Section A-Research paper

OPTIMIZATION AND *IN-VIVO* CHARACTERIZATION OF TOPICAL GEL CONTAINING COMBINATIONS OF ANTIFUNGAL DRUGS

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Abstract

The goal of this study is to create a topical gel for drug delivery with antifungal effects. The preparation containing Fluconazole and Clindamycin was selected for preparation. The system delivers the drug at the specified time required by the patient to achieve maximum efficacy and improve drug efficacy with minimal side effects. To combat both topical and systemic fungal infections, doctors use fluconazole, an imidazole derivative. Because of its many negative side effects, fluconazole should not be used orally. Clindamycin is most commonly used to treat anaerobic infections, which are caused by anaerobic bacteria that are amenable to clindamycin's treatment and have very little effect. The purpose of the current investigation was to develop and assess a variety of topical gel formulations including fixed dose combination of fluconazole with Clindamycin mainly accelerate the anti-fungal effect by acting on fungus as well as reduce development of bacteria's associated with that. Carbopol 940, Hydroxypropyl methylcellulose E4M, Methyl cellulose, Pectin, and Pluronic P407 were used to manufacture the gel at varying concentrations. The simultaneous determination of medicine in dose form was developed using the simultaneous equation approach. DSC and FT-IR analyses confirmed that the drug and excipients were compatible with one another. A modified Franz diffusion cell was used to evaluate drug penetration across a cellulose membrane and drug release in vitro in a phosphate buffer at pH 5.5. To test the efficacy of the developed formulations against *Candida albicans*, a model fungus, we employed Nizoral® cream as a gold standard. The greatest values came from F3 (91.3% of drug released after 2 hr) in in vitro drug release and permeation experiments. Even in terms of antifungal action, F3 is superior than the others. Also, for the chosen formula, the stability analysis found no noticeable change between the two time points.

Keywords: Carbopol 940, Fluconazole, Clindamycin, antifungal, topical gel, Candida albicans, Hydroxypropyl methylcellulose E4M, Methyl cellulose, Pectin, and Pluronic P407.

1. Introduction: Skin fungal infections are frequent today. Physicians can prescribe solid, semisolid, or liquid dosages. Transparent gels are increasingly used in cosmetics & pharmaceuticals as semisolid formulations [1-2]. Systemic & superficial fungal diseases are typically classified. Hence, systemic and topical antifungals dominate. Chemical structure classifies antifungals as polyene, azole, allylamine, and others. [3-4]

Topical drugs can treat skin disorders locally or systemically. Cosmetics and pharmaceuticals employ transparent gels in semisolid formulations. Gels release drugs faster than creams and ointments, regardless of water solubility. They are biocompatible, simply applied, and require no removal. [5-6]

Gels release drugs faster than creams and ointments, regardless of water solubility. They are biocompatible, simply applied, and require no removal. Dermatological gels are water-soluble, miscible, thixotropic, greaseless, spreadable, easily removed, emollient, non-staining, and compatible with numerous excipients. [7-8]

Triazole antifungal fluconazole is synthetic. It treats superficial and invasive fungal diseases. Fluconazole has unique pharmacokinetics. Bistriazole renders this molecule less lipophilic and more hydrophilic than other azoles antifungals. Halogenated phenyl rings boost antifungal action. [9-10]

Despite its nausea, vomiting, bloating, and stomach pain, fluconazole is exclusively sold as pills and injections. Gel compositions for topical use can overcome these drawbacks.

Clindamycin cures osteomyelitis, pelvic inflammatory disease, strep throat, pneumonia, acute otitis media, and endocarditis. It cures acne and MRSA (MRSA). It treats malaria with quinine. It can be eaten, injected, applied, or vaginalized. [11-12]

Our research seeks to produce and test polymers with different proportions to make a safe, effective, and stable gel containing Fluconazole and Clindamycin and evaluate its in-vitro performance, stability, and antifungal activity. Drug delivery devices are needed because none are sold. Combination treatment surpasses monotherapy dosage increases.

