RESEARCH ARTICLE

Formulation, Stability, and Antifungal Potential of Lemongrass Oil-Loaded Organogel in BALB/c Mice

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ABSTRACT

The current work intended to develop a stable, lemon grass oil (LGO) amalgamated organogel with sustained release of drug profile and superior antimicrobial potential. The LGO organogel were further analyzed for organoleptic character, diffusion, spreadability, and antifungal potential. The organogels possessed slight viscous texture of yellowish pale colour. The pH of the gels (5.65 ± 0.05 to 5.81 ± 0.05) was adjusted to be in close proximity with the skin pH. The LGO loading capacity (97.10 to 101.30%) of organogels was within established and acceptable limits. The textural and rheological profile were within optimum values. A complete 90-100% of LGO diffusion from the organogel required 9-12 hr. The organogels exhibited excellent prolonged release drug profile in all optimization batches.The F4-optimized organogel displayed remarkable sustained release pattern of drug release. The viscosity in formulation batches were in the range of from 2548 (F6) to 3412 cP (F3) while the spreadability ranged between 9.85 to 13.84 gm. cm/sec. At a concentration of 30µg/ml, the optimised batch of organogel (F4) exhibited the maximum antifungal efficacy based on the zone of inhibition (33 mm). Mice in Group 6 exhibited a 165% reduction in erythema severity compared to Group 5, indicating the superior efficacy of the LGO loaded organogel in treating superficial mycosis and promoting lesion recovery. Moreover, organogel exhibited excellent physical and chemical stability under accelerated conditions, making it a promising formulation for long-term use. This enhanced therapeutic effect is likely attributed to the formulation's immediate drug availability, ensuring a robust local antifungal action at the infection site. The intended work exhibits the efficacious development of LGO-loaded sustained-release organogel with superior antifungal potential.

Keywords: Lemon grass oil, organogel, antifungal, spredability, viscosity, diffusion

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INTRODUCTION

The chronic and recurring character of fungal infections, especially those caused by *Candida albicans*, presents a significant difficulty in dermatology[1]. The limitations of traditional antifungal therapies, such creams and ointments, might result in patient non-compliance[2]. These drawbacks include inadequate skin penetration, quick drug clearance, and frequent dose needs[3]. Interest in innovative drug delivery methods like organogels has grown as a result of the requirement for an antifungal formulation that is efficient, biocompatible, and has a sustained release[4]. An organic phase confined in a 3D network of suitable gelators results in organogels formulation, which are semi-solid in nature. These formulations are perfect for topical antifungal therapy because they provide a number of benefits, including as better skin retention, controlled release, and increased drug stability[5].

Lemongrass oil (LGO), one of several natural antifungal agents, has drawn interest because of its strong antibacterial properties. Bioactive substances with broad-spectrum antifungal qualities, including citral, geraniol, and limonene, are found in LGO, which is produced from *Cymbopogon citratus*[6]. According to earlier research, LGO efficiently inhibits *Candida albicans*, which makes it a viable substitute for traditional antifungal medications. However, LGO's therapeutic potential is limited when taken alone due to its volatile nature and quick breakdown. LGO's stability, antifungal efficacy, and prolonged drug release at the infection site can all be improved by encasing it in an organogel matrix[7].

The increasing need for alternative antifungal treatments that provide longer-lasting effectiveness and better patient compliance serves as the justification for this investigation. Even while synthetic antifungal medications like econazole are readily available, long-term usage of these medications might result in drug resistance, decreased effectiveness, and unfavorable side effects[8]. A possible solution to these problems is the use of natural antifungal agents, such as LGO, in a controlled-release organogel system. Additionally, evaluating such a formulation's stability and effectiveness under accelerated settings guarantees that it is suitable for long-term usage[9].

