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Formulation and Evaluation of Topical Liposomal gel Containing Antifungal Drug

Shivani Fotedar [(1)], Anshul Namdev [(2)], Priyanka Gupta [(3)] Sakshi S Chouhan [(4)]
Chameli Devi Institute of Pharmacy, Indore Gram Umrikheda, Khandwa Road, Indore- 452020
1. Ms. Shivani Fotedar
Assistant Professor (Department of Pharmaceutics)
Chameli Devi Institute of Pharmacy, Indore
2. Mr. Anshul Namdev
Assistant Professor (Department of Pharmacology)
Chameli Devi Institute of Pharmacy, Indore
3. Mrs. Priyanka Gupta
Assistant Professor (Department of Pharmacognosy)
Chameli Devi Institute of Pharmacy, Indore
4. Ms. Sakshi S Chouhan
Assistant Professor (Department of Pharmaceutics)
Shivaji Rao Kadam Institute of Pharma. Edu. & Research

ABSTRACT
Superficial fungal infection in immunocompromised patients can lead to many disorders and complications. Currently, new topical treatment options are critically needed to treat these fungal infections. The present study was undertaken with an intention to develop a stable and effective topical formulation containing luliconazole. Luliconazole, an FDA sanctioned novel azole antifungal drug that combats fungal contagions. It exhibits highest antifungal activity against Trichophyton spp. Candida albicans, Malassezia spp., and Aspergillus fumigatu, which are major causative agents of dermatophytosis. It acts by inhibiting lanosterol demethylase, which is major component of fungus cell wall. Prior to the formation of a gel, medication pre-formulation tests were conducted to obtained a desire antifungal gel

KEYWORDS: *Luliconazole, Antifungal, Liposome loaded gel, Topical formulation*

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I. INTRODUCTION

Topical drugs are administered to the skin for effects on the surface, localized, or systemic. Fungus is responsible for a variety of skin illnesses. Nowadays, fungus infection is one of the most common skin concerns. For the topical treatment of skin disease, a variety of solid, semisolid, and liquid preparations are available. A wide range of products are used to apply to the skin. Topical treatment of fungal infections has various advantages, including the ability to target the infection site, reduced risk of systemic adverse effects, improved treatment efficacy, and high patient compliance. A range of topical antifungal agents have been employed in the treatment of various dermatological skin infections. The focused class of topical antifungals in this research work is azoles. When antifungals are applied topically, the drug components must penetrate through the stratum corneum, the skin's outermost layer, to reach the lower layers, particularly the viable epidermis. New carrier systems for authorized and investigational medications are being developed as alternate techniques for topical treatment of fungal infections of the skin. Antifungal chemicals can be delivered more effectively into the skin via carriers such as colloidal systems, vesicular carriers, and nanoparticles. Various types of nanocarriers have recently been developed to increase drug delivery through the dermal and transdermal routes. To aid in the penetration of the incorporated agents, lipophilic and hydrophilic medicines can be incorporated into liposomal vesicular systems. The purpose of this research is to create and characterise a Luliconazole-loaded liposomal gel as a viable topical delivery.

II. METHODOLOGY

Material

Luliconazole was gift sample obtained from Hema Pharma Laboratories Pvt. Ltd, Gujarat and other analytical grade chemicals and reagents used from the laboratories.

Method

Liposome was prepared by method reported by Riaz et al (1996) with some modification. Phosphate dyclonine, Cholesterol and Luliconazole were dissolved in chloroform/methanol (2:1, v/v) mixture and subsequently transfer red into appear-shaped flask connected to a Rota vapor (Buchi-type). Speed was maintained at 150r/min, vacuum applied and the thin film were formed by slow removal of the solvents at 40°C.The lipid film was maintained under vacuum for 12 hr in a desiccator to remove solvent traces and subsequently it was hydrated with a Saline Phosphate Buffer of pH 7.4 solution at 40°C under continuous rotation of the flask until a dispersion was formed (about1h).