2. MATERIALS & METHODS

2.1. Materials. Fluconazole with Clindamycin was a present from the Atra Pharmaceuticals Aurangabad (Maharashtra, India), IPCA Laboratories Ltd., SEZ Pithampur, Indore (M.P., India), provided free samples of Carbopol 940, Hydroxypropyl methylcellulose E4M, Methyl cellulose, Pectin, and Pluronic P407.

2.2Drug-ExcipientsCompatibilityStudies

A-Differential scanning calorimetry (DSC): The drugs, polymers, and 1:1 drug-polymer physical combinations were all studied using DSC. Using a differential scanning calorimeter, we placed the samples (3-4 mg) on an aluminium pan and heated them to 200 °C at a rate of 10 ℃/min. (TA-501; shimadzu corporation, Japan).

B. Infrared spectrophotometer with Fourier transform: Drugs, polymers, and the drug-polymer physical combination in a ratio of 1:1 were individually mixed with IR grade KBr in a ratio of (100:1), and discs corresponding to this mixture were formed by applying 5.5 metric tonnes of pressure in a hydraulic press using an FTIR Spectrophotometer. Across a certain range of wave numbers, the discs were examined (4000 - 400cm).

2.3. Method of simultaneous equations. To estimate a fixed-dose medication combination simultaneously. Fluconazole was diluted to 2–6 g/ml and Clindamycin to 5–15 g/ml using double distilled water. The overlapping spectra yielded two wavelengths, 261 and 210 nm, for a simultaneous equation. Both medicines had E (1%, 1 cm)

absorptivity ratings at both wavelengths. Five binary combination solutions of Fluconazole and Clindamycin were prepared at a ratio of 2:5, which is quite near to the therapeutic dosage ratio of 2.5:6.25. Drugs were quantified by solving simultaneous equations.

$$Cx = (A_2ay_1 - A_1ay_2) / (ax_2ay_1 - ax_1ay_2) \quad - (1)$$

$$Cy = (A_1ax_2 - A_2ax_1) / (ax_2ay_1 - ax_1ay_2) \quad - (2)$$

Where, Cx is the conc. of Fluconazole& Cy is the conc. of Clindamycin, & A1 & A2 are the mixture's absorbance at 261 & 210 nm, respectively. Similarly, ax1 & ax2, ay1 & ay2 are the absorptivities of x & y at 261 & 210 nm, respectively. [13]

2.4. Formulas for making topical gels containing fluconazole and clindamycin are laid forth in the following table. Propylene glycol (20% w/w) and glycerin (10% w/w) were heated together to dissolve the moistening agent and the antifungal agents fluconazole and clindamycin (1% w/w each). Polyacrylic acid polymer (carbopol 940), cellulose polymers (HPMC, MC), and polysaccharide polymer (Pectin) gel were created by dispersing the estimated quantity of polymer in the warm water with steady stirring using magnetic stirrer at a reasonable speed. The drug-infused mixture from before should be added now. The acidity of the carbopol gel was neutralised by adding TEA. The cold approach called for the gradual dispersion of the transition polymer Pluronic in cold water at 4oC with steady stirring. Last but not least, preservatives methyl and propyl paraben were added gradually while swirling constantly until gel formation occurred. The gels were then put in wide-mouth glass jars with screw-capped plastic lids and kept in a dark, cold room with the openings covered with aluminum foil.

Table 1: Describes the ingredients of a topical gel (% w/w)

Ingredients	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10
Fluconazole	1	1	1	1	1	1	1	1	1	1
Clindamycin	1	1	1	1	1	1	1	1	1	1
Carbopol 940	**0.5**	**1**	—	—	—	—	—	—	—	—
HPMC	—	—	**1.5**	**2**	—	—	—	—	—	—
Methyl Cellulose	—	—	—	—	**2**	**4**	—	—	—	—
Pectin	—	—	—	—	—	—	**3**	**4**	—	—
Pluronic F-127	—	—	—	—	—	—	—	—	**15**	**18**
Glycerin	10	10	10	10	10	10	10	10	10	10
Propylene Glycol	20	20	20	20	20	20	20	20	20	20
Methyl Paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Propyl Paraben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified water to	100	100	100	100	100	100	100	100	100	100

2.5 Physicochemical Evaluation [14]

2.5.1 Visual examination: When the gels had set in the container, visual examination was used to check for homogeneity, colour, syneresis, and the presence of lumps in each of the created gel formulas.

