DEVELOPMENT, CHARACTERIZATION AND IN VITRO - IN VIVO EVALUATION OF EFINACONAZOLE LOADED NIOSOMAL NAIL LACQUER FOR THE TREATMENT OF ONYCHOMYCOSIS

Vibhavari Chatur*[1], Shashikant Dhole[2], Nilesh Kulkarni[3]

[1*]PhD Scholar, PES Modern College of Pharmacy, Moshi, Pune 412105.

[2]Principal, PES Modern College of Pharmacy, Moshi, Pune 412105

[3]Associate Professor in Pharmaceutics, PES Modern College of Pharmacy, Moshi, Pune 412105

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Address for Correspondence:

Vibhavari M. Chatur

Rasiklal M. Dhariwal Institute of Pharmaceutical Education and Research, Pune-19.

Pune- 411 019, Maharashtra, India

Email ID- vibhavaric@gmail.com

Contact No: +91 9595478590

Abstract:

Objectives: The goals of the study were to develop, describe, and test the efinaconazole-loaded niosomes in Nail Lacquer so that they could be used to treat onychomycosis.

Methods: Using different ratios of non-ionic surfactants (Span 60 and Pluronic L121) and cholesterol, Efinaconazole niosomes were made using the probing sonication method. This was done to try to improve the poor penetration of topical medications into the skin and reduce the negative side effects that come with them. The niosomes that were made were tested for their size, how well they trapped drugs, and how well they released drugs in a test tube. The results showed that niosomes made with a ratio of 1:2 (Span 60: cholesterol) had smaller particle sizes and a high Entrapment Efficiency. Niosomal nail polish was made by using different polymers in a good way. The modified formulation was tested for stability, resistance to water, drug content, drug release in a test tube, antifungal effectiveness, and the ability to flow.

Results:Niosomes that had been loaded with efinaconazole were round and ranged in size from 95 to 135 nm. In vitro, the amount of drug that was released in 24 hours ranged from 25% to 86%, while the amount of drug that was trapped ranged from 40% to 90%. When efinaconazole niosomes were mixed with Span 60 and CHO in a ratio of 1:2, the results were promising and were used to make nail polish. Compared to the other formulations, the efinaconazole-loaded niosomal nail polish showed the best drug release (91.34 ±1.34), antifungal effectiveness, and smoothness. Most drugs that don't work well when taken by mouth can be put on the nails with nail polish. This method will make it easier for the medicine to get into the body through the nail. So, the created ENNL could be used as a system for putting drugs on the skin to treat onychomycosis.

Keywords: Efinaconazole, Niosomes, Nail Lacquer, Onychomycosis

Introduction:

Onychomycosis is the most common fungal infection of the nails. It affects the area under the fingers and toes. Onychomycosis can now be treated with oral and topical drugs, either alone or in combination. Oral antifungal therapy has been linked to low bioavailability and possible side effects in the gut and throughout the body. Onychomycosis is a fungal infection of the nail plate that is caused by yeasts, non-Dermatophyte fungi, and dermatophytes. It is a common disease. About 10–12% of people in the United States have it. Even though some people with nail dystrophy feel pain, discomfort when wearing shoes, infections, and find it hard to do daily tasks because their nails don't look good, this condition can cause both physical and mental problems. The goal of treatment is to get rid of the fungus and help the nail grow in a healthy way. Since the 1990s, therapeutic advances have led to better results, shorter treatment times, and fewer drug-drug interactions. Still, even with these improvements, the rate of recurrence is high, and 20% to 25% of people do not respond to treatment **(Jo Siu et al., 2013; Ogura et al., 1999).**

Efinaconazole, a recently approved antifungal triazole drug with low water solubility, shows low permeability. Efinaconazole is an azole drug that used to be called IDP-108 and KP-103. It has antifungal activity against dermatophytes similar to that of Terbinafine and amorolfine, more activity against Candida species than itraconazole, and antimicrobial activity against non-Dermatophyte moulds similar to that of Terbinafine and stronger than amorolfine, ciclopirox, and itraconazole. Triazoles were once taken by mouth to treat onychomycosis, but never put on the skin (such as fluconazole and itraconazole). They are interesting because they have a small effect on fungi and a wider spectrum than allylamines and morpholines. Efinaconazole is the first azole that has been approved by the FDA for use on the skin. Onychomycosis is treated with it **(Piraccini et al., 2010; Scher and Baran, 2003; Ghannoum et al., 2000).**

Niosomes are made in a number of ways, including by injecting ether, using reverse phase evaporation, and rehydrating thin films. These steps take a long time and cost a lot of money. They also require the removal of organic solvents. To solve these problems, the probe sonication method was made. Since the probe sonication method doesn't use chemical solvents, it is safe for the environment and doesn't harm it. This method is also quick and cheap. In this method, only the watery part of the drug is mixed with surfactant, cholesterol, and other surface enhancers. The mixture is then ultrasonified with a probe. In past studies that compared the two methods, spherical vesicles were made by both probe sonication and thin film hydration. Also, niosomes made with the probe sonication method had smaller diameters, more uniform sizes, and released drugs faster than those made with the traditional method **(Kanaani et al., 2017).** As was already

said, both hydrophilic and hydrophobic drugs can be put inside niosomal structures. However, niosomal formulations are especially helpful for hydrophobic drugs that don't dissolve well. Drugs that work better when they are released slowly and in the right place are good candidates for niosomal drug delivery. These include chemotherapeutic drugs used to treat different types of cancer **(Amiri et al., 2018).** These drugs are hard to give because they have bad side effects, don't dissolve well in water, and people are resistant to them **(Alemi et al., 2018).** Niosomes are made with the best non-ionic surfactants available so that their solubility, bioavailability, toxicity, and time spent in the bloodstream are all at their best **(Sayed et al., 2018).**

The goal of this study was to make a niosomal formulation using a less common, environmentally friendly, and economically beneficial probe sonication technology. This was done so that the drug release profile of a poorly soluble drug could be optimized. To improve the effectiveness of efinaconazole niosomal nail polish against fungus and to get patients to use it more often by giving them a medicine with a slow release. The current work involves in vitro assessment, including the production and characterization of efinaconazole niosomes, as well as in vitro release tests and in vivo testing of the optimized efinaconazole niosomal nail lacquer.

