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COMPARATIVE ASSESSMENT OF NAIL PERMEATION, DRUG RETENTION AND ANTIFUNGAL ACTIVITY OF EFINACONAZOLE LOADED INVASOMAL AND CONVENTIONAL GELS FOR TREATMENT OF ONYCHOMYCOSIS

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ABSTRACT
Onychomycosis is a widespread fungal infection of the nails that is challenging to treat since the dense keratinised nail plate hinders the penetration of topical antifungal drugs. This study aimed to develop and optimize an efinaconazole-loaded invasomal gel and to compare its nail permeation, drug retention and antifungal activity with those of a conventional gel formulation for the treatment of onychomycosis. Invasomal formulations were developed with the use of phospholipid, ethanol and terpene-based components and optimized according to the criteria of vesicle size, entrapment efficiency and polydispersity index. The optimized invasomal gel was characterized in terms of physicochemical properties, in vitro drug release, nail permeation, drug retention and antifungal activity. The optimized invasomal gel formulation had the following characteristics: vesicle size of 135 ± 3 nm, entrapment efficiency of 91.8 ± 0.7% and good physical stability. As compared to the conventional gel, the invasomal gel showed significantly higher values of cumulative drug release, nail permeation, drug retention and antifungal activity. Nail permeation increased twice and drug retention was 122% higher for invasomal gel as compared to the conventional gel. Moreover, invasomal gel caused larger zones of inhibition, low minimum inhibitory concentrations and higher reduction in the fungal burden in the course of in vivo assessment without causing any irritation. The improvement in the performance of invasomal gel is explained by the synergistic action of phospholipids, ethanol and terpenes. This increased the deformability of vesicles, drug transport through the keratinized nail plate and maintained the prolonged drug release from the vesicles. Overall, the optimized efinaconazole-loaded invasomal gel demonstrated significant potential as an advanced topical formulation for improving drug delivery and antifungal efficacy in the management of onychomycosis.

KEYWORDS: Onychomycosis, Efinaconazole, Invasomal gel, Transungual drug delivery, Nail permeation, Antifungal activity

1. INTRODUCTION

1.1 Background of Onychomycosis

Onychomycosis is one of the most common fungal infections of the nail which is caused by dermatophytes, non-dermatophyte molds and yeasts. The common causative organisms include Trichophyton rubrum, T. mentagrophytes, Aspergillus spp., Fusarium spp and Candida spp. The worldwide prevalence of this fungal infection is 5.5% and the risk factors include poor nail grooming, prior dermatologic conditions such as tinea pedis and psoriasis [1]. Moreover, comorbidities including diabetes, immunosuppression and obesity can also enhance the risk of onychomycosis. This infection is characterised by nail plate discoloration, thickening, brittleness, subungual hyperkeratosis, and separation of the nail plate from the nail bed. Although onychomycosis is not life threatening, it significantly impacts the quality of life of the patients by causing pain, discomfort and cosmetic concerns. Furthermore, untreated infections may persist for many years which results in disease progression, and recurrent infections, thereby highlighting the need for effective therapeutic interventions.

1.2 Current Treatment Approaches

There are various kinds of treatment approaches for onychomycosis including topical, oral, laser, photodynamic light and procedural treatments. Oral therapies are regarded as the gold standard for the treatment of onychomycosis. The oral agents including terbinafine, itraconazole, and fluconazole are considered to provide most effective therapy. However, oral therapy requires prolonged use due to restricted capacity and bioavailability to maintain proper drug concentrations in the nail bed. On the other hand, topic treatments have minimal side effects and low systemic exposure. The topical antifungal formulations such as ciclopirox, amorolfine and efinaconazole are often considered as most suitable for therapeutic interventions for onychomycosis. Moreover, efinaconazole shows the highest probability of cure after oral treatment with a mycologic cure rate of 54 percent and a complete cure rate of 17 percent [2]. Despite the availability of

multiple treatment approaches, the achievement of complete clinical and mycological cure remains challenging due to poor drug penetration into the nail plates. Hence, there is a need to explore novel drug delivery systems to enhance nail permeation and improve therapeutic outcomes in onychomycosis treatment.

1.3 Efinaconazole in Nail Drug Delivery

Efinaconazole is an azole antifungal agent which belongs to the 1,2,4 - triazole chemical class (Figure 1). The heterocyclic nature of the drug molecule is basic and exists in hydrochloride salt form with hydrochloric acid. Efinaconazole has demonstrated "potent broad spectrum of activity" against Trichophyton species and Candida albicans as compared to other antifungal agents [3]. It exerts antifungal activity through inhibition of lanosterol 14α-demethylase which is a key enzyme involved in the biosynthesis of ergosterol, an important component of fungal cell membranes. However, the effectiveness of topical antifungal therapy largely depends on the ability of the drug to penetrate through the highly keratinized nail plate. In this regard, efinaconazole and tavaborole do not bind to keratin which enhances the topical therapeutic effectiveness to treat onychomycosis [4]. However, the dense keratinised structure of the nail plate significantly restricts the diffusion of the drug into the deeper infected tissues. Therefore, further research is required to compare the nail permeation, drug retention, and antifungal activity of efinaconazole-loaded invasomal and conventional gels for the treatment of onychomycosis.