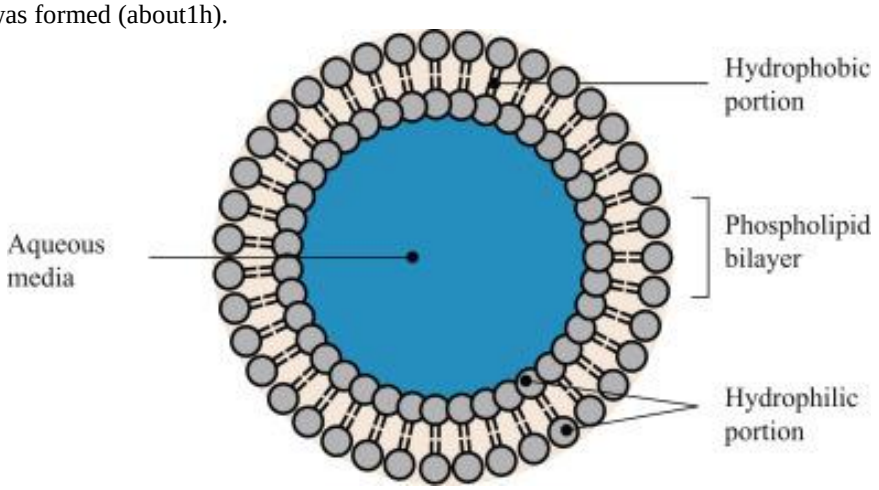

Figure 1: Structure of Liposome

Preparation of Gel Base

Carbopol 934 (1-3% w/v - Liposome based gel formulation i.e., LG-1 of 1% w/v, LG 2 of 2% w/v, LG-3 of 3% w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution 28. The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Liposome containing gel in which previously prepared Liposome suspension was added. Liposome preparation corresponding to 0.1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Figure 2: Prepared Carbopol Gel

Table 1: Composition of Liposomal Formulation

S.No.	Batch Code	Soya lecithin (mg)	Cholesterol (mg)	Luliconazole (mg)
1.	F-1	150	30	100
2.	F-2	150	40	100
3.	F-3	200	50	100
4.	F-4	200	30	100
5.	F-5	250	40	100
6.	F-6	250	50	100

Table 2: Composition of Liposome Loaded Formulation

S.No.	Ingredients	F1	F2	F3	F4	F5	F6
1.	Liposome Formulation (%)	1	1	1	1	1	1
2.	Phosphatidylcholine (%)	1.0	1.5	2.0	1.0	1.5	2.0
3.	Terpenes (%)	0.25	0.25	0.25	0.5	0.5	0.5
4.	Ethanol (ml)	5	5	5	5	5	5

EVALUATION

Physical appearance

It is the initial evaluation during Pre-formulation studies which assess the color, odor and taste of the substance.

Identification of drug and drug excipient interaction study

By UV Spectroscopy - The identification of pure drug the Pre-formulation studies were carried out for the drug using the UV spectrophotometer. The calibration curve was plotted for different concentration for drug in 6.8 pH buffer solution using Shimadzu 1800 UV at 296 nm.

Melting Point Determination - range of substance is defined as those point of temperature within which or the point at which, the substance begins to coalesce and is completely melted. Melting point of luliconazole is determined by capillary melt method using melting point apparatus. A small amount of drug sample was kept at open end of capillary tube and placed in melting point apparatus. The sample was observed continuously. The melting range was recorded from starting of first melt to completely melt of sample. Melting point of drug was determined by Open capillary method.