1 Material and Method:

1.1 Material:

Efinaconazole was sold by the Hyderabad, India-based Company Aurobindo Pharma Ltd. From SD Fine Chemicals Ltd. in Mumbai, Span 60, Nitrocellulose, and Ethyl cellulose were bought. The cholesterol came from a company in Mumbai called Sigma-Aldrich. Merck, Bombay sent Pluronic L121, dicetylphosphate, salicylic acid, and salicylic acid. SD Fine Chemicals Ltd., which is based in Bombay, gave them the ethanol, the methanol, and the sodium dihydrogen phosphate. The remainder of the items were suitable for examination.

1.2 Preparation of Efinaconazole Niosomes (EFN)

Using the probe sonication method, 15 mL of water and 15 mg of efinaconazole were mixed with a magnetic stirrer to make niosomes. After that, cholesterol, Span 60, Pluronic L121, and dicetylphosphate were added. To make the best niosomes, the concentrations of Pluronic L121 and DCP were used. Based on preliminary research, the central point of the central composite design was set at 290 mg of Pluronic L121 and 1 mg of Dicetylphosphate. The mixture was then sonicated for 5 minutes at a probe temperature of 57 °C in a pulsed way (50 s of sonication followed by a 10 s break) with 30% amplitude using a Vibra cell (Sonics & Materials, Inc., USA). Niosomes were collected after probe sonication and kept at 4 °C for physicochemical analysis **(Sharma et al., 2018; Khan et al., 2020; Khan et al., 2020).**

Table 1: Various concentrations of surfactants in which Niosomes are prepared

Formulations	Span 60 (mg)	Pluronic L121 (mg)	Cholesterol (mg)	DCP (mg)	Drug (mg)	Milli-Q H_2O (ml)
EFN1	40	290	80	1	10	15
EFN2	**35**	**250**	**70**	**1**	**10**	**15**
EFN3	30	334	60	1	10	15
EFN4	35	318	70	2	10	15

1.3 Evaluation of Efinaconazole niosomes

1.3.1 Morphology

The size, shape, and roughness of the niosomes' surfaces were measured using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (TEM). One drop of Niosome solution was put on a silicon wafer to make a sample. The sample was then fully dried in a desiccator overnight before SEM analysis. After sputtering, samples with a thin layer of gold were looked at with a field emission scanning electron microscope (HITACHI S-150). For TEM examination, a drop of Niosome suspension was put on the copper grids right away, and it was left to dry overnight so that all the water was gone. A 200 kV field emission TEM was used in the experiment (FE-TEM, Eindhoven, Netherlands).

1.3.2 Size and zeta potential measurements

For each formulation, the average Niosome diameter (z-average), polydispersity index (PDI), and zeta-potential were measured with Zetasizer Nano ZS (Malvern Instruments Ltd., USA). Before the tests, the 20 μL of niosomal formulations were diluted with 15 mL of water to avoid a phenomenon called "multi-scattering." Three separate measurements were taken. Using zeta potential to look at how stable the Niosomes colloidal property is. The decrease in electrostatic repulsion caused by ions in solution shielding the particle's surface charge is caused by particles coming together. By looking at how particles move in an electrophoretic field, one can figure out the surface charge of a vesicle, which is called the Zeta potential. A Malvern Zeta Analyzer is used in this investigation.

1.3.3 Fourier-Transform Infrared Spectroscopy (FT-IR)

Fourier Transform Infrared Spectroscopy (FTIR) (Spectrum Two, U.S.A.) was used to study how molecules in efinaconazole and niosomes interact with each other. For this test, lyophilized samples from each person were mixed with KBr and then pressed into pellets with a hydraulic

press. At room temperature, FTIR measurements were done with a constant resolution of 4 cm^{-1} and a scanning range of 4000 to 400cm^{-1}.

1.3.4 Entrapment Efficiency

The unbound medicine is taken out of the efinaconazole niosomes by using a cooling spinner and cooling centrifugation at 5000 rpm for 30 minutes at 4°C (Union 32R, Korea). The niosomal pellets are spun again after being rinsed with 10 mL of PBS. The supernatant is filtered with a 0.22 m Millipore filter (Millipore, USA). By measuring the absorbance at 230 nm, a UV spectrophotometer is used to figure out how much free medicine is in the supernatant. This is done three times to make sure that all of the free drugs are gone. After centrifugation, n-propanol is used to break up the niosomes. The absorbance at 230 nm is then used to figure out how much drug is inside the niosomes.

Percent drug entrapment = Total drug - drug in supernatant/ Total drug × 100

1.4 In vitro release study

To test how much efinaconazole came out of niosomes, a Franz diffusion cell with a 25 mL receptor volume was used. Each sample was put on its own dialysis membrane in two different diffusion cells. The donor chamber was between the receptor chamber and a dialysis membrane (MWCO 12 KDa) **(Ravalika et al. 2017).** The medium used for the release was PBS with 0.05 percent SDS. The water bath was kept at 37°C, and a magnetic stirrer was put between it and the Franz cell. During the dialyzation process, 1 mL of the sample was taken out of the receptor compartment at set times (0.5, 1, 3, 6, 9, 12, 24, 48, and 72 h) and replaced with an equal amount of buffer at the same temperature to keep the volume the same. Lastly, the samples' optical densities were measured with a UV-visible spectrophotometer at 230 nm and the buffer mentioned above as a blank. The total amount of drug that was released was calculated and shown against time **(Basak et al. 2013; Essal et al. 2010).**

1.5 Development of Efinaconazole Niosomal Nail lacquer (ENNL)

By following the recipe, the formula was put together. The mixture has a film-forming substance called nitrocellulose, a keratolytic agent called salicylic acid, a permeabilityenhancer called 2-H-β-CD, an antifungal agent called Efinaconazole Niosomes, and a solvent called ethanol. To make formulations, basic mixing methods are used.Several amounts of nitrocellulose, a polymer that forms films, were used to make films and improve them. Researchers looked at a range of concentrations from 2% to 8%. By increasing the polymer concentration, the film's thickness and strength were found to improve by up to 6%. When the concentration went up to more than 6%, sticky films were made. A concentration of 6% polymer was used to improve the plasticizer even

more. Plasticizers that had 10% propylene glycol in them were tested. Propylene glycol was very flexible and good at making a thin film. Because they make better films, 6% nitrocellulose and 12% propylene glycol were chosen for more testing to improve the formula.