Using BALB/c mice, a well-known in vivo model for cutaneous infections, this work attempts to create and assess an LGO-loaded organogel for its physicochemical stability, prolonged-release characteristics, and antifungal activity against *Candida albicans*. The goal of this research is to create a new, stable, and powerful antifungal

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Table 1: Formula composition of organogel

S.no.	Ingredients	Quantity (mg)				
		F3	F4	F5	F6	F7
1.	Lemongrass oil	250	250	250	250	250
2.	Span 80	500	250	500	500	500
3.	Tween 80	500	500	500	750	750
4.	Carbopol 934	60	40	40	40	60
5.	Methyl paraben	3	3	3	3	3
6.	Propyl paraben	1	1	1	1	1
7.	Water (q. s. to 10 gm)	8686	8956	8706	8456	8436

formulation that increases the therapeutic potential of naturally occurring bioactive substances by incorporating LGO into a controlled-release organogel. The results of this study might lead to the creation of substitute antifungal medications, lowering reliance on synthetic medications and addressing problems with side effects and drug resistance.

MATERIALS AND METHODS

Materials

Lemon grass oil was procured from local market. Span 80 and polysorbate 80 were acquired from Loba Chemie Mumbai. Carbopol 934, Propyl paraben and Methyl paraben were obtained from Sigma Aldrich.

Methods

Formulation of Lemon grass-loaded organogel

Span 80 and lemon grass oil were weighed accurately and mixed uniformly in beaker. Tween 80 was weighed as per batch quantity and added in another beaker containing pre weighed Carbopol 934. Span 80 and lemon grass oil mixture was then added to the mixture of Carbopol 934 and Tween 80 under continuous stirring till cream-like mixture forms. Dropwise addition of water was done during mixing of both phases. Methylparaben and Propylparaben as per batch quantity was added to above mixture under continuous stirring. The pH of the formulated organogel was adjusted to a range of 5.4 to 5.9 using 0.1 N hydrochloric acid, resulting in a pale yellow creamy organogel[10]. Formula composition of the organogel is presented in Table 1.

