Pharmacological Activity Of Antifungal Drug Loaded Solid Lipid Nanoparticle For The Treatment Of Dermatitis

Simran Singh[1*], Naresh Kalra[2]

[1*]Research Scholar, Lords university, Alwar, Rajasthan, 301028
[2] Professor, Lords university, Alwar, Rajasthan, 301028

***Correspondent Author:**Simran Singh
Research Scholar, Lords university, Alwar, Rajasthan, 301028

ABSTRACT
Globally, fungus-related infections are on the rise. For fungal infections, oral medication is associated with side effects, a protracted course of treatment, and patient intolerance. In contrast, low drug solubility, skin irritation, and limited permeability via the skin are associated with superficial fungal infections and topical therapy. The goal of top topical therapy is to reduce adverse effects like irritation and provide quick symptomatic relief from fungal diseases while also enhancing the penetration of poorly soluble drugs. The current study formulated Terbinafine- and Co-loaded SLN to increase therapeutic action and improve penetration via the stratum corneum. A gel of solid lipid nanoparticles was made via high shear homogenization. There were excipients in the formulation. While continual release offers a long-lasting anti-fungal effect, the initial burst release offers a quick start to activity. Following the OECD's requirements, research on skin irritation was conducted, and the formulations were found to be safe and non-irritating for topical application. The anti-fungal activity and stability of the Terbinafine and Co-trimoxazole Solid Lipid nanoparticles gel were improved. Thus, solid lipid nanoparticles loaded with terbinafine and co-trimoxazole show promise as a drug delivery vehicle.

Keywords: - Fungal infection, Terbinafine and Co-trimoxazole, Solid Lipid nanoparticles gel

1. INTRODUCTION

Numerous regions of the natural environment are prone to fungal diseases. Fungal infections in humans occur when an invasive fungus colonizes a body part that is too big for the immune system to handle.[1,2] There are two categories for fungal infections: superficial and invasive. Internal organs, other tissues, and the agents that allow the infection to penetrate the tissue or organ are all susceptible to invasive fungal infections. Up to 20% to 25% of people worldwide suffer from superficial fungal infections, which impair daily functioning, lower quality of life, and increase healthcare costs.[3,4] In clinical practice, superficial fungal skin disorders are the most prevalent infectious disease in humans. Candida albicans or dermatophytes are the culprits behind superficial fungal infections, which affect the skin, scalp, and nails. According to epidemiological studies on fungal infections, people of all ages are susceptible to the fungi that cause surface mycoses, which are common. The most frequent cause of infection is dermatophytes, or fungi, which act by degrading keratin, a protein that covers the skin's outer layer. The classification of fungal infections is based on the affected body part.[5,6] Rashes in non-affected areas of the body can be caused by fungal infections in one place. A rough, itchy rash on the foot could be the result of a fungal infection on the fingertips. Usually, an allergic reaction to the fungus is the cause of this. They do not result from contact with the contaminated area. 1 and 2. Fungal infection causes:It is possible that fungi are not a part of the kingdoms of plants or animals. They fall within their kingdom classification. Most infections in humans are caused by certain fungus. Microscopic spores are dispersed by fungi to multiply. These spores are frequently found in soil and the air, where they can enter the body through the skin or be inhaled. Consequently, fungal infections typically begin on the surface of the skin or in the lungs.[7,8]

Topical drug distribution is the process of applying medication-containing formulations to the skin's surface or inside it in order to treat disorders like fungal infections or acne as well as cutaneous or subcutaneous diseases. Of all the many methods of administration, the topical route is supposedly the most popular one for local drug delivery. There are numerous advantages to the topical delivery route over the conventional oral method. When applying the drug topically, it is important to prevent or decrease its diffusion into the bloodstream.[9,10] Drug localization in the skin is crucial for topical distribution, which involves distributing medications to different layers of the skin with the least amount of systemic absorption. A novel class of therapeutic carrier known as solid-lipid nanoparticles is made up of a solid matrix made of lipids that are solid at body temperature as well as room temperature, with a mean particle size that ranges from 50 to 1000 nanometers overall.[11,12] SLNs are often prepared using complicated glyceride combinations, highly purified triglycerides, or waxes. Solid Lipid nanoparticles were developed as an alternative to emulsions, liposomes, and polymeric nanoparticles for controlled drug delivery.

Three main models are used to integrate bioactive elements into self-ligand nanoparticles (SLNs): the homogeneous matrix model. A drug-enhanced center model and a drug-enhanced shell model.[13,14]

2. MATERIAL AND METHODS

2.1 *Ex vivo* skin hydration study

The skin hydrating effect of the selected SLN formulation was investigated *ex vivo* and compared with the conventional cream. The topical formulations were applied to the rat cadaver skin and after 24 hrs skin was isolated, vertically sliced using microtome, and stained with dye carbol fuchsin. The slides were observed under optical microscope and thickness of stratum corneum was observed. The photomicrographs were taken using optical microscope with image analyser.

2.2 *In Vitro* drug diffusion study

In Vitro drug release study was performed on the franz diffusion cell using cellulose acetate membrane. Phosphate buffer pH 6.8 and methanol (60:40) was used as diffusion medium and previously put in contact with membrane 30 min before placing the sample. Phosphate buffer pH 6.8 and methanol (60:40) mixture (10 ml) was placed in receptor compartment of franz diffusion cell. The receptor compartment was continuously stirred using magnetic bar and the temperature was kept at 37°C using water bath. The experiment was started with the even application of 0.5 gm of SLN gel on the surface of cellulose acetate membrane from donor compartment side. Sampling was performed after 0, 1, 2, 5, 7, 9, 12, 24, 36 and 48 hr and the fresh diffusion medium was added with each withdrawal of sample. The samples were diluted and analysed UV Visible spectrophotometrically.

