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FORMULATION AND EVALUATION OF CREAM CONTAINING ANTIFUNGAL AGENTS, ANTIBACTERIAL AGENTS AND CORTICOSTEROIDS

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Keywords: Miconazole nitrate, Mupirocin, Hydrocortisone, Antifungal, antibacterial, Skin irritation studies.

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ABSTRACT

Plan: *The main aim of our research was to develop a novel cream formulation consisting of combination of Miconazole nitrate, Mupirocin and Hydrocortisone for the treatment of secondary skin infections.*

Prologue: *Topical route is most suitable route for skin infections. The development of topical drug delivery systems designed to have systemic effects appears to be beneficial for a number of drugs on account of the several advantages over conventional routes of drug administration.*

Methodology: *A novel cream formulation consisting of combination of Miconazole nitrate, Mupirocin and Hydrocortisone was prepared. The formulation was subjected to in-vitro diffusion studies. Microbiological studies and in-vivo skin irritation studies were performed to find out the safety of materials used in the formulation.*

Outcome: *The developed cream consisting of combination of Miconazole nitrate, Mupirocin, and Hydrocortisone was found to be safe and effective for the treatment of skin infections.*

INTRODUCTION

The development of topical drug delivery systems designed to have systemic effects appears to be beneficial for a large number of drugs on account of the several advantages over conventional routes of drug administration in order to optimize both the release of the drug from the topical vehicle and skin permeation[1]. The topical antifungal agents have varying mechanisms of action and different spectrums of activity and have few adverse reactions or drug interactions.

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Steroids have systemic effects such as anti-inflammatory and anti-allergic effects. Mupirocin is a naturally occurring antibiotic which inhibits bacterial protein synthesis reversibly by binding to bacterial isoleucyl transfer - RNA synthetase. It is active against Gram positive aerobes including staphylococcus aureus, staphylococcus saprophyticus, staphylococcus epidermidis, streptococcus pyogenes, streptococcus viridans, streptococcus agalactiae, and streptococcus pneumoniae[2]. Miconazole works by stopping the fungi from producing a substance called ergosterol, which is an essential component of fungal cell membranes. The disruption in production of ergosterol disrupts the fungal cell membrane, causing holes. It is active against fungal infections of the skin such as ringworm, candidiasis, athlete's foot, scalp infections, fungal nappy rash, groin infections and fungal infections of the nails[3]. Hydrocortisone is a corticosteroid with both glucocorticoid and to a lesser extent mineralocorticoid activity. It tends to be preferred for the long-term systemic therapy of auto-immune and inflammatory diseases[4]. When applied topically, it is used in the treatment of various skin disorders. Hydrocortisone and its acetate, butyrate, and valerate esters are commonly employed in the preparation of creams, ointments, and lotions.

Human infections, particularly those involving skin and mucosal surface constitute serious problems. The drug resistant bacterial and fungal pathogens have further complicated the treatment of skin infections. Topical route is most suitable route for skin infections. Numerous topical treatments are currently used for the treatment of bacterial, fungal skin infections and skin inflammations along with presence of corticosteroids. The main aim of the present study was to develop an effective and novel cream formulation consisting of combination of Miconazole nitrate, Mupirocin. Mupirocin and fusidic acid both are indicated as equally effective. Fusidic acid acts as a bacteriostatic whereas mupirocin has got both bacteriostatic and bactericidal action.

In Market, mupirocin were available in ointment base and fusidic acid is available in either ointment or cream base. Ointment formulation has poor patient compliance in nature due to its greasiness, since improve the patient compliance of mupirocin, cream formulation is adopted for mupirocin combination. Considering novel combination mupirocin is chosen along with Miconazole nitrate and hydrocortisone and Hydrocortisone for the treatment of secondary skin infections for the population suffering from skin diseases.

