Formulation and Evaluation of Cimetidine as a Topical Preparation

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الخلاصة:

السميتيدين كدواء مضاد لمستقبل الهستامين 2 يستعمل بشكل واسع لعلاج قرحة المعدة بالاضافة الى تاثيراته على جهاز المناعة. تهدف هذه الدراسة الى أيجاد افضل صيغة تركيبية للسميتيدين كمستحضر موضعي. تمت دراسة تحرر السميتيدين من عدة قواعد و هي: مستحلب زيت / ماء (أ و ب), هلام الصوديوم كاربوكسي مثيل سيليلوز, و مستحلب ماء / زيت. أفضل تحرر تم الحصول عليه من قاعدة مستحلب زيت / ماء (ب) (قاعدة الكريم), لذلك تم اختيارها لاجراء باقي التجارب.

تاثير تركيز السميتيدين على سرعة نفاذ الدواء قد تمت دراسته واظهرت النتائج زيادة في سرعة النفاذ مع زيادة تركيز السميتيدين باستخدام قاعدة الكريم. تضمنت الدراسة ايضاً عملية نفاذ السميتيدين من السميتيدين 10% في قاعدة الكريم خلال قطعة مستأصلة من جلد الفأر مع عدة عوامل مسرعة للنفاذ هي: صوديوم لوريل سلفايت, بروبيلين كلايكول, صمغ الزانثان, حامض الاوليك, مثيل سالسليت, يوريا, و بولي اثيلين كلايكول 1000. وقد تبين ان الصوديوم لوريل سلفايت بتركيز 3% أعطى تأثير محسوس عالي جداً على سرعة النفاذ. أظهرت النتائج ايضا ان الحرارة و فترة الخزن أدت الى قلة في سرعة النفاذ و زيادة في اللزوجة لكريم السميتيدين 10% و تغير طفيف في الاس الهيدروجيني. كما أظهرت النتائج ان استخدام الداي صوديوم اي دي تي أي 1.0% كمادة مثبتة أعطت ثبوتية اكثر للصيغة التركيبية للسميتيدين 10% في قاعدة الكريم حيث ان تاريخ النفاذ اصبح 4.09 سنة مقارنة ب 2.74 سنة عند استخدام 1.0% دل – الفا – توكو فيرول كمادة مانعة للأكسدة و 1.35 سنة للسميتيدين كريم لوحده (اي خالي من مادة مثبتة او مانعة للأكسدة).

تم اجراء دراسة تمهيدية سريرية على 15 مريض, 10 منهم مصابون بالثأليل و 5 مصابون بداء الثعلبة النسائي لارتفاع الاندروجين الكظري.و أظهرت النتائج ان 80% من المرضى المصابين بالثأليل قد اعطوا استجابة ايجابية و 40% من المصابين بداء الثعلبة النسائي لارتفاع الاندروجين الكظري قد اعطوا استجابة ايجابية.

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ABSTRACT

Cimetidine is a H_2 – receptor blocking drug, widely used for the treatment of stomach ulcer in addition to its immune response modifying activity. This study is carried out to formulate cimetidine in a stable topical preparation.The in vitro release of cimetidine from different semisolid bases was studied utilizing: o/w emulsion base A and B, sodium carboxymethylcellulose gel, and w/o emulsion base. The results showed that the best release was obtained from the o/w emulsion base B (i.e. cream base); hence it was selected for extensive studies.

The effect of cimetidine concentration on its diffusion through excised mouse skin was also investigated and the data showed an increase in the diffusion rate with increasing cimetidine concentration using cream base. The study also involved the diffusion of the drug from cimetidine 10% w/w cream base through excised mouse skin with different penetration enhancers utilizing: sodium lauryl sulphate, propylene glycol, xanthan gum, oleic acid, methyl salicylate, urea, and PEG_{1000}. The study showed that 3% w/w sodium lauryl sulphate had a very highly significant effect on the diffusion rate of cimetidine. The study also showed that the temperature and the storage period led to a decrease in the diffusion rate, increase in the viscosity of cimetidine 10% w/w cream formula and a little change in the pH. The use of EDTA disodium 1.0% w/w as a stabilizer gave a more stable formula for cimetidine 10% w/w cream formula where the expiration date was 4.09 years compared to 2.74 years when using 1.0% w/w dl – α tocopherol as an antioxidant, and 1.35 years for the blank formula (i.e. formula free from antioxidant and stabilizer).

Preliminary clinical study was performed among fifteen patients, ten with warts, and five with adrenal androgenic female – pattern alopecia. The results indicated that 80% of patients with warts gave positive response, while, 40% of patients with adrenal androgenic female – pattern alopecia gave positive response.

INTRODUCTION

Cimetidine [N – cyano – N' – methyl – N'' – (2 – ((5 – methyl – 4 – imidazol – 4 – yl) methyl) thio) ethyl) guanidine] (CMT) is a drug largely used in medicine for its protective action on stomach walls in ulcer diseases, due to its H_2 receptor blocking effect. The drug is orally and intravenously administered and reaches H_2 – receptors via blood – stream [(1)]. CMT has an immune response modifying activity. It enhances both humoral and cell – mediated immunity by preventing activation of T – suppressor cells through blocking histamine action[(2)]. Oral administration of CMT showed antiandrogenic effect through competition and displacement of these androgens from androgen receptors [(3)].