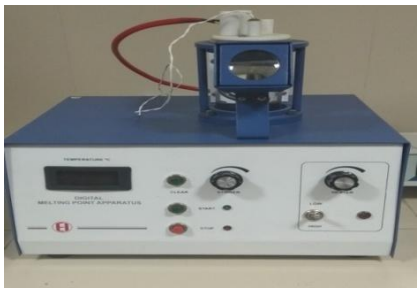

Figure 3: Melting Point Apparatus

Determination of Partition Coefficient -

50 mg of drug was taken in three separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phases was calculated by using formula:

KPC = Concentration of Drug in Oil Phase / Concentration of Drug in Water Phase

Determination of λmax -

A solution of drug containing the concentration 10 µg/ ml was prepared in 0.1 N HCl. The solution was scanned in the range of 200 – 400 nm UV spectrums using Systolic double beam spectrophotometer.

Standard Calibration Curve of Luliconazole-

Determination of absorption maximum (λmax) –

100 mg of Luliconazole was accurately weighted into 100 ml volumetric flask, dissolved in small volume of acetone and volume was made up with 0.1M HCL. Pipette 1ml of this solution into another 10 ml volumetric flask and the volume was made with 0.1M HCL and marked as Stock. The resultant solution is scanned in the range of (200-400nm) by UV Spectrophotometer (UV-1700Shimadzucorporation, Japan) to get absorption maximum (λmax). And, also an absorption maximum is estimated similarly using phosphate buffer pH (7.4).

Preparation of Calibration Curve-

Luliconazole standard stock solution (1000µg/ml), 1ml solution was diluted to 10 ml using phosphate buffer (pH 7.4) to get concentrations of 100 µg/ml. from this solution, aliquots of,

0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml from standard drug solution were diluted to 10 ml with phosphate buffer (pH 7.4) and the absorbance of these solutions was measured Spectro photo metrically using phosphate buffer (pH 7.4) as a blank.

A stand r curve was plotted using concentration on X-axis and the absorbance obtained on Y- axis.

Entrapment Efficiency- The liposome suspension was ultra-centrifuged at 5000 rpm for 1 hr. by using ultra centrifuge to separate the free drug. Supernatant contained liposome in suspended stage and free drug at the wall of centrifugation tube. The supernatant was collected and again centrifuged at 5000 rpm at for 30 minutes. A clear solution of supernatant and pellets of liposome were obtained. The pellet containing only liposome was resuspended in distilled water until further processing.

The liposome free from unentrapped drug were soaked in 10 ml of methanol and then sonicated for 10 min. The vesicles were broken to release the drug, which was then estimated for the drug content. The absorbance of the drug was noted at 393.7 nm. The entrapment efficiency was then calculated using following equation.

Amount of drug entrapped = Amount of drug present in supernatant – total amount of drug added.

In vitro Drug Release Study-

The in vitro drug release study indicates the nature of drug release from the formulations and predicts the release kinetics. In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane (HiMedia molecular weight 5000) was placed between receptor and donor compartments. Luliconazole liposomal suspension was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (18 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 200rpm throughout the experiment. At fixed time intervals, 1ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible Spectrophotometer to determine the in vitro release amount of drug.

Antifungal Activity- The antifungal activity was screened by disc diffusion method. The Sabouraud‟s dextrose agar plates were inoculated with the fungal culture of Candida albicans (72h culture). Sabouraud Dextrose Broth cultures were prepared in test tubes. Using sterile cotton swab, the surface of Sabouraud‟s Dextrose Agar plates were swabbed to prepare lawn cultures. 5 min after the agar surface had dried, wells were dug using sterile cork borer aseptically. Wells were saturated with topical gels six formulations. Without liposome-based formulation was used for comparison. The plates were incubated at 28 ºC for 24-48 hrs. The diameters of zones of inhibition were measured using a scale to the nearest millimeter.

Stability Studies – As soon as the product is developed, it is subjected to ageing; as a result, its physical properties, chemical composition and even its biological availability may be changed. To assess long-term stability, nail liquor-based formulations were stored in tube at (4°C±1°C, 25°C±1°Cand 40°C±1°C), 75% relative humidity (RH) ±5% for a period of up to 3 months.

