

Clotrimazole for Treatment of Fungal Skin Infections Disease

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Abstract

The research includes the preparation of a pharmaceutical composition of Clotrimazole at a concentration of 2.5%, which is used to treat bacterial and fungal infections of the skin of field animals, which mainly work on ring worms, eczema (atopic dermatitis) and candidiasis. The initial biological laboratory tests were conducted for the preparation and it was 100% in conformity with the pharmaceutical specification and within the constitutional limits (110-90) % according to the British Pharmacopoeia. The stability was studied at temperatures (40, 30) °C and relative humidity (70, 75) %, respectively, for a period of six months. The prepared composition proved its ability to heal the wound by acting as an anti-oxidant to preserve the tissue from external influences that hinder the healing process and activate the formation of fibroblasts and collagen in the damaged layer and thus the formation of granulation tissue that accelerates healing. The results of the research were enhanced by sending samples to the veterinary department/ veterinary hospital, where it was tested on a cases of skin fungi in the face and legs in poultry, about 24, and the response to treatment was good through the improvement of the condition, and an acceptance certificate was obtained from the veterinary hospital.

1. Introduction

Clotrimazole, antifungal, chemical name: 1-[(2-Chlorophenyl)diphenylmethyl]-1H-imidazole is shown in Figure (1). Clotrimazole is a white or light yellow crystalline substance that is insoluble in water and belongs to a broad-spectrum synthetic imidazole used to treat skin infections caused by various types of dermatophyte infections. It is an antifungal agent used to treat various organisms from *Dermatophytes sp.*, *yeast* and *sp. Malassezia* caused mild skin infection, *Sporotrix schenki* causing severe skin infections and *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Candida albicans* may cause life-threatening [1] Therefore, it is considered one of the anti-fungal drugs that is used to treat and prevent fungi such ringworm, candidiasis (thrush), and serious systemic infections such as streptococcal meningitis and others, which are spread among field animals such as calves, sheep and poultry fields [2].

Clotrimazole mechanism of action: The substance works to kill the fungi by interacting with the cell membranes of the fungus cells causing the disease, leading to a hole in the membrane and the leakage of its components to the outside, leading to the death and decomposition of the fungus [3]. Clotrimazole is used to treat a number of infectious skin diseases, including the ringworm, which affects cows and calves in particular [4]. In addition to

Candidiasis caused by the fungus (*Candida albicans*), where the infection is superficial and sometimes may extend to the mucous membranes in field animals [5].

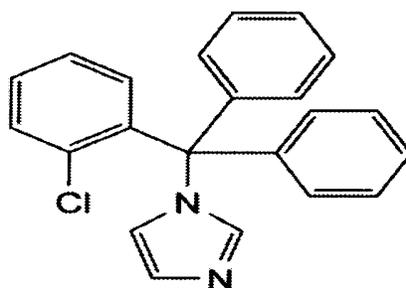


Figure (1). Shows the molecular structure of clotrimazole.

2. Materials and Method

The chemicals and devices used in the composition as shown in Table (1).

Table (1). The specifications of the active substances and additives.

Substance name	Description	Solubility
Clotrimazole	It appears as a white Crystalline	Ethanol
Ethanol	colorless liquid	
Propylene glycol	colorless liquid which is nearly odorless	
D.M.SO(<i>Dimethyl sulfoxide</i>)	colorless liquid	
Glycerin	colorless, odorless	

Materials used in the composition and their weights are shown in Table (2). Each 100ml of contains:

Table (2). Composition and their weights.

No.	Material	Weight
1	Clotrimazole	2.5g
2	Ethanol	15ml
3	Propylene glycol	30ml
4	D.M.CSO (<i>Dimethyl sulfoxide</i>)	10ml
5	Glycerin	42.5

The composition was prepared by the following steps:

1. I weighed 2.5 g of the active substance (Clotrimazol).
2. The active substance was dissolved in 15 ml of Ethanol.
3. Adding the following materials with continuous stirring and heating at a temperature of 60C°:
 - 30ml of Propylene glycol.
 - 10 ml of Dimethyl sulfoxide.
4. Complete the volume with Glycerin to 100ml.