The aim of this work includes: formulation of CMT in different topical bases and studying the release and diffusion of the drug from these bases. The effect of drug concentration, also the type and concentration of different penetration enhancers on the diffusion rate, in addition to the effect of storage time and temperature on the viscosity, diffusion, physical properties (color and odor), and pH were also studied. Determination of shelf life, and preliminary clinical trials were carried out in a hope to have stable and effective topical preparation of the drug.

MATERIALS AND METHOD

Materials

CMT, methyl hydroxybenzoate, propyl hydroxybenzoate, dl – α tocopherol, EDTA disodium, and white bees wax (supplied by S. D. I. / Iraq). PEG1000, trypan blue, disodium hydrogen phosphate, propylene glycol, liquid paraffin, and white soft paraffin (Merck – Schuchardt / Germany). Cetyl alcohol, sodium lauryl sulphate, urea, glycerol, histamine dihydrochloride, diethyl ether, and sodium carboxymethylcellulose (BDH chemicals LTD, Poole / England). Wool fat (Riedel – De – Haen AG, Seelze – Hanover / Germany). Potassium dihydrogen phosphate and oleic acid (Hopkins and Williams LTD / England). Xanthan gum (supplied by Al – Safa factory / Iraq). Methyl salicylate (W. J. Bush Lab. / France).

Method

Preparation of Bases

The general method employed for the preparation of bases was the fusion method. The drug was incorporated by trituration. Four types of semisolid bases (4) were selected to study their effects on the release of the drug:-

A-Water – in – Oil emulsion base (wool fat and water).

B- Oil – in – Water emulsion base A (white bees wax, cetyl alcohol, propylene glycol, sodium lauryl sulphate, and water).

C- Oil – in – Water emulsion base B (white soft paraffin, liquid paraffin, cetostearyl alcohol, methyl hydroxybenzoate, propyl hydroxybenzoate, and water).

D- Sodium carboxymethylcellulose gel (sodium carboxymethylcellulose, glycerol, methyl hydroxybenzoate, and water).

In Vitro Release of Cimetidine from the Semisolid Bases

A small funnel with a diameter of 3 cm was modified in order to be filled with 5 grams of each base containing 5% w/w of CMT. The mouth of the funnel was covered with a filter paper, which was secured in place with a rubber band. The dialysis cell was inverted and immersed to within 0.5 cm of its surface in 500 ml phosphate buffer pH 7.4 contained in a flask of the dissolution apparatus (collecting medium). The flask was partially immersed in a large water bath at a constant temperature of 37Cº inside the dissolution apparatus. The stirrer was immersed in the collecting medium and the stirring rate was maintained at 100 r.p.m. (5). The release of CMT was followed by monitoring the receiver medium concentration for 6 hours. Five milliliters samples were withdrawn with a pipette from the collecting medium after 1/4, 1/2, 1, 2, 3, 4, 5, and 6 hours and replaced with an equal volume of fresh phosphate buffer pH 7.4 at 37Cº. The samples were then analyzed for their drug (CMT) content using U. V. spectrophotometer at its λmax (260 nm).

Preparation of Mouse Skin (i.e. Diffusion Membrane)

The preparation of mouse skin was carried out according to Skelly and co – workers method (6). The full thickness skin from abdominal surface of 15 – 20 grams, 4 – 6 weeks old, male mouse was taken, prepared, defatted, and frozen until use.

In Vitro Diffusion of Cimetidine through Mouse Skin from the Semisolid Bases

The diffusion cell (small test tube with a diameter of 1.4 cm) was used in this study. The excised skin was placed on the diffusion cell with the stratum corneum facing the donor compartment (the diffusion cell) and the dermis facing the receiver compartment (phosphate buffer pH 7.4). A quantity of 2.5 grams of each base containing 10% w/w of CMT was spread on the stratum corneum surface of the mouse skin. The skin was stretched over the mouth of the test tube. Care was taken to bring the entire exposed surface of the base in contact with the membrane. The skin was then legated with a cotton thread in order to be fixed on the surface of the diffusion cell. The diffusion cell was immersed (to within 0.5 cm of the surface) in 500 ml of phosphate buffer pH 7.4 contained in a flask of the dissolution apparatus (collecting medium). The flask was also immersed in a large water bath at a constant temperature of 37Cº inside the dissolution apparatus. The stirrer was immersed in the collecting medium and the stirring rate was maintained

at 100 r. p. m. (7). The diffusion of CMT was also followed by monitoring the receiver medium concentration for 6 hours. Five millimeter samples were withdrawn with a pipette from the receiver compartment after 1/4, 1/2, 1, 2, 3, 4, 5, and 6 hours and replaced with an equal volume of fresh phosphate buffer PH 7.4 at 37C°. The samples were then analyzed spectrophotometrically for their drug (CMT) content using U. V. spectrophotometer at its λmax (260 nm).

Effect of Different Concentrations of Cimetidine on the Diffusion Process:

Different concentrations of cimetidine (5%, 10%, and 15% w/w) were used to study the effect of cimetidine concentration on the diffusion process, using o/w emulsion base (B).

Effect of Different Possible Enhancers and their Concentrations on the Diffusion Process of Cimetidine 10 % w/w Cream:

For the selected base, the effect of sodium lauryl sulphate (SLS) (1%, 2%, and 3% w/w), propylene glycol (PG) (5%, and 20% w/w), xanthan gum (XG) (2%, and 4% w/w), oleic acid (OA) 10% w/w, urea 10% w/w, methyl salicylate (MS) 15% w/w, and polyethylene glycol (PEG 1000) (5%, and 10% w/w) on the diffusion of CMT 10% w/w cream was investigated.