III. RESULT & DISCUSSION

Physical Appearance-

The drug was obtained as a kind gift from Hema Pharmaceuticals Pvt. Ltd, India. The supplied powder of Luliconazole was light yellow powder.

Melting Point-

Melting point of Luliconazole was determined by melting point apparatus (Tempo) and found to be 151.5 ± 2°C

Partition Co-efficient

Table 3: Partition coefficient value of Luliconazole

S.No.	Solvent system	Partition Coefficient
1.	n-Octanol/PBS (pH 7.4)	5.45 ±0.26.

Standard Curve of Luliconazole in Phosphate Buffer Solution (pH 7.4)-

All dilutions and measurements were made as above in phosphate buffer solution of pH 7.4 made as per formula (I.P.). The absorbance was taken at λ_{max} 293.7 nm against a reagent blank. The standard curve was plotted between absorbance and concentration.

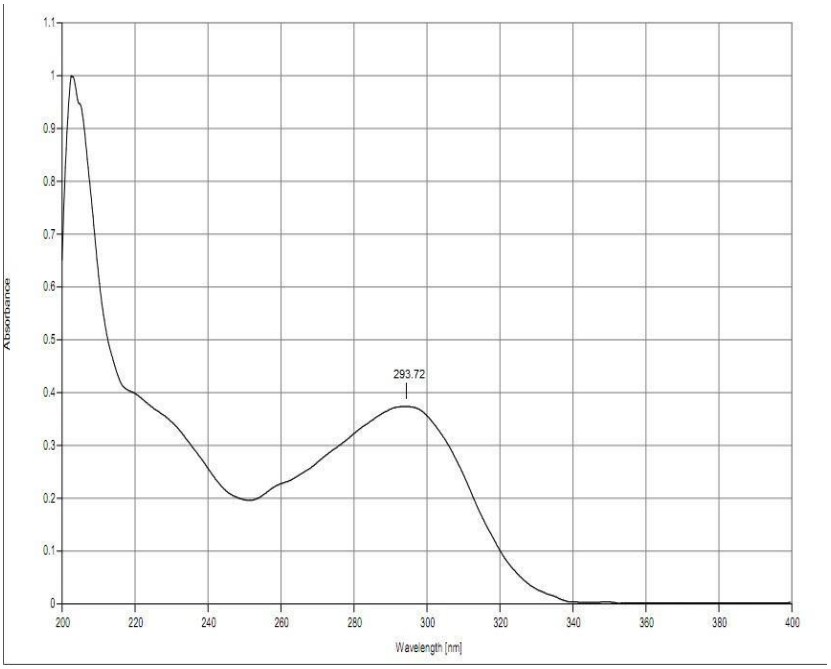

Figure 4: UV Spectra of Luliconazole in PBS Buffer (pH 7.4)

Table 4: Standard Curve of Luliconazole in Phosphate Buffer Solution (pH 7.4)

S.No.	Drug Conc. (µg/ml)	Absorbance at 293.7 nm
1.	5	0.112
2.	10	0.287
3.	15	0.452
4.	20	0.543
5.	25	0.672

