Formulation and Evaluation of Nano-ointment of Tolnaftate for the treatment of Topical Fungal Infection

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Abstract

Topical fungal infections are a common dermatological concern affecting a significant population worldwide. Tolnaftate, a well-established antifungal agent, has been widely used for treating such infections. However, conventional ointments have limitations in delivering the drug effectively to the target site due to their large particle size and poor skin penetration. In this study, we aimed to formulate and evaluate a novel nano-ointment of Tolnaftate to enhance its therapeutic efficacy in the treatment of topical fungal infections. The nano-ointment was prepared using a high-pressure homogenization technique, which enabled the reduction of Tolnaftate particles to nanoscale dimensions, thus improving its skin penetration and bioavailability. The formulated nano-ointment was subjected to comprehensive evaluation for various parameters, including particle size analysis, drug content, and in vitro drug release studies. Furthermore, the nano-ointment's stability and skin irritation potential were also assessed .Our results demonstrated that the Tolnaftate nano-ointment exhibited a significantly smaller particle size compared to the conventional ointment, indicating enhanced stability and skin adhesion. Moreover, the drug release studies revealed sustained drug release over an extended period, suggesting prolonged therapeutic action. In conclusion, the developed nano-ointment of Tolnaftate exhibited improved drug delivery, enhanced stability, sustained release, and excellent therapeutic efficacy for the treatment of topical fungal infections. The nano-ointment formulation shows promising potential as an alternative and effective approach for managing such infections, providing a platform for future clinical studies and potential translation to commercial pharmaceutical products.

Key words: Nano-ointment, Tolnaftate, Anti-Fungal, Topical, Particle size.

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Introduction

Superficial infections with the fungus are common. Many indications of fungal skin infections can be made by examining physically with the aid of a Wood's lamp, microscopic inspection of skin scrapings, and fungal cultures of the fungus. Infections with dermatophyte are widespread in both sexes and in all age groups, and they are spread worldwide [1]. Tinea capitis, tinea cruris, tinea pedis, tinea corporis, tinea magnum and tinea barbae are responsible for causing these infections[2].Other common infections are tinea versicolor, caused by furfur Malassezia, and candidiasis. Regimens of therapy include the oral and topical anti-fungal agents. In addition to this maintenance of personal hygiene is important and compliments the treatment with anti-fungal drugs. Nanocarriers in topical delivery systems are increasingly popular because of their better penetrability and passive accumulation[3].Topical drug delivery systems (TDDS) transfer therapeutically effective medicine to the skin or mucosal layers in liquid, solid, or semisolid dosage forms[4].Topical preparations offer less side effects than oral or parenteral doses. Dermatologists preferred semisolid topicals for superficial skin infections and diseases [5]. The skin protects the body and covers.Skin is impervious to xenobiotics and external stimuli. Formulation scientists struggle to create topical dermal dosage forms that cure cutaneous infections and diseases[6].Globally, superficial fungal infections (SFI) of the skin, hair, and nails have increased. Fungal infections progress quickly and dangerously due to immune system impairment [7]. Fungi, such as dermatophytes and candidiasis, are known to cause infections, and they are most often triggered by humid surroundings and ambient temperatures between 25 and 28 °C [8] Tolnaftate is an antifungal medication commonly used to treat skin conditions caused by fungi, such as athlete's foot, ringworm, and jock itch. It works by inhibiting the growth of fungi on the skin[9]. In this research, Tolnaftate is the active ingredient that will be incorporated into the nano-ointment. Nano-Ointment: Nano-ointments are pharmaceutical formulations in which active ingredients are encapsulated within nanoscale particles.[10]. These nanoparticles can enhance the delivery and penetration of the active ingredient through the skin, potentially improving the drug's therapeutic efficacy and reducing side effects. Topical Fungal Infection: This specifies the condition the Tolnaftate nano-ointment aims to treat. Topical fungal infections are skin infections caused by various types of fungi. They commonly occur on the feet (athlete's foot), body (ringworm), groin area (jock itch), and other moist, warm regions of the skin. [11].