Evaluation of organogel

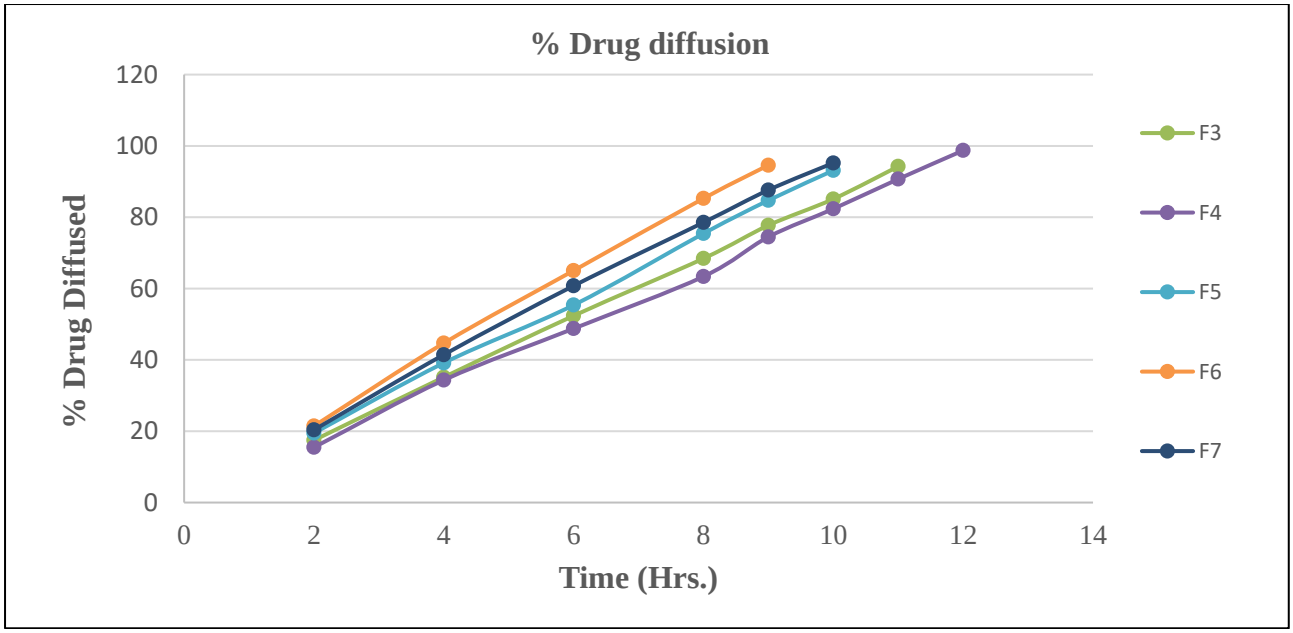

Figure 1: Comparative in vitro drug diffusion of organogel formulations (F1 to F8) in phosphate buffer solution pH 5.8

Table 2: Physicochemical characterization studies of the developed organogels

Batches	Color	Texture	pH	% Assay	Spreadability (gm. cm/sec)	Viscosity (cP)	Diffusion time (hr) (90-100% diffusion)
F3	Pale yellow	Viscous	5.65 ± 0.05	98.12	9.85	3412 ± 11.15	11
F4	Pale yellow	Smooth	5.74 ± 0.05	99.60	11.86	3105 ± 13.50	12
F5	Pale yellow	Smooth	5.79 ± 0.05	97.33	12.67	2845 ± 20.46	10
F6	Pale yellow	Smooth	5.81 ± 0.05	101.30	13.84	2548 ± 19.50	9
F7	Pale yellow	Viscous	5.74 ± 0.05	97.10	11.98	2874 ± 11.14	10

Table 3: % Cumulative Drug Release from organogel batches

Time (Hrs.)	% Drug Release				
	F3	F4	F5	F6	F7
2	17.48	15.37	19.59	21.3	20.21
4	36.06	34.29	39.89	44.64	41.55
6	51.81	48.73	55.17	64.94	60.47
8	68.05	64.76	74.79	84.19	78.22
9	77.35	74.27	84.74	94.22	87.83
10	85.21	83.74	93.8	-	95.59
11	94.29	90.49	-	-	-
12	-	98.73	-	-	-

Color and Texture: All organogel batches were checked for color against white background and texture was analysed by taking organogel between two fingers.[11,12]

pH: Organogel (0.5 g) was dissolved in water (50mL) in a beaker. The pH of resulting solution was measured using Calibrated pH meter.

Drug content: First, a 100 mL volumetric flask was thoroughly cleaned and dried. Then, 800 mg of the test sample (equivalent to 20 mg of lemongrass oil) was accurately weighed and transferred into the flask. Next, methanol was mixed and subjected to sonication for 5 to 10 minutes to ensure proper dissolution. After sonication, the volume made up using methanol (q.s). The resultant sample was passed through through a 0.45 µm PVDF membrane, discarding the first 3 to 5 mL of the filtrate to ensure accuracy. From the filtered solution, 1 mL of aliquot was taken further mixed with methanol to 20 mL. Finally, the sample was analyzed at 238 nm in a single replicate, and the % assay was calculated against the standard.

Viscosity: The Brookfield viscometer (20 rpm and spindle 64) was used to measure the viscosity of organogel. Torque (%) and viscosity (cP) were measured.

Diffusion study: A Franz diffusion cell setup was utilized to evaluate drug release from the developed organogel. The setup consisted of a donor and receiver section, partitioned by a cellophane sheet. The receptor compartment was designed for sampling, while the donor compartment (24 mm inner diameter) remained open to the atmosphere. Phosphate buffer solution (PBS, pH 5.8) was used as the diffusion medium. A 200 mg dosage form containing 5 mg of equivalent drug was kept over the pre-soaked cellophane membrane (immersed in PBS for 24 hours, donor chamber). The compartments were clamped together, ensuring the membrane made slight contact with the diffusion medium. The receiver chamber held 25 mL of PBS, sustained at 37 ± 0.5°C, and agitated unceasingly using lab stirrer at 100 rpm. At predetermined time points, 1 mL of aliquots were collected, diluted to 20 mL with methanol, and analyzed at 238 nm using a UV spectrophotometer. After each sampling, an equivalent aliquots of fresh PBS was reconstituted in dissolution media to continue sink state.