2.3 Drug permeation study

In vitro skin penetration and permeation experiments were performed on franz diffusion cells using pig ear skin. Amongst rodents, the most relevant animal model for human skin is the pig. The histological and biochemical properties, the vascular anatomy and collagen fiber arrangement in the dermis, as well as the contents of SC glycosphingolipids and ceramides of pig skin have been proven to be similar and give comparable results to human skin. Fresh pig ears were obtained from a local market. Pig ears were washed with distilled water and adipose subcutaneous tissue was removed. The skin was placed horizontally on franz diffusion cells, between the donor and receptor compartments exposing dermal side to receptor compartment and stratum corneum in contact with donor compartment. Sink conditions were obtained in the receptor compartment with phosphate buffer pH 6.8 and methanol (60:40) mixture. The receptor fluid 10 ml was continuously homogenized using magnetic stirrer. The temperature of cell was maintained at 37.0±0.1°C. The 0.5 gm gel preparation was applied at an amount equivalent to 1% of drug was applied on the membrane in donor compartment ensuring an intimate contact with the skin. Sampling was done at 0, 1, 2, 5, 7, 9, 12, 24, 36 and 48 hr. At each point, 1 ml aliquots were drawn from the receiver compartment and simultaneously an equivalent volume of receptor fluid was replaced. Amount of Terbinafine Hydrochloride in diffusion medium was determined by UV Visible spectrophotometry. A graph of cumulative % release and time was plotted. Another graph of amount of drug diffused per unit area (Q/A) versus time (hr) was plotted. At the end of 48 hr the excess formulation from the surface of the skin and the entire dosing area was collected and drug content in the skin was determined. The drug remained unabsorbed on the skin surface was also quantified.

2.4 *In vivo* skin retention studies

SLN gel (0.25 g) was applied on the shaved skin area of albino rat. After 24 hr, the animal was humanely sacrificed and the skin was collected. Applied formulation was removed and stratum corneum layer was removed by stripping with an adhesive tape while the epidermis layer was separated using heat separation technique. Presence of Terbinafine Hydrochloride in different skin strata was extracted and quantitatively determined.

2.5 Skin irritation study

The primary skin irritation of the SLN-based gel was evaluated according to OECD guideline by acute skin irritation test. The protocol for the study was approved by the Institutional Animal Ethical Committee.

2.5.1 Selection of animal

Animals used for the study were healthy male New Zealand white rabbits weighing between 2.5 and 3.0 kg. 12 animals were used for the study and categorized into four groups. The four formulations plain gel, plain drug gel, marketed formulation, and SLN gel were studied for skin irritation test.

2.5.2 Preparation of the animals

The animals were weighed 24 hr before the test. The fur was carefully removed from the dorsal area of trunk of the animal without abrading the skin. The animals with intact skin were further used for the study.

2.5.3 Housing and feeding conditions

The housing conditions were typical and conventional. The temperature was maintained at 25°C with relative humidity between 35-70%. The artificial light with 12 hr light and dark cycle was provided. The animals were feed with conventional laboratory diet and drinking water.

2.5.4 Test substance

0.5 g of the test samples was applied to the hair-free area of skin i.e right side of the trunk and covered with a gauze patch. The left side of trunk was kept as untreated skin areas which serve as the control. After 1 hr exposure of test sample, the rabbits were observed for signs of erythema, edema and irritation. The degree of irritation, erythema and edema were read and scored at specified time intervals. Data was recorded at interval of 24 hr, 48 hr and 72 hr after patch removal.

Erythema and Eschar Formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2

Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Maximum possible:	*4*
Oedema Formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum possible:	*4*

Table 1. Grading of skin reactions

2.6 *In vitro* antifungal activity

In vitro antifungal activity study was performed against *Candida albicans* species using modified agar diffusion method. Sabouraud's dextrose agar (SDA) was used for the preparation of cultures and incubation of fungal species. Cultivation/incubation media was prepared and sterilized (by autoclaving at 15 psig pressure, 121°C for 15 min). Fresh cultures of *C. albicans* were prepared and incubated at 37±2°C for 48 hr in dark condition. Sterilized SDA plates were prepared and a spherical ditch was made with a sterile borer in an aseptic area. Each of the formulation (blank gel, SLN gel, plain drug gel, and marketed formulation) was mixed thoroughly with the medium and was poured in the ditch made on agar plate under sterile conditions. The plates were dried and incubated at 37±2°C for 48 hr. Zone of inhibition was measured at the end of incubation. The details of experimentation were as follows:

I) Preparation of Standard solution

1. The standard solutions of pure drug were prepared in dimethyl sulfoxide of the strengths 2000 µg/ml, 1500 µg/ml, 1000 µg/ml, 500 µg/ml and 250 µg/ml and sonicated for 2 min to ensure complete solubilisation of drug.

All dilutions were made in DMSO.

II) Preparation of Test Sample

1. Prepared SLN formulation was extracted in DMSO. (Equivalent to 1000 µg/ml of pure drug)
2. Blank Preparation for respective Formulation.

III) Preparation of Media

Sabouraud's dextrose agar medium

Dextrose	4.0%
Peptone	1.0%
Agar	2.5%

1. Sabouraud Dextrose Agar (SDA): SDA media was prepared according to standard procedure written on the container. Then the media was autoclaved at 121°C for 30min, 15pound per sq inch.
2. Normal saline solution: 0.9% sodium chloride was dissolved in distilled water and autoclaved at 121°C for 30 min, 15 pound per sq inch.

IV) Fungi strain for the test:

Candida albicans NCIM 3417

V) Inoculum Preparation:

1. Organisms were subcultured from fresh slants on sterile Sabouroud Dextrose Agar aseptically. Then incubated at 25°C for 24 hrs.
2. The inoculum was prepared by picking same colonies of fresh cultures from petridish plate.
3. The colonies were suspended in 5 ml sterile saline solution and matched with Mcfarland Standard.
4. The resulting suspension was vortexed for 15-20 sec.

VI) Procedure for Test

1. After autoclaving of the media it was kept aside for cooling.
2. Before starting experimental process, laminar air flow work station was cleaned using the 70% alcohol. Then airflow was started and UV light was kept on for 20-30 min.
3. All prepared dilutions of drug were kept under laminar air flow.
4. Round petridish were used for the test.
5. To the cooled SDA media, a sufficient amount (100 µg) of inoculum of *candida* strain was added with vigorous shaking.
6. This media was poured in petridish plate.
7. After solidification, plates were kept in cold room for half an hour.
8. Sterile borer was used to make the wells on completely solidified plates.

9. The standard solutions and the test Sample were added into the respective premarked wells.
10. The plates were kept at the room temp i.e. at 25°C for 3 days.
11. The plates were identified for zone of inhibition after completion of 3 days.
12. All the processes were performed aseptically and carried out in triplicate.

The activity was performed in stringent conditions and by avoiding all the possible experimental error.