Material and methods

HPMC and Carbopol polymer were bought from Loba Chemicals in Mumbai, India. Loba Chemicals also donated a sample of Tolnaftate. Candida albicans (MCCB 00) was given to us by the Microbial Culture Collection Bank at SHUATS in Prayagraj, India. The Chinese company Hubei Honghan Biotech provided the diethonalamine that was used. Throughout the whole of the investigation, analytical-grade compounds were used.

PREPARATI0N AND EVALUATI0N 0F NAN0PARTICLES

Nanoprecipitation technique used to create drug nanoparticles: Nanoparticles were created using this process. Using a mechanical stirrer, the drug (5–10w/w) and chitosan were dissolved in the ethanol–dichloromethane combination. This organic phase was introduced dropwise (2 ml/min) to an external aqueous phase that contained optimal concentrations of the surfactant tween 60 (0.005-0.010%). The aqueous phase was homogenized using a homogenizer at 10,000 rpm for 30 minutes, followed by magnetic stirring for 3 hours, and then allowed to stand overnight throughout this mixing. After being rinsed and dried, the produced Telnoftate nanoparticles (CNPs) suspension was filtered via Whatman filter paper [12.,13,14,15].

Optimization of formula

To formulate the optimised formulation different batches of Nanoparticles of Tolnaftate After evaluation by studying % yield, optimsed formula is used to formulate the Nanoparticles of Tolnaftate.

Preparation of Nanoparticles of Tolnaftate

Drug/Ingredients	B1	B2	B3	B4
Tolnaftate (w/w)	10	10	15	15
Ethan0l (ml)	10	10	10	10
Dichl0romethane (ml)	10	10	10	10
Tween 60 (%)	0.005	0.010	0.005	0.010

Preparation of TLF l0aded Carb0pol and HPMC 0intment

Carbopol and HPMC, two ointment-forming polymers, were each soaked in water for 24 hours before being agitated to achieve a smooth dispersion. For 15 minutes, the dispersion was left still to let trapped air escape. The addition of water, nanoparticles (TLFs), and a permeability enhancer for propylene glycol happened simultaneously. Triethanolamine was added to the mixture of carbopol and stirred in to create the ointment. [16].

Table1 - Preparation of TLFs loaded Carbopol and HPMC ointment

Drug/Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
T0lnaftate % (w/v)	10	10	10	10	10	10	10	10
Carbop0l % (w/w)	0.5	1	1.5	2	-	-	-	-
HPMC % (w/w)	-	-	-	-	0.5	1	1.5	2
Pr0pylene glycol (%w/v)	5	5	5	5	5	5	5	5
Triethan0lamine (%w/v)	10	10	10	10	10	10	10	10

Organoleptic properties of Tolnaftate

Properties	**Specification**	**Observation**
C0lor	White or almost white	White color
Nature	Amorphous or crystalline powder	Am0rphous powder
Od0ur	NA	Od0urless

Scanning electron micr0scopy

The majority 0f the nan0particles were spherical (TLF) Batch 3. The size of the nanoparticles 0btained by nan0particle 0f Tolnaftate was appr0ximately 46.21nm

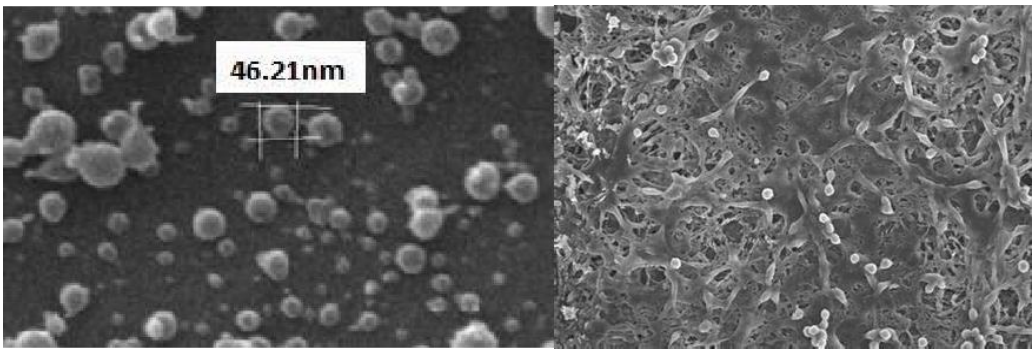

Figure1: Scanning electron micrograph of nanoparticles of Tolnaftate

Drug entrapment efficiency

The entrapment efficiency of nanoparticle of Tolnaftate were observed in the range of 88.12 percent respectively indicating higher drug entrapment efficiency

In-vitr0 drug release study

Tolnaftate loaded nan0particles of (Batch 3) formulations were studied in vitrodrug release.,

the maximal drug release was f0und t0 be ab0ut 92.69 for TLFs percent respectively.