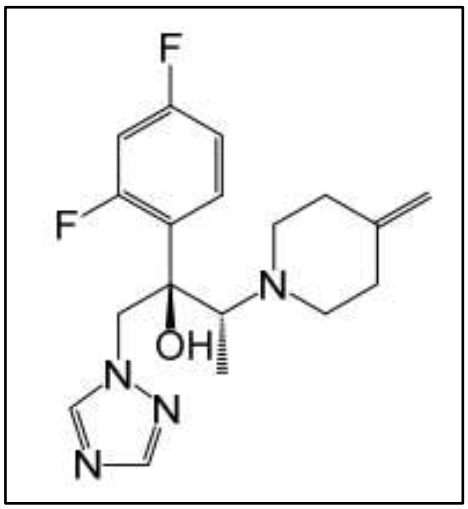

Figure 1: Molecular structure of Efinaconazole [3]

1.4 Invasomal Drug Delivery Systems

Invasomes are advanced vesicular drug delivery systems which are composed of phospholipids, ethanol and terpenes. All the components of the invasomes work in a synergistic way to enhance vesicle flexibility and improve drug penetration through biological barriers. Phospholipids form the vesicular structure, ethanol increases membrane fluidity and drug solubility while terpenes act as permeation enhancers by disrupting the constrained arrangement of keratin [4]. Furthermore, invasomes possess high deformability which allows them to penetrate deeper into the nail structure as compared to conventional formulations. Thus, the ability to enhance drug permeation and retention makes invasomal drug delivery systems quite promising to improve therapeutic effectiveness of antifungal agents against onychomycosis.

1.5 Research Gap, Aim and Significance of the Study

Despite the availability of several antifungal therapies, the treatment of onychomycosis remains challenging because of the poor permeability of the nail plate and inadequate drug delivery to the site of infection. Efinaconazole has shown potential as an antifungal agent, but its optimal nail penetration and retention are rarely achieved, thereby reducing its therapeutic use [2]. Furthermore, limited studies have investigated the use of invasomal carriers for enhancing the transungual delivery of efinaconazole. Also, the comparative assessment between invasomal and conventional gel formulations for treatment of onychomycosis remains limited. Therefore, there is a need to evaluate whether invasomal technology can improve nail permeation, drug retention, and antifungal efficacy. The aim of this study is to compare the nail permeation, drug retention and antifungal activity of efinaconazole loaded invasomal and conventional gels in treatment of onychomycosis. The results of this study have the potential to influence the formulation of more effective topical antifungal products and the use of nano vesicular systems in transungual drug delivery.

Research Objectives

- To compare the nail permeation efficiency of efinaconazole-loaded invasomal gel and conventional gel using an in vitro transungual permeation model.
- To evaluate and compare the nail drug retention of efinaconazole-loaded invasomal gel and conventional gel following topical application.
- To compare the antifungal efficacy of efinaconazole-loaded invasomal gel and conventional gel against onychomycosis-causing fungal pathogens through in vitro and in vivo evaluations.

2. LITERATURE REVIEW

2.1 Onychomycosis and Challenges in Topical Drug Delivery

Onychomycosis is a persistent fungal disease of the nail plate, nail bed and surrounding tissues that occurs in almost 50% of the nail disorders in the world [5]. The prevalence of the disease is estimated to be about 5.5% worldwide, with higher prevalence in older adults, patients with diabetes mellitus, peripheral vascular disease (PVD) and immunocompromised patients [6]. The major organisms responsible are the dermatophytes, mainly Trichophyton rubrum and Trichophyton mentagrophytes, although Candida species and non-dermatophyte moulds, including Aspergillus and Fusarium are also implicated in chronic infections [7].

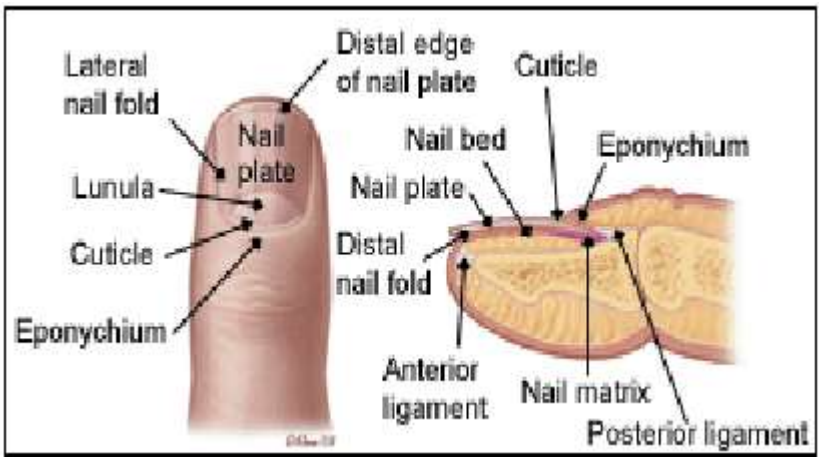

Figure 2: Structure of a nail [8]

Its clinical presentations include a change in the coloration, thickening, brittleness, subungual hyperkeratosis and onycholysis, which can result in pain, aesthetic concerns and decreased quality of life. Although topical antifungal medications are available, successful treatment is difficult since the nail plate is an effective biological barrier. The nail is composed of densely packed keratinized cells interconnected by disulfide bonds with low lipid content and minimal porosity, substantially restricting drug diffusion into the infected nail bed [8]. The amount of transungual drug transport is also dependent on molecular weight, lipophilicity, keratin affinity, nail hydration and disease severity which all affect drug permeation. As a result, the therapeutic drug levels in the nail area are often not reached with conventional topical formulations, thus there is a great need for advanced drug delivery systems to improve permeation of the nail and improve the effect of the antifungal treatment.