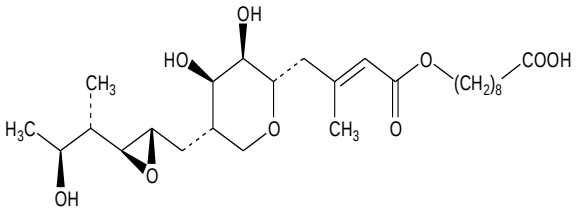


Figure 1: Mupirocin

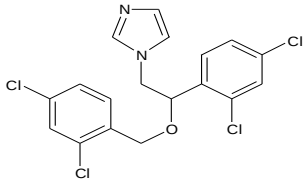


Figure 2: Miconazole

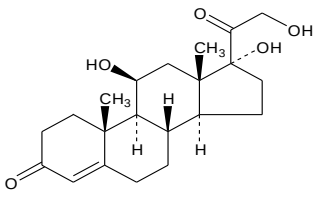


Figure 3. Hydrocortisone

2. MATERIALS AND METHODS

2.1. Materials

Mupirocin was obtained from Teva pharmaceuticals, Ahmedabad. Hydrocortisone was procured from Tinjin Jinjin Pharmaceuticals, Tianjin, China. Miconazole nitrate was purchased from Gufic bioscience, Gujarat. Cetomacrogol 1000 was purchased from India Glycols, Uttar Pradesh. Isopropyl myristate, Benzoic acid, Cetostearyl alcohol, Propylene glycol, White soft paraffin, Glyceryl monostearate, Butylated hydroxyl anisole, Liquid paraffin, Ethanol (AR grade) Acetonitrile (HPLC grade), Methanol (HPLC grade) were obtained from Merck, Germany. Triple distilled water was obtained from Milli Q unit.

2.2. Preparation of cream formulation [5]

2.2.1. Preparation of oil phase

Cetomacrogol 1000, Cetostearyl alcohol, white soft paraffin and Glyceryl monostearate were melted in a stainless steel vessel. To this mixture Isopropyl myristate, Liquid paraffin, Butylated hydroxyl anisole were added and allowed to melt. The temperature of oil phase maintained between 65 – 70°C and mupirocin is introduced into the oil phase just prior to addition into the aqueous phase (Table 1).

2.2.2. Preparation of Aqueous phase

Water was heated to 65 – 70°C. In this weighed benzoic acid were added the temperature of the phase was maintained at 65 – 70°C.

2.2.3. Dispersion part

Miconazole nitrate and hydrocortisone were sieved through appropriate mesh and dispersed in propylene glycol

2.3. Development of Cream formulation

Oil portion was then slowly incorporated into the aqueous phase at 65-70°C and mixed for 10 to 15 minutes. Then dispersion part was added into the above part slowly when temperature reaches to 40°C. pH of cream kept between 3.5 – 4.5

Table 1. Formulation table

Ingredients	*MMH01 g*	*MMH02 g*	*MMH03 g*	*MMH04 g*	*MMH05 g*	*MMH06 g*
Miconazole nitrate	2.000	2.000	2.000	2.000	2.000	2.000
Mupirocin	2.000^	2.000^	2.000^	2.000^	2.000^	2.000*
Hydrocortisone	0.500^	0.500^	0.500^	0.500^	0.500^	0.500*
Cetostearyl alcohol	2.500	3.000	3.000	7.500	10.000	10.000
Cetomacrogol 1000	1.200	1.500	1.000	1.500	1.600	1.600
Glyceryl monostearate	-	0.500	0.250	0.750	1.100	1.100
Liquid paraffin	5.000	5.000	5.000	5.000	3.760	3.760
Propylene glycol	25.000	25.000	35.000	35.000	20.000	20.000
Isopropyl myristate	3.000	3.000	3.000	2.280	2.280	2.280
Benzoic acid	0.100	0.100	0.100	0.100	0.100	0.100
Butylated hydroxy toluene	0.040	0.040	0.040	0.040	0.040	0.040
White soft paraffin	2.500	2.000	1.000	1.000	5.280	5.280
Purified water	q.s to 100 g	q.s to 100 g	q.s to 100 g	q.s to 100 g	q.s to 100 g	q.s to 100 g

**Raw material sieved through 200# sieve, ^Raw material sieved through 100# sieve*

2.4. Evaluation parameters

Take about 1 gram of cream in a clean petri dish and observe visually.

