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Research Article

DESIGN OF CLOTRIMAZOLE SOAP BARS FOR SKIN DISEASES

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ABSTRACT

Background: Fungal infections like ringworm (tinea), candidiasis and dermatitis is always a worry and uneasiness to the suffering patients because of their recurrence. For their treatment many topical drug delivery systems are available like medicated liquid soap, shampoos etc. The disadvantage being their economy, wastage, no accuracy in dosing, formation of foam etc.

Objectives: In the present work, it was planned to prepare medicated soap bars to counter all the above disadvantages effectively.

Methods:Soap bars were prepared by heating, emulsification, congealing and moulding technique using reported formulas and procedure (Cold Process Method). During the preparation the 2% drug was dispersed uniformly on continuous stirring.

Conclusion: The results of present study revealed that the prepared medicated soap bars of Clotrimazole are convenient for use, gives good foam on application, showed uniform drug content and no skin irritation on animals as well as on human volunteers. The project work has industrial importance.

Keywords: Fungal infections, Antifungal drugs, Clotrimazole, Medicated Soap Bars.

INTRODUCTION

Fungal infections are infections caused by a fungus, a type of microorganism. Some very common types of fungal infections are caused by the fungus tinea. An increasing incidence of fungal infections could merely reflect greater laboratory expertise in the detection and identification of fungi.[1] Some of these infections are contagious, which means they easily spread from person to person. People at risk for fungal infections include those taking strong antibiotics, especially for a long period of time. Taking antibiotics can cause some persons to get a yeast infection. Antibiotics get rid of germs that make us sick, but they can also kill many of the harmless bacteria in our body. These harmless bacteria normally fight with the yeast for a place to live, but when antibiotics kill them, the yeast is free to grow.[2] Patterns of antimicrobial use, particularly prolonged administration of prophylaxis against not only fungi but also other opportunistic pathogens may also be contributing to the changing epidemiology of fungal infections.[1] Fungal infections are broadly classified as, either superficial or systemic.[3] Systemic fungal infections need extensive treatment by oral or I.V. administration of antifungal drugs. Whereas, superficial fungal infections of the skin and mucous membrane respond readily to topical application of antifungal agents.[4] Common Types of superficial fungal infections are ringworm (Tinea), Candida, Dermatitis etc.[5] Medicated soap bars is a novel approach for the treatment of superficial fungal infections affecting the skin. In proposed work it was planned to prepare medicated soap bar formulations to treat fungal infections which are very handy to use, can be carried easily by patients.

MATERIALS AND METHODS

Coconut Oil, Palm Oil, Olive Oil, Cellophane paper (Local Market, Gulbarga), Sodium Hydroxide, Methanol (Qualigens Fine Chemicals, Mumbai), Potassium dihydrogen orthophosphate (S.D. Fine Chemicals Ltd., Mumbai).

Preparation of Medicated Soap Bars: Cold Process Method

1. Sodium Hydroxide (NaOH) pellets were dissolved in distilled water (250 ml beaker) then was put aside to let them cool down (27-38^{0}C).
2. The mixture of oil and water was heated to 82^{0}C on hot plate and then the NaOH solution was drizzled on continuous stirring but gentle to create the uniform mixture. Now the drug was dispersed on continuous stirring. Initially the mixture looks like water shimmering with unsaponifiable oil, but after 10-15 minutes, it gradually become thick and uniform. Temperature of the soap was monitored so it was not exceed 82^{0}C or fall below 71^{0}C; then the beaker was removed from the heat occasionally and returned to the hot plate as needed.
3. Pour the mixture into suitable mould, cover it and placed aside for 18-24 hours for saponification.
4. After saponification, cover was removed and set aside for another 10-12 hours. Then by using rubber gloves the soap was cut into bars of different shapes.

Evaluation of Soap Bars

The Soap Bar was evaluated for Size and Shape, Thickness, Weight, Foam Test, pH, Drug Content, Stability Studies, microbiological studies, *In-vitro* drug release and primary skin irritation test on experimental animals and healthy human volunteers.