2.2 Current Therapeutic Approaches and Role of Efinaconazole

Management of onychomycosis involves oral antifungal therapy, surgical nail removal and topical formulations, laser-assisted treatment, depending on disease severity. Oral antifungal drugs (OADs) (terbinafine, itraconazole and fluconazole) are still considered as first-line drugs due to their high mycological cure rates in moderate to severe infections [9]. Their long-term use is limited by a long duration of treatment, hepatotoxicity, potential drug-drug interactions and poor patient adherence. Topical agents such as ciclopirox, amorolfine, tavaborole and efinaconazole are safer, have low systemic absorption yet often less effective as they are poorly absorbed through the keratanized nail plate [10]. The use of laser therapy and surgical avulsion of the nails may improve response to treatment in select cases though these treatments are expensive, technique sensitive and are typically used as adjunct procedures rather than definitive therapy. Efinaconazole is a triazole antifungal and belongs to the azole class which inhibit lanosterol 14-α-demethylase to prevent ergosterol production, which prevents disruption of the integrity of the fungal cell membranes [11]. It has a broad spectrum of activity against dermatophytes, Candida species and a few non-dermatophyte moulds and has low keratin binding to increase drug availability within the nail than older topical anti-fungal drugs. In clinical studies, efinaconazole has demonstrated good efficacy and safety, with minimal systemic absorption, which makes it a promising topical treatment option [9]. However, its clinical efficacy remains limited due to poor penetration into deeper layers of the nail necessitating the development of new carrier systems with better ability to enhance drug permeation, retention and prolonged antifungal activity.

2.3 Nano-Based Drug Delivery Systems for Onychomycosis

The drawbacks of existing topical antifungal products have led to the creation of nanotechnology-based drug delivery systems for enhancing transungual drug transport [12]. The unique features of these nanocarriers would be to facilitate drug permeation, retention and controlled release across the dense keratinized nail plate. Liposomes are the earliest system, which are relatively rigid with vesicles that are biocompatible and can carry both hydrophilic and lipophilic drugs yet they are unable to penetrate through the compact nail barrier [13]. Both ethosomes containing high ethanol concentration and transfersomes with highly deformable vesicles have better flexibility of the membranes and drug diffusion compared to other vesicles, and also transfersomes have deeper penetration in biological barriers.

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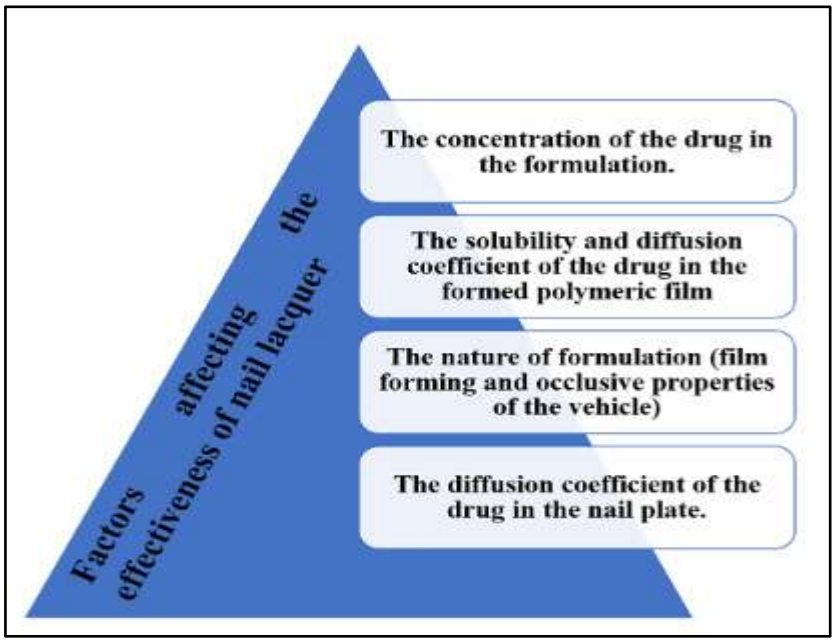

Figure 3: Factors influencing the nail lacquers effectiveness [15]

In addition to their stability and cost-effectiveness, niosomes have been evaluated for their chemical stability and cost-effectiveness, while solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are known for delivering controlled release of drugs and maintaining stability and drug retention in the nail matrix [14]. Among these nanosystems, invasomes have received significant interest, as they are composed of phospholipids, ethanol and terpenes, which interact synergistically to improve the deformability of the vesicles and disruption of keratin [15]. This novel formulation enhances the permeation of drugs into the nail plate and the accumulation of the drug at the infection sitey. Therefore, the use of invasomes as one of the most promising nanocarriers for the transungual delivery of antifungal agents, especially in enhancing the therapeutic performance of topical efinaconazole formulations.

2.4 Invasomal Gel versus Conventional Gel Formulations

Invasomal gels are the next-generation vesicular systems that are developed to achieve the transungual delivery of antifungal agents. They are made up of phospholipids, ethanol and terpenes, which all have an important function in enhancing the transport of drugs. The phospholipids are used as flexible vesicular bilayers that surround the drug, as ethanol makes the membrane more fluid and the drug more soluble and terpenes are used to break the tightly bound keratin structure of the nail for better penetration of the drug [16]. This synergic association results in an improvement of the deformability of the vesicles, which translates to better nail permeation, more drug retention, slow drug release and greater antifungal activity. Compared to this, conventional gels are semi-solid gels formed by using polymeric gelling agents such as Carbopol or hydroxypropyl methylcellulose [17]. While these formulations are easy to produce, cheap and patient-friendly, much of the drug release is by passive diffusion. This diffusion is prevented to a great extent due to the dense keratinized nail plate which leads to insufficient drug permeation and retention in infected nail tissues [18]. The results of comparative studies indicate that the performance of invasomal gels is superior to the conventional gels in terms of transungual permeation, sustained drug release, intranail drug accumulation and antifungal activity. These advantages make invasomal formulations an attractive approach to the topical treatment of onychomycosis and to solve some of the drawbacks of conventional gel formulations [19]. In addition to physicochemical characterisation and permeation studies in vitro, preclinical testing via Wistar rats in vivo is extensively used for evaluation of antifungal activity, local safety and tolerability to skin or nails, and biocompatibility of topical nanocarrier formulations. These studies serve as an important source of information on therapeutic activity and possible toxicity prior to clinical trials.