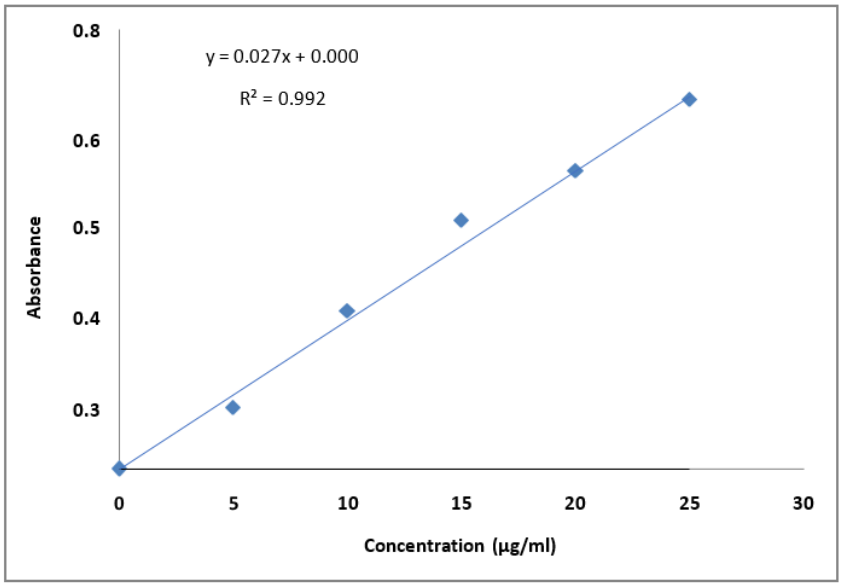

Figure 5: standard curve of Luliconazole in phosphate buffer solution (pH 7.4) at 293.7nm

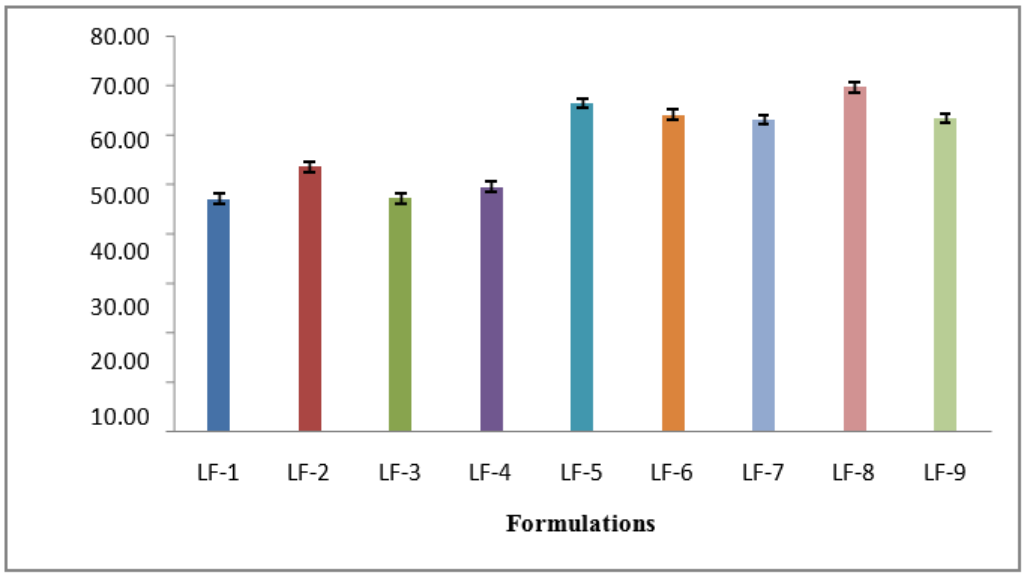

Figure 6: % Drug Entrapment of Luliconazole Loaded Liposomal Formulation

Table 4: Cumulative % of Drug Release of Luliconazole Loaded Liposomal Formulation

F. Code / Time	Cumulative % of Drug Release				
	LF-1	LF-2	LF-3	LF-4	LF-5
2	2.24±1.56	2.8±1.33	2.88±1.18	4.43±2.23	4.26±1.66
4	7.48±1.32	9.11±1.67	9.22±2.09	11.72±1.86	12.53±1.53
6	12.42±1.98	15.27±1.54	16.09±1.08	19.67±2.06	20.96±1.79
8	18.31±2.15	22.39±1.17	23.18±2.62	29.23±1.56	29.95±1.43
10	25.34±2.28	28.74±2.15	31.15±1.32	37.69±2.98	40.13±3.08
24	47.89±1.06	59.68±2.18	64.51±2.67	71.61±1.54	67.79±1.69

Figure 7: Cumulative % of Drug Release of Luliconazole Loaded Liposomal Formulation