Size and Shape Determination

The soap bases of size of 5.0 cms x 3.0 cms with thickness of 0.5 cms, which is a rectangular shaped, was chosen for preparation of Soap Bars. This was chosen, as this size as ideal in regular usage to apply on the effected skin parts of the body.

Thickness Determination

The thickness was determined with the help of "screw gauge" which is precalibrated. The thickness was measured, by observing thickness at five different parts of the soap bars. Then taken the average of five readings.

Weight Determination

The weight was determined by using "Digital weighing balance".

Foam test[6,7]

For this purpose "modified vibratory flask shaker" was used. 500ml. measuring cylinders were clipped to the arms of the apparatus. Prepared medicated soap bars were hydrated in 20ml. distilled water for 1 hr. Then with slight stirring, the soap solution was prepared and the remaining soap bar was removed. Then the 20 ml. soap solution was transferred to the 500ml. measuring cylinder and shaken with a speed of 1000 rpm vibrations for 10 minutes and initial height of foam was noted from the surface of solution to its

height. The apparatus was kept open for 5 minutes and then second reading was noted and difference in the height of foam gave the main reading.

pH Tests[8]

The pH test was performed for all the formulations. Each formulation of soap solution was made with 20ml. distilled water and tested for pH with the help of digital pH meter.

Drug Content Estimation[9,10]

The drug equivalent to 25 mg of formulation was taken and dissolved in small quantity of methanol. Then the formulation is warmed on the water bath so that the drug present in the formulation was completely dissolved. Then the solution was filtered through Whattman filter paper in 25 ml. volumetric flask and volume was made up to the mark by methanol to give concentration of 1000 µg / ml. for Clotrimazole. Then 1 ml. was pipetted out in 25 ml. volumetric flask to give concentration of 40 µg/ml and then the last dilution was made by taking 1.5 ml. from 40 µg/ml. concentrations and diluted upto 10 ml. by methanol, to give concentration of 6 µg/ml. Then the absorbance was measured 262.2 nm.

Stability Studies[11, 12]

The prepared formulations according to the formula contain 2% Clotrimazole Drug. These formulations were stored at room temperature (30°C & 40°C) for a period of 6 months and studied for viz., physical appearance, foam formation, pH, drug content.

Microbiological Studies[13]

In the present work antifungal activity of prepared formulations were tested. The yeast cultures were isolated from the top most layer of skin of the diseased patient suffering from Malassezia furfur infection by a qualified dermatologist. The media used for the isolation include Sabouraud's dextrose broth (Hi media). The skin scraping of patient was immediately inoculated from the peptone water to broth medium. Medium used for isolation of yeast was incubated at 30°C for 5-7 days. One loopful broth culture from each tube was then streaked on the plate (in triplicate) on Sabouraud's Dextrose Agar (SDA) and incubated at 30°C for 5-7 days to obtain isolated colonies. Morphologically different colonies were selected from the plates and subculture on respective agar slopes. The (Agar-well diffusion) standard cup plate technique was used to determine the antimicrobial activity by using Sabouraud's dextrose agar (Hi-media). The melted media were seeded with the suspension of microorganisms and allowed to solidify. The formulations were aseptically transferred to the Hi media in petridish with the help of sterile forceps. The medicated soap bar were kept for incubation in an incubator at 30°C for 5-7 days. The assessment of antimicrobial activity was based on the measurement of diameter of zone of inhibition in mm. The values were recorded as mean of triplicate observation.

IR Spectral Analysis

Infrared spectroscopy is one of the most powerful analytical technique, which offers the possible chemical identification. In the present work, IR spectra of Clotrimazole pure drug and with other excipients in various formulations were determined for their interactions using FT-IR spectrophotometer.