2.5 Research Gap and Conceptual Basis of the Present Study

Although significant efforts have been made in tropical antifungal agents, the successful treatment of onychomycosis is still limited by an inadequate delivery of effective drug to the infection site which is barrier for topical agents [6] [7]. Efinaconazole shows promising broad-spectrum antifungal efficacy, safety and decreased binding to keratin yet its clinical impact remains limited due to lack of penetration and retention in deeper nail layers [20]. Recent investigations have revealed that these drug delivery systems based on nano-technology such as invasomes can significantly increase the transungual drug delivery by increasing the permeation and prolonging the release of the drug. However, there are not many comparative studies to evaluate efinaconazole-loaded invasomal gels and traditional gel formulations [21]. Thus, the purpose of the present study is to comparatively assess the invasomal formulation of efinaconazole gel to the conventional formulation of efinaconazole gel to observe whether the invasomal technology could enhance the delivery of drugs topically and therapeutic outcome in the treatment of onychomycosis. Also, the study asses antifungal efficacy and safety using an in vivo Wistar rat model to establish the preclinical therapeutic potential and biocompatibility of the developed formulations.

3. MATERIALS AND METHODS

3.1 Materials

Efinaconazole served as the active pharmaceutical ingredient and antifungal agent in the formulations of both the invasomal and conventional gels. Soya phosphatidylcholine (SPC), ethanol and limonene are used for the preparation of invasomes due to their vesicle forming and permeation enhancing properties [22]. Carbopol 934 was selected as the gelling agent while propylene glycol acts as the co-solvent and humectant to improve the consistency of formulation. Triethanolamine is used to adjust the pH of the gel formulations to an appropriate range. Power of hydrogen or pH measures the acidity of basicity of the gel formulations [23]. All the chemicals, solvents and reagents used in the study are of analytical grade and did not require any further purification. Double-distilled water was employed throughout the formulation and evaluation procedures.

3.2 Preparation and Optimization of Efinaconazole-Loaded Invasomes

Efinaconazole-loaded invasomes were prepared using a modified thin-film hydration technique. Soya phosphatidylcholine and efinaconazole were dissolved in a mixture of organic solvent to obtain a homogenous lipid solution. After the process of solvent evaporation is carried out under reduced pressure, a thin lipid film is formed and hydrated with an aqueous phase containing ethanol and limonene. The resulting vesicular dispersion was sonicated to minimise vesicle size and simultaneously improve uniformity. A Design of Experiments (DoE) based factorial design has been used to optimise the formulation by varying phospholipid and ethanol concentrations while maintaining other formulation variables constant. The optimised invasomal formulation was selected for selected for subsequent physicochemical and biological evaluation.

3.3 Preparation of Invasomal and Conventional Gels

The optimised invasomal dispersion was incorporated into a Carbopol 934 gel base to produce the invasomal gel formulation. Carbopol was dispersed in double-distilled water and allowed to hydrate completely before the gradual addition of the invasomal suspension. Propylene glycol was incorporated as a co-solvent to improve the consistency of the formulation and triethanolamine was added to adjust the pH in order to obtain an uniform gel [23]. A conventional gel containing the same concentration of efinaconazole was prepared using the identical gel base but the invasomes were not incorporated. Both the formulations were homogenised and stored under refrigerated conditions until further evaluation.

3.4 Characterization of Formulations

The developed formulations were characterised to evaluate their physicochemical properties and formulation quality. The invasomal dispersion was assessed to determine vesicle size, polydispersity index, zeta potential and drug entrapment efficiency using appropriate analytical techniques. The prepared gel formulations were further evaluated for appearance, pH, viscosity, spreadability and drug content to determine their suitability for topical application. These parameters were selected to ensure formulation stability, uniform drug distribution and acceptable application characteristics. The optimised formulation was subsequently selected for comparative evaluation with the conventional gel based on the obtained characterisation results.

3.5 Nail Permeation, Drug Retention and Antifungal Evaluation

The performance of the developed formulations was investigated through a series of in vitro, ex vivo, and in vivo experiments. Drug release was determined using a Franz diffusion cell to assess the release behaviour of efinaconazole from both formulations [24]. Nail permeation and drug retention studies were conducted to compare the ability of the gels to deliver and retain the drug within the keratinised nail tissue. Antifungal efficacy was assessed against fungal pathogens which are associated with onychomycosis and further confirmed through an in vivo Wistar rat model using the approved experimental protocol. Clinical observations and fungal reduction were recorded throughout the study.

3.6 Statistical Analysis

All experiments were run in triplicate, and the data were presented in terms of mean ± SD. Statistical analysis was done using statistical software to assess the performance of the invasomal and gel-based formulations. Variations between experimental groups were analysed using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons wherever needed. A p-value less than 0.05 was considered statistically significant. The statistical analysis ensured the reliability of the experimental findings and supported the comparative assessment of formulation performance across all evaluation parameters.