Table 2: Formulation of Efinaconazole Niosomal Nail lacquer

Ingredients (%)	**ENNL -1**	**ENNL -2**	**ENNL -3**	**ENNL -4**	**ENNL -5**	**ENNL -6**
Efinaconazole Niosomes	10	10	10	10	**10**	10
Nitrocellulose	4	4	5	5	**6**	6
Salicylic acid	-	4	8	16	**20**	20
2-HPβ-CD	-	-	6	8	**10**	12
Ethyl cellulose				0.3	**0.6**	0.9
Propylene glycol	12	12	12	12	**12**	12
Ethanol q.s	100	100	100	100	**100**	100

1.6 Evaluation of ENNL

1.6.1 Thickness (µm)

A micrometer screw gauge was used to measure the thickness of each film.

1.6.2 Folding endurance

The polymer film shows how flexible it is by how long it can be folded. The made films were put through endurance folding tests to see how flexible they were. The number of folds a film can take without breaking is a measure of its folding endurance.

1.6.3 Water resistance

This number shows how well the film stops water from getting through. Three glass plates should be given a thin coating, left to dry, and then weighed. The plates must then be put in a water bath with distilled water heated to 370°C for 24 hours. The panels should then be taken out, dried by putting them between two sheets of absorbent material, and weighed again. Gain in weight is taken into account. Water resistance goes down as weight goes up.

1.6.4 Non-volatile content

In a glass Petri dish with a diameter of 8 cm, a sample was taken. Tarred wire was used to spread the samples out evenly. The dish was taken out of the oven after an hour at 105°C, let cool, and then weighed. After the sample was dried, the difference in weight was calculated.

1.6.5 Drying time

A sample film was put on a glass petri plate with a brush. We used a timer to keep track of how long it took for the film to become dry to the touch.

1.6.6 Smoothness

A piece of nail polish was put on a glass plate, and the plate was then lifted up to see how smooth it was.

1.6.7 Gloss

The film's sheen was evident and fulfilled specifications. Onscreen, the film's individuality could be detected.

1.6.8 Viscosity

The thickness of the nail polish was measured with a Brookfield viscometer13. So that the solvent doesn't evaporate, samples are put in a jar that can't be opened. Put the spindle into the sample up to the line that was scored while the motor is running at 60RPM and shaking hard at 25°C. After reading the instrument for ten minutes, change the speed control to 6 RPM, then read it again after another ten minutes. By multiplying the reading on the dial by the following: 60rpm X 20; 6rpm X 200 to produce centipoises. At 25 °C and 60 rpm, the relationship between viscosity and thixotropy for nails should be between 375 and 500 cps.

1.7 Drug Content Estimation

At 7.4 degrees Celsius, 50 cc of phosphate buffer solution with a pH of 7.4 was used to dissolve 100 mg of nail polish. After 15 seconds, ultrasonic waves were used to treat the solution. At the end, there was 100 ml of filtered phosphate buffer solution with a pH of 7.4. Mix 10 ml of the solution we just talked about with 100 ml of PBS with a pH of 7.4 to make a total of 100 ml. Then, using the diluted solution and spectrophotometry at a wavelength of 230 nm, the drug concentration was found.

1.8 In vitro drug diffusion study

The thing is a glass tube with holes at both ends. One end of the cylinder has a dialysis membrane stuck to it. This membrane has been soaked in distilled water for 24 hours before it is used. The cell is put in a small beaker with 100 ml of PBS pH 7.4 and 10% v/v methanol (to keep the sink condition) and then a gel with 10 mg of efinaconazole is added (donor compartment). Set up so that the bottom of the gel with the cells is only 1 to 2 mm below the surface of the diffusion medium. Use a magnetic stirrer to move the medium at 37 ± 0.5 degrees Celsius. Aliquots of 5 ml are taken out of the receptor compartment and replaced with new

buffers of the same volume at regular intervals. The samples are looked at with a UV visible spectrophotometer set to 230 nm. In all, there are three tests.

1.9 Antifungal Activity

In Vitro Studies by Agar diffusion methods

Using the cup plate method, the in vitro activity of different doses of Efinaconazole niosomal Nail Lacquer (ELNNL) was tested. The medium was made by mixing 1 litre of distilled water with 10 grammes of peptone, 20 grammes of agar, and 40 grammes of dextrose powder. Then, to make sure it was clean, it was put in an autoclave for 20 minutes at 121 degrees Celsius. The dishes were cleaned in an oven with hot air at 160°C for an hour. After the Candida albicans culture was put on the plate, 20 ml of sterile Sabourauds dextrose agar was added. By carefully shaking the plate, the test organism and the Sabourauds dextrose agar were spread out evenly. After letting the plates dry for a while, three 6-mm-wide cups were drilled into the media. The standard was the product that was sold (Daktarin® Gel 2% w/w). Poured into a cup were the standard, pure Efinaconazole and the tested, optimized formulations (ELNNL). The plate was kept at 25 °C for 48 hours. After 48 hours, the zones of inhibition were measured in millimeters for the test, reference, and pure Efinaconazole medicines **(Kanaani et al., 2017).**

1.10 In Vivo Studies

1.10.1 Animal used for in vivo antifungal activity

In this study, 150–200 g male Wistar rats that were raised locally and were 12–14 weeks old were used. The rats were kept in separate cages made of propylene at room temperature (27 ± 2 degrees Celsius). Animals were given pellets to eat and could drink as much water as they wanted.

1.10.2 In vivo antifungal assay

The treatment groups for the animals were made up of control, placebo, and treatment groups. Before abrading each animal, the back hair was shaved and the area most likely to get infected was cleaned with a cotton swab soaked in 70% ethyl alcohol. The area most likely to get infected was then abraded. A 23–25 mm square on the back of each animal was washed and shaved with a pathological scalpel. The T. rubrum cultures that were 7 days old and floating in sterile Tryptic soy broth were used to start the fungus. With Whatman No. 1 filter paper, the leftover pieces of agar and hyphae are taken out. After the animal was depilated, 0.2 ml of inoculum was put on the area that had been depilated and left for eight days. The concentration of the final suspension was then changed so that there were 1 x 107 conidia per milliliter. On day four, pathogens were found in skin scales from infected areas that had grown on SDA plates with antibiotics

(penicillin and streptomycin). This showed that the infection was still going on. When the animals were looked at on days 4 through 8, it was clear that they had infections. Animals with a confirmed active infection were treated starting on day 8 after infection and going until day 25 after infection. Even though different kinds of finaconazole niosomal nail polish were applied topically to each group, the skin was checked every day. As a standard, 1% tioconazole cream was used to measure how well Efinaconazole niosomal Nail Lacquer worked as a medicine. The medicines were given every day, and the infected areas were looked at to see if they had edoema or scaling. For a clinical exam of the diseased skin area, the lesion ratings from Table 3 were used.