Irritancy Study

Since most of the useful skin medications have a low level of irritancy, the detection and estimation of low levels is very important. For the estimation of irritancy of substances applied repeatedly to the skin of humans, the following test has given a good correlation between the results obtained with human volunteers (8). A group of three male albino rabbits weighing approximately 1kg were used to do the irritancy test on the selected formula. The ventral side of the animal was carefully shaved, and four circular areas of 2.5 cm in diameter were drawn on the animals' abdomen. The circumscribed areas were painted with 20% aqueous solution of formaldehyde. The solution was allowed to evaporate and this process was repeated three times. The cimetidine 10% w/w cream, cream base alone, and cimetidine powder were impregnated separately on 2.5 cm circular cotton pads, these pads were applied on three of circular areas and a substance of known irritancy (histamine) was injected intradermally into the fourth circular area for the purpose of calibration. The back of the animal's ear was shaved carefully, xylene was used to dilate the superficial ear vein, and approximately 1ml of (0.5%) trypan blue was slowly injected through the selected vein of the animal. The degree of irritancy of the substance is estimated by the accumulation of trypan blue at the treated site. The degree of blueness at the treated site is visually ranked to provide a relative order of irritancy of the substances used. The treated sites were observed after 1, 6, 12, and 24 hours.

Effect of Storage Time and Temperature

To 10% w/w CMT in the selected base; 0.2% w/w methyl hydroxybenzoate and 0.1% w/w propyl hydroxybenzoate were added as preservatives. The formulas were kept in airtight collapsible tubes at 40Cº, 50Cº, and 60Cº (in addition to room temperature), for suitable period of time (9). The effects of the followings were studied:-

A-Effect of storage Time and Temperature on the Diffusion of the Drug through mouse skin:

Samples from the selected formula at 40Cº, 50Cº, and 60Cº were subjected to the in vitro diffusion study after 15, 30, 45, and 60 days.

B-Effect of storage Time and Temperature on the Physical Properties (Color and Odor) of the Selected Formula

The physical properties (color and odor) of the selected formula were observed over the storage period of 120 days at room temperature.

C-Effect of storage Time and Temperature on the pH of the Selected Formula

The pH of the cream was measured at room temperature using pH meter at time intervals of 1st day, 15, 30, 45, and 60 days by taking 2 gm of the cream and shaking up with 10 ml of water.

D-Effect of storage Time and Temperature on the Viscosity of the Selected Formula

The viscosities of the selected formula which contains 10% w/w CMT and blank formula (formula free from CMT) were determined at room temperature, 40Cº, 50Cº, and 60Cº for 15, 30, 45, and 60 days using rotational viscometer by spindle no. L4.

E- Shelf – Life Determination

CMT concentration in the stored formulas which contained EDTA disodium as an antioxidant and dl – α tocopherol as a stabilizer separately in addition to the blank (original) formula at three different temperatures (40Cº, 50Cº, and 60Cº) was checked every 15 days for 120 days. This was done by taking one gram from the formula and shaked with 25 ml of distilled water for 5 minutes. The mixture then filtered and 1 ml of the filtrate was taken and diluted to 10 ml with distilled water and analyzed spectrophotometrically for drug content at its λmax (260 nm) (10). Working standard curve for CMT in the cream base was prepared following the same procedure.

Preservative Efficacy Test

Challenge test was used to determine the efficacy of the preservatives methyl hydroxybenzoate 0.2% w/w and propyl hydroxybenzoate 0.1% w/w added to the selected formula. The U.S.P. 24 procedure of the test was applied (11).

Preliminary Clinical Study

A total of fifteen patients, ten with warts, and five with adrenal androgenic female – pattern alopecia were enrolled in the study. Patients were classified according to disease into:-

a- Patients with Warts

Ten patients with different wart types (common and plane warts), their ages range between 7 – 26 years old were given the selected formula containing 10% w/w CMT to be applied topically once at night. The patients were instructed to soak the warts in water for about five minutes in order to soften the keratin surface. Then remove the keratin surface with an abrasive material. After that, the selected formula applied with a tape to cover the area and facilitate the penetration (12). The patients were instructed to visit the clinic every week to follow up the treatment.

b- Patients with Adrenal Androgenic Female – Pattern Alopecia

Five female patients with adrenal androgenic female – pattern alopecia were selected, their ages range between 23 – 37 years old. The patients were instructed to apply the selected formula three times daily and to visit the clinic every week to follow up the treatment.

Statistical Analysis

Statistical comparisons were made using student t – test (two – tailed t test analysis) and P – value for the test:-

- Significant ($P < 0.05$).

- Highly significant ($P < 0.001$).

- Very highly significant ($P < 10\text{-}5$).

Bar chart was used to show the differences in mean values for each formula. The correlation coefficient (r) was used to test the relation between two parameters.