4. RESULTS AND ANALYSIS

4.1 Preformulation Studies

Table 1: Preformulation Parameters of Efinaconazole

Parameter	Observation
Exterior	White to off-white crystalline powder
Melting Point (°C)	124–126

λmax (nm)	210
Solubility	liberally soluble in methanol and ethanol; poorly soluble in water and buffer
Calibration Curve (R^2)	0.9992

Table 2: Solubility of efinaconazole in selected excipients used for invasomal formulation

S. No.	Excipient	Solubility (mg/mL)
1	Phospholipid (Soya lecithin)	52.3
2	Cholesterol	15.8
3	Ethanol	92.4
4	Transcutol® P	68.7
5	Propylene Glycol	45.6
6	Span 80	12.6
7	Tween 80	18.9
8	Carbopol 934	4.3
9	HPMC K4M	6.2

The preformulation studies revealed the suitability of efinaconazole for use in the invasomal gel formulation. From Table 1, efinaconazole presented itself as a white to off-white crystalline powder with a melting point of 124 -126 °C which indicated its purity and stability. The drug possessed a maximum absorbance (λmax) at 210 nm while the calibration curve exhibited a high linearity (R^2 = 0.9992) which ensured the accuracy of the analytical method for estimating the drug. In terms of solubility, efinaconazole was found to be highly soluble in methanol and ethanol but not in water and buffer, showing the lipophilic property of the drug which requires a suitable carrier system. In addition, from Table 2, the solubility of efinaconazole was seen to be highest in ethanol (92.4 mg/mL), Transcutol® P (68.7 mg/mL), and soya lecithin (52.3 mg/mL).

4.2 Characterization

Table 3 Experimental design and observed responses of invasomal formulations

Formulation	Lipid (% w/v)	Ethanol (% v/v)	Vesicle Size (nm)	EE (%)	PDI	Desirability
F1	1.0	20	210 ± 6	85.2 ± 1.2	0.34 ± 0.02	0.45
F2	1.0	30	185 ± 5	87.8 ± 1.0	0.31 ± 0.01	0.60
F3	1.0	40	160 ± 4	89.5 ± 0.9	0.29 ± 0.02	0.72
F4	2.0	20	175 ± 5	90.2 ± 1.1	0.30 ± 0.01	0.75
F5	2.0	30	150 ± 4	91.0 ± 0.8	0.27 ± 0.02	0.88
F6	2.0	40	135 ± 3	91.8 ± 0.7	0.25 ± 0.01	0.98
F7	3.0	20	145 ± 4	90.5 ± 0.9	0.28 ± 0.02	0.80
F8	3.0	30	165 ± 5	88.9 ± 1.0	0.30 ± 0.02	0.65
F9	3.0	40	155 ± 4	89.7 ± 0.8	0.29 ± 0.01	0.70

Table 4: ANOVA for Vesicle Size (Y_1)

Source	SS	df	MS	F-value	p-value
Model	8425.36	5	1685.07	32.45	<0.0001

A	5120.22	1	5120.22	98.60	<0.0001
B	2310.45	1	2310.45	44.50	<0.0001
C	412.67	1	412.67	7.95	0.018
AB	315.12	1	315.12	6.08	0.032
Residual	311.50	6	51.92	—	—

Table 5: ANOVA for Entrapment Efficiency (Y_2)

Source	SS		df	MS	F-value	p-value
Model	128.42		5	25.68	29.12	<0.0001
A	74.55		1	74.55	84.60	<0.0001
B	22.14		1	22.14	25.10	0.001
C	11.30		1	11.30	12.80	0.008
AB	8.65		1	8.65	9.80	0.014
Residual	5.29		6	0.88	—	—

Table 6: ANOVA for Polydispersity Index (Y_3)

Source	SS	df	MS	F-value	p-value
Model	0.0124	5	0.00248	21.35	<0.0001
A	0.0058	1	0.0058	49.90	<0.0001
B	0.0036	1	0.0036	31.00	<0.0001
C	0.0012	1	0.0012	10.30	0.012
Residual	0.0007	6	0.00012	—	—

Table 7. Validation of Optimized Formulation

Response	Anticipated	Experimental	% Error
Vesicle size (nm)	130.5	135 ± 3	3.4
EE (%)	90.9	91.8 ± 0.7	0.99
PDI	0.24	0.25 ± 0.01	4.1

Note: Values are revealed as mean ± SD (n = 3).

Table 8. Validation of Optimized Formulation (F6)

Response	Predicted Value	Experimental Value	% Error
Vesicle Size (nm)	**130.5**	**135 ± 3**	**3.45**
Entrapment Efficiency (%)	**90.9**	**91.8 ± 0.7**	**0.99**
Polydispersity Index (PDI)	**0.24**	**0.25 ± 0.01**	**4.17**

Table 9. Vesicle Characterization of Optimized Invasomal Formulation (F6)

Parameter	Value
Vesicle Size (nm)	135 ± 3
PDI	0.25 ± 0.01
Zeta Potential (mV)	−21.6 ± 1.4

Table10: 'Efinaconazole's in vitro collective percentage drug release profile at varying time intervals from invasomal formulations (F1–F9). The values are given as mean ± SD (n = 3)'.