2.5.2 During the spreadability test, 0.5 g of each formula was pushed between two slides (split into squares with 5 mm sides) and left for around 5 minutes, after which no further spreading was expected19. The diameters of the resulting circles were measured in centimetres and used as a basis for comparison of the spreadability of various materials. The findings are an average of three separate calculations.

2.5.3 The gels' pH levels were measured using a digital pH metre.

2.5.4 To test the gel's drug content, we removed a measured volume and dissolved it in 100 millilitres

of a phosphate buffer with a pH of 5.5 [14]. To ensure full drug solubility, the volumetric flask holding the gel solution was agitated on a mechanical shaker for 2 hours. The Millipore filter (45 m) was used to purify this solution. Drug absorbance was measured using a UV-visible spectrophotometer after appropriate dilution, (UV – 1700, Shimadzu, Japan).

2.6 The research was conducted utilising *in-vitro* release studies [15]. One gramme of Gel is in the aluminum-mesh watch glass. 500 cc of release medium (phosphate buffer pH 5.5 at 37oC 0.5 °C, paddle speed 50 rpm) was introduced to the watch glass jar. A 5 ml aliquot was changed with fresh dissolving media every 10 minutes for two hours. Using the standard curve, spectrophotometers measured drug concentration in samples. Each experiment was repeated three times, averaged, and included "blank" experiments with untreated bases.

2.7 Drug Release Kinetics Study [16]:

a. Zero–order equation: $Q=kot,$

Given that Q is the dose released at time t and k0 is the initial rate of release

b. First – order equation: $In\ (100–Q)=In100–k1t$

Where Q is the percent of drug release at time t, and k1is the first–order release rate constant.

c. Higuchi's equation: $Q=kt^{1/2}$

Where Q is the percent of drug release at time t, and k is the diffusion rate constant

2.8 *In-vitro* Drug Diffusion Study [17]: This study used 0.45µm Sigma Chemicals cellulose membrane. 1g of the preparation was spread using a cellulose membrane soaked overnight in release medium. The loaded membrane was firmly stretched over a 2-cm glass tube and rubber-tied to prevent leaking. In the dissolving vessel, tubes were submerged in 50 ml of release liquid, phosphate buffer pH 5.5, at 37oC ± 0.5oC. The shafts rotated at 50 rpm and 3 ml aliquots were extracted from the release medium at predetermined times. New media replaced withdrawn samples. Calibration curve spectropotometry determined drug concentration. Data was averaged three times. Four-hour in vitro release experiments. Solubility studies established sink conditions. Fluconazole and Clindamycin flow, lag time, and permeability over synthetic membrane.

2.9 Skin irritation test: Draize patch tests were performed on rabbits to compare the irritation strength of gels containing free and entrapped drugs to commercialised gels. Animal handling during experiments followed CPCSEA guidelines. The IAC approved the experiments. Swiss albino rats (2.5-3 kg) were acclimatised before the trial. [18]

2.10 Antifungal study: The formulae were evaluated in triplicate using agar cups and the *Candida albicans* strain. After spreading an inoculum of the tested fungal suspension strain throughout the surface of the Sabouraud dextrose agar, sterile cups with a diameter of 10 mm were formed. Containers were refilled with each formula using sterile syringes. Each cup's zone of inhibition was then measured to determine its radius and compared to that of the control formula, Nizoral® cream. [19]

2.11 In Vivo Method: Male albino rats were tested in-vivo (400-500g). Three groups of nine rats were created. Animals were fed commercial pellet food and water ad libitum. Animals were acclimated to lab conditions for 10 days before the trial. Institutional Animals Ethics approved all animal studies.