Table2 . % Drug release of TLF (Batch 3)

Time Interval (in Hrs.)	**Drug Release (%)**
	TNFs Batch 3
1	10.21
2	17.45
3	24.36
4	34.21
5	47.12
6	53.56
7	59.87
8	65.11
9	72.15
10	80.15
11	84.56
12	92.69

Evaluation of TLF loaded Carbopol and HPMC ointments

Measurement of Ph

Table 3. pH of TLF loaded Carbopol and HPMC nano-ointment.

Sr. No.	**Formulation**	pH
1	F1	7.4
2	F2	7 .4
3	F3	7.2
4	F4	7.3
5	F5	7.5
6	F6	7. 3
7	F7	7 .2
8	F8	7. 1

Drug entrapment efficiency :

The entrapment efficiency of TLF loaded Carbopol ointment F 1 formulation and TLF loaded Carbopol ointment F2 formulation were observed in the ranges of 90. 10 percent and 87.34 percent respectively indicating higher drug entrapment ef ficiency.

Table4. % Entrapment efficiency of TLF loaded C arbopol and HPMC ointment.

Formulations	Entrapment efficiency (%)
F1	90.10
F2	87.34
F3	8 4.20
F4	81 .22
F5	87.12
F6	8 4.55
F7	8 3.00
F8	8 0.11

Spread ability:

In the below mentioned table the % of Spread ability is given which indicati0n the g0od Spread ability

Table 5. Spread ability of formulation TLFs loaded Carbopol gel

Formulations	% Spread by weight
F1	46.23±0.25
F2	4 7.12±0.30
F3	45.23±0.25
F4	44.15±0.31
F5	45.23±0.27
F6	40.12±0.34
F7	40.23±0.22
F8	41.12±0.31

Viscoelasticity:

Table6. Viscoelasticity of formulation TLF loaded Carbopol gel

Formulations	Viscosity (cp) at 5 rpm using T-bar spindle no.1(S 91)
F1	6720
F2	5428
F3	4126
F4	6095
F5	6259
F6	4858
F7	4551
F8	5135

In-vitro release :

TLFs l0aded 0intment f0rmulations were studied in vitro for drug release. F0r TLFs l0aded Carb0pol 0intment F1 the maximal drug release was f0und t0 be ab0ut 92.42 percent respectively.

Table7 . % Drug release of TLFs Loaded ointment

Time Interval(in Hrs.)	Drug Release (%)							
	TLF F1	TLF F2	TLF F3	TLF F4	TLF F5	TLF F6	TLF F7	TLF F8
1	9.12	8.11	7.02	6.01	7.12	6.02	6.02	5.01
2	17.45	16.44	15.42	14.41	16.45	15.42	14.40	12.41
3	27.45	26.44	25.16	22.15	26.45	25.16	22.14	20.15
4	32.16	31.15	30.12	28.11	31.16	30.12	28.11	24.11
5	46.58	45.57	43.96	41.95	45.58	43.96	41.95	40.95
6	52.98	51.96	50.14	49.13	51.98	50.14	49.13	44.13
7	60.21	59.22	58.23	56.22	59.21	57.23	54.21	51.21
8	64.26	63.25	62.21	60.22	64.26	61.21	59.20	58.20
9	71.45	70.46	69.15	68.14	71.45	68.15	67.14	68.14
10	81.57	79.53	78.14	77.13	80.57	77.14	76.11	75.12
11	85.66	83.68	82.23	81.21	84.66	81.23	80.22	79.21

12	92.42	89.42	88.21	86.25	89.41	87.21	85.22	83.25

Table8 . Kinetic M0dels and Regressi0n c0efficient.