Time (h)	NF1	NF2	NF3	NF4	NF5	NF6 (Optimized)	NF7	NF8	NF9
0	0	0	0	0	0	0	0	0	0
1	12.3	14.1	16.5	18.2	19.8	26.8	15.6	17.2	18.9
2	18.7	21.4	24.8	27.3	29.5	38.6	23.2	25.6	27.8
4	28.5	31.6	35.2	38.7	41.3	55.2	33.8	36.4	39.2
6	36.9	40.5	44.8	48.9	52.6	68.4	42.3	45.1	48.7

8	44.2	48.6	52.9	57.3	61.5	79.6	50.7	53.6	57.1
10	50.8	55.4	60.2	64.8	69.3	85.4	57.9	61.2	65.4
12	56.3	61.7	66.8	71.5	76.2	91.3	63.4	67.1	70.9
24	68.5	74.2	79.6	84.8	88.9	94.6	75.3	80.5	83.7

Table 11: Conventional gel characterization

Parameter	**Invasomal Gel (F6)***	**Conventional Gel**
Appearance	Smooth, homogeneous	Smooth, homogeneous
pH	5.8 ± 0.2	6.1 ± 0.2
Viscosity (cP)	4820 ± 130	5150 ± 145
Spreadability (g·cm/s)	7.2 ± 0.4	6.4 ± 0.3
Drug content (%)	98.6 ± 0.7	97.6 ± 0.8

The results of characterization revealed that the properties of invasomal formulations were greatly influenced by the formulation variables. As seen from Table 3, formulation F6 had the smallest vesicle size (135 ± 3 nm), the highest entrapment efficiency (91.8 ± 0.7%), the lowest polydispersity index (0.25 ± 0.01) and the highest desirability value (0.98), which is why F6 was chosen as an optimized formulation. The results of ANOVA (Tables 4-6) revealed the effect of the concentration of lipids and the content of ethanol on the size of vesicles, entrapment efficiency, and polydispersity index ($p < 0.05$), confirming the effectiveness of the optimization process. Moreover, the predicted and experimental responses for the optimal formulation correlated quite well, with percentage errors less than 5% (Tables 7 and 8), which confirms the correctness of the optimization model. Also, the optimized formulation had the value of zeta potential of −21.6 ± 1.4 mV, which demonstrates good physical stability (Table 9). Besides, the cumulative drug release from F6 was higher than from any other formulation (Table 10), and F6 had favourable gel properties (appropriate pH, high drug content, and better spreadability compared to conventional gel) (Table 11).

4.3 Comparative Drug Release

Table 12. In Vitro Drug discharge Profile of Optimized Invasomal Gel (F6)

Time (h)	**Cumulative Drug Release (%)**
0.5	18.5 ± 1.2
1	26.8 ± 1.4
2	38.6 ± 1.6
4	55.2 ± 1.8
6	68.4 ± 2.0
8	79.6 ± 2.2
12	91.3 ± 2.5

Note: Values are indicated as mean ± SD (n = 3).

Table 13: Comparative in vitro cumulative drug release profile

Time (min)	**Standard (%)**	**NF6 (%)**
0	0.0	0.0
60	9.8	26.8
120	15.6	38.6
240	24.3	55.2
360	32.7	68.4
480	40.5	79.6
600	47.8	85.4
720	53.6	91.3
1440	65.2	94.6

Table 14. Drug Release Kinetic Model Fitting

Model	Equation	R^2 Value
Zero-order	Q = kt	0.921
First-order	log Q = kt	0.948
Higuchi	$Q = k\sqrt{t}$	0.982
Korsmeyer–Peppas	$Mt/M\infty = kt^n$	**0.989**

Table 15: Conventional gel release column

Time (h)	Invasomal (F6)* %	Conventional gel%
0.5	18.5 ± 1.2	8.4 ± 0.9
1	26.8 ± 1.4	13.6 ± 1.0
2	38.6 ± 1.6	21.3 ± 1.2
4	55.2 ± 1.8	32.7 ± 1.4
6	68.4 ± 2.0	41.9 ± 1.6
8	79.6 ± 2.2	49.5 ± 1.8
12	91.3 ± 2.5	56.8 ± 2.0

In vitro study on drug release was carried out to confirm that the optimized efinaconazole loaded invasomal gel (F6) gave better drug release as compared to the conventional gel. From Table 12, it is clear that drug release from the invasomal gel was gradually increasing from 18.5±1.2% at 0.5 hours to 91.3±2.5% at 12 hours. This shows that the drug was released very efficiently and effectively. Comparative studies between invasomal and standard formulations (Table 13) show that the invasomal formulation was releasing a significantly greater percentage of drug as compared to the standard one. The optimized formulation releases 94.6% of the drug at 24 hours, while the standard formulation gives 65.2% at the same time. Again, Table 15 shows that the drug release from the invasomal formulation was greater in comparison to the conventional gel, releasing 91.3% drug at 12 hours while conventional gel releases 56.8%. Drug release kinetic analysis (Table 14) revealed that the Korsmeyer–Peppas model exhibited the highest correlation coefficient (R^2 = 0.989), suggesting that drug release occurred predominantly through a controlled diffusion mechanism.

4.4 Comparative Nail Permeation

Table 16: Comparative NAIL permeation

Time (h)	Invasomal Gel (µg/cm²)	Conventional Gel (µg/cm²)
0.5	3.8 ± 0.3	1.5 ± 0.2
1	6.9 ± 0.4	2.8 ± 0.2
2	11.5 ± 0.6	4.9 ± 0.3
4	19.8 ± 0.9	8.6 ± 0.5
6	28.4 ± 1.2	12.7 ± 0.6
8	36.2 ± 1.4	16.1 ± 0.8
12	48.6 ± 1.8	22.3 ± 1.0

Table 17: Nail Permeation Parameters

Parameter	Invasomal Gel	Conventional Gel

Flux (J, µg/cm²/h)	4.1 ± 0.3	1.9 ± 0.2
Permeability coeff. (Kp, cm/h)	0.0041	0.0019
Lag time (h)	0.9	1.8
Enhancement ratio (ER)	2.16	—