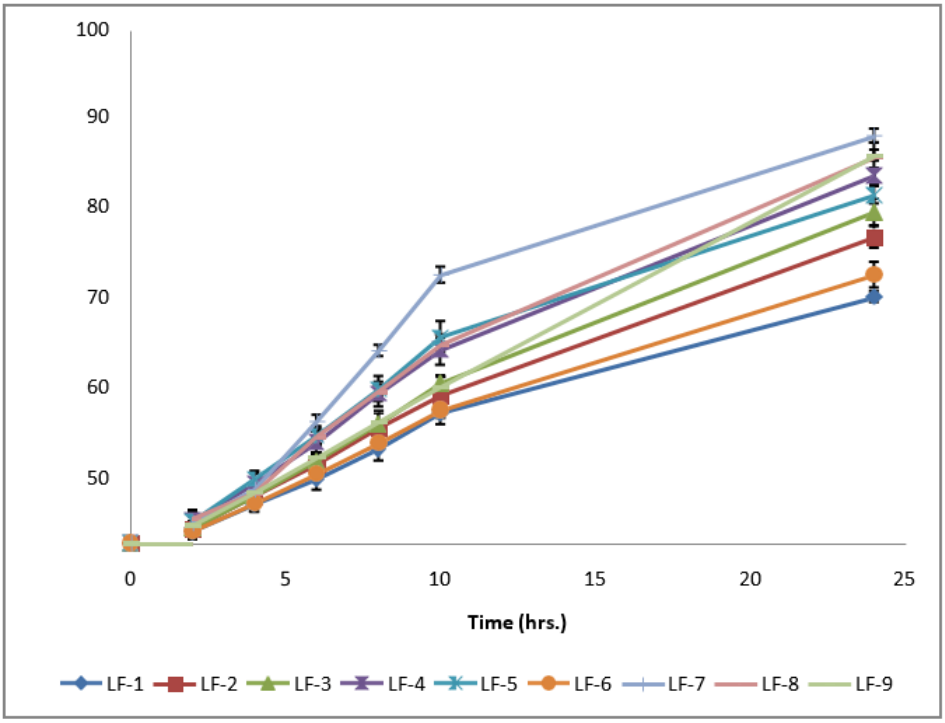

Table 5: Cumulative % in Vitro Drug Release Study of Luliconazole Loaded Liposomal Based Formulation

Time (hr.)	Cumulative % In Vitro Drug Release						
	LLNLF-1	LLNLF-2	LLNLF-3	LLNLF-4	LLNLF-5	LLNLF-6	Plain Formulation
0	0	0	0	0	0	0	0
1	5.12± 1.63	5.16± 1.51	6.86± 1.42	7.95± 1.16	2.24± 2.42	2.74± 1.85	2.6±3.31
2	14.23 ±1.59	12.92 ±1.55	15.46 ±1.15	6.78± 1.05	7.48± 1.36	8.96± 3.46	4.47±1.15
3	26.87 ±3.63	27.69 ±1.54	26.22 ±1.36	20.18 ±2.91	15.16 ±1.34	12.45 ±2.36	5.26±1.89
4	34.55 ±1.67	32.27 ±1.55	33.45 ±1.34	38.19 ±2.68	32.92 ±1.35	23.21 ±2.93	6.77±1.38
5	42.67 ±1.73	47.65 ±2.65	41.88 ±2.35	42.54 ±1.75	45.65 ±2.11	38.16 ±1.98	8.63±1.78
6	54.72 ±2.87	52.15 ±1.95	49.75 ±2.11	53.54 ±1.54	50.71 ±1.97	44.97 ±1.44	13.57±1.28
8	62.87 ±1.02	65.44 ±2.34	55.97 ±2.97	62.17 ±1.08	67.54 ±2.43	52.26 ±1.83	18.22±1.41
12	78.71 ±1.72	73.26 ±2.15	66.45 ±1.51	76.13 ±1.26	79.3± 1.05	73.28 ±1.21	22.01±1.29
14	88.65 ±1.68	89.06 ±1.89	82.29 ±1.54	81.82 ±1.67	84.52 ±1.23	81.18 ±2.15	26.94±1.34
16	92.67 ±1.55	91.64 ±2.34	96.72 ±2.86	97.38 ±3.64	93.05 ±1.56	91.41 ±1.24	28.39±2.34
18	-	-	-	-	-	-	30.11±2.59
20	-	-	-	-	-	-	32.65±1.36
22	-	-	-	-	-	-	35.94±3.21