Spredability: To assess the spreadability of the organogel, 1 g of the organogel was positioned between two glass slides. A 100 g weight was placed on the top slide to ensure even distribution of the gel. After a few moments, the weight was removed, and any excess gel was wiped off. The bottom slide was fixed onto a stable board, while the top slide was attached to a non-flexible string supporting a 20 g weight. The tenure required for the top surface to slip off was recorded, and the distance it moved was measured using a digital vernier caliper.

Spreadability (S) was evaluated utilizing the calculation:

$S=L\times D/T$

Where:

S = Spreadability

L = Load on the upper surface (20 g)

D = Distance moved by the upper surface

T = Time required for the slide to move

In vitro anti-fungal activity

Antifungal activity of optimized organogel, and Econazole (std drug) was determined using well diffusion method against microbial strain Candida albicans. Sensitivity of microorganism towards the anti-fungal formulation was studied at the dose of 30 µg/ml. The dose of 10, 20 and 30 µg/ml was used for comparative study.

In vivo animal studies

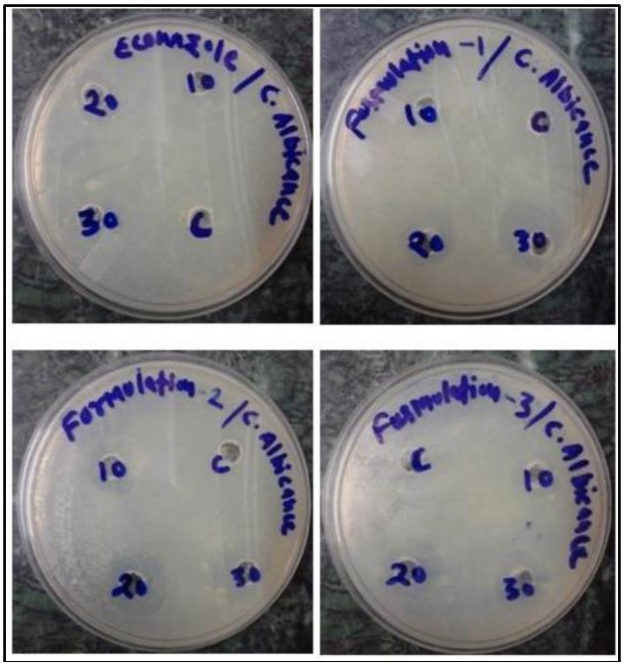

Figure 2: Photograph showing antifungal activity

Table 4: Antifungal activity of different formulations against Candida albicans

Sr.no	Sample	Zone of inhibition (mm)		
		10µg/ml	20µg/ml	30µg/ml
1.	Econazole	10±0.22	12±0.12	15±0.11
2.	Organogel (Containing Lemon grass oil) Formulation F4	19±0.15	27±0.23	33±0.25