2.7 Stability studies of SLN gel

Stability of the prepared SLN gel was determined in terms of drug retained in SLNs, pH, appearance, color and organoleptic properties like viscosity, strength and firmness of gel. SLN gel of given drugs were subjected to stability study for a period of 12 month at different conditions of storage (refrigeration, 25±2°C/60% RH, 30±2°C/65 % RH ± 5% RH and 40±2°C/75 % RH ± 5% RH) as per ICH guidelines.

3. RESULT AND DISCUSSION

3.1.1 *Ex vivo* skin hydration study of Terbinafine Hydrochloride

The skin hydrating effect of the selected SLN formulation was studied in rat cadaver skin and compared with the conventional cream using optical microscope with image analyzer. Skin hydration of skin is directly related to the occlusion and moisturization of skin shows the photomicrograph of untreated human cadaver skin. The photomicrograph of rat cadaver skin (RCS) treated with marketed cream and B9 TH-SLN gel respectively. The stratum corneum (SC) appears dark brown in the photomicrographs represents the top layer of the skin. Skin hydration was studied by observing the thickness of stratum corneum. The stratum corneum of RCS treated with B9 TH-SLN gel was found to be thicker as compared SC of RCS applied with marketed cream revealing the greater hydrating potential of B9 TH-SLN gel.

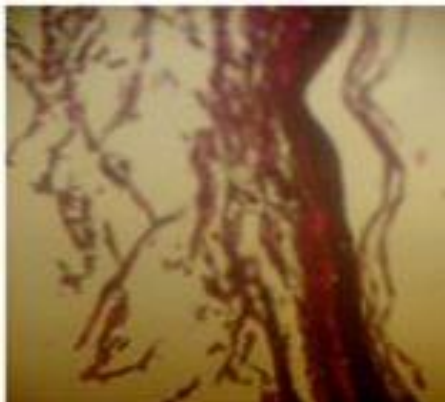

Figure 1. before application of gel

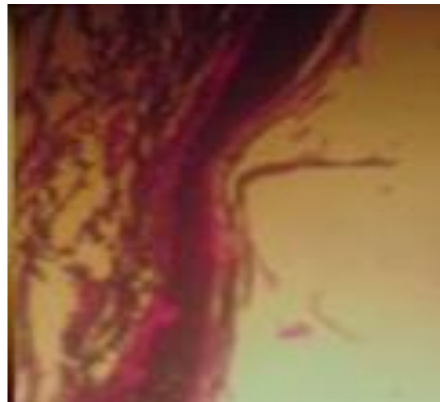

Figure 2. after application of market cream

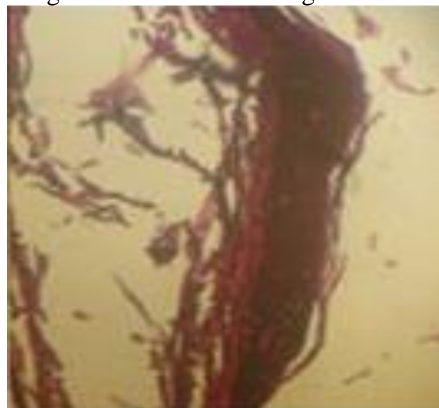

Figure 3. after application of TH-SLN gel

3.1.2 *In-Vitro* drug diffusion study

In-vitro drug release study of B9 TH-SLN was compared with marketed cream FINTOP (reference). Release pattern of Terbinafine Hydrochloride from SLN was initially faster which becomes sustained after 2 hr. The sustained release from gel was further useful for maintaining the depot for drug release. The marketed cream showed comparatively controlled release than the SLN gel.

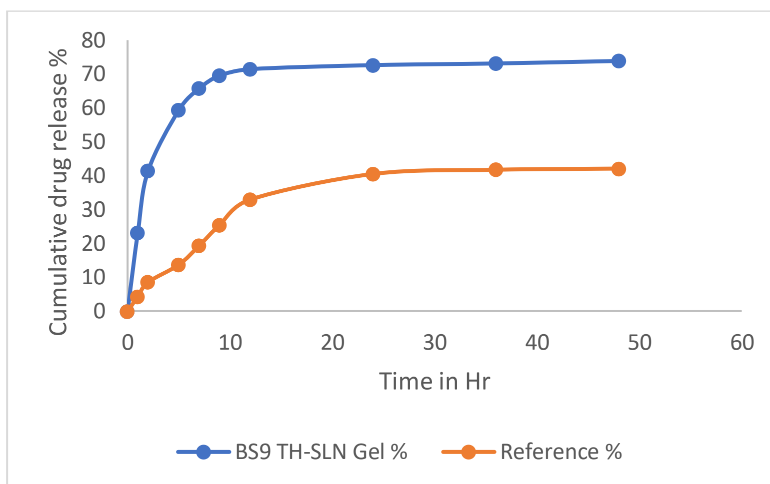

Figure 4. In vitro cumulative percent drug release of B9 TH-SLN gel and reference marketed Fintop cream

3.1.3 Drug permeation study

The ability of B9 TH-SLN to permeate through pig ear skin was studied. The plot of cumulative amount of drug permeated showed 5.42 times higher drug release for the B9 TH-SLN gel when compared with reference cream. The flux value calculated from the linear portion of graph Q/A vs time from SLN gel was found to be 1666.7 ± 0.198 ng/cm2hr and 818.181 ± 0.392 ng/cm2hr for reference cream. After 24 hr the amount of Terbinafine Hydrochloride undiffuse, deposited and permeated through skin was determined.