Table 3: Description of lesion scores for clinical assessment of animals

Score	Description
0	Novisiblelesion
1	Fewerythematouslesions
2	Welldefinedvesicles
3	Largeareas ofmarkedredness,incrustation,scaling
4	Mycoticfociwelldeveloped inaddition toscore3lesions

1.10.3 Histopathology of the skin of animal post-treatment

The skin of an animal after it has been treated Periodic acid schiff staining (PAS), a method used in histopathology to look for fungal hyphae, was used to test how well the cream worked as a treatment. Hematoxylin and eosin (H&E) staining was also done to see how the skin's dermis and epidermis were changing over time **(Mahtab et al., 2016; Li et al., 2022).**

2 Result and Discussion

2.1 Evaluation of Efinaconazole niosomes

Morphology

Morphology is important because it affects how we understand shape, size, and structure. It gives you a general idea of how the surface of the generated formulation looks. Surfactants like Span 60 and Pluronic L121 were used to put efinaconazole into niosomes. All of the different kinds of niosomes had the same amounts of Span 60, cholesterol, and medicine. To find the best formulation composition, the concentrations of Pluronic L121 and DCP were changed. Three possible values for these two variables were looked at in the core composite design. Transmission electron microscopy lets you see complex structures that are the right size and shape (TEM). Variable magnification SEM pictures of the EFN2 formulation of niosomes loaded with efinaconazole. SEM micrographs of multiple Efinaconazole-loaded Niosome formulations (EFN2) and one EFN2 were taken at a magnification of 5000X. The SEM picture of the new

formula showed that the vesicles were almost round and all the same size (Figure 1 and Figure 2).

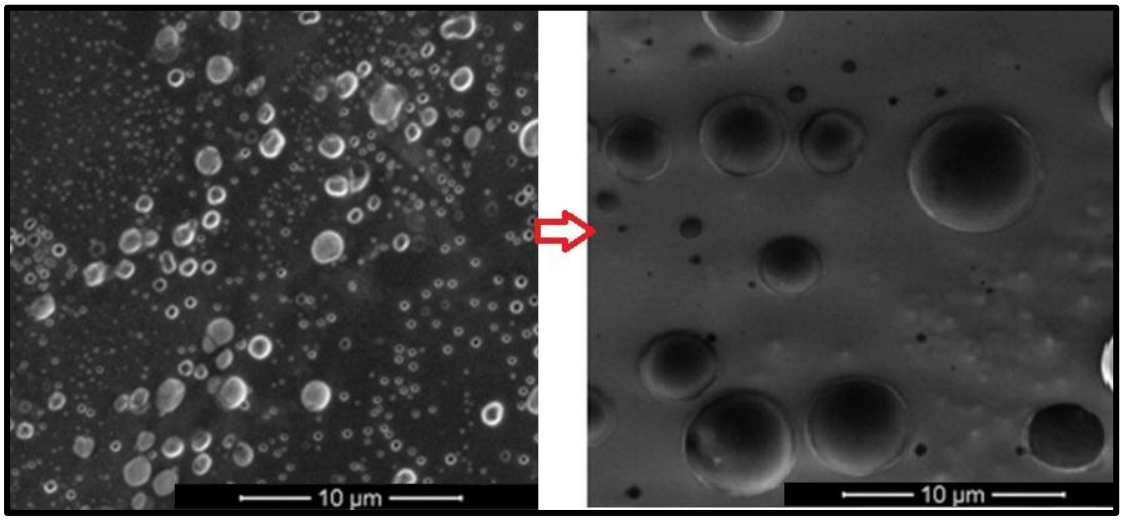


Figure 1: SEM of Efinaconazole niosomes

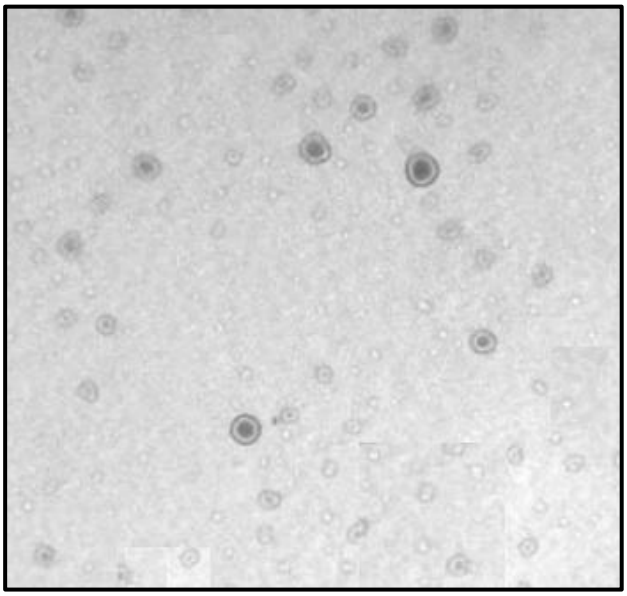


Figure 2: TEM of Efinaconazole niosomes

2.2 Size and zeta potential measurements

These two variables are very important for stability and give a quick look at how the particles in a formulation are spread out. Stability is strongly affected by zeta potential. Previous research has shown that formulations without cholesterol look like gels, but formulations with cholesterol have Niosome structures that are rigid, stable, and whole, and their PDI values are low. The best entrapment efficiency is with Span 60, and vesicles can't form when the HLB value is more than 11. Pluronic L121 effectively binds the drugs that don't dissolve well in water. This lowers the

PDI and makes the vesicular niosomes more stable. The membrane additive DCP is a negative agent that helps make Monodispersed niosomes with low PDI values by reducing their tendency to stick together because of strong electric repulsion between particles. Figure 3 shows that the zeta potential of Span 80, 60-derived niosomes was more than -30 mV, which is a sign that the systems are stable.