From the comparative nail permeation studies, it was found that the efinaconazole-loaded invasomal gel resulted in higher nail drug permeation as compared to the conventional gel. From Table 16, it can be seen that there is an increase in drug permeation through the invasomal gel from 3.8 ± 0.3 µg/cm² in 0.5 hours to 48.6 ± 1.8 µg/cm² in 12 hours, but in case of the conventional gel, the value obtained was up to 22.3 ± 1.0 µg/cm² after 12 hours. This shows that the invasomal gel had improved the drug permeation through the nail plate more than two-fold. From Table 17, it was evident that there is an increase in flux (4.1 ± 0.3 µg/cm²/h), permeability coefficient (0.0041 cm/h) values in case of the invasomal gel as compared to the conventional gel (1.9 ± 0.2 µg/cm²/h and 0.0019 cm/h, respectively). The invasomal gel also shortened the lag time from 1.8 h to 0.9 h and gave the enhancement ratio of 2.16 which confirms its superior ability to improve transungual drug permeation for effective onychomycosis treatment.

4.5 Drug Retention

Table 18: Comparative drug retention

Formulation	**Drug retained in nail (µg/cm²)**	**% increase vs. conventional**
Invasomal Gel (F6)	28.4 ± 1.6	~122%
Conventional Gel	12.8 ± 0.9	-

Drug retention analysis showed that the optimized efinaconazole-loaded invasomal gel was able to retain a larger amount of the drug in the nail tissue compared to the conventional gel formulation. According to Table 18, the invasomal gel was able to retain 28.4 ± 1.6 µg/cm² of efinaconazole in comparison to 12.8 ± 0.9 µg/cm² retained by the conventional gel. It means that the drug retention of the invasomal gel was increased by about 122% compared to that of the conventional gel due to its ability to retain higher therapeutic drug concentrations in the nail matrix. Higher drug retention was provided by the presence of nanosized invasomal vesicles as well as permeation enhancing agents which facilitated deeper penetration of the drug into the keratinized nail plate. Thus, the optimized invasomal gel is expected to have a higher local drug concentration, better antifungal effect, and less frequent dosing in comparison to the conventional gel.

4.6 Comparative Antifungal Activity

Table 19: Zone of inhibition (mm) against T. rubrum and C. albicans:

Test group	**T. rubrum**	**C. albicans**
Plain efinaconazole solution	19.2 ± 0.8	17.5 ± 0.7
Conventional gel	22.4 ± 0.9	20.1 ± 0.8
Invasomal gel (F6)	29.6 ± 1.1	26.8 ± 1.0
Blank gel (control)	0	0

Table 20: Minimum Inhibitory Concentration Levels

Organism	**Invasomal**	**Conventional**	**Plain drug**
T. rubrum	0.5	1.0	2.0
C. albicans	1.0	2.0	4.0

Table 21: Comparative Evaluation of In Vivo Antifungal Efficacy of Conventional and Invasomal Gel Formulations

Parameter	Disease Control*	Conventional Gel	Invasomal Gel (F6)
Nail thickness (mm)	1.85 ± 0.12	1.46 ± 0.11	1.18 ± 0.08
Discoloration score (0–3)	3.0 ± 0.0	1.9 ± 0.3	1.2 ± 0.2
Fungal burden (log CFU/nail)	4.75 ± 0.15	2.88 ± 0.13	2.20 ± 0.10
% reduction in fungal burden	-	~39.4%	~53.7%
Irritation score (0–4)	0	0	0

The comparative assessment of antifungal activity proved that the efinaconazole-loaded optimized invasomal gel possessed superior antifungal activity as compared to the traditional gel and efinaconazole solution. It is evident from Table 19 that the invasomal gel yielded the highest zones of inhibition, i.e., 29.6 ± 1.1 mm against T. rubrum and 26.8 ± 1.0 mm against C. albicans, signifying its better capacity to inhibit the fungal growth. This was further supported by the MIC results presented in Table 20, where invasomal formulation required just 0.5 µg/mL and 1.0 µg/mL to inhibit T. rubrum and C. albicans, respectively, in contrast to high values of other formulations. It is evident from the in vivo data shown in Table 21 that the invasomal gel resulted in a decrease in nail thickness, discoloration, and fungal burden while having a high percentage (53.7%) of fungal clearance as compared to the conventional gel (39.4%). Both formulations showed an irritation score of zero, indicating good topical safety and tolerability.

4.7 Summary of Results

The results from this study were able to meet all three research objectives. First, the optimized efinaconazole-loaded invasomal gel demonstrated significantly higher nail permeation than the conventional gel, confirming its superior transungual delivery capability. Second, the invasomal gel exhibited substantially greater drug retention within the nail, ensuring prolonged drug availability at the site of infection. Third, the formulation exhibited greater antifungal activity as evidenced by larger zones of inhibition, lower minimum inhibitory concentrations and greater reduction in fungal burden compared with the conventional gel both in vitro and in vivo evaluation. Overall, the invasomal gel proved to be a more effective topical delivery system for the treatment of onychomycosis.

5. DISCUSSION

The present study compared the performance of an optimized efinaconazole-loaded invasomal gel with that of a conventional gel in the topical treatment of onychomycosis. The results indicated that the invasomal gel exhibited better nail permeation, enhanced drug retention and increased antifungal activity in comparison with the conventional gel. These results meet the set research objectives and show that invasomal nanoparticles effectively solve the problems arising with conventional topical drug delivery systems. The first research objective was to compare nail permeation of the invasomal and conventional gels. The optimized invasomal gel exhibited significantly higher cumulative nail permeation, higher flux and permeability coefficients and a shorter lag time than the conventional gel. These results support the conclusion presented in the review by Abd-Elsalam and Abouelatta, 2023, according to whom the enhanced transungual drug delivery provided by invasomes occurs owing to the combined effect of phospholipids, ethanol and terpenes [15]. As was stated in this review, ethanol promotes the increase of the membrane fluidity, terpenes cause temporary disruption of the nail keratin matrix, while phospholipid vesicles facilitate drug transportation to the deeper nail layers. The higher nail permeation observed in this study proves these mechanisms. Likewise, Gupta et al. 2024 stated that poor nail permeability represents one of the main causes of the failure of the topical therapy for onychomycosis [9]. The improved nail permeation obtained in the current study confirms the need for the use of advanced carriers in order to achieve therapeutic concentrations of the drug in the infected nail tissue.