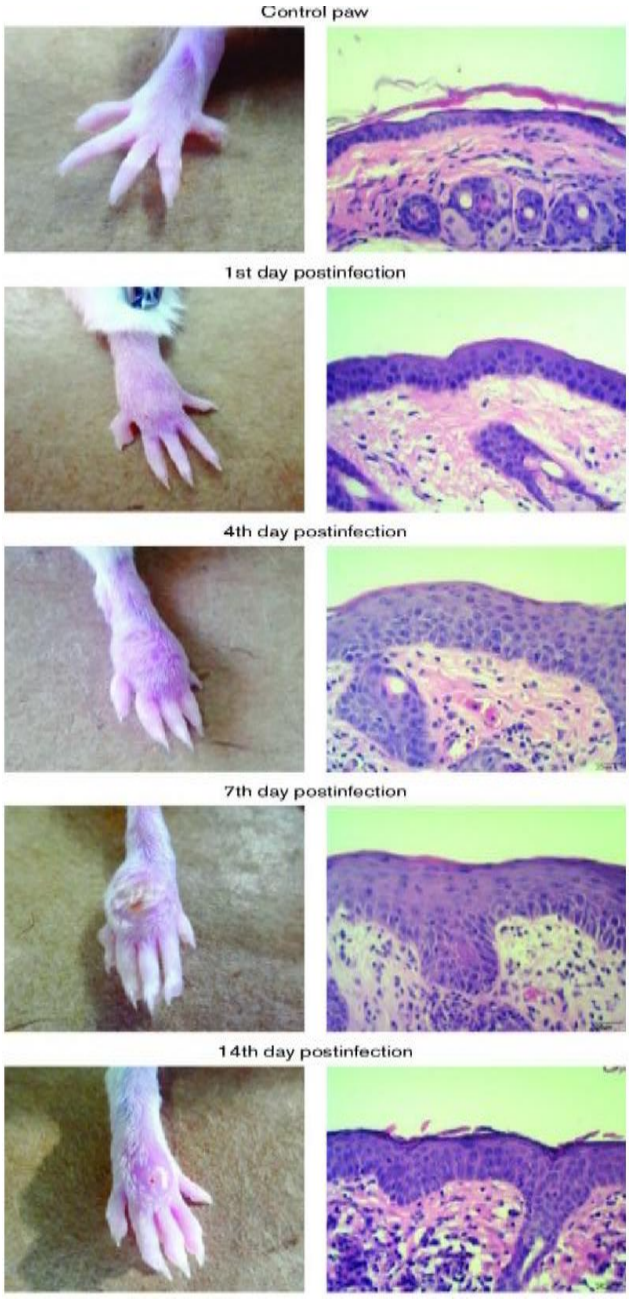

Figure 3: *In vivo* antifungal potential of organogel formulation

Female BALB/c mice weighing approximately 20g and 42 days old were utilized in this experiments. Mice were caged in a controlled environmental conditions at standard conditions of temperature and humidity (23ºC, 55±10%), and alternate light dark cycles. All studies were accomplished in agreement with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals and the guidelines of the institutional animal care and use committee.

In Vivo Skin disruption and Antifungal potential of Organogel

Animals were randomly subdivided into 6 groups with 15 animals each. Groups 3, 4, 5, and 6 were intraperitoneally introduced dexamethasone sodium phosphate in aqueous phase (5mg/kg/day) for 3 days to induce immunosuppression. On the 2nd day of induction, nearly 2X2 cm2 of posterior hair was trimmed in all rodents. Subsequently, disinfected finely-meshed sandpaper was used to create a wound of approximately 1.75 X 1.75 cm^2 in groups 3, 4, 5, and 6. *C. albican*s (10^7CFU) fungal strain was continuously exposed to the scratched surface of mice in groups 4, 5, and 6 for next 3 days. Microbial infiltration in groups 4, 5, and 6 was proved by assessment of scientific scores (based on the presence of exudate, erythema, and a white substance over the infected area) and compared with the outcomes in group 3. The symptoms were individually scored as follows: 0 (complete absence), 1 (slightly visible), 2 (clearly visible), 3 (substantially visible in a small area), and 4 (substantially visible in a large area). Furthermore, to investigate the antifungal ability, the lesion area was captured by means of a digital camera and analyzed through ImageJ software (LOCI); the rate of healing was articulated as a fraction of the remanant wound area. Moreover, skin (2X2 cm2) from wound sites (n = 5 mice per group) were isolated for fungal infection examination in groups 3,4,5,6. The isolated skin were reduced to attain excisions of 8 mm in diameter. Each excision was subjected to vortexing in SDA medium (without antibiotics) for 1 min to allow isolation of remnant viable microbe. Later, isolates was burdened onto a SDA plate and allowed to rest at 37^0C. The number of colonies grown were examined and the average values (CFUs/1 cm2 skin) were reported. Itraconazole was administered for 10 days in all mice for therapy induction. Treatment of mice with Organogel(Containing Lemon grass oil) at concentration equal to 40 mg/kg/day was accomplished as follows: groups 1, 3, and 4 Organogel(Containing Lemon grass oil) groups 2 and 3 Organogel(Containing Lemon grass oil) and group 5 [Econazole (oral)].