RESULTS AND DISCUSSION

Figure (1) shows the differences in the release of CMT from different semisolid bases containing 5% w/w of the drug. The differences in the release as well as the diffusion were significant and in the following order:- o/w emulsion B > sodium carboxymethylcellulose gel > o/w emulsion A > w/o emulsion. The presence of white soft paraffin and liquid paraffin in the content of o/w emulsion B (Cream Base) makes the concentration of CMT in the aqueous phase greater than the oil phase, since there will be reduction in CMT partition coefficient (4). Since o/w emulsion B base gave the highest release and diffusion for the drug, hence it was selected for extensive studies. The amount of cimetidine diffused increased with increasing concentration of cimetidine and in the following order 15% > 10% > 5%, and this indicates that the penetration rate is proportional to concetration and it is in a good agreement with reported for other drugs (13).

The effect of possible penetration enhancers on the diffusion of CMT through mouse skin is shown in figure (2). There was no significant effect of propylene glycol (PG) 20% w/w as a co solvent, while, PG 5% w/w showed a significant increase in the diffusion process of CMT, and the diffusion decreased as the concentration of PG increased in the following order:- 5% PG > zero% PG > 20% PG. This is because; concentrated PG solutions interact with skin causes dehydration of corneocytes due to the hygroscopic nature of PG, while, the diluted PG solutions offer the possibility to PG and water to incorporate into the stratum corneum through the lipid domain, the protein domain, or both to modify the solubility of permeants in the tissue (14).

Xanthan gum (XG) 2% w/w led to a significant increase, while, 4% w/w gave a highly significant effect on the diffusion of CMT. The increase in diffusion rate constant could be due to the micro – emulsification of the drug and the effect of XG as a thickening agent (15). The addition of 10% w/w oleic acid (OA) and 15% w/w methyl salicylate (MS) led to a significant decrease in the diffusion of the drug and this could be attributed to the changes in the texture consistency of the selected base and formation of fluidly cream that resembles lotions (16, 17). The addition of 10% w/w urea leads to a significant effect on CMT diffusion. When 5% w/w and 10% w/w of PEG1000 were added to the selected base led to a significant and a highly significant increase of CMT diffusion, respectively, and this attributed to the significant effect of polyethylene glycols on drug penetration when skin structures were hygroscopically manipulated (18).

The addition of sodium lauryl sulphate (SLS) at 1% and 2% w/w led to a highly significant effect, while, 3% w/w gave a very highly significant effect on the diffusion process of CMT. This can be attributed to the ability of SLS to alter drug solubility and affecting drug concentration in the vehicle as well as altering the skin barrier through surfactant – membrane interaction (19). Therefore, 3% w/w SLS was chosen to be added to the selected base, to which methyl hydroxybenzoate 0.2% w/w and propyl hydroxybenzoate 0.1% w/w were added as preservatives, hence the selected formula was subject for further studies. The irritancy test showed that the selected formula was not irritant when applied topically.

This study showed a significant decrease in the diffusion of CMT from the selected formula with the increase of storage time at higher temperature (40Cºand 50Cº) as shown in figures (3) and (4), this might be due to the degradation of CMT. There was no change in the color and odor with a negligible change in the pH of the selected formula stored at room temperature. This study showed an increase in the viscosity of the selected formula upon storage at room temperature and at 40Cº and 50Cº (Table 1), and this is in agreement with that reported for emulsions viscosity which generally increases upon aging (20).

It was found that the degradation of CMT in all formulas that contain EDTA disodium as an antioxidant agent, dl – α tocopherol as a stabilizer agent, and the blank one, follow first order kinetic, since straight lines were obtained when Log % remaining concentration of CMT was plotted versus time as shown in figures (5), (6), and (7). The observed degradation rate constants (K) at 40Cº, 50Cº, and 60Cº were calculated from the slopes of the lines for the three different temperatures. Arrhenius plot (21) was utilized to determine the expiration date at 25Cº for all formulas and it was found that the expiration date of CMT 10% w/w cream with 1.0% w/w EDTA disodium (4.09 years) is higher than that of formula with 1.0% w/w dl – α tocopherol (2.74 years) and the blank formula (1.35 years). This indicates that the addition of EDTA disodium increases the stability of cimetidine cream. Same result obtained with the addition of dl – α tocopherol but to a less extent. The preservatives used were effective and they met the requirements of U.S.P. 24.

The results of the preliminary clinical study indicated that, 80% of patients with warts showed positive response, whereby 60% completely cured after 35 days of treatment, while, 20% completely cured after 45 days. The clinical study also showed that 40% of patients with adrenal androgenic female – pattern alopecia who applied CMT 10% w/w cream on their scalp showed regrowth of hair after two months of treatment.

The overall results showed that suitable, stable, effective and safe topical formula for CMT can be prepared and used successfully for treatment of some dermatological problems without adverse effect. Further clinical studies are required to study the effectiveness of the selected formula for the treatment of other skin diseases.

Table (1): Effect of storage time and temperature on viscosity of cimetidine 10% w/w cream.