Candida albicans was challenged. *C. albicans* for albino rat cutaneous candidiasis was grown on Sabouraud dextrose agar for two days at 35°C. 108 cells/ml were obtained by dispersing yeast colonies in saline. Control group I. Scissors cut group II and III rats' dorsal hair. The skin was cleansed with damp cotton after 10 minutes of

Anne French depilation. Before and after the injection, rats received 30 mg/kg prednisolone subcutaneously. Once rats developed cutaneous candidiasis, they were treated with several gel formulations. Topical therapy, 100 mg containing 0.1 mg equivalent of Fluconazole & Clindamycin of the F3 formulation, free drug solution in ethanol with similar medicine was given to animals in group II and III once a day for three days starting on day after infection. Group I was infected but untreated. To avoid licking or swallowing the topical medicine, treated rats were kept apart. After 2 days, a cotton swab and 70% ethanol cleaned the area. The treated skin was cut with scissors and homogenised in a tissue homogenizer in 4 ml of saline with 0.05% Tween 80. Sabouraud dextrose (10 ml) was added to part of the homogenate (10 ml). Five days at 35°C incubated all plates. The log of CFU/infected site was derived using agar plate counts. [20]

2.12 Stability studies: The most effective formulation underwent a stability study. The best-performing Gel was housed in pristine, lacquered, collapsible aluminium tubes, and several replicas were kept in a humidity room at a temperature of 40 ±2 °C and a relative humidity of 75 ±5%. Gel was evaluated at an interval of 30, 60 and 90 days for alteration of appearance, pH, and in vitro release profile. [21]

3. RESULTS & DISCUSSION

3.1. Drug-excipient Interaction study

A. FTIR Study

Fluconazole and Clindamycin shows peaks at 1652 cm^{-1} & 1235 cm^{-1}, respectively. Carbopol 940 maxima were 2959 & 1942cm^{-1}, HPMC at 1304 & 1482cm^{-1}, Methyl Cellulose exhibited peaks at 3649& 2854 cm^{-1} Pectinat 1443 & 1304cm^{-1} and Pluronic F127 at 2342 & 2113 cm^{-1}respectively. According to spectra, there is no chemical interaction between the medication and polymer. The cross-linking of polymers caused a couple of the bands in the formulation to disappear and combine. Drugs had spectra at the 1652 and 1235 cm-1peaks, proving they were pure and unaltered structurally.