2.1. Biological Examination

The biological examination was carried out to show the extent of the biological activity on different types of bacteria and yeast and the diameter of inhibition according to Table (3) and enhanced with Figures (4, 5, 6, & 7) and a culture medium (Potato dextrose agar) was used with a comparison of the prepared composition with the standard substance Blank. That contain all additives in the formulation except the active ingredient Clotrimazole to determine the effect of the active ingredient only [6, 7].

2.2. Laboratory Animals

The white mice approved by the Animal House / Al-Razi Research Center were randomly divided into 3 groups consisting of (5) mice in each group, their weights ranged between 23-25 g, each of them was placed in a separate cage with the provision of appropriate conditions. The treatment continued daily until recovery, as follows: The first group: 5 mice were treated with clotrimazole 1% swabs combination daily for two weeks. The second group: 5 mice were treated with an artificial formulation of cephalixin ointment 0.5% daily for two weeks. The third group: contains 5 mice, the control group was left untreated for two weeks. The effectiveness of the formulation was examined compared to the control group in treating open wounds in laboratory animals (mice), the duration of the examination lasted 15 days, starting from the stage of preparing laboratory animals (mice), which lasted for a week, and then the stage of making the wound and noticing bleeding, then treatment with the ointment, with continued treatment daily until The state of healing occurs and the skin of the laboratory animal returns to its normal position, as follows: the method of wounding: First: The mice were placed in cages with the provision of appropriate conditions and making sure that there was no injury or accident that would prevent the examination process. Second: A wound was made in the dorsal region of the rats, measuring 3 cm, using a surgical scalpel. Third: leaving the wound open with continued bleeding until treatment. Fourth: treating mice with ointment and according to the groups until healing occurs, recording the observations that occur during the treatment process and observing the behavioural changes shown by the animal during it compared to the control group as shown in Figures (8 & 9).

Stability study: The stability of the product was monitored at (30 - 40) °C for a period of 6 months, the initial evaluation was zero time (100%).

3. Results and Discussion

The pharmaceutical composition was prepared within a group of homogeneous materials in terms of its biological effect on bacterial and fungal infections of the skin, and these substances include alcohol (Ethanol) as a solvent for the active substance because it is insoluble in other solvents and glycerin as an antibacterial in addition to being a moisturizer and disinfectant for the skin and to complete the size of the prepared formula Also, Propylene glycol has been added, which gives greater stability to the active substance because it is a preservative. The initial biological examination of the preparation was carried out and it was 100%, which is considered within the constitutional limits of veterinary drugs (90 – 110) %. The stability of the composition was studied by biological examination after storing the composition in follow-up ovens for six months with different temperatures at 30°C and 40°C and relative humidity (70, 75) %.

Where the results showed the stability of the composition at 99.8%. Table (4) which is evidence of the efficiency of the preparation and the ability of the composition to withstand the heat of the ovens with continued storage for 6 months at temperatures ranging between (30, 40) °C with a slight change in the level. This was explained by the sliding Figures (2 & 3) that the homogeneity of the substances in the formulation led to an increase in the bonding between the components of the drug formulation and its tolerance to storage conditions. By conducting the calculations for the stability study to calculate the validity date, it was found that the validity period is (three years and five months) from the date of preparation.

The toxicity examination of clotrimazole preparation was carried out in the treatment of open wounds in mice after 15 days of daily use of the substance, where the combination proved its therapeutic efficacy through the results obtained in comparison with an external product and in the presence of a control model as shown in the Figure (2) of the experiment, and it represents Effects of the active substance Clotrimazole. A field evaluation of the formulation was also conducted in the Veterinary Hospital / Baghdad, and the report proved the efficacy of

Clotrimazole in treating cases of exogenous skin fungi, especially ringworm, in poultry. The results were very good with a quick response and within a period of seven days from the start of treatment.

Table (3). Inhibition zone diameter according to bacteria and yeast compared to the standard substance.