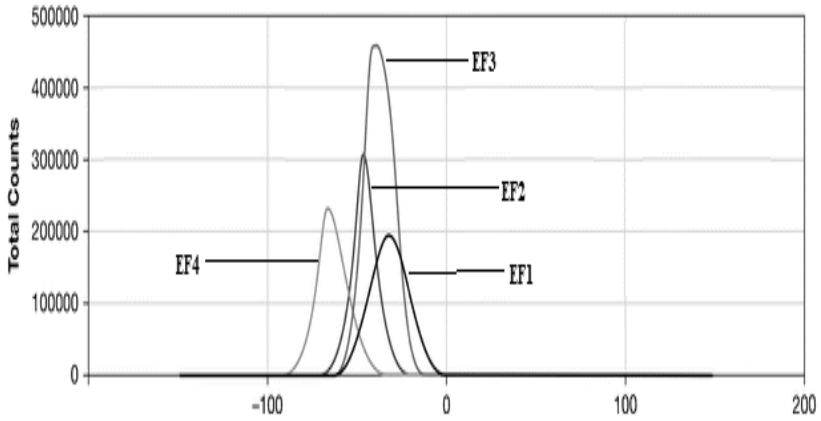


Figure 3: Zeta potential of efinaconazole niosomes

2.3 Fourier-Transform Infrared Spectroscopy (FT-IR)

In Figure 4, the FT-IR data for the different parts of the niosomal formulation are shown. It was found that the best drug-free niosomal formulation has most of the peaks of its components, such as span 60 and cholesterol. The FTIR spectra of efinaconazole showed a significant band of functional groups, such as the carbonylic C=O stretch of the ester at 1730 and 1164 cm-1, the O-H stretching at 3552 and 3749 cm-1, the C-H stretching at 3010 and 2871 cm-1, and the O-H stretching at 3552 and 1467 cm^{-1} (C-C stretching). C=O stretching reached its peak at 2939 cm^{-1}, C-H stretching reached its peak at 1717 cm^{-1}, CH2 bending reached its peak at 1377 cm-1, C-C stretching reached its peak at 1506 cm-1, and C=C stretching reached its peak at 1674 cm^{-1}. The C-O peak happened at 1172 cm-1, the C-H peak happened at 2928 cm^{-1}, the C=O peak happened at 1730 cm-1, and the 1460 cm^{-1} peak happened at 1460 cm^{-1} (C-C stretching). But the FT-IR spectra of niosomes show that the C=C stretching peaks at 1674 cm^{-1} have disappeared. This supports the idea that cholesterol molecules get stuck in the lipid bilayer shell when niosomes form. The successful encapsulation of the drug by niosomes is shown by the fact that the main peaks of efinaconazole disappear in the final, optimum niosomalformulation product.

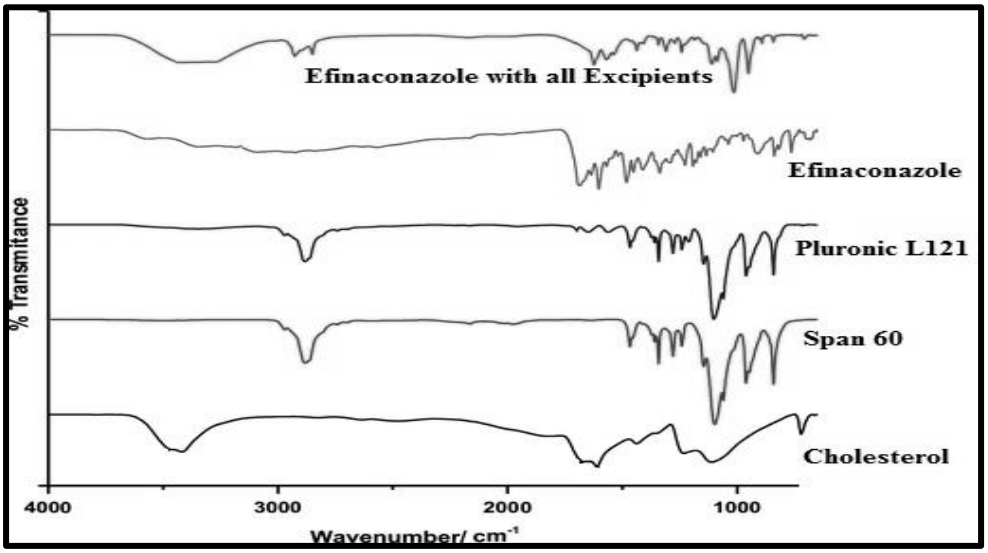


Figure 4: FT-IR spectra of efinaconazole niosomes and their Excipients

2.4 Entrapment Efficiency

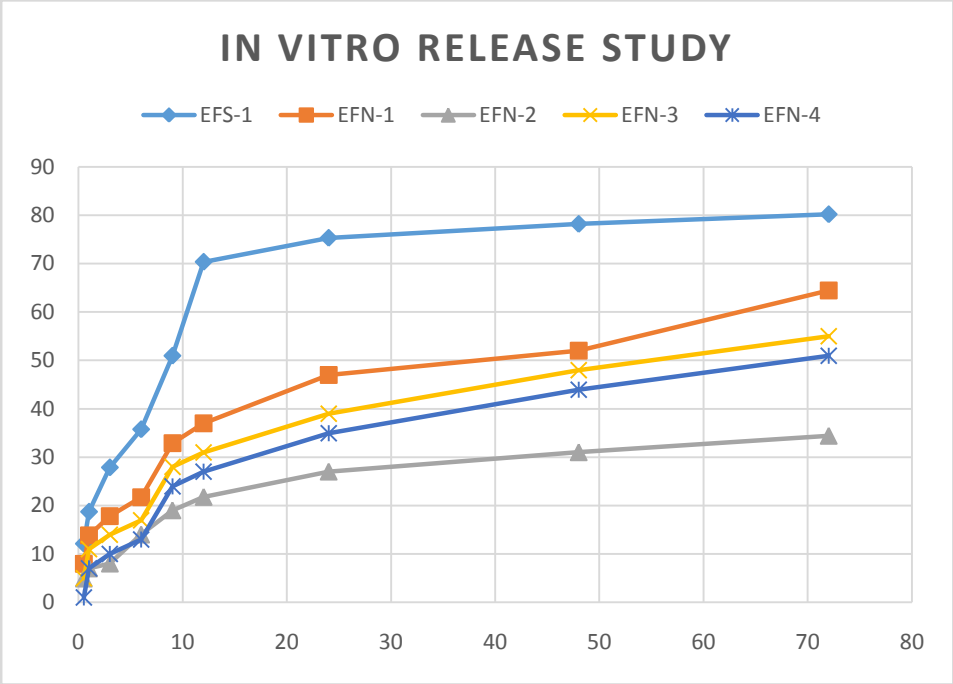
Often, this parameter is used to figure out how much of the drug is in the formulation. Entrapment efficiency, which can also be used to figure out how often a formulation needs to be given as a dose, is a key part of drug delivery. So, increasing the amount of drug in the formulation could reduce the number of times the therapeutic portion needs to be taken.