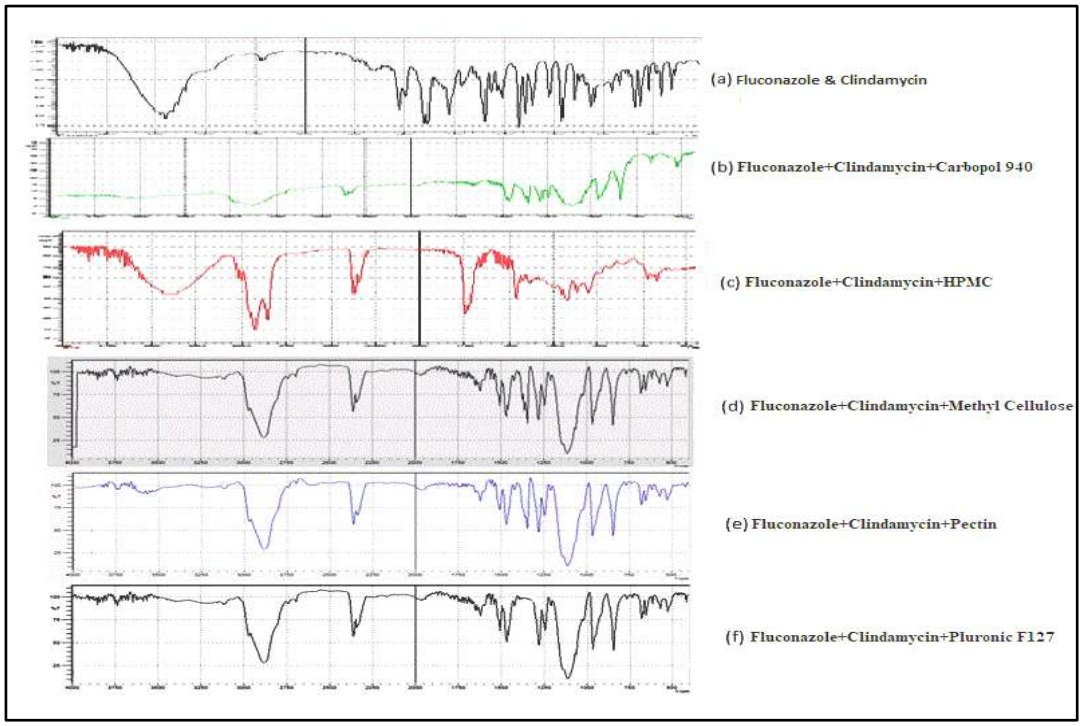

Figure 1: IR study of drugs &polymers

B. DSC (Differential Scanning Calorimetry). Figure displays the DSC Thermogram for the Fluconazole, Clindamycin, and Physical combination. The melting point of a pure medicine is between 110°C and 140°C, according to the thermographs generated by the DSC investigation, but that of a formulation is between 204°C and 292°C. There is a little but discernible variation between the preparations of the drug and the pure drug in terms of melting points. This demonstrates that even after being created, the medications do not respond. This proves that there are no chemical interactions between the medication and the polymer.

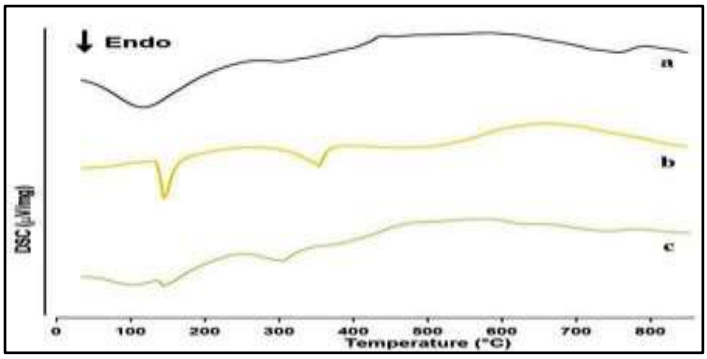

Figure 2: DSC Thermogram for: (a) Clindamycin, (b) fluconazole (c) Physical mixture (Drugs + polymer)

3.2Evaluation of Topical Gel

3.2.1 Visual examination: Visually inspecting gel formulas for color and syneresis. Only pectin gel was opaque. All gel formulas were homogeneous and syneresis-free.

3.2.2 Spreadability: As gel comes out of the tube, spreadability is crucial. The table shows that all polymers produced gels with low shear. Pluronic F127gel spread circles were 3 cm, carbopol and HPMC gel 5 cm. The table showed that increasing any gelling agent concentration invariably decreased spreadability, as measured by the spreaded circle diameter.

3.2.3 pH Determination: To avoid skin irritation, all formulations had pH values of 5-6. Excluding pectin gel; pH was 3.5.

3.2.4 Drug Content determination: After gel formulation, the estimated drug level was 9.5 to 9.99 mg/gm gel, within statutory limits. The gel's medication content was likewise uniform.

Table 2: Displays the Topical Gels' Physical Characteristics

Batch	Color	Syneresis	Spreadability (in cm)	pH	Drug content (Combined mg/gm gel)
F-1	Shiny transparent	Negative	4.5	6.1	9.55
F-2	Shiny transparent	Negative	4	5.99	9.7
F-3	transparent	Negative	5	5.60	9.99
F-4	transparent	Negative	5	5.67	9.78
F-5	Translucent yellowish	Negative	4.5	6.1	9.99
F-6	Translucent yellowish	Negative	3.6	6.13	9.7
F-7	Buff, Opaque	Negative	5	3.6	9.7
F-8	Buff, Opaque	Negative	3.5	3.7	9.98
F-9	transparent	Negative	3.6	6.22	9.93
F-10	transparent	Negative	3	6.3	9.89

3.3 In-Vitro Release Studies: The Figure showed the topical gel formulae's in-vitro release profile. With

Fluconazole, it was noted that the following rankings of the drug's release from its various formulations may be made in decreasing order: F 3>F 4>F 5>F 7>F 9>F 8>F 1>F 10>F 2>F 6 and for Clindamycin F 3>F 4>F 5>F 7>F 9>F 8>F 1>F 10>F 2>F 6; where the quantities of the drug released after 2 hours were 92.3%, 90.2%, 83.4%, 80.1%, 80.8%, 78.4%, 72.4%, 74%, 68.6% and 57.5%respectively. Drug release is largely influenced by polymer type and concentration.