2.4.1. Physical examination

The prepared topical creams were inspected visually for their color, homogeneity, consistency, spreadability and phase separation. The pH was measured in each cream, using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted in to the sample 10 min priors to taking the reading at room temperature.

2.4.2. Viscosity

The viscosity of formulated creams was measured by Brook field Viscometer LVD using spindle S 94 at varying speed and shear rates [6]. The measurements were done over the range of speed setting from 0.10, 0.20, 0.30, 0.40 and 0.50 rpm in 60 s between two successive speeds as equilibration with shear rate ranging from 0.20 s^{-1} to 1.0 s^{-1}. Viscosity determinations were performed at room temperature.

2.4.3. Tube extrudability

In the present study, the method adopted for evaluating cream formulation for extrudability was based upon the quantity in percentage cream extruded from tube on application of finger pressure [7]. More quantity extruded better was extrudability. The formulation under study was filled in a clean, lacquered aluminium collapsible 5 gm tube with a nasal tip of 5 mm opening and applied the pressure on the tube by the help of finger. Tube extrudability was then determined by measuring the amount of cream extruded through the tip when a pressure was applied on a tube.

2.4.4. Determination of Drug content

To ensure uniform formulation of the cream, it was sampled from the different locations in the mixer and assayed for the drug content [6, 7]. Drug content of the cream was determined by following method using liquid chromatography.

2.5. Microbiological studies

Topical formulation with broad, non-resistance promoting activity against staphylococci, streptococci, dermatophytes or yeast or molds can be of great use in dermatology preparation were infections are often mixed. Since formulation containing antimicrobial agents as active moiety, it is likely to protect from microbial growth [8, 9]. To determine the activity of formulation is subject to study the prepared formulation with standard method called standard cup plate method and the inhibition zone diameters were measured with the help of zone reader. Soya bean agar media was used for aerobic culture and incubated at temperature of 37° C for 48 hours.

2.6. In-vivo skin irritation test

The experimental protocol was approved by the Institutional Animal Ethics Committee. Proposal No: JSSCP/ IAEC/PHD/ PHARMACOLOGY/ 02/2013-14.Although all the materials used for preparation of cream formulation were under GRAS, concentrations of all materials are critical issue for this formulation[10, 11]. Since it contains surfactant, preservatives and other excipients usually irritant to skin when contact time increase according to drug delivery. Therefore skin irritation test was performed to confirm concentration of materials used for cream is safe.

The experiment was carried out using 3 adult male white Newzealand rabbits, weighing about 1.5-2.5 kg to test for the skin irritation. The animals were housed in the animal house facility, with environmental conditions set to a temperature of 25± 2º C, a humidity of 60-90% RH and a 12-h light/dark cycle and provided with ad-labium access to a commercial rabbit-diet and drinking water. The back of each rabbit was shaved into 2 areas, each of 6 cm^2. 0.5 g of sample was topically applied as test to one of the shaved areas of each animal whereas other area was left blank as control. Both the areas were covered by gauze and the back of the rabbit was wrapped with a non-occlusive bandage. The animals were returned to their cages.

The reactions, defined as erythema and edema, were observed at 24, 48 and 72 hours after application, and evaluated according to the scoring system for skin reactions (Table 2 & 3). Photos were taken at time of observation and documented properly.