Table 4: Evaluation parameters of Efinaconazole Niosomes

Formulation	Percentage Yield	Entrapment Efficiency	Particle size (nm)	Zeta potential (mV)
EFN1	25.77±0.17	45.25±0.87	130.13	-5.7
EFN 2	**84.22±1.12**	**89.21±0.67**	**97.21**	**-4.4**
EFN 3	71.82±0.74	71.55±0.82	113.82	-6.2
EFN 4	68.14±0.53	86.23±0.85	124.21	-5.1

2.5 In vitro release study

An in-vitro drug release study is a type of quantitative analysis that can be used to figure out how much medicine is released from the formulation at different times. With the help of in vitro drug release experiments, the Cmax, Tmax, and AUC of the drug in the systemic circulation are given. In simulated intestinal fluid, the rate at which efinaconazole was released from Niosome preparations was much slower than in free solution. After 3 hours, the most amount of drug is delivered, and it stays at that level for the next 5 hours. In the Niosome system, only 21% of the efinaconazole was released in 12 hours, while about 70% was in free solution. At the end of the experiment, 80% of the simulated stomach fluid was released into free solution, while only 35% was released from the Niosome system **(Chouhan et al., 2021; Thatai et al., 2018).**



Graph 1: In-vitro release study of efinaconazole niosomes along with efinaconazole solution (Mean ± SD, n=3)

2.6 Evaluation of Niosomal Nail lacquer (ENNL)

2.6.1 Thickness (µm)

Consistent thickness reflects the homogeneity of the formulations and, by extension, the acceptability of the technique being carried out. Findings revealed that the thickness of each formulation varied between 50 and 65 µm.

2.6.2 Folding endurance

Folding endurance is a very important test that shows how flexible a film is. The more numbers there are for the number of times the film can be folded, the more flexible it will be. The 6% film **(ENNL-5)** was hard to fold, which made sure it was flexible.

2.6.3 Water resistance

When weight grows, water resistance lowers. In this case, the 6% Nitrocellulose Film **(ENNL-5)** is rather lightweight and gives increased water resistance.

2.6.4 Non-volatile content:

After all the volatile substances had evaporated, a thin layer of non-volatile substances was found. Between 25% and 36% is the range. As the polymer concentration went up, so did the amount of non-volatile material, and vice versa. The amount of nonvolatile material depends on the amount of polymer and changes as it does.

2.6.5 Drying time:

The drying time of nail polish was determined to be between 60 and 70 percent. The drying time increased as polymer concentration increased.

2.6.6 Smoothness:

ENNL1–ENNL4 were smooth enough because they didn't have too much polymer, but ENNL5 and ENNL6 are very smooth because they have more polymer.

2.6.7 Gloss:

The film's uniqueness could be seen on the screen. When compared to ENNL5 and ENNL6, the gloss of ENNL1–ENNL4 was better. The formulation with a higher polymer concentration had a duller sheen than those with a lower polymer concentration.

2.6.8 Viscosity:

Viscosity measurements are done as per the procedure mentioned in the methodology and results are given in the above Table 4. Viscosity is an important parameter for characterizing the nail lacquer as it effects the spreadability and release of drug. All formulation showed increased viscosity ranging from 240-320 cps. As the polymer concentration increased viscosity also increased and vice-versa

Table 4: Evaluation of Efinaconazole Niosomal Nail lacquer

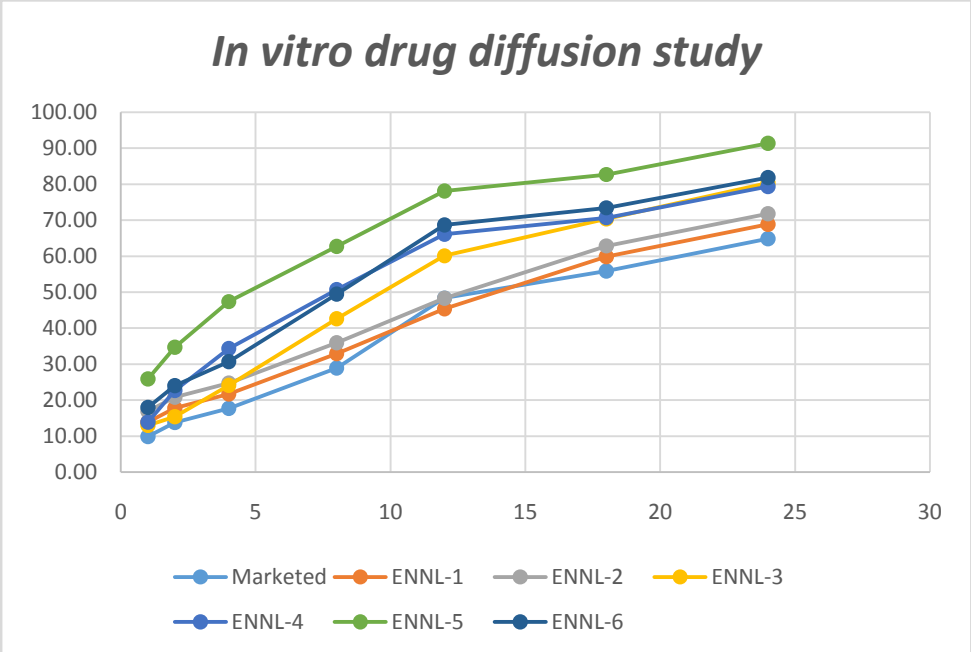
Ingredients (%)	**ENNL-1**	**ENNL-2**	**ENNL-3**	**ENNL-4**	**ENNL-5**	**ENNL-6**
Thickness (µm)	57±0.01	58±0.02	60±0.02	59±0.02	55±0.04	56±0.02
Folding endurance	153	158	164	162	174	169
Water resistance	0.07	0.06	0.05	0.06	0.03	0.04
% Non-volatile content ±SD	26%±0.32	28%±0.54	26%±0.13	27%±0.54	33%±0.83	32%±0.52
Drying time (Sec) ±SD	62±0.25	65±0.20	60%±0.30	64%±0.10	68%±0.20	67%±0.15
Smoothness	++	++	++	++	+++	+++
Gloss	+++	+++	+++	+++	++	++
Viscosity (CPS) ±SD	240.6 ±0.3	255.3±0.4	278.6 ±0.4	284.6 ±0.3	319.2 ±0.5	321.2 ±0.1

2.7 Drug Content Estimation

As shown in Table 3, the acceptable range for the percentage of drug content in all of the lacquers was between 92% and 97%. The highest percent of drug content was determined to be 96.28% **(ENNL-5)** and the lowest was 93.01% **(ENNL-2).** Drug content more than 90% in the formulation indicates a large quantity of drug present in the formulation, guaranteeing that formulation processes and chosen components do not impair drug stability. A high medication concentration also provides confidence that a positive therapeutic effect may be anticipated.