Sample	<i>E.Coli</i> ATCC 10536	<i>Staphylococcus aureus</i> ATCC 6538	<i>Pseudomonas</i> ATCC 15442	<i>Candida albicans</i> ATCC 10231
Formula 1	33mm	31mm	32mm	26mm
Formula 2	32mm	31mm	31mm	25mm
Clotrimazole st	32mm	32mm	31mm	25mm

Table (4). The concentration of the active substance during the stability period after storing the product at temperatures (30 - 40) °C and (70, 75) % humidity, respectively.

No	Time	30°C and 70% Humidity	40°C and 75% Humidity
1	After three month	100.5%	100%
2	After six month	100.3%	99.8%

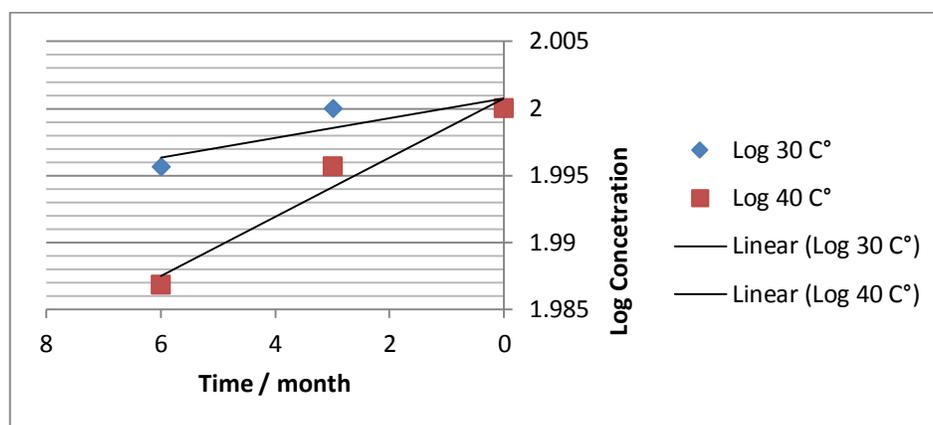


Figure (2). Clotrimazole storage at (30 - 40) °C and relative humidity (70 - 75) %, respectively.

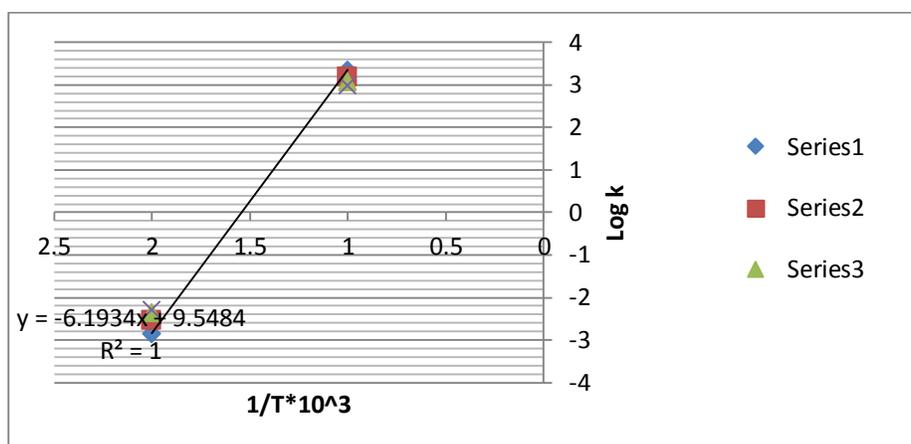


Figure (3). Relationship diagram between Log k and $1/T \times 10^3$ of Clotrimazole formula.

Through the calculations of the stability study and knowledge of (intercept and slope) according to Figure (2), it was found that the age of the product from the date of preparation is three and a half years, which is equivalent to three and a half years from the date of preparation.

3.1. Field Assessment

Samples were sent to the Veterinary Hospital/Baghdad for the purpose of evaluating the formula on cases of fungal infections of the skin, where it was tested on chickens infected with fungi, and the treatment results were very good in response to the treatment as shown in Table (5).