The Score of Primay Irritation (SPI) was calculated for test and control in each rabbit as the following.

$$SPI \text{ for test or control in each rabbit} = \Sigma \frac{\text{Erythema and edema grade at 24, 48 and 72 hrs}}{\text{Number of observation}}$$

The Primary Irritation Index (PII) was calculated as follows

$$PII = \frac{\Sigma SPI\ (Test) - \Sigma SPI\ (Base)}{\text{Number of animals}}$$

The irritation degree was categorized as negligible, or slight, moderate or severe irritation based on the PII (Table 3).

Table 2. Classification system for skin reaction

Reaction	*Score*
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation	4
Edema	
No edema	0
Very slight edema	1
Well defined edema (edges of the area well defined by define raising)	2
Moderate edema (raising approximately 1mm)	3
Severe edema (raised more than 1 mm and extended beyond the area of exposure	4
Total possible score for primary irritation	8

Table 3. Response categories of irritation in rabbit

Category	*Primary Irritation Index (PII)*
Negligible	0-0.4
Slight irritation	0.5-1.9
Moderate irritation	2-4.9
Severe irritation	5-8

2.7. In vitro diffusion studies

Formulations were subjected to *invitro* diffusion studies carried out using Electrolab Diffusion cell apparatus, EDC07, 8 stations, semi-automatic instrument[12, 13]. Cellulose nitrate membranes were soaked in distilled water for 24 h prior to use. About 100mg of cream kept in donor compartment. The entire surface of cellulose nitrate membrane was in contact with the receptor compartment containing 5 ml of water: ethanol. The receptor compartment was continuously stirred at 100 rpm using the magnetic stirrer. The temperature was maintained at 37ºC ± 1ºC. The diffusion studies carried out with surface area was calculated and found to be 0.6359cm^2. The study was carried out for 6hours and the sample was withdrawn at every 1 hour time interval and replaced with same media. The content of miconazole nitrate, mupirocin and hydrocortisone from withdrawn sample was measured after suitable dilution. The diluted samples were analyzed using High performance liquid chromatography with method described in drug determination part.

3. RESULTS & DISCUSSION

Semisolid dosage forms have been the subject of wide research in the past few years. Attempts have been made to improve the performance of these systems, be it the therapeutic efficacy of the incorporated drug or the cosmetic acceptability of the formulation. Greater emphasis has been placed on achieving comparable drug release with new drug-carrier systems, eliminating the cosmetically unfavorable qualities of the conventional semisolid dosage forms. Significant attention has been placed on the exploitation of semisolid dosage forms for systemic delivery of a topically applied drug on the skin. All formulations were evaluated for physical examinations. Cream not developed successfully in the initial trials, it is due to concentration of emulsifier added in the formulation. Trial no. 1, 2, 3 & 4 were found to be physically not good, hence other parameters not evaluated for those trial formulation.

Drug's physicochemical properties are responsible for successful drug permeation. Great opportunities for the development of semisolid dosage forms exist because of the diverse class of drugs, with unique characteristics, that are proposed for topical delivery. Prepared topical formulations are subjected to physical stability for centrifugation and freeze thaw cycle [14]. During physical stability testing trial no 1 and 2 found to be phase separation and coalescence, hence those withdrawn from further studies.
The compositions of cream formulation were shown in Table I. From the results of *In vitro* release studies, it is clearly proved that particle size of drug plays a vital role in drug permeation. Other parameters showed good physical characters, homogeneity, spreadability and viscosity.

Trial 3 and 4 found to be not stable in Heating and cooling studies and same was not studied for further studies. Only those formulations, which showed no phase separation, creaming, turbid, cracking, coalescence, and phase inversion during stress stability tests, were selected for further studies. The results of physical stability studies were shown in table 4.