2.8 In vitro drug diffusion study

The creation of Niosomal Nail Lacquer was witnessed. The maximum release of efinaconazole niosomal formulations **ENNL-5** was found to be 91.34 percent after 24 hours, whereas the minimum release of formulations **ENNL-2** was assessed to be 71.44 percent. During in vitro drug release experiments, it was determined that both formulations exhibited release kinetics of the first order. Graph 2 depicts the drug release from each of the five formulations **(Akhtar et al., 2016; Kataria et al., 2016).**



Graph 2: Comparison of %release of Marketed drugs and In-House Niosomal Nail lacquer

2.9 Antifungal Activity

2.9.1 Agar diffusion methods

According to the table below, Efinaconazole niosomal Nail Lacquer was effective against fungus when tested on a number of dermatophytes species (ENNL).

Table 5: Antifungalactivity of Efinaconazole Niosomal Nail lacquer

Antifungalactivities (Zone of inhibition)		
Samples	**Concentration in (µl/ml)**	**Zone of inhibition in mm**
Daktarin® Gel 2%	**100**	**25**
Efinaconazole API	100	17
ENNL (Placebo)	100	20
ENNL	50	20
ENNL	**100**	**21**

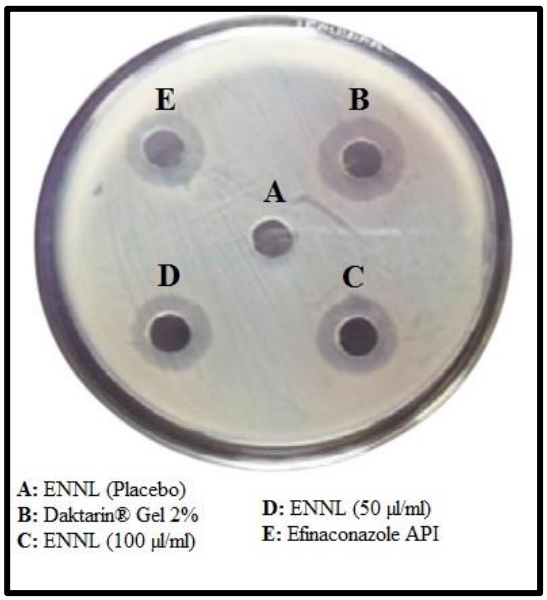


Figure 5: Antifungalactivity of Efinaconazole Niosomal Nail lacquer

2.9.2 In Vivo Studies of Antifungal formulation

In the table below, you can see clinical evaluations of infected rats before and after they were given a cream. The results showed that infection had already started to happen before the cream was used (Figure, plates A–C) **(Tabata et al., 2016; Valdes et al., 2017).** After 16 days of using the specially made cream (16 days),

I= 1% Tioconazole cream

II= No treatment (Control Group)

III= Placebo ENNL

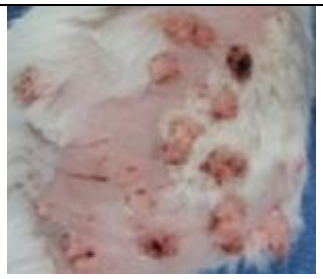
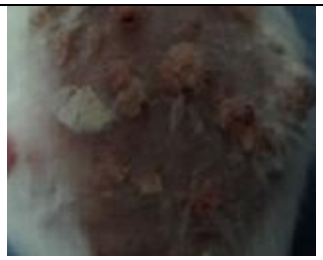
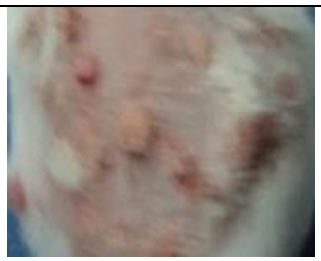
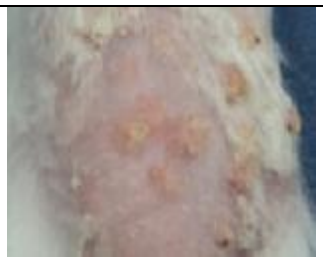
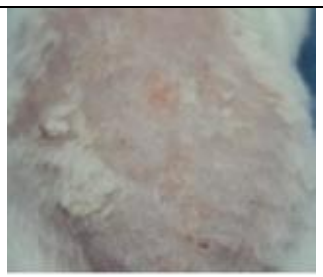
IV= ENNL

Table 6: The clinical assessments of infected rats before and after applying treatment in vivo

AnimalGroup /Treatment Day	**I**	**II**	**III**	**IV**
Infected days				
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	1±025	1±0.25	0	1±0.50
5	2±0.20	2±0.20	1±0.50	2±0.40
6	2±0.25	2±0.50	2±0.20	3±0.25
7	3±0.20	3±0.20	2±0.25	3±0.20
8	3±0.25	3±0.25	3±0.20	3±0.02
Treatment Days				
9 (PTD1)	3±0.25	3±0.25	3±0.25	3±0.25
10 (PTD2)	3±0.25	3±0.25	3±0.25	3±0.20
11 (PTD3)	3±0.25	3±0.25	3±0.25	3±0.20
12 (PTD4)	3±0.25	2±0.25	3±0.25	2±0.25
13 (PTD4)	0	3±0.50	3±0.25	2±0.25
14 (PTD5)	0	3±0.25	3±0.25	1±0.25
15 (PTD6)	0	3±0.25	3±0.20	1±0.25
16 (PTD7)	0	3±0.20	3±0.22	1±0.25
17 (PTD8)	0	3±0.02	2±0.25	0
18(PTD9)	0	3±0.02	2±0.20	0
19 (PTD10)	0	2±0.02	2±0.20	0
20 (PTD11)	0	2±0.02	2±0.22	0
21 (PTD12)	0	2±0.02	2±0.22	0
22 (PTD13)	0	2±0.00	2±0.20	0
23 (PTD14)	0	2±0.00	2±0.20	0
24 (PTD15)	0	2±0.00	2±0.20	0
25 (PTD16)	0	2±0.00	2±0.20	0