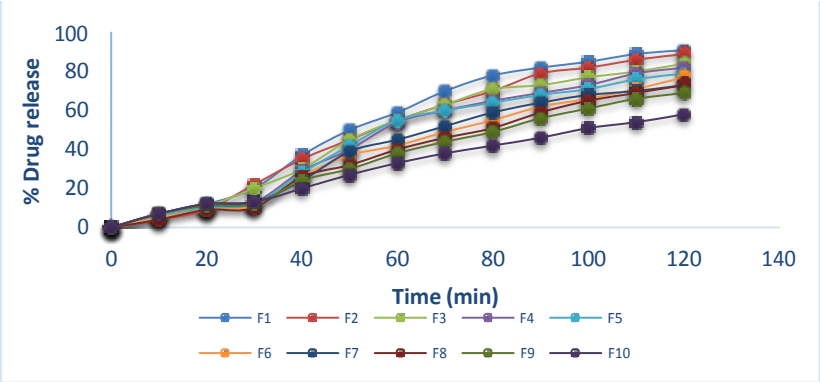

Figure 3: Shows Release profile of Fluconazole from its gel formulae

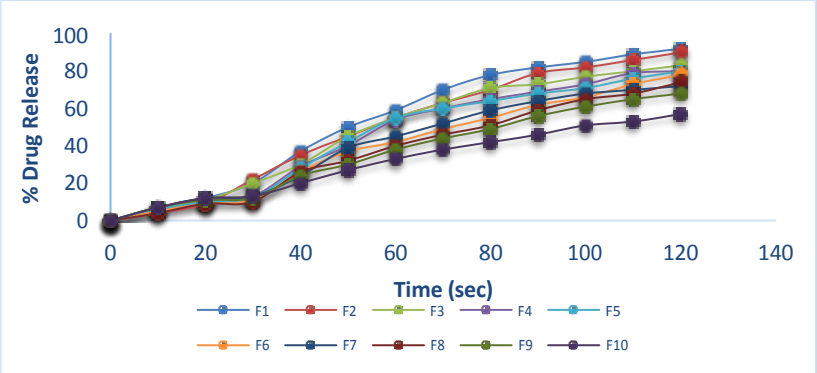

Figure 4: Shows Release profile of Clindamycin from its gel formulae

3.4 Drug Release Kinetic Study: To analyse the release data, several kinetic models were used, including cumulative % drug release vs. time (zero order kinetic model), log cumulative % drug remaining vs. time (first order kinetic model), and cumulative% drug release vs. square root of time (Higuchi model). In the table, the R2 values are tabulated. The Higuchi model kinetics suited all formulas the best.

Table 3: Kinetic evaluation of in vitro release data in various formulas

Formula	Correlation Coefficient(R^2)					
	Zero order		First order		Diffusion	
	Fluconazole	Clindamycin	Fluconazole	Clindamycin	Fluconazole	Clindamycin
F-1	0.9339	0.9456	0.9896	0.9906	0.9929	0.9949
F-2	0.954	0.952	0.9958	0.9842	0.9960	0.9991
F-3	0.8727	0.8711	0.9722	0.9769	0.9938	0.9968
F-4	0.9268	0.9201	0.9928	0.9889	0.9994	0.9982
F-5	0.9651	0.9623	0.9893	0.9897	0.9975	0.9887
F-6	0.903	0.910	0.9438	0.9269	0.9649	0.9786
F-7	0.7823	0.7936	0.9255	0.9301	0.9255	0.9352
F-8	0.9666	0.9768	0.9876	0.9799	0.9950	0.9889
F-9	0.9593	0.9687	0.9905	0.9899	0.9964	0.9992
F-10	0.9833	0.9910	0.9774	0.9879	0.9935	0.9854

3.5 In-vitro Drug Diffusion Study: Table exhibits topical gel formula penetration over cellulose membrane in vitro. All gel formulae release Fluconazole and Clindamycin linearly (r >0.9). At 4 hours, F1, F3, F5, F7, and F9 penetrated 220.63, 246.2, 174.23, 243.8, and 144.44 µg/cm2/h. Vehicle composition impacts medication release and skin permeability. F3 penetrated epidermis the greatest after 4 hours, indicating its topical efficacy.