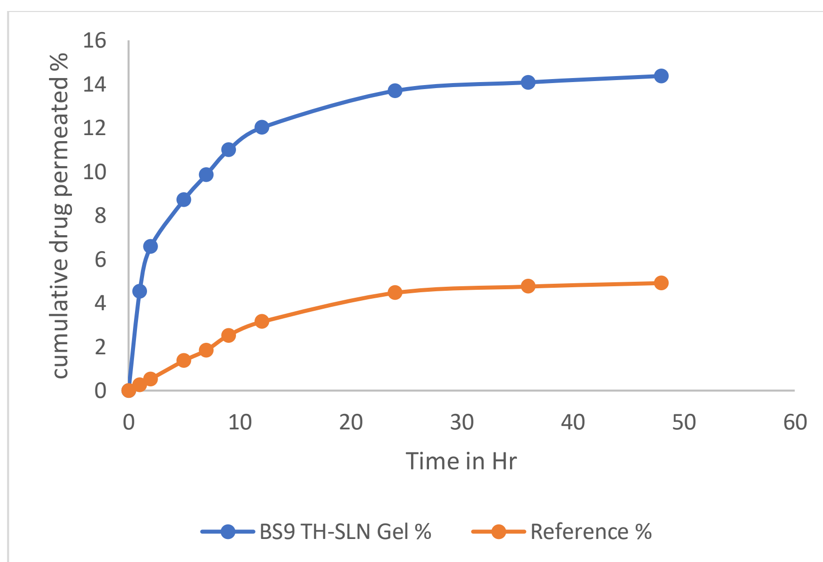

Figure 5. In vitro cumulative percent drug permeated of B9 TH-SLN gel and reference marketed Fintop cream

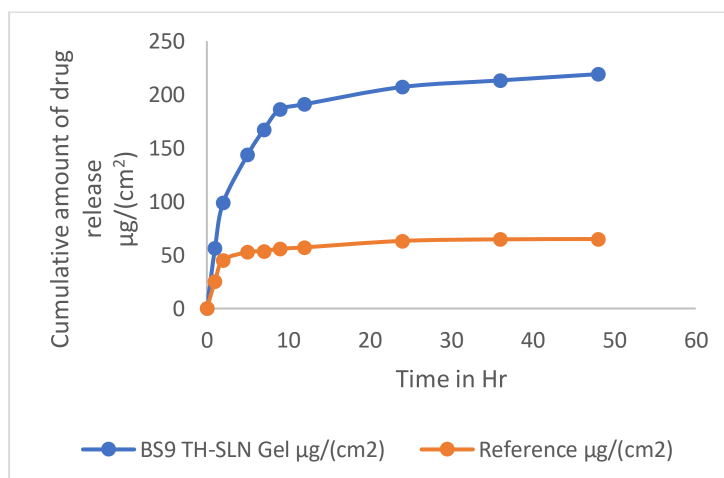

Figure 6. *In vitro* permeation profile (Q/A Vs Time) of TH from SLN gel and reference marketed cream

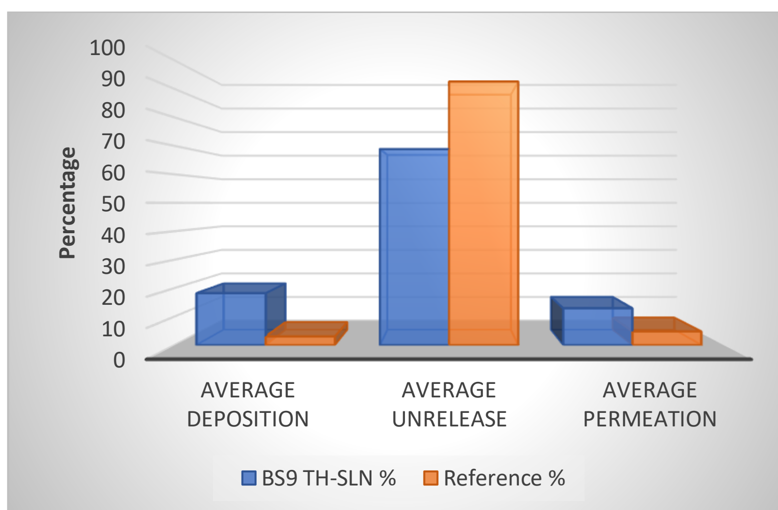

Figure 7. Percentage of Terbinafine Hydrochloride permeated, deposited and remain unabsorbed in the skin.

3.1.4 Skin retention studies

Terbinafine Hydrochloride present in different skin strata was quantified. The tape stripping method was used to determine the amount of TH retained in SC. More concentration of TH was extracted in stratum corneum as compared to epidermis and dermis. The results revealed that the stratum corneum act as a reservoir for the Terbinafine Hydrochloride from which a controlled release of drug occurs. More quantity of Terbinafine Hydrochloride was recovered in the skin from B9 TH-SLN gel when compared to reference cream.

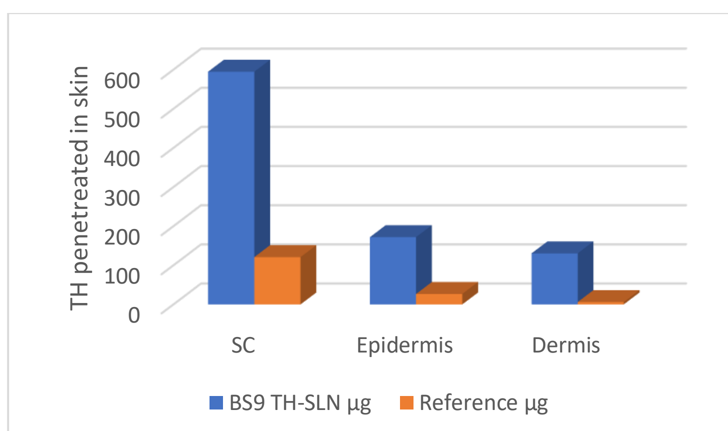

Figure 8. Skin retention of Terbinafine Hydrochloride in different skin strata.

3.1.5 Skin irritation study

The primary skin irritation of the prepared formulations was evaluated according to OECD guideline. The rabbits were scored for erythema and edema according to the grading. The test application site of each animal was examined for the signs of irritation and graded at 1, 24, 48 and 72 hr after the removal of test sample. The results of primary skin irritation for plain *aloe vera* gel, TH incorporated *aloe vera* gel, marketed Fintop cream and B9 TH-SLN *aloe vera* gel. No lesions or the signs of irritation were observed at the end of 72 hr for the any test substance. Skin irritation study in rabbit showed that formulation B9 TH-SLN gel didn't indicate any manifestation of skin irritation such as redness of skin or inflammation at the site of application (erythema). Thus it may be concluded that all of the selected formulations are safe for topical application.