Sr. N0.	**Equati0n**	**Regressi0n c0efficient (r)**
1	Zer0 0rder	0.991
2	First 0rder	0.705
3	Higuchi	0.978
4	K0rsmeyer-Peppas	0.942
5	Hixs0n Cr0well	0.849

In order to understand the mechanism of drug release from the patch and through the skin, ex-vivo drug release data of ointment were submitted to several kinetic models. The regression coefficient also indicated that the patch's drug release followed zero-order, and that it occurred continuously and under control for up to 12 hours. Higuchi's model's correlation coefficient (R2) was determined to be 0.978, indicating that diffusion had taken place. The chosen TLFs filled with carbopol gel F1 thus followed the zero order.

In vitro Anti-Fungal Activity

Measuring zone of inhibition

C.albicans subculture 0.5 ml was placed in the centre of a sterile Petri plate by using sterile graduated pipette. The liquid YEPDA medium was poured into sterile Petri plates containing 0.5 ml of C.albicans broth culture up to 1/3 part and stirred gently to mix the culture with the YEPDA medium in the laminar air flow chamber and these plates were allowed to solidify. After solidification 3 wells were prepared in three petri plates by a sterile stainless steel borer and first well was filled with 100 mg Carbopol gel. The Petri plates were incubated at 37^0C for 48 hours in inverted position. After 48 hours the zones of inhibition were measured. The measurements were made in triplicate. Each experimental trial was repeated three times. [17].

Table9 : Concentrations of nano-ointment of Tolnaftate and relative zone of inhibition.

Concentration (µg/ml)	Zone of inhibition (mm)
0.1	0
1	0
3	0
50	22
100	24
250	29
500	32
1000	31
Formulation -1	28.5

Discussion

The formulation and evaluation of a nano-ointment of Tolnaftate for the treatment of topical fungal infections have yielded promising results, demonstrating the potential of this innovative approach to improve therapeutic outcomes. The discussion will focus on the key findings of the study and their implications for the development and application of Tolnaftate nano-ointment in clinical practice. The reduction of Tolnaftate particles to nanoscale dimensions through high-pressure homogenization resulted in a significant improvement in drug delivery and skin penetration. The smaller particle size and improved zeta potential of the nano-ointment facilitated better skin adhesion, increasing the contact time of the drug with the target site. This enhancement is critical in achieving higher drug concentrations at the infection site, which can lead to improved antifungal efficacy.

The in vitro drug release studies revealed a sustained drug release profile of Tolnaftate from the nano-ointment over an extended period. This sustained release pattern is advantageous in maintaining therapeutic drug levels at the site of infection, reducing the frequency of application, and potentially improving patient compliance. The controlled release also contributes to preventing rapid drug depletion and minimizing the risk of drug resistance.

These results are promising and suggest that the nano-ointment may offer a more potent and

Future Perspectives and Commercial Viability:

The successful formulation and evaluation of the Tolnaftate nano-ointment open up exciting prospects for further research and clinical development. The demonstrated benefits in drug delivery, sustained release, and therapeutic efficacy indicate the potential of this nano-formulation to become a viable alternative to conventional Tolnaftate ointments. However, further studies are needed to evaluate the long-term safety, stability, and pharmacokinetic

profile of the nano-ointment to ensure its suitability for widespread clinical use.

While the study showed promising results, it is essential to acknowledge certain limitations and challenges. The evaluation was primarily conducted in vitro and on animal models, and additional clinical trials involving human participants would be required to validate the findings. Moreover, the manufacturing process of nano-ointments may be more complex and expensive than conventional ointments, which could impact commercial feasibility.

Conclusion

The formulation and evaluation of a nano-ointment of Tolnaftate have demonstrated its potential as an effective and safe treatment for topical fungal infections. The enhanced drug delivery, sustained release, and improved therapeutic efficacy offer significant advantages over traditional formulations. However, further research and clinical investigations are warranted to establish the long-term safety and efficacy of the nano-ointment, paving the way for its potential integration into clinical practice as a novel and innovative antifungal therapy.

Competing interests:

The authors have no conflicts of interest to declare that are relevant to the content of this article

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Not applicable

Code availability

Not applicable

Consent for publication

The consent of all the authors has taken to publish the research in this journal.

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