24	-	-	-	-	-	-	38.45±1.48

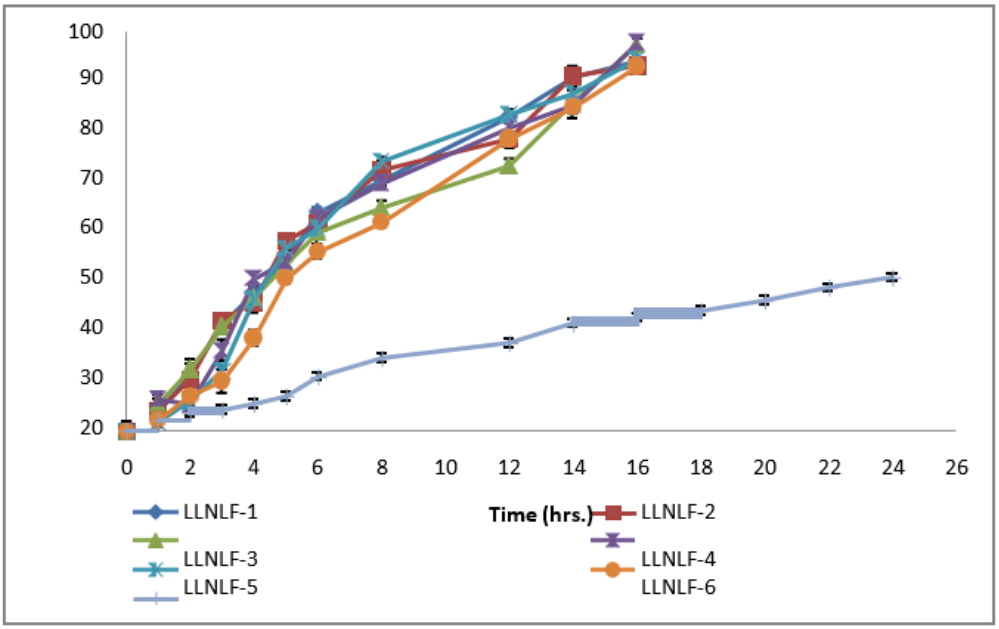

Figure 8: Cumulative % in Vitro Drug Release Study of Luliconazole Loaded Liposomal Based Formulation

Table 6: Antifungal activity of Luliconazole Loaded Liposomal Based Formulation

S.No.	Formulation	Zone of inhibition in mm (Organism: Candida Albicans)
1.	LLNLF-4	47
2.	Plain Luliconazole	36