Table (5). Field assessment.

Type of animals	Poultry
No. of animals	24
Pathological case	Fungal infection on the face and legs
Treatment Result	Very good
Side effect	non
Committee opinion	Recommend the use of the treatment for its effectiveness



Figure (4). Inhibition zone of clotrimazole formula against *Pseudomonas sp.*



Figure (5). Inhibition zone of clotrimazole formula against gram negative bacteria (*E. coli*).

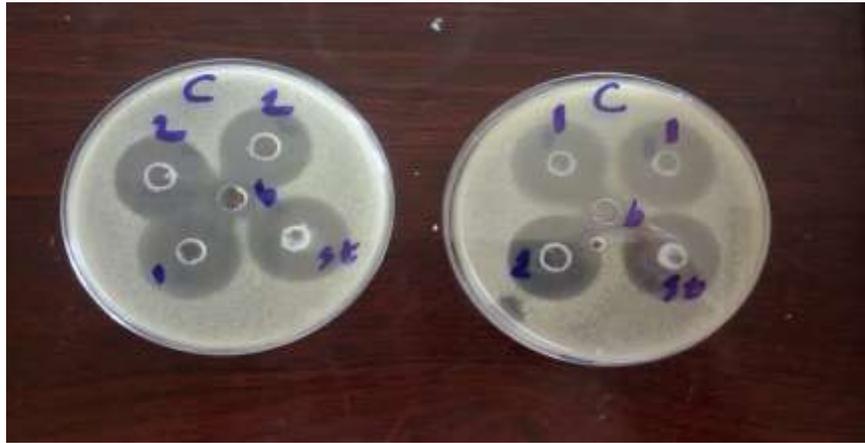


Figure (6). Inhibition zone of the preparation on *Candida albicans* yeast.



Figure (7). The inhibition zone of the preparation on *Staphylococcus aureus* bacteria.

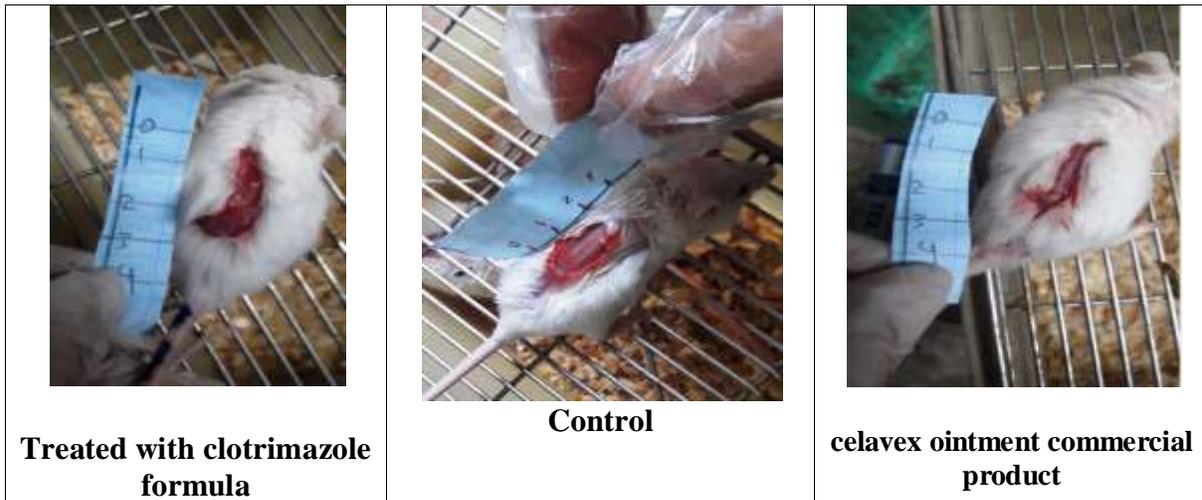


Figure (8). First day: Checking the speed of wound healing a wound with a distance of 2 cm has been created, we wait for the whole day until the treatment.



Figure (9). Fifteenth day after treatment: Mice were treated with clotrimazole swabs combination.

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