Table 4: In vitro Drug Diffusion Investigation of Various Topical Gels

Batch	Js (µg cm^{-2} hr^{-1})	P(cm hr^{-1})	K	r
F 1	220.63	0.022	36.94	0.9475
F 3	246.37	0.024	146.53	0.9999
F 5	174.23	0.017	6.76	0.99999
F 7	243.83	0.024	61.66	0.9865
F 9	144.44	0.004	-428.71	0.9945

3.6 Skin irritation study: All formulations were tested for skin irritation, including itching, minor and moderate erythema. No sign of any reaction was found in any formulation.

Table 5: Skin irritation studies of various batches of Topical gel

Batch	Skin irritation studies
F-1	A
F-2	A
F-3	A
F-4	A
F-5	A
F-6	A
F-7	A
F-8	A
F-9	A
F-10	A

A: No reaction, B: Slight erythema, C: Moderate erythema

3.7 Antifungal study: The antifungal activity of gel from its different gel formulae compared with Nizoral® cream as control. The inhibitory zone measured antifungal activity. F3 having the most activity at 37 mm and F2 the lowest at 19 mm. In vitro release research results match these. The chosen dissolving model matches in vitro antimicrobial susceptibility testing.

Table 6: Displays the topical gel formula's inhibitory zone.

Batch	Inhibition zone(mm)
Nizoral® cream	22
F-1	22
F-2	19
F-3	37
F-4	36
F-5	36
F-6	27.5
F-7	35
F-8	32
F-9	32.5
F-10	30

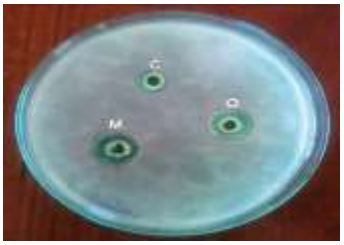

Figure 5: In vitro Antifungal Studies

C = Control, M = Marketed Formulation, O = Topical gel (F3)

3.8 *In-Vivo* Antifungal Studies: Rat model was used to investigate the in-vivo efficacy of topical gel formulation. *C. albican* isolate was employed for the induction of cutaneous candidiasis in animals used (rats). The potency of gel formulation is compared with plane drug solution in rats induced with cutaneous candidiasis. Viable *C. albicans* were retrieved from lesions of all the treated rats. Treatment with formulation containing Fluconazole & Clindamycin showed 88.8%negative culture while that with the drug solution gave 36.2% result. Moreover, Quick healing from fungal infection was reported in gel formulations considerably significant ($p<0.001$) difference in comparison to drug solution and that of formulation is attributed to the enhanced capacity of Fluconazole & Clindamycin containing gel formulation to penetrate the skin and therefore transfer agents into the subcutaneous areas.

Table 7: In vivo antifungal studies of F3 formulation

Group	Treatment	Total animals with positive culture/ No. of animals%	Log of CFU/ Infected Site
Group I	Control	9/9 (100%)	3.78± 0.60
Group II	F-3	2/9 (22.2%)	2.64 ± 0.40
Group III	Drug Solution	7/9 (77.8%)	3.62 ± 0.55