* = Average result of experimental animals in each group; PTD= Post treatment day;

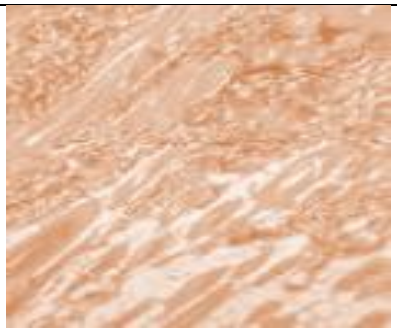
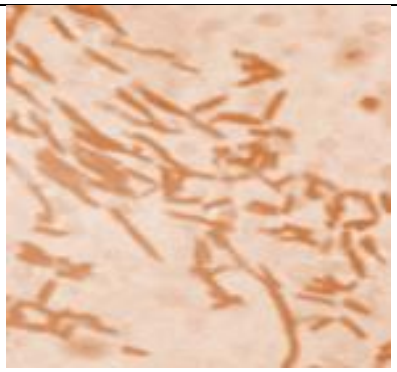
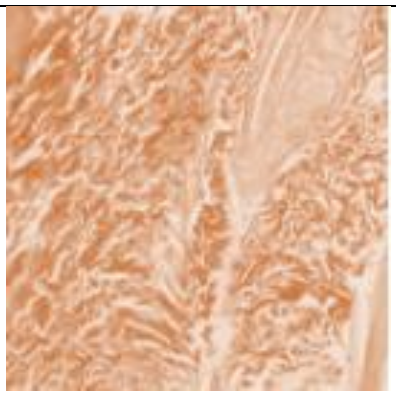
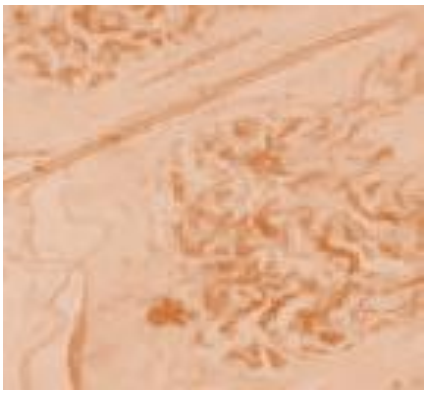
Table 7: Steps in animals infected with T. rubrum and treated with formulations.

STEPS	ENNL
Skin of a freshly-shaved animal displaying the area of abrasion prior to infection	
On day 4 after infection, depilated skin displaying infection development	
On day 7, before to starting therapy, depilated regions of infected skin foci showed the development of visible vesicles and the creation of rashes.	
On day 13 (four days after therapy with the antifungal formulation), the depilated portions of the diseased skin began to heal along with the elimination of erythema, vesicles, and rashes.	
On day 15 (six days after therapy), recovered depilated skin foci on infected skin foci seemed to have fur.	

2.10 Histopathology of the skin of animal post-treatment of the ENNL

Histopathology was used to figure out how well Efinaconazole Niosomal Nail Lacquer (ENNL) worked as a treatment by using periodic acid Schiff staining to find fungal hyphae (PAS). The PAS results showed that the hair follicle, shaft, and epidermis were all growing back, and there were no fungal hyphae (see figure). However, there was no sign of a fungal infection (plates A-D).

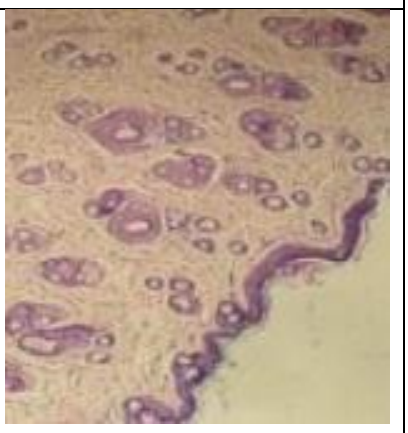
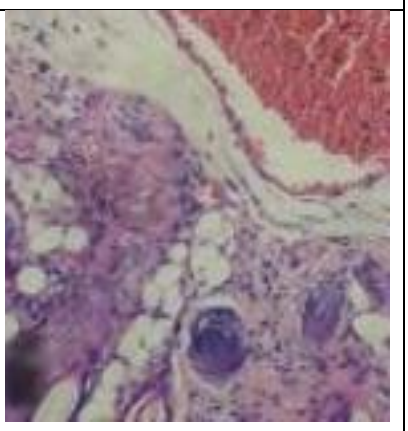
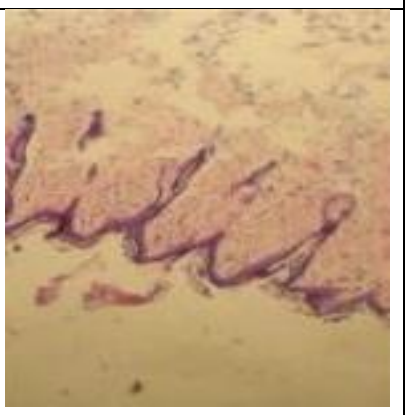
Table 8: Histopathology PAS staining of the skin sections of before and after treatment with ENNL

Histopathology	**ENNL**
Normal-appearing skin with a prominent epidermis, subcutaneous fat deposit, and hair follicles.	
Periodic acid staining (PAS) results showed that albino rat skin had fungal hyphae on it prior to treatment.	
A Periodic Acid result Schiff reported that on the fourth day of using the prescribed cream, the hyphae had cleared, the hair follicle and shaft had begun to regenerate, the epidermis had recovered, and the epidermis' cells had begun to inflate.	
Upon application of the cream's formulation, the Periodic Acid Schiff (PAS) test results show that the hair follicle, shaft, and epidermis have all recovered. Fungal hyphae have also completely vanished.	

Hematoxylin and eosin staining (H&E) was also done