Stability study

F4 batch was charged for stability study at 40° C / 75% RH and stability study was conducted for 6 months. Analysis performed at 3 and 6 months.

RESULTS AND DISCUSSION

Physicochemical properties of organogels

The developed organogels were evaluated for various physicochemical properties. All gels were possessed smooth to viscous texture of pale colour. Span 80, Tween 80, and Carbopol 934 played excellent role in maintaining the smooth texture of the gels[13]. The pH of the gels ranged from 5.65 ± 0.05 to 5.81 ± 0.05, aligning well with the natural skin pH (4.7 to 5.75), indicating their suitability for topical application. The drug content of the organogels (97.10% to 101.30%) was within the acceptable range of 85% to 115%. Additionally, the spreadability and viscosity values were estimated to be within limits. The comparative physicochemical parameters of developed organogels are displayed in Table 2.

In vitro diffusion study

Organogels enable controlled drug release, enhancing bioavailability and minimizing side effects by maintaining therapeutic levels. Their prolonged release reduces dosing frequency, improving patient compliance[14]. Additionally,

Table 5: Stability study results at 40° C / 75% RH

Sr. no.	Parameter	Initial	3 M	6 M	Acceptance criteria
1	% Diffusion at 12 hrs	98.73	98.87	95.60	NMT 5 %
2	Viscosity (cP)	3105 ± 13.50	3028 ± 12.06	2982 ± 11.24	NMT 10% w.r.t. to initial
3	Spreadability (gm.cm/sec)	11.86	11.63	11.45	NMT 10% w.r.t. to initial
4	Assay (%)	99.60	97.75	97.29	NMT 5.0
5	pH	5.74 ± 0.05	5.65 ± 0.05	5.59 ± 0.05	NMT 0.2
6	Colour and Texture	Yellowish pale colour and lack of grittiness	Yellowish pale colour and lack of grittiness	Yellowish pale colour and lack of grittiness	Unnoticeable phase separation, colour change or deterioration

they provide stability, protecting drugs from degradation. In Table 2 the time required for 90-100% drug diffusion is captured. The diffusion of lemongrass oil from the organogel formulation required approximately 9–12 hours, with F6 achieving 90% diffusion and F4 reaching 100%. Span 80 and Tween 80 exhibited an inverse relationship with diffusion time, decreasing it as their concentrations increased. Conversely, Carbopol 934 showed a direct correlation, where higher concentrations led to prolonged diffusion times. The comparative drug diffusion profiles of all organogel formulations (F3 to F7) in PBS (pH 5.8) are detailed in Table 3 and Figure 3. All formulation batches exhibited excellent prolonged release behavior. Span 80 and Tween 80 enhanced emulsification and drug dispersion, while Carbopol 934 played a key role in forming a gel-like matrix, increasing viscosity, and affecting hydration and swelling behavior.

Spredability

The spreadability of organogels is crucial across industries, impacting application ease and user acceptance. In cosmetics, it enhances user experience and product performance, while in pharmaceuticals, it ensures uniform drug distribution and absorption[15]. In industrial applications, it aids in coatings and lubrication. Table 2 presents spreadability observations with varying independent variables. The spreadability across all batches ranged from 9.85 g.cm/sec (F3) to 13.84 g.cm/sec (F6), significantly influenced by Span 80 and Tween 80, which exhibited a direct correlation—higher concentrations increased spreadability. In contrast, Carbopol 934 showed an inverse relationship, where increased concentration led to reduced spreadability.

In vitro anti-fungal activity

Candida albicans was inhibited by the standard antifungal drug in present work i.e., oregano gel with Lemongrass oil. The concentration (10, 20 and 30 µg/ml) was used for comparative study. The results of anti-fungal activity are shown in table 4.