Animal Code	**Grading and time intervals**			
	1 hr	**24 hr**	**48 hr**	**72 hr**
D1	0,0	0,0	0,0	0,0
D2	0,0	0,0	0,0	0,0
D3	0,0	0,0	0,0	0,0
Total Score	0=erythema, 0=edema			

Table 2. Grading of erythema and eschar formation and edema formation for the TH-SLN aloe vera gel

3.1.6 *In vitro* antifungal activity

In-Vitro antifungal activity was performed against *Candida albicans*, with the objective to determine the effect of process parameter on the strength (potency) of Terbinafine Hydrochloride and to study the antifungal activity of Terbinafine Hydrochloride in terms of zone of inhibition. In this study the extract of B9 TH-SLN equivalent to 500 µg/ml was compared with the marketed cream dilution equivalent to 500 µg/ml and different standard drug dilutions. From the present investigation the test sample with the strength equivalent to 500 µg/ml showed the greater zone of inhibition (32 mm) when compared with the standard 500 µg/ml butenafine solution (30 mm) shows antifungal activity study showing Petri plates. The study was carried out in triplicate and the same results were obtained for the zone of inhibition Hence B9 TH-SLN formulation was found to have greater antifungal activity as compared to marketed cream.

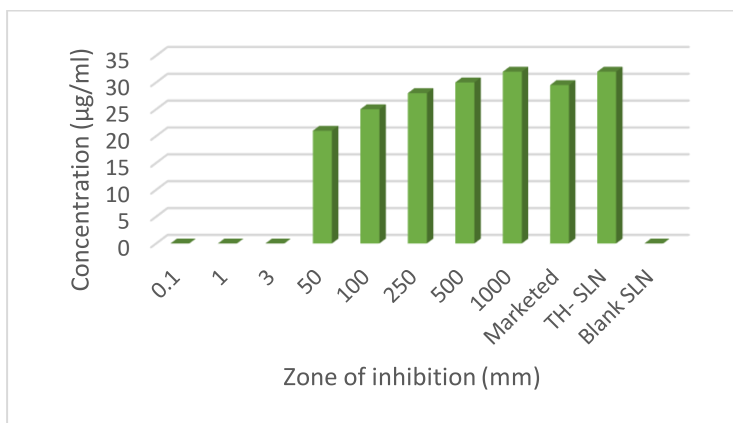

Figure 9. Comparative Antifungal activity of different dilutions of Terbinafine Hydrochloride with marketed and B9 TH-SLN formulation.

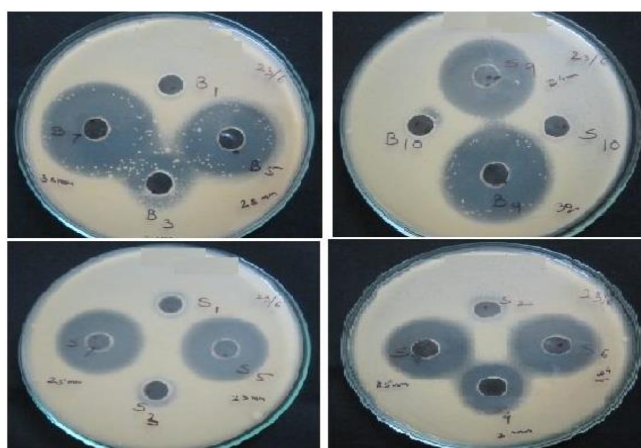

Figure 10. Petri Plates showing zone of inhibition of standard drug, marketed preparation and B9 TH-SLN formulation.

3.1.7 Stability studies of B9 TH-SLN gel

Stability of the prepared BS9 TH-SLN gel was calculated in terms of Terbinafine Hydrochloride retained in SLN gel. The result reveals that no significant change was observed at studied temperature. While among the different storage conditions, the refrigerated storage gives more constant results for drug retention. The formulations were evaluated for the color, appearance, physical stability, pH, and organoleptic properties like viscosity of gel, gel strength and firmness etc. Thus, the prepared formulations were found to be stable at studied temperatures.

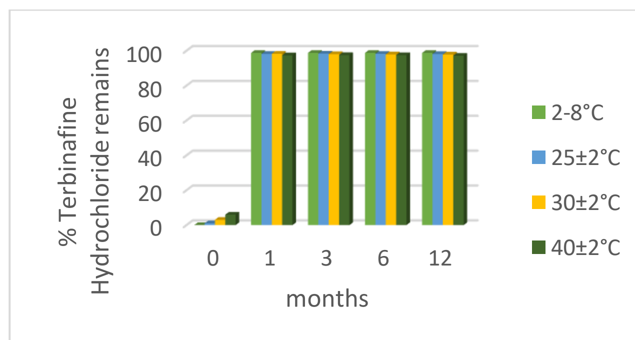

Figure 11. Mean retention of Terbinafine Hydrochloride from B9 TH-SLNs Gel

3.2.1 Ex-vivo skin hydration study of Co-trimoxazole

The ability of drug carrier system to penetrate through stratum corneum is depends on its skin hydrating potential. It represents the untreated rat cadaver skin (RCS), treated with marketed cream and treated with F12 CT-SLN gel respectively. The skin hydration potential was determined qualitatively on the basis of thickness of stratum corneum (SC) viewed as dark brown layer. The thickness of SC was found to be 2.9-fold greater for marketed cream and the 3.6-fold greater for F12 CT-SLN *aloe vera* gel when compared with untreated rat cadaver skin. The nano size SLN *aloe vera* gel forms a compact layer on the surface of skin which moisturizes the skin. This layer prevents the trans epidermal water loss hence increases the gap between the compact corneocyte which lead to skin hydration. Hydration allows the SLN and drug to partition between the hydrophilic corneocytes and lipophilic layer between corneocytes which consist of ceramides and phospholipids. F12 CT-SLN gel forms an impermeable barrier and hence leads to better skin hydration as compared to marketed cream, due to this mechanism more amount of drug has been diffused through stratum corneum and deposited in the underlying layers of skin. The epidermis forms the depot for the further drug release and lead to controlled drug release.