***In-vitro* Drug Release Studies**

In vitro dissolution studies of Clotrimazole were carried out using USP XXIII dissolution test apparatus-II (Electrolab), employing a basket at 100 rpm using 900 ml of pH 7.4 phosphate buffer at 37±0.5°C as dissolution medium. One soap bar (1 gm) was used in each test. At predetermined time intervals 5ml of the samples were withdrawn by means of a syringe fitted with a pre filter. The volume withdrawn at each interval was replaced with same quantity of fresh dissolution medium maintained at 37±0.5°C. The samples were estimated by measuring the absorbance of clotrimazole at 262.0 nm in a 1700 UV Shimadzu spectrophotometer. All the studies were conducted in triplicate.

Primary Skin Irritation Test

Laboratory experimental animals[14]

3 healthy guinea pigs (2 male and 1 female) and 3 healthy rabbits (2 male and 1 female) were selected for the study. They were kept in different cages and supplied with fresh food and water during the test period. 24 hours prior to test, the hair from the upper portion of wiest was shaved to expose sufficiently large test area. The test site was cleaned with surgical spirit. By dipping medicated Soap Bars in distilled water it was applied to test area. The test site was observed for erythema and edema for 24 hrs., 48 hrs. & 72 hrs after application. This test was conducted to evaluate the irritancy of the prepared medicated Soap Bars on the intact skin of guinea pigs and rabbits. None of the prepared medicated Soap Bars showed any erythema and / or edema, indicating that the prepared formulations were non-irritant on the skin of guinea pigs and rabbits. These studies were carried out in the animal house of M.R. Medical College, Gulbarga.

Healthy Human Volunteer Studies

The prepared formulations showed high compliance in animal studies, thereby prompting to carry out skin irritation studies on healthy human volunteers. The study was conducted under the supervision of staff, Dept. Of Dermatalogy, M.R.medical and General Hospital, Gulbarga.

Test procedure

The skin irritation test was performed on three healthy human volunteers for each formulation (2 male and 1 female) by applying Soap Bar formulations. The volunteers were of age group between 22-28 years and weighing 50-70 Kgs. The test was performed primarily by examining each volunteer to notice any changes after application of formulations. Then photographic imaging of hands of human volunteers were taken out before and after subsequent application for 72 hrs i. e. at completion of study period and these images were compared determining the difference with the images taken at 0^{th} hr of study i.e. prior to first application of formulation. Moreover, skin irritation was evaluated by questioning the human volunteers at regular interval of time about the feeling of irritancy, which appears to be highly subjective for the study.

RESULTS AND DISCUSSION

Clotrimazole is a drug of choice for the treatment of various fungal infections. In the present study an attempt has been made to prepare soap bar of clotrimazole. The prepared Clotrimazole Soap Bars showed a uniform thickness of 5.95 ± 0.005 mm and weight of 1.00 ± 0.005 mm. The foam height of pepared formulation was found to be 52 ± 0.57 ml. The percentage drug content of prepared saop bar formulation was found to be 98.16%. pH of the formulation was shown in range of skin pH (8.2).Results showed that at the end of 30 min the percentage amount of drug released from F was found to be 45.45% with respective to the marketed formulation (MF) having 40.42% drug release in 30 min (Table-2). The release of drug from these formulations were found to be governed by dissolution process since the plot of percentage cumulative drug release Vs square root of time were found to be linear. The prepared soap bar formulation passed stability studies with no much significant changes in physical appearance, pH and drug content (Table-3). The antimicrobial activity of prepared Soap Bars was studied. It was found that formulation showed good zone of inhibition (Figure-1). IR study concluded that all the peaks of the pure drugs are also observed in different formulation with slight modification. The result concludes that there is no drug-excipient interaction. The results shown that the formulation was devoid of any primary skin irritation or sensation or erythema, or edema even after 72 hrs of application (Figure-2,3&4).