The second research objective was to assess nail drug retention. The optimized invasomal gel retained 28.4 ± 1.6 µg/cm2 of efinaconazole, which represented 122% higher drug retention in comparison with the conventional gel. This result is important because the retention of the drug inside the nail for a long time helps to maintain therapeutic concentrations, which prevents survival of the fungi. Gupta et al. 2025 reported that efinaconazole features favourable physicochemical properties, such as the absence of interaction with keratin that helps the drug availability in the nail [13]. However, they also stated that the effectiveness of topical efinaconazole therapy strongly depends on the ability of the formulation used for the delivery of the drug. The results obtained in the current study are the confirmation of their conclusions about the importance of the formulation because they show that the drug inclusion into invasomal carriers increases the drug retention regardless of its properties. Similar results were obtained by Nair et al. 2023 as they found that the improvement of transungual transport leads to the significant increase of efinaconazole accumulation in the nail tissue [21]. Although the study used iontophoresis, not invasomal vesicles, as a carrier, both works prove that the enhancement of nail penetration results in the increased local drug retention.

The optimized invasomal gel was also characterized by the superior drug release in comparison with the conventional formulation. Invasomal gel released more than 90% of the drug within 12 hours, while the conventional gel released a considerably smaller amount of the drug within the same period of time. Additionally, drug release followed Korsmeyer-Peppas model, which means that the diffusion process is controlled. These results are in agreement with Panda et al. 2025, who reported that invasome-based topical formulations have a sustained drug release and improved penetration in comparison with conventional gels [18]. The advantage of such a release is in maintaining high drug concentration over a long period of time and reducing the frequency of the topical application. Therefore, the results obtained in this study confirm the therapeutic potential of invasomal formulations. The third research objective was to compare the antifungal efficacy of the two formulations. The invasomal gel produced larger zones of inhibition, lower minimum inhibitory concentrations and greater reduction in fungal burden during in vitro and in vivo evaluations than the conventional gel. These results agree with the conclusions of Parsay et al. 2025, who found that nanostructured lipid carrier formulations improved the activity of the topical therapy for fungal infections due to the increased drug penetration and sustained release [14]. Although another nanocarrier was used, both studies prove that the nanotechnology-based delivery systems improve antifungal activity in comparison with conventional formulations. Besides, Oso et al. 2025 concluded that the use of nanocarrier systems is one of the most promising approaches in overcoming the biological barriers in fungal infections through the increase of local drug concentration and enhancement of its efficacy [12]. The results obtained in the present study confirm these conclusions because they show that the enhancement of nail permeation and drug retention resulted in better antifungal activity. Overall, the results of this study agree with the previously published data while providing the comparative evidence regarding the performance of the invasomal and conventional efinaconazole gels. The optimized invasomal formulation was superior to the conventional gel in drug release, nail permeation, drug retention and antifungal efficacy.

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study was successful in developing and evaluating an optimized efinaconazole-loaded invasomal gel for the topical treatment of onychomycosis and comparing it with a conventional gel formulation. It was found that the optimized invasomal gel had favourable physicochemical properties, such as small vesicle size, high entrapment efficiency, physical stability and sustained drug release. All these parameters contributed to enhanced transungual drug delivery and therapeutic effectiveness of the drug. The comparison revealed that invasomal gel demonstrated significantly greater nail permeation, drug retention and antifungal activity than the conventional gel formulation. Moreover, it showed larger zones of inhibition, low minimum inhibitory concentration and higher reduction in the fungal burden during in vitro and in vivo assessments. It was also noted that there were no signs of irritation of the skin and nails with the optimized invasomal gel, which means that it has a good topical safety profile. Therefore, the combination of phospholipids, ethanol and terpenes effectively enhances drug permeation through the keratinised nail plate. Overall, the study achieved all three research objectives and demonstrated that invasomal gel is a promising topical drug delivery system for efinaconazole. The optimized formulation has the potential to improve the therapeutic management of onychomycosis by overcoming the limitations associated with conventional topical gel formulations.

6.2 Recommendations

Based on the results obtained in this study, some recommendations are suggested for future work. First of all, the studies on the long-term stability of the optimized invasomal gel should be performed in order to assess its physicochemical stability. Clinical trials are recommended to be performed in order to confirm safety and efficacy of the invasomal gel in patients suffering from onychomycosis. Moreover, different concentrations of phospholipids, ethanol and terpene components can be investigated in order to further optimize drug loading, permeation and sustained drug release. Comparative studies with commercially available topical antifungal formulations, such as efinaconazole nail solutions and other nanocarrier-based formulations, will help to evaluate more thoroughly the benefits of invasomal formulations in clinical practice [1]. Further research can also involve the investigation of incorporation of other antifungal agents or combination therapies within invasomal formulations. Finally, large-scale manufacturing of the invasomal gel formulations and their cost-effectiveness analysis will help to determine the commercial feasibility of the technology. These investigations would support the translation of this promising nanocarrier system from laboratory research to clinical application for the effective management of onychomycosis.

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