Figure 12. untreated skin

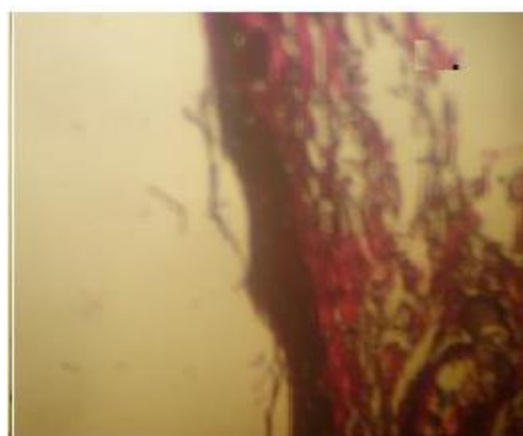

Figure 13. After application of marketed cream

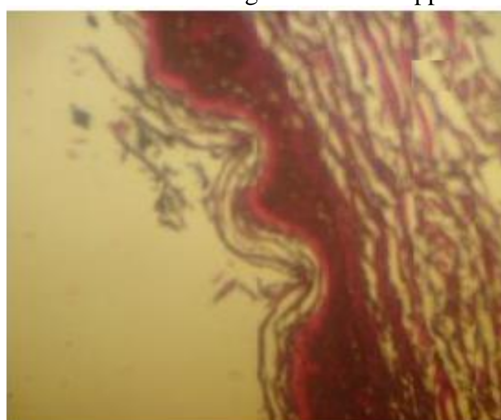

Figure 14. After application of CT-SLN gel

3.2.2 *In Vitro* drug release study

Comparative release of F12 CT-SLN and marketed cream (Sertakon) was studied by using Franz diffusion cell. The release pattern for F12 CT-SLN gel was found to be typical for topical drug delivery. The release profile showed biphasic release pattern which commences with burst release for first few hours and there after sustained release for long period of time was observed. Marketed cream showed more controlled release than F12 CT-SLN which limits the targeting and maintenance of effective drug concentration at the affected site for action.

In vitro drug release study of F12 CT-SLN was compared with marketed cream (Sertakon) (reference). Release pattern of cotrimoxazole from SLN was initially faster which becomes sustained after 2 hr. The SLN gel showed comparatively faster release than the marketed cream revealing its potency for faster onset of action. The sustained release was further useful for maintaining the depot for drug release.

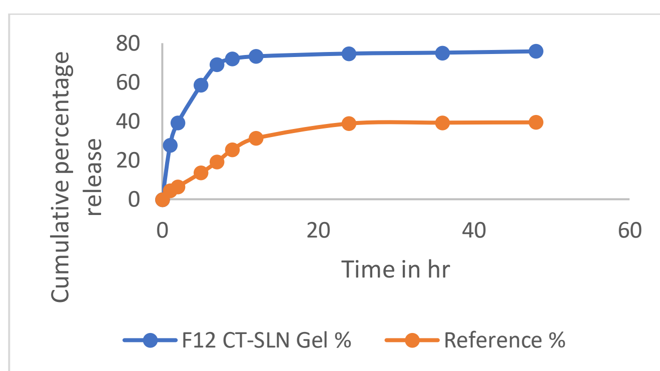

Figure 15. In vitro drug release profile of F12 CT-SLN gel and reference marketed cream.

3.2.3 Drug permeation study

The permeation study mimics the behaviour of F12 CT-SLN after application on skin. The F12 CT-SLN formulation showed remarkably higher permeation rate as compared to marketed reference. The plot of cumulative amount of drug permeated showed 3.74 times higher drug release rate when compared with reference cream. The flux value calculated from the linear portion of graph Q/A vs time from SLN gel was found to be 7.5 ± 0.392 mcg/cm2hr and 4 ± 0.198 mcg/cm2hr for reference cream. This type of release pattern is required for the targeting to skin fungal infection, first burst release will affectively target to fungal cell wall and sustained release will maintain the depot of drug. The possible sequence of events that F12 CT-SLN might have followed was i) Diffusion and Release of superficial cotrimoxazole present on SLN, ii) Diffusion of cotrimoxazole from matrix containing nanodroplets of DMSO and TPGS with solid lipid iii) Erosion of solid lipid iv) Partitioning and diffusion of drug from the drug reservoir to the superficial layer of skin. Here the point has to be considered regarding the presence of various enzymes and minerals present in the stratum corneum, body temperature which can lead to polymorphic transformation and the presence of disease state causes the degeneration of the layers of skin.

Results of quantification of cotrimoxazole after 24 h was expressed as in percentage of drug un diffused, deposited and permeated through skin. The data reveals that the F12 CT-SLN gel showed maximum penetration of drug in skin as compare to reference, the amount of drug deposited for F12 CT-SLN gel was 3.12 times higher than reference cream explaining its higher potential to treat the infection.

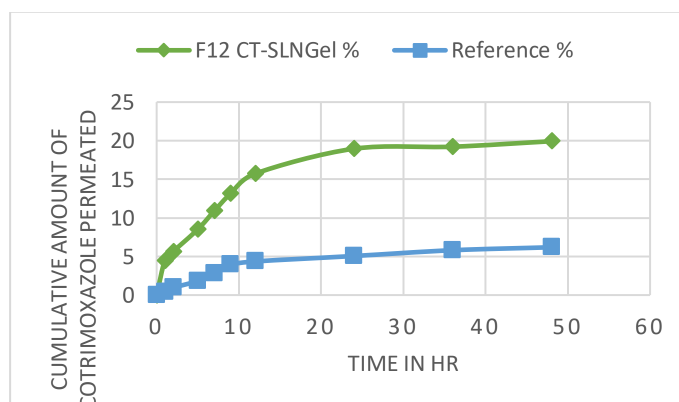

Figure 16. In vitro permeation profile of CT from SLN gel and reference marketed cream

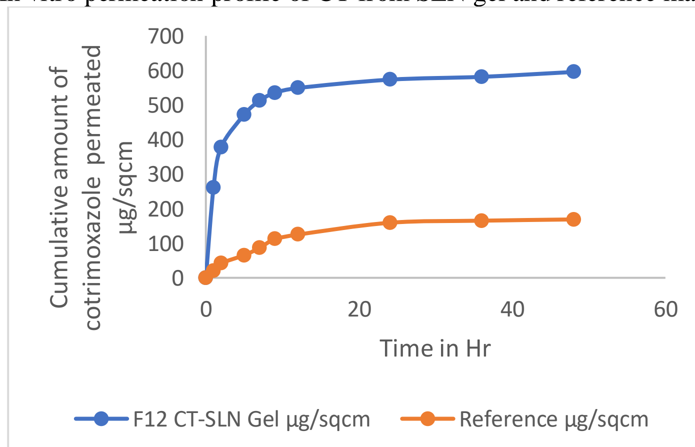

Figure 17. In vitro permeation profile (Q/A Vs Time) of CT from SLN gel and reference marketed cream