Table 1: Formulation of 2% Clotrimazole Soap Bars (F)

Sr No.	Ingredient	Quantity Given	Quantity Taken
1.	Clotrimazole	2 gm	1 gm
2.	Coconut Oil	44 gm	22 gm
3.	Palm Oil	14 gm	7 gm
4.	Olive Oil	8 gm	4 gm
5.	Lye	12 gm	6 gm
6.	Distilled Water	20 ml	10 ml
	Total	100 gms	50 gms

Table 2: Comparative *In-vitro* Drug Release Profile of Clotrimazole (2%) Soap Bar (F) With Marketed Formulation (MF)

Sr No	Time (min)	Square root of time	Cumulative Percent Drug Released		Cumulative Percent Drug Remaining		Log Cumulative Percent Drug Remaining	
			F4	MF	F4	MF	F4	MF
1	0	0	0	0	100	100	2	2
2	5	2.2360	13.32 ± 0.30	14.02 ± 0.37	86.68	85.98	1.9379	1.9343
3	10	3.1622	20.61 ± 0.39	19.11 ± 0.16	79.39	80.89	1.8997	1.9078
4	15	3.8729	27.19 ± 0.24	25.88 ± 0.28	72.81	74.12	1.8621	1.8699
5	20	4.4721	35.16 ± 0.21	31.34 ± 0.40	64.84	68.66	1.8118	1.8367
6	25	5.0000	40.56 ± 0.21	37.57 ± 0.21	59.44	62.43	1.7740	1.7953
7	30	5.4772	45.45 ± 0.37	40.42 ± 0.24	54.55	59.58	1.7367	1.7751

Each reading is a mean of three replicates; Each sample of 1 gm Soap Bar contains 20 mg of drug.

Table 3: Stability Studies of Clotrimazole Soap Bars (F)

Sr. No	Storage Temp.	Time of Analysis (Days)	Physical Appearance	pH	Drug Content (%)
1.	(30°C)	30	White	8.2	98.16 %
2.		60	White	8.2	98.16 %
3.		90	White	8.0	98.14 %
4.		120	White	8.2	98.16 %
5.		150	White	8.1	98.10 %
6.		180	White	8.2	98.16 %

Each reading is a mean of three replicates; All above formulations contain 2 % of Clotrimazole.

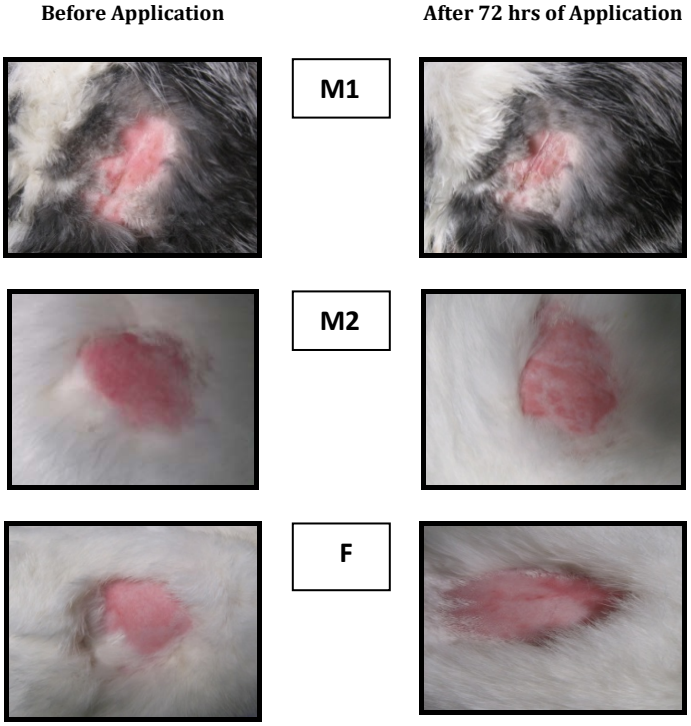
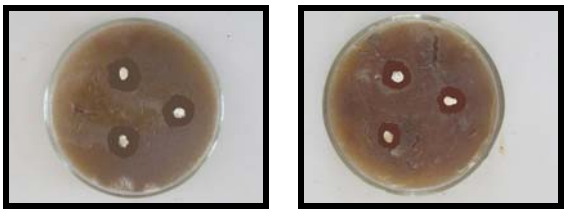