The antifungal efficacy of the optimized organogel formulation (F4) containing lemongrass oil was evaluated against *Candida albicans*. The study assessed the zone of inhibition at three concentrations (10, 20, and 30 µg/mL) for both the organogel and the standard antifungal agent, econazole. The optimized formulation (F4) exhibited significantly larger inhibition zones of 19±0.15 mm for low dose**,** 27±0.23 mm for medium dose**,** and 33±0.25 mm for high dose, respectively, compared to econazole, which demonstrated inhibition zones of 10±0.22 mm**,** 12±0.12 mm**,** and 15±0.11 mm at the corresponding concentrations. These results indicate that the organogel formulation (F4) possesses enhanced antifungal activity against *C. albicans* relative to the marketed econazole formulation. The findings suggest that the incorporation of lemongrass oil in the organogel formulation contributes to its superior antifungal efficacy.

In vivo animal study in BALB/c Mice

The outcomes of mice skin irritation observations and antifungal assays were estimated based on lesion size, as well as the presence or absence of exudate, skin redness (erythema), and dry scaly skin. Mice in groups 1 [normal skin treated with Organogel(Containing Lemon grass oil) (vehicle)] and 2 [normal skin treated with Organogel(Containing Lemon grass oil)] did not exhibit any signs of skin disruption, signifying compatibility of developed Organogel (Containing Lemon grass oil) (vehicle) and Organogel (Containing Lemon grass oil) in normal mice skin. The isolate severity scores for mice in groups 4 [fungal-infected skin treated with Organogel (Containing Lemon grass oil) (vehicle)], 5 [fungal-infected skin treated with ITZ (oral)], and 6 [fungal-infected skin treated with Organogel(Containing Lemon grass oil)] were estimated to be higher than the exudate severity score for mice in group 3 [scratched skin treated with Organogel(Containing Lemon grass oil) (vehicle)] on day 0. Groups 4, 5, and 6 reveal efficacious fungal infiltration based on the outcomes obtained. On day 0, the exudate and erythema severity scores in groups 4, 5, and 6 indicate analogous levels of microbial infiltration. Post 10 days of constant therapeutic exposure, animals in group 4 displayed unnoticeable changes in the wounded region. Conversely, rodents in groups 5 displayed 77.5% and group 6 illustrated 93.1% reduction of lesion area in infiltrated region. Presence of exudates were missing after 7 days of therapy among mice in group 6 (Figure 3). Additionally, a inferior erythema score of around 165% in group 6 was obtained compared to group 5. This greater therapeutic outcome of Organogel (Containing Lemon grass oil), in association to superficial mycosis and lesion recovery at the infested region, may have been due to the

immediate drug release that allowed superior therapeutic activity.

Stability study

Stability testing of the organogel was performed at 40°C / 75% RH over a tenure of half years, and the results indicate that the formulation remained stable with minimal variations in key parameters (Table 5). The % diffusion at 12 hours exhibited a slight decline from 98.73% (initial) to 95.60% (6M), which remained within the acceptance criteria of NMT 5.0% variation. The viscosity showed a minor reduction from 3105 cP to 2982 cP, which was within the permissible limit of NMT 10% deviation. Similarly, spreadability decreased marginally from 11.86 to 11.45 gm.cm/sec, adhering to the NMT 10% acceptance criteria. The assay values showed a slight decline from 99.60% to 97.29%, remaining well within the acceptable NMT 5.0% variation. The pH values exhibited minimal fluctuations from 5.74 to 5.59, staying within the NMT 0.2 limit. Additionally, no changes in colour or texture were observed, and the formulation maintained its pale yellow and smooth appearance without any phase separation or deterioration. These findings confirm that the organogel exhibits excellent physical and chemical stability under accelerated conditions, making it a promising formulation for long-term use.

CONCLUSION

In conclusion, this study successfully developed sustained-release organogel incorporating lemongrass oil with enhanced antifungal efficacy. The optimized formulation exhibited prolonged antifungal activity, stable over period of 6 months at accelerated conditions demonstrating its potential for dermatological applications requiring sustained and effective therapy against fungal infections. These findings contribute to the advancement of controlled-release pharmaceutical formulations, offering a novel approach for improving antifungal treatment efficacy and expanding the scope of organogel-based drug delivery systems.

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