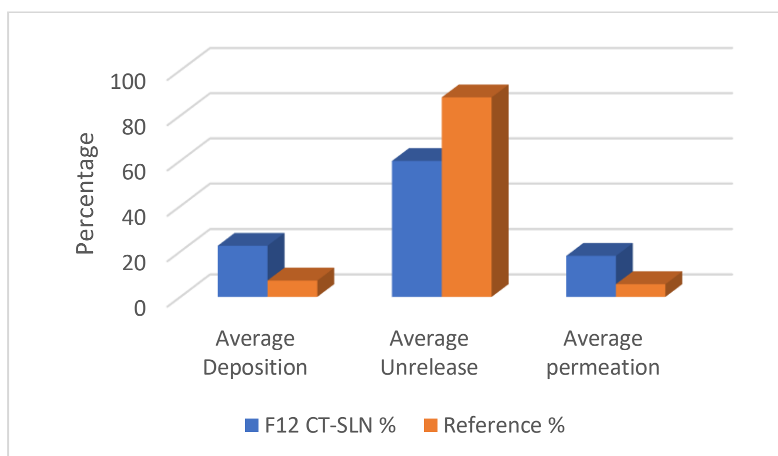

Figure 18. Percentage of cotrimoxazole permeated, deposited and remain unabsorbed in the skin

3.2.4 Skin retention studies

F12 CT-SLN gel showed more permeability and localization of drug than the reference cream. The amount of F12 CT-SLN in stratum corneun was 4.48 times higher than the reference cream. The drug accumulates in the stratum corneum and act as depot for further drug release.

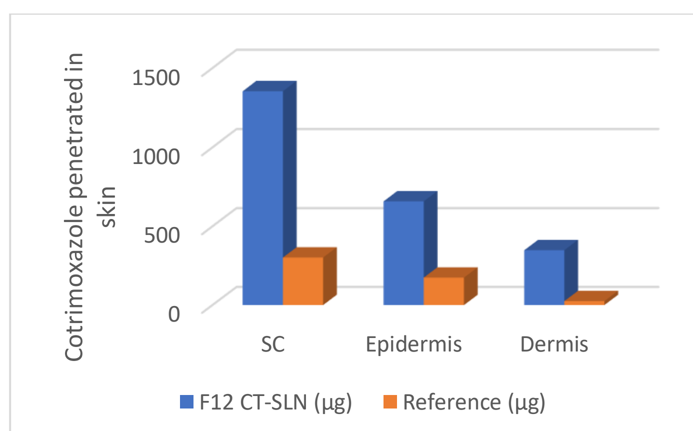

Figure 19. skin retention of cotrimoxazole in different skin strata

3.2.5 Skin irritation study

The skin irritation potential of plain *aloe vera* gel, CT incorporated *aloe vera* gel, marketed (Sertakon) cream and F12 CT-SLN *aloe vera* gel was studies according to the OECD guideline. The site of application was scored and graded according to the marking system of OECD. The application site was examined after the removal of test sample and graded for irritation, erythema and edema at 1, 24, 48 and 72 hr. The tabulated grading results were given for the plain *aloe vera* gel, CT incorporated *aloe vera* gel, marketed cream and F12 CT-SLN *aloe vera* gel. The results interpret that all the formulation showed no signs of irritation, erythema and edema at the end of 72 hr hence all the formulation were found to be free of any irritation when applied to the skin. There was no sign of erythema and edema and showed improved safety.

Animal code	Grading and time intervals			
	1 hr	24 hr	48 hr	72 hr
H1	0,0	0,0	0,0	0,0
H2	0,0	0,0	0,0	0,0
H3	0,0	0,0	0,0	0,0
Total score	0=erythema, 0=edema			

Table 3. Grading of erythema and eschar formation and edema formation for the F12 CT-SLN aloe vera gel

3.2.6 *In vitro* antifungal activity

The antifungal activity was evaluated on the basis of zone of inhibition. Zone of inhibition interprets the effectiveness of formulation against the microbial species taken. Here *C Albican* is used as a reference as it is responsible for various skin

infections. The zone of inhibition measured after 72 h for F12 CT-SLN was found to be higher when compared with marketed preparation (figure 5.66 and table 5.50). This is due to the permeability of SLN to cross the fungal cell membrane and releases the drug inside the cell. The result reveals the enhanced potential of F12 CT-SLN-SLN gel to act against *Candida* species as compared to marketed cream and the standard dilutions of cotrimoxazole. The study was carried out in triplicate and the same results were obtained for the zone of inhibition.

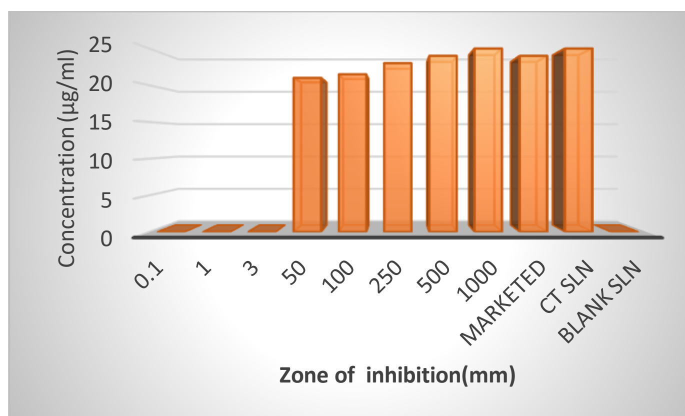

Figure 20. Comparative Antifungal activity of different dilutions of Co-trimoxazole with marketed and F12 CT-SLN formulation.

3.2.7 Stability studies of F12 CT-SLN gel

Stability of the prepared F12 CT-SLN gel was calculated in terms of Cotrimoxazole retained in SLNs, from the amount of drug leaked out of SLNs over a period of 12 month at different conditions of storage (2-8°C, 25°C, 30°C and 40°C). The formulations were evaluated for the physical stability, organoleptic properties, color, pH and appearance. The results were depicted in the table. Stability was calculated in terms of percent cotrimoxazole retained in SLNs. All the formulations were found to be stable at all temperature for the period of 12 months.