Table 4. Physical stability studies

Trial No.	*H/C Cycle*	*Centrifuge*	*Freeze thaw*	*Results*
MMH01	↓	↓	↓	Fails
MMH02	↓	↓	↓	Fails
MMH03	↑	↓	↑	Fails
MMH04	↑	↑	↑	Passes
MMH05	↑	↑	↑	Passes
MMH06	↑	↑	↑	Passes

H/C – Heating and cooling

All formulated creams were subjected to physical nature of creams by studying viscosity. The viscosity of formulated creams was measured by Brook field Viscometer LVD using spindle S 94 at varying speed and shear rates. Viscosity of formulated creams with different emulsifier and concentration of stiffening agent and other solvents shows differ in formulation characteristics and viscosity. And the results of formulated creams were compiled and graphs shown in figure 4.

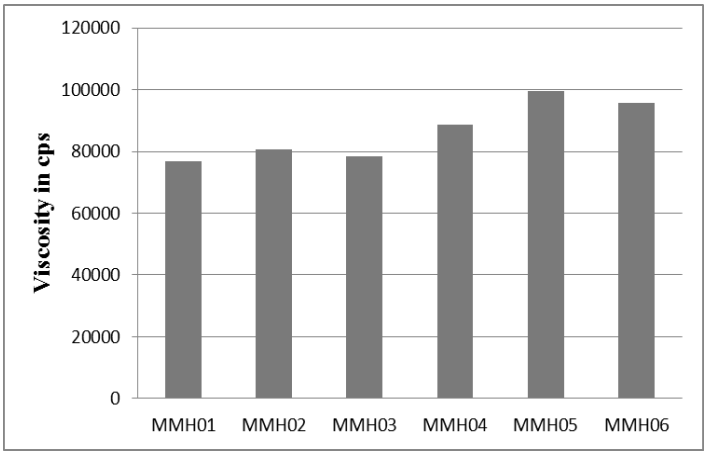


Figure 4 Effect of different concentration of excipients with respect to Viscosity in cps

All formulation was analyzed for Drug content (table 5), determined from the standard area with sample area using method specified in methodology part. Drug content in the formulation reveals that the accuracy of formulation and quality of product. Drug permeation studies reveals that each formulation shown higher drug release in MMH06 when compared to the MMH05. In each formulation drug release based on particle size of dispersed drug in the formulation. Higher the drug permeation noted in the formulation where drug sieved through 200#.

Table 5. Percentage assay of formulations

Batch no.	% Assay Hydrocortisone	Mupirocin	Miconazole
MMH01	98.40	98.37	100.47
MMH02	99.15	101.23	102.45
MMH03	100.45	98.34	101.23
MMH04	100.56	99.78	99.01
MMH05	97.34	99.12	100.23
MMH06	99.49	99.37	99.35

Topical formulation with broad, non resistance promoting activity against staphylococci, streptococci, dermatophytes or yeast or molds can be of great use in dermatology preparation were infections are often mixed. The antibacterial activity of various cream formulations of miconazole, mupirocin and hydrocortisone against various strain of anaerobic and aerobic microorganisms were evaluated by the standard cup plate method and inhibition zone diameters were measured. Organism such as E. coli, S. aureous, B. subtilis were studied and results are shown in table 6.

Table 6. Microbial studies for MMH05 and MMH06

	Zone of Inhibition		
Formulation	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
MMH05	45.87	34.89	40.12
MMH06	43.81	37.11	39.63

Dermal irritation studies proved that cream formulation was found to be non irritant, the Score of Primary Irritation (SPI) is found to be 0.3 and Primary Irritation Index (PII) is calculated and found to be negligible limit as 0.1 and indicates safe for human use. Management of Some skin diseases requires introducing treatment approaches that are most effective, safety and do not produce harmful effect (table 7). It concludes from this work is focusing on inflammation, redness, purities, edema, psoriasis, and secondary infection.

Table 7. Result of skin irritation test.

