.

Ethanolic gel the next one showed a higher release due to the presence of ethanol as an ingredient in the base that may enhance the solubility of the drug ,ease of its partitioning through the membrane and acts as enhancer to increase its permeability. The presence of glycerin in ethanolic gel may increase viscosity and decrease evaporation of ethanol. Also, PEG base showed a high release which may be due to that PEG may increase solubility of the drug where PEG is considered as water-soluble base and increase the release of the drug. In contrast, slow release was noticed from o/w cream duo to the presence of mixture of oil phase (paraffin oil , cetyl alcohol, span80) they increase affinity of the drug towered the base but the presence of the aqueous phase (Tween80, glycerin) enhanced the release of drug from this formulation in amounts less than that observed by emulgel, gel and PEG ointment. The oleaginous base gave the smallest release because it is completely lipophylic and held the drug firmly .

Kinetics parameters were calculated such as correlation coefficient (r) and half life time of different topical formulations through tea bags and cellophane membrane were demonstrated in table(1)and (2) respectively. From determination of correlation coefficient (r), it is clear that the order of the drug follow Higuchi-model in case of tea bag and zero-order in case of cellophane membrane.

Tables (3) showed the results of antifungal activity of econazole released from different bases, that the radius of inhibition zones can be arranged according to the following descending order:

Emulgel (13mm) > gel (11.5mm) > PEG ointment (11mm) > o/w cream (10mm) > market's cream (7.3mm) and these results were in a good agreement with the results obtained in vitro release.

Table (4), showed the results of antimicrobial activity of econazole released from different bases, that the radius of inhibition zones can be arranged according to the following descending order:

Emulgel (5mm) > gel (4mm) > PEG ointment (4mm) > o/w cream (2.5mm) > market's cream (2.3mm).

It is clear that; econazole nitrate is mainly antifungal agent, but it showed activity against some gram-positive bacteria, this may be attributed to the high degree of lipophilicity of econazole nitrate and its ability to penetrate the cell wall of gram-positive bacteria.

These results of microbiological inhibition zones were compatible to the amount of drug released obtained in case of in vitro release study.

Conclusion & Recommendation

From the previous studies (in vitro release & microbiological test) it can be concluded that:

Emulgel > gel > PEG ointment > o/w cream > market's cream

Accordingly, we recommended that:

- Econazole better to be formulated in emulgel formulation.
-Further stability study must be done for those new formulations.
- Researches must be proceeded for clinical trails on humans.

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