Storage time (days)	Viscosity (centipoise)					
	Room temperature		40C°		50C°	
	Test	Blank	Test	Blank	Test	Blank
15	30700	52470	32550	53400	34670	69700
30	34110	54746	35446	55090	40530	71110
45	36010	55610	41446	56350	60900	73170
60	50700	63470	55430	64400	62900	81900

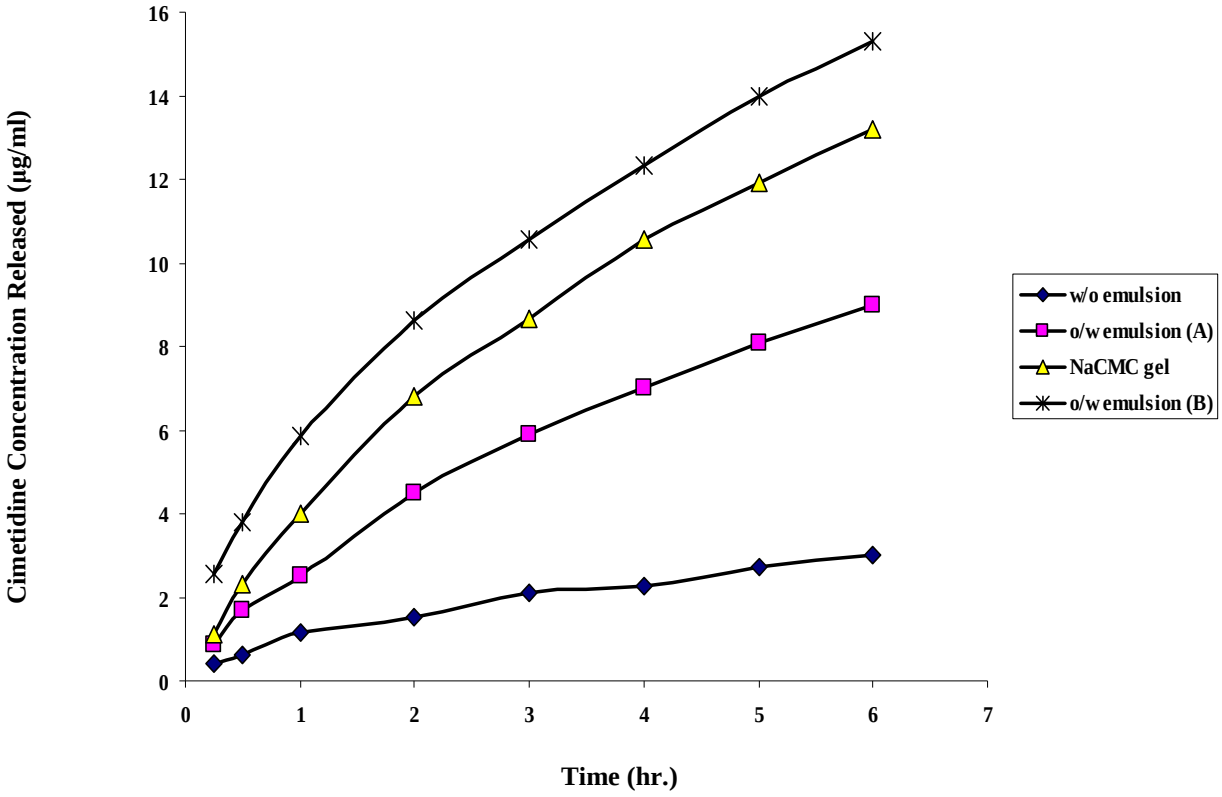

Figure (1): Effect of different bases on the release of cimetidine 5% w/w

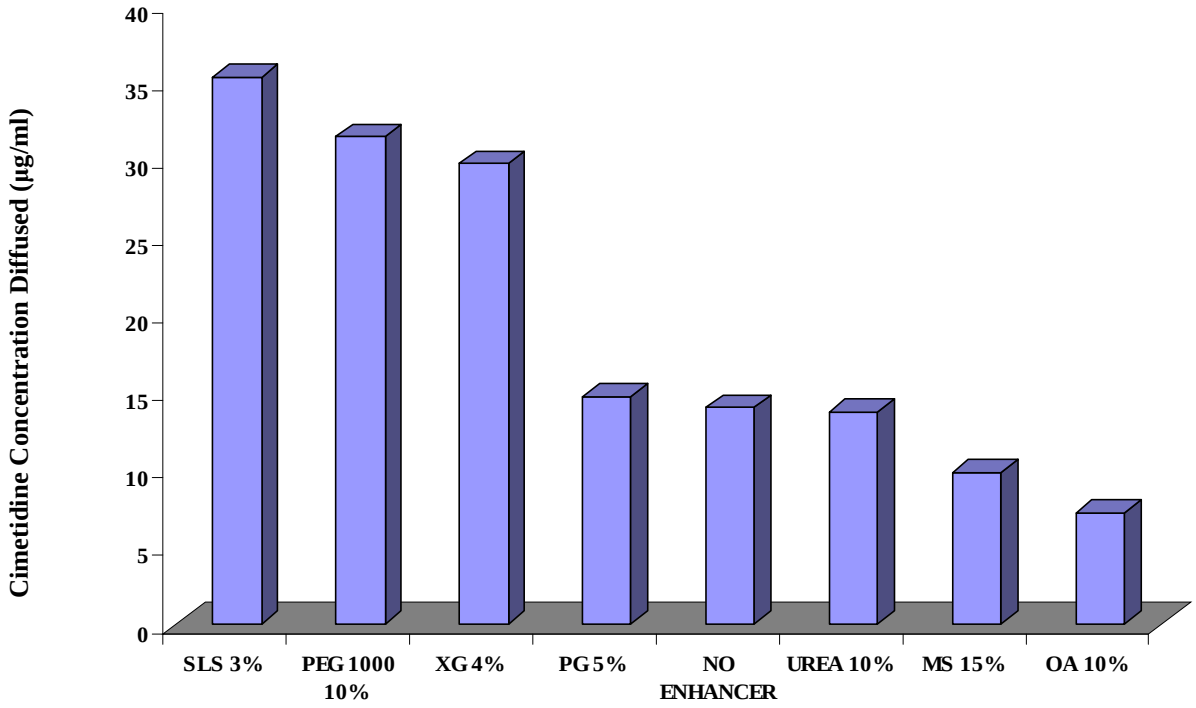

Figure (2): Effect of different enhancers on the diffusion of cimetidine 10% w/w through mouse skin.