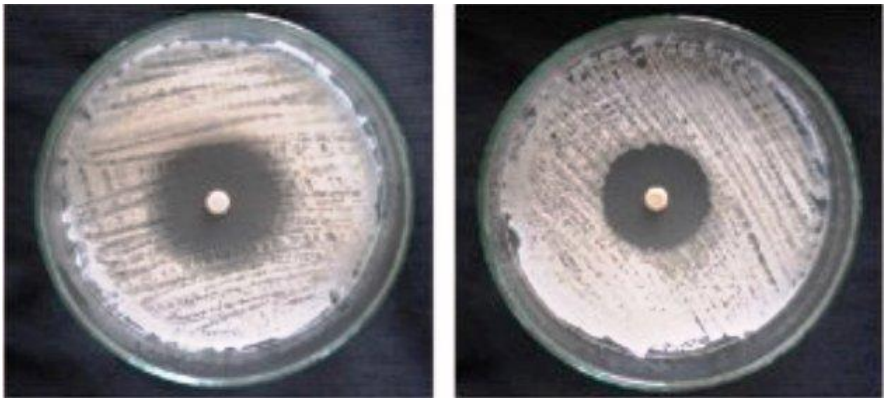

Figure 9: Antifungal activity of Luliconazole Loaded Liposomal Formulation- LLNLF-4 (a) and Plain Luliconazole (b)

Table 7: Stability Study of Luliconazole Loaded Liposomal Formulation- LLNLF-4.

	Stability Condition / Parameters		
Time	**4°C± 2°C**	**25°C± 2°C**	**40°C± 2°C**
1.	**Smoothness**		
Initial	+++	+++	+++
30 Days	+++	+++	+++
2.	**Color**		
Initial	Transparent	Transparent	Transparent
30 Days	Transparent	Transparent	Transparent
3.	**% Drug Release (After 16 hr.)**		
Initial	97.38	Initial	97.38
30 Days	97.26	30 Days	97.26

IV. SUMMARY & CONCLUSION

Conventional treatments for fungal infection are clinically not efficient, as formulations must permeate the skin barrier in order to deliver therapeutic levels to the target site. In the present study, topical gel containing a luliconazole loaded liposomal based formulations are tried out and successfully prepared. Initially, researchwork started with a wide and through literature survey followed by Pre-formulation and formulation development studies, Pre-formulation studies were done to evaluate the purity of drug by physical/morphological examination, melting point, partition coefficient, and λmax determination. Luliconazoledrug was found to be Orange to Green powder to crystal powder. All parameters were found tobe concordant with standard.

The Next step was the preparation, optimization and characterization, Luliconazole loaded liposome were successfully formulated by **modified film hydration technique**. Luliconazole has poor water solubility, instability, and also low bioavailability; thus, therefore, we encapsulate the luliconazole into liposome which may have potential application and further thisliposomal suspension was used to prepare topical gel. The formula LF-7 had the smallest size (5.53±0.21µm) with good entrapment efficiency (62.93±0.35) while the formula LF-8 showedsignificantly the highest percent of entrapment efficiency approaching 69.61±1.09 %. But largerparticle size (9.36±0.12). So, considering to other parameters, formulation LF-7 was chosen forthe further formulation of topical gel instead of LF-8.

Further optimized formulation of liposomal suspension (LF-7) was used to prepare nail liquorformulation. Topical gel formulation LLNLF-1-LLNLF-4 showed good smoothness due to lower polymer concentration while formulation LLNLF-5-LLNLF-6 shows very good smoothness due to presence of increased polymer concentration. FormulationLLNLF-4 showed highest amount of drug release (97.38±3.64) from the formulation and after considering the other parameters the formulation it was chosen for the further study. In anti- fungal activity studies, formulation LLNLF-4 showed better anti-fungal activity by LLNLF-4 The results of Stability studies for color and smoothness and % drug release (After 16 hr.) of formulation which were stored at 4°C, 25°C, and 40°C over a period of 3 months showed no significant changes. The findings of stability study suggested that prepared formulation showed good stability.

It can be concluded from the above research findings that the permeation rate of luliconazole loaded liposomal topical gel formulation showed good results in in-vitro conditions. Significantly enhanced results were observed when delivered through liposome-loaded topical gel formulation in comparison to medicated topical gel with a penetration enhancer. Theluliconazole loaded liposomal topical gel proved to be a better tool as a drug delivery system for the ungual drug delivery of an antifungal in the treatment of onychomycosis and other fungal infections. This improves patient compliance and acceptability. The designed drug delivery system thus would serve as an efficient tool for transungual permeation and can further be explored for the delivery of drug substances used specifically in nail disorders.