Fig. 2: Primary Skin Irritation Test of Rabbits



Pure Drug **Formulation (F)**

Fig. 1: Comparative Zone of Inhibition Studies of Drug in the Formulations with Pure Drug

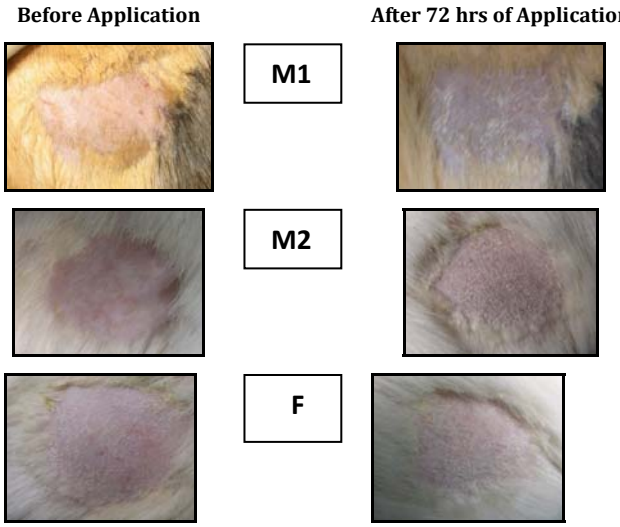


Fig. 3: Primary Skin Irritation Test of Guinea Pigs

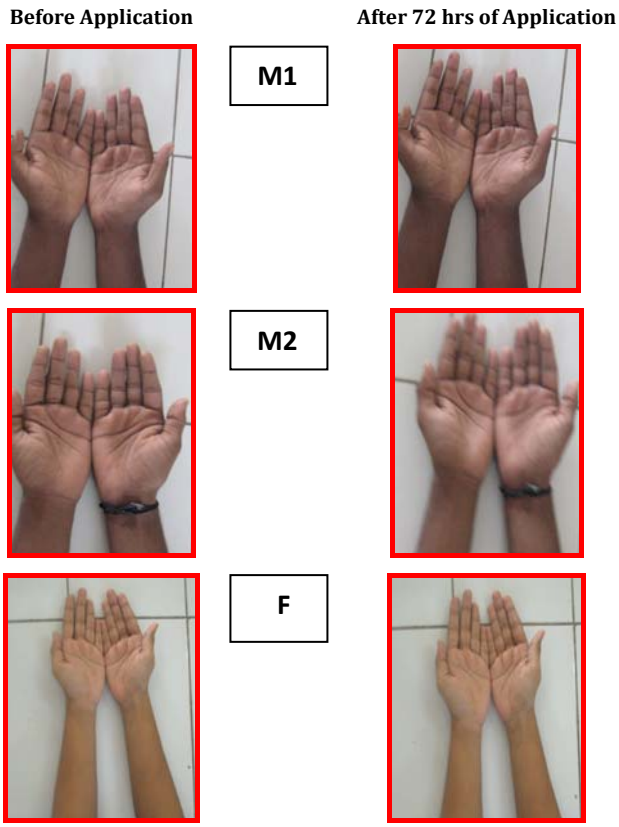


Fig. 4: Primary Skin Irritation Test of Healthy Human Volunteers

CONCLUSION

From present study it is revealed that the prepared clotrimazole Soap Bars with formulation of foam remained in contact with the applied body part for a prolonged period of time and avoided the moistening of the body part which in turn inhibited the further fungal growth and controlled the initial symptoms effectively. This is a very Novel concept of drug design of uniform dosage in the Medicated Soap Bars for the patients suffering from topical fungal infections especially on hands and legs.

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