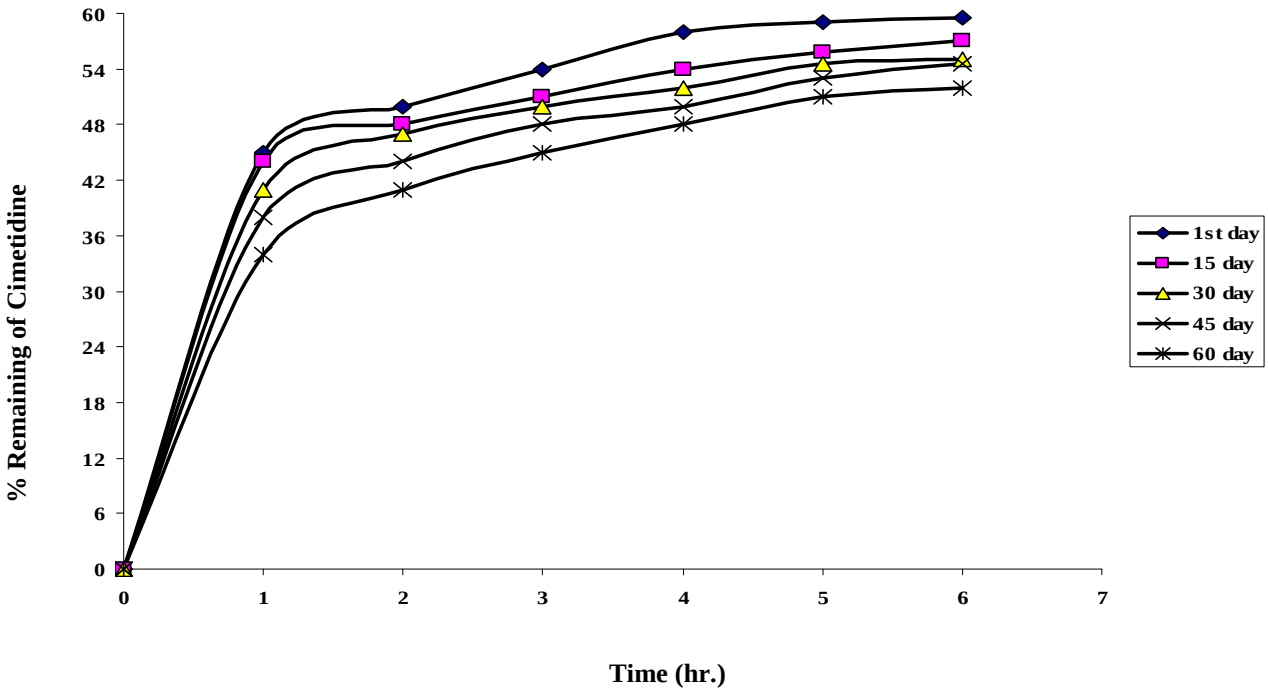

Figure (3): Effect of storage time on the diffusion of cimetidine from the selected formula through mouse skin at 40C°.