Rabbit No.	*Reactions*	*Score for Skin reaction*					
		Sample			*Control*		
		24 hr	*48 hr*	*72 hr*	*24 hr*	*48 hr*	*72 hr*
1.	Erythema	0	0	0	0	0	0
	Edema	0	0	0	0	0	0
2.	Erythema	0	0	0	0	0	0
	Edema	0	0	0	0	0	0
3.	Erythema	0	0	0	0	0	0
	Edema	0	0	0	0	0	0

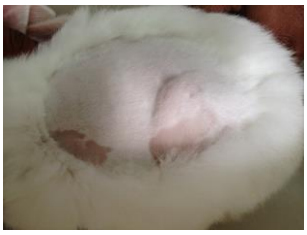
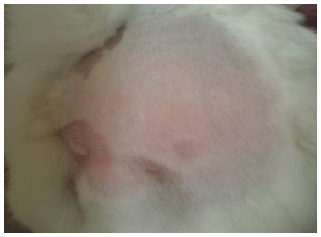
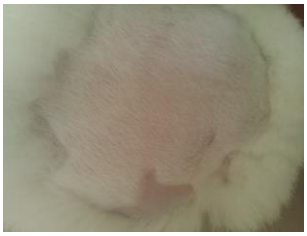
Time (hrs)	*Test*	*Control*
24 hrs		
48 hrs		
72 hrs		

Figure 5 Images of skin irritation test.

In vitro Diffusion studies reveals that both the formulation were found to be drug permeation into cellulose nitrate membrane, especially drug permeation was directly plays role in particle size of dispersed drug in formulation. *In vitro* studies for selected formulation were studied and results were shown in figures 6-8, all the three drug release was studied by intercepting cumulative drug release versus square root of time.

CONCLUSION

The formulation of antimicrobial agents along with corticosteroids exhibited enhanced rate of diffusion and antibacterial activity. The results of different chemical and physical tests of cream showed that it could use topically in order to protect against skin infections caused by fungus or bacteria.

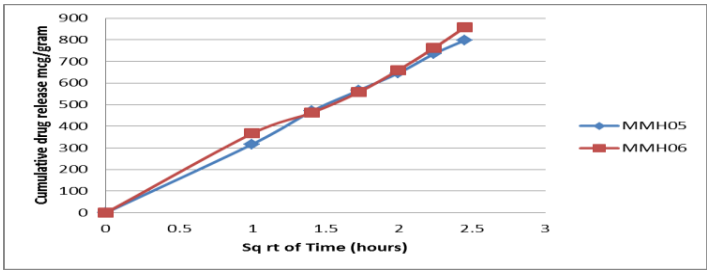


Figure 6. Cumulative drug release of hydrocortisone permeated through cellulose nitrate membrane

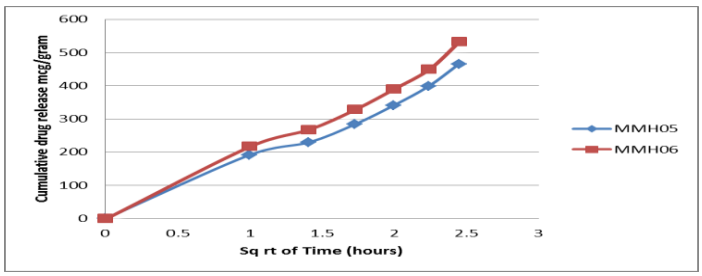


Figure 7. Cumulative drug release of Miconazole nitrate permeated through cellulose nitrate membrane

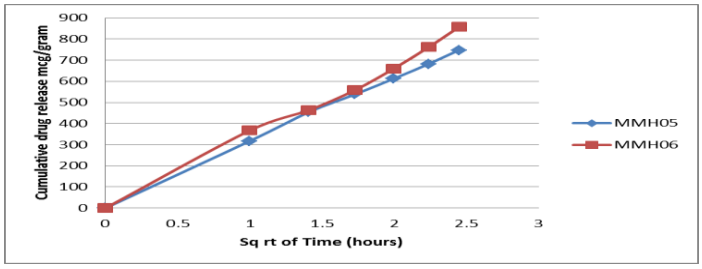


Figure 8. Cumulative drug release of Mupirocin permeated through cellulose nitrate membrane

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