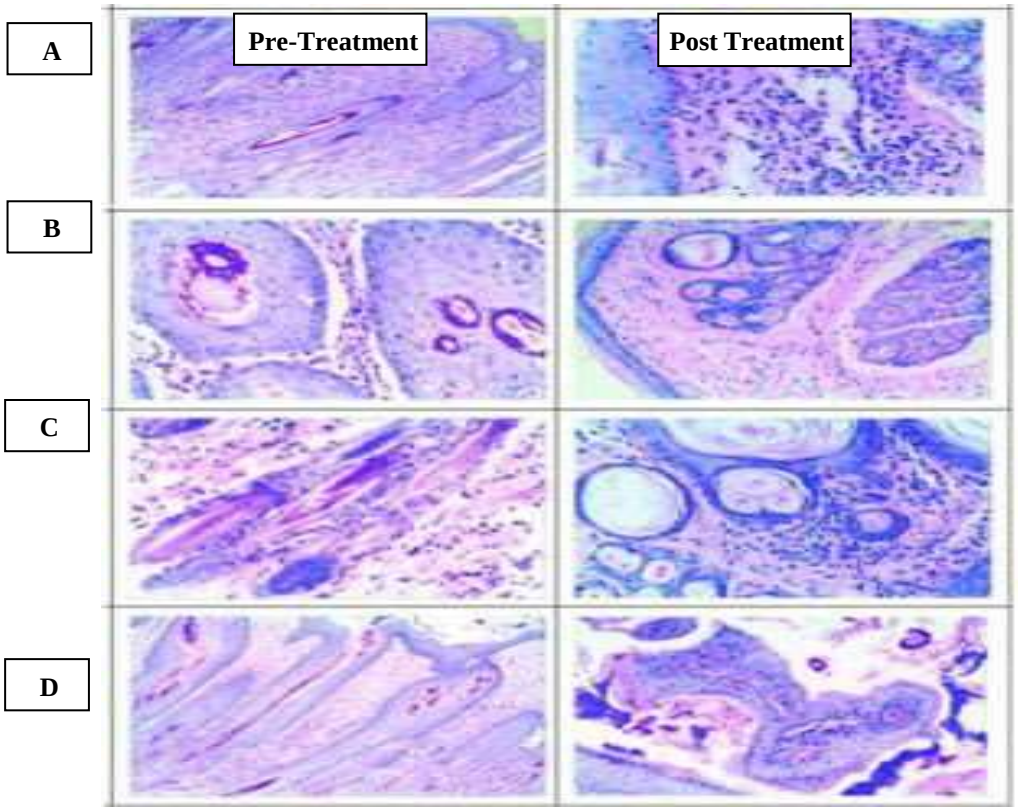

Figure 6: Skin histology after PAS staining. Red spots represent *C. albicans* on the skin. Less fungi were found after treatment with- A: Topical gel, B: free drug solution in ethanol. C: Only solvent ethanol. D: No Treatment

3.9 Stability Studies: For the optimized Gel formulation F3 was carried out over a span of six months. Evaluation of the formulation was done for the content of drug, pH and percent release over the duration was done. The formulation was attributed to have average 92.9 ± 1.5 mg drug content at the starting 0 month, which found to be 92.8 ± 1.8 at the 6th month. There is no significant difference between the drug content in mean time interval. This

justifies the standardized parameters of evaluation and there is no engulfing and loss of drug content. In the duration of the study the pH and physical appearance of the formulation remained constant. Drug formulation % drug release does not alter much. At the day 0 it was found to be 61 ± 1.24 and at 3rd month it was found to be 64.23 ± 1.9 %.

Table 8: Stability Studies of Optimized Gel formulation

Parameters	Months			
	0	1	2	3
Drug content (mg)	92.9 ± 1.5	91.1 ± 0.50	94.7 ± 1.2	92.8 ± 1.8
pH	6.2	6.1	6.2	6.3
(%) Drug release	61 ± 1.24	63.5 ± 0.23	65 ± 1.24	64.23 ± 1.9
Physical appearance	Transparent gel			

Mean ± SD, N= 3 p<0.05

4. Conclusion

Based on earlier findings, topical gel was successfully incorporated into several topical gel compositions. Formula F 3 is the most spreadable, viscous, drug-releasing, and antifungal. F-3 had the best viscosity, pH, ex-vivo drug permeability through rat skin, skin irritation, and drug content. Optimized batch F3 release kinetics indicated zero-order kinetics. Three groups were created and discovered that the formulation outperformed the negative control and the medication solution-only group. Gel enhances drug permeability. This shows that Fluconazole and Clindamycin gel can cure fungal infections topically. The study shows that a topical polymeric formulation for patient compliance is possible. In vivo experiments are needed to determine their efficacy. This study may assist explore pharmacological and polymer treatments for fungal infections. This research suggests treating fungal infections with topical Fluconazole & Clindamycin gel.

5. Acknowledgments

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