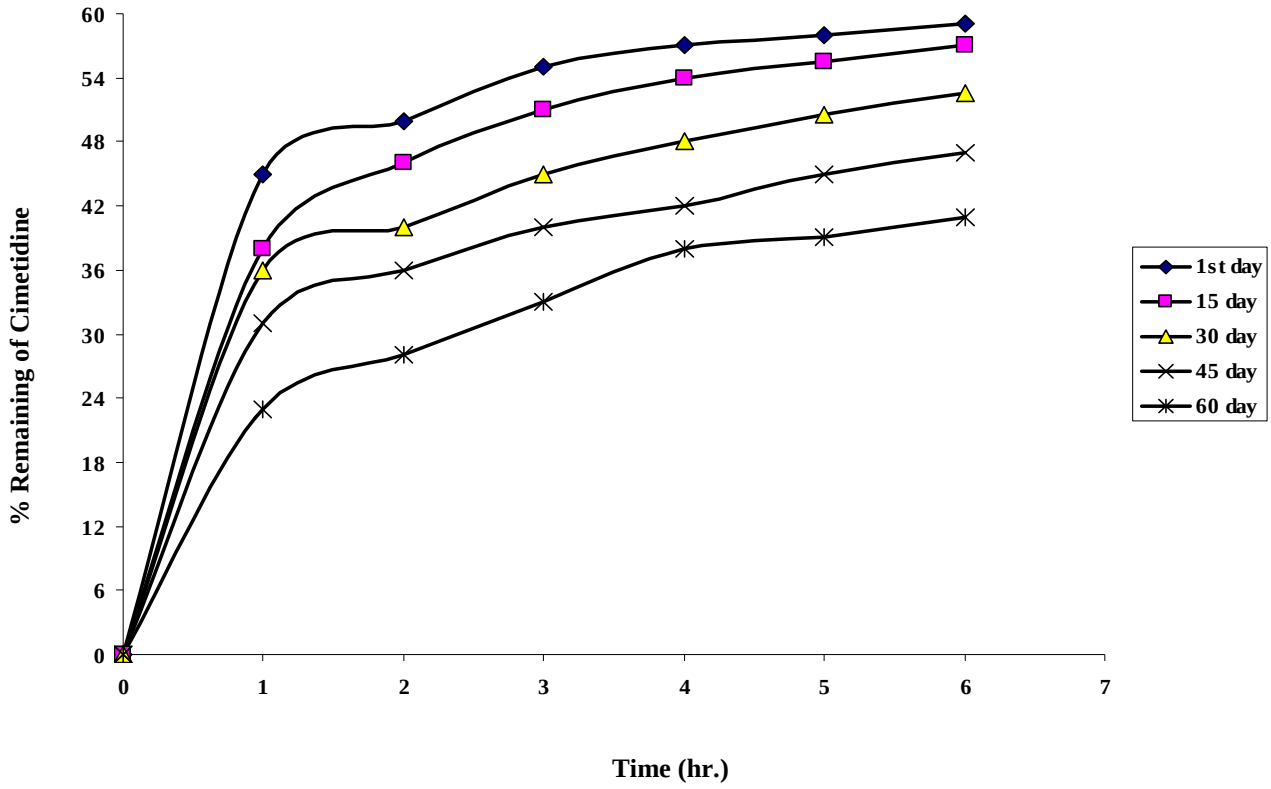

Figure (4): Effect of storage time on the diffusion of cimetidine from the selected formula through mouse skin at 50C°.

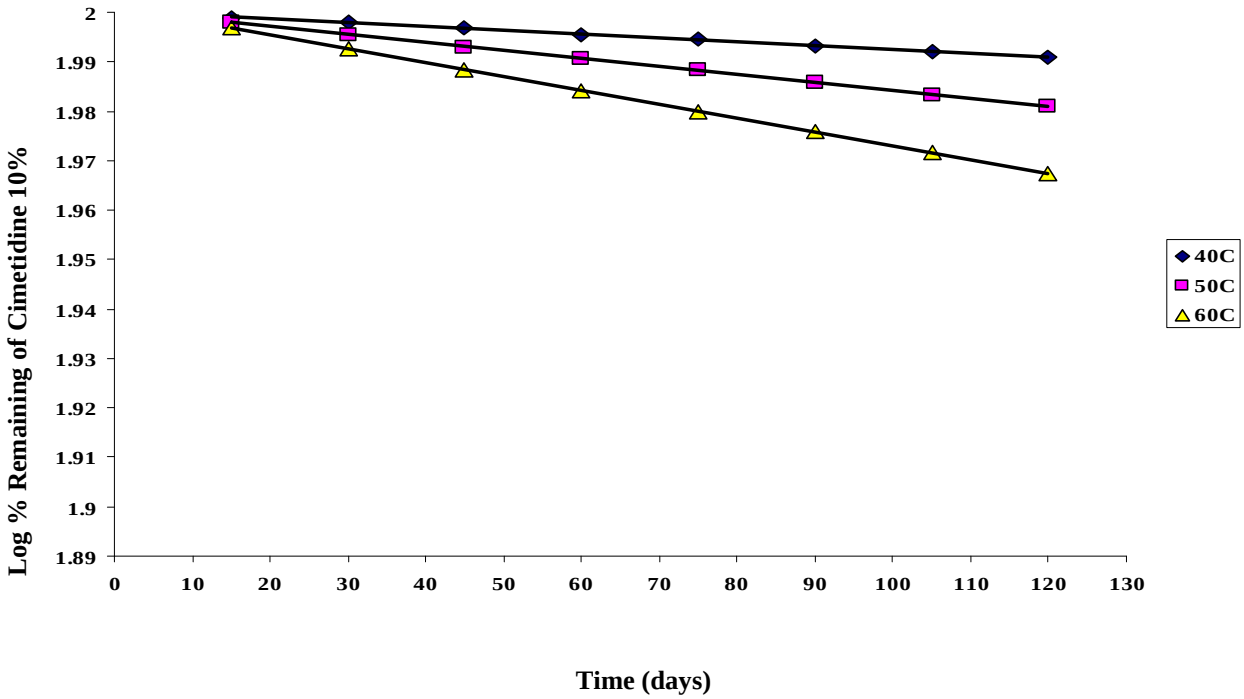

Figure (5): Degradation curve of cimetidine at 40C°, 50C°, and 60C° for formula with 1.0% w/w EDTA disodium.