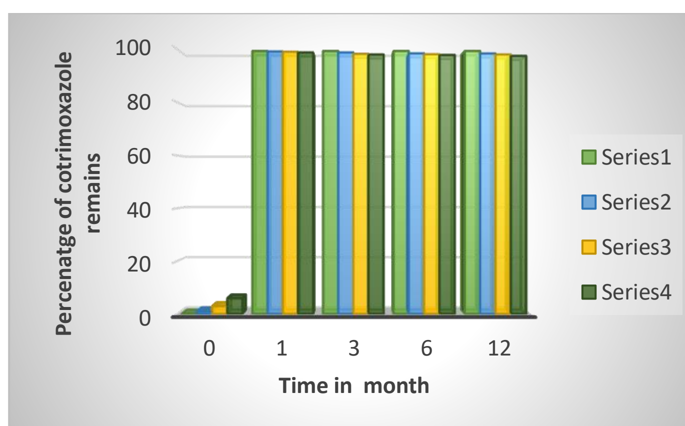

Figure 21. Mean retention of cotrimoxazole into SLNs from F12 CT-SLNs Gel

4. CONCLUSION

The present study gives emphasis on the formulation of novel drug delivery system for the treatment of topical fungal infection. By considering the limitations of conventional dosage form and antifungal drugs, here attempt has been made to formulate solid lipid nanoparticles of Terbinafine Hydrochloride and Cotrimoxazole made up of natural excipients for the effective monitoring of fungal infections. B9 and F12 TH-SLN and CT-SLN was incorporated into *Aloe vera* gel. The rheological study of B9 BUTE-SLN *aloe vera* gel was found to show non-Newtonian pseudoplastic flow behavior. The ease of spreading, uniformity, consistency, and adhesion on skin were shown by the B9 and F12 TH-SLN and CT-SLN gel. The occlusion factor for B9 and F12 TH-SLN and CT-SLN gel at the end of 72hr, proving the impermeable layer on the stratum corneum. In accordance to the occlusion study, skin hydration study reveals the more hydration of skin as compared to marketed cream.

In vitro drug release study of B9 and F12 TH-SLN and CT-SLN gel was compared to FINTOP cream. Retention of Terbinafine Hydrochloride and Cotrimoxazole in different layers of skin was quantified and stratum corneum showed maximum localization of drug as compared to reference cream.

Primary skin irritation potential was determined for B9 and F12 TH-SLN and CT-SLN *aloe vera* gel and it showed no erythema and edema scores. B9 and F12 TH-SLN and CT-SLN *aloe vera* gel was found to be safe and non-irritant for topical application. *In vitro* antifungal activity reveals the improved antifungal property of B9 and F12 TH-SLN and CT-

SLN *aloe vera* gel as compared to marketed and standard solution. B9 and F12 TH-SLN and CT-SLN *aloe vera* gel was subjected to different storage condition and found to be more stable at all storage conditions.

5. REFERENCES

1. Jain, S., Jain, S., Khare, P., Gulbake, A., Bansal, D. and Jain, S.K., 2010. Design and development of solid lipid nanoparticles for topical delivery of an anti-fungal agent. *Drug delivery*, *17*(6), pp.443-451.
2. Orthaber, K., Pristovnik, M., Skok, K., Perić, B. and Maver, U., 2017. Skin cancer and its treatment: novel treatment approaches with emphasis on nanotechnology. *Journal of Nanomaterials*, *2017*.
3. Naguib, Y.W., Rodriguez, B.L., Li, X., Hursting, S.D., Williams III, R.O. and Cui, Z., 2014. Solid lipid nanoparticle formulations of docetaxel prepared with high melting point triglycerides: *in vitro and in vivo* evaluation. *Molecular pharmaceutics*, *11*(4), pp.1239-1249.
4. Geetha, T., Kapila, M., Prakash, O., Deol, P.K., Kakkar, V. and Kaur, I.P., 2015. Sesamol-loaded solid lipid nanoparticles for treatment of skin cancer. *Journal of drug targeting*, *23*(2), pp.159-169.
5. Londhe, V. and Save, S., 2017. Zaltoprofen Loaded Solid Lipid Nanoparticles for Topical Delivery: Formulation Design.
6. Tosta, F.V., Andrade, L.M., Mendes, L.P., Anjos, J.L.V., Alonso, A., Marreto, R.N., Lima, E.M. and Taveira, S.F., 2014. Paclitaxel-loaded lipid nanoparticles for topical application: the influence of oil content on lipid dynamic behavior, stability, and drug skin penetration. *Journal of nanoparticle research*, *16*(12), p.2782.
7. Kakadia, P.G. and Conway, B.R., 2014. Solid lipid nanoparticles: a potential approach for dermal drug delivery. *American Journal of Pharmacological Sciences*, *2*(5A).
8. Ekambaram, P., Sathali, A.A.H. and Priyanka, K., 2012. Solid lipid nanoparticles: a review. *Sci Rev Chem Commun*, *2*(1), pp.80-102.
9. Sonawane, R., Harde, H., Katariya, M., Agrawal, S. and Jain, S., 2014. Solid lipid nanoparticles-loaded topical gel containing combination drugs: an approach to offset psoriasis. *Expert opinion on drug delivery*, *11*(12), pp.1833-1847.
10. Chen-yu, G., Chun-fen, Y., Qi-lu, L., Qi, T., Yan-wei, X., Wei-na, L., Guang-xi, Z. (2012). Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. *Int J Pharm* 430: 292–98.
11. Chow, K. T., Chan, L. W. and Heng, P. W. S. (2008). Characterization of spreadability of nonaqueous ethylcellulose gel matrices using dynamic contact angle. *J Pharm Sci* 97: 3467–82.
12. Cirri, M., Bragagni, M., Mennini, N. and Mura, P. (2012). Development of a new delivery system consisting in ‘‘drug–in cyclodextrin–in nanostructured lipid carriers’’ for ketoprofen topical delivery. *Eur J Pharm Biopharm* 80: 46–53.
13. Cohen-Avrahami, M., Libster, D., Aserin, A. and Garti, N. (2012). Penetratininduced transdermal delivery from HII mesophases of sodium diclofenac. *J Control Release* 159: 419–28.
14. Cui, Z., Hsu, C. H. and Mumper, R. J. (2003). Physical characterization and macrophage cell uptake of mannan-coated nanoparticles. *Drug Dev Ind Pharm* 29: 689–700.