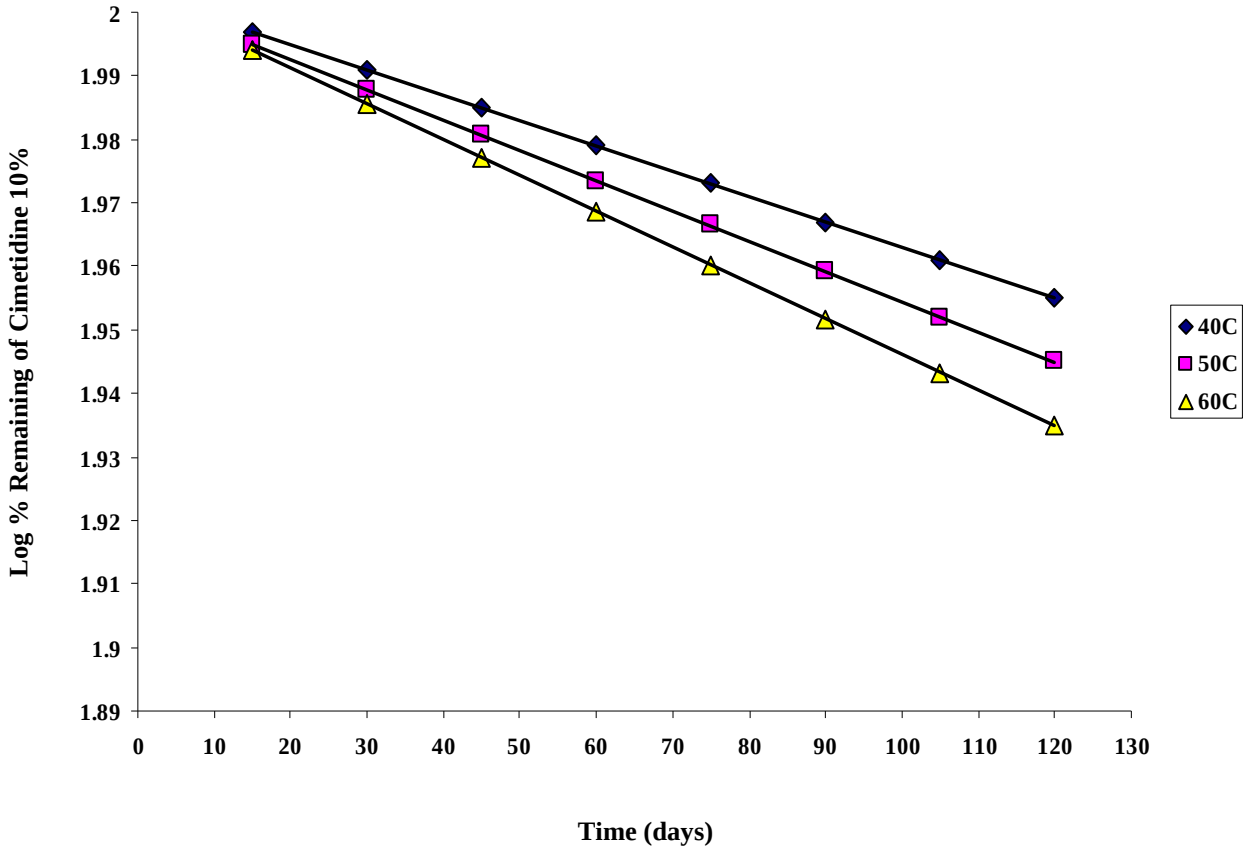

Figure (6): Degradation curve of cimetidine at 40C°, 50C°, and 60C° for formula with 1.0% w/w dl – α tocopherol.

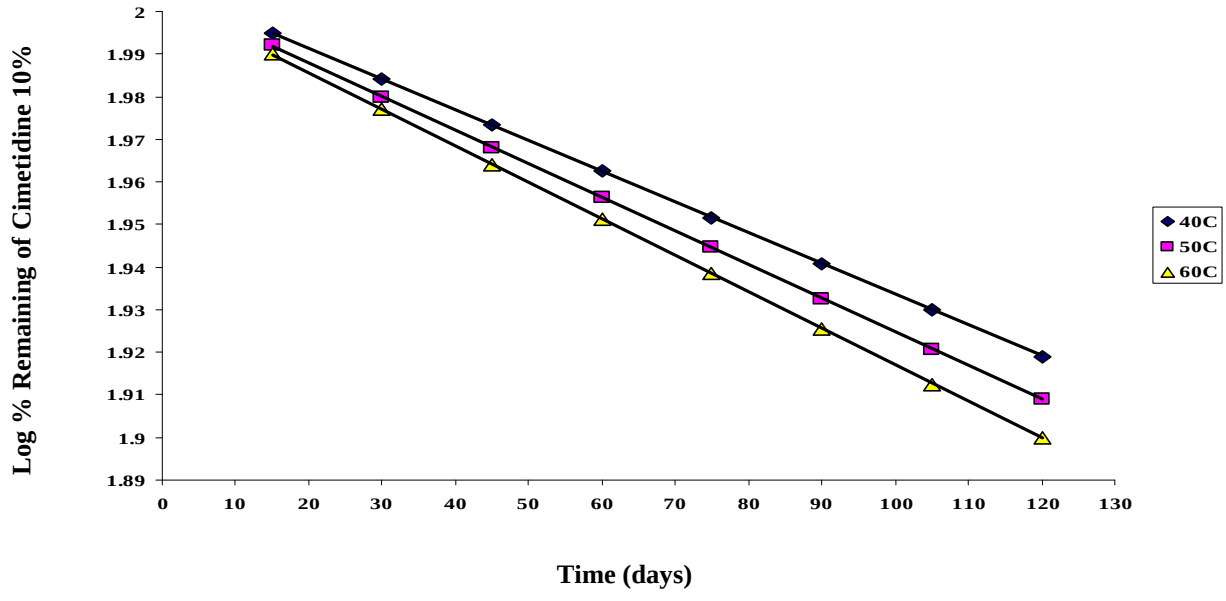

Figure (7): Degradation curve of cimetidine at 40C°, 50C°, and 60C° for blank formula.

A case of 24 years old female suffering from androgenic alopecia:

Figure (8): Before treatment.

Figure (9): After two months of treatment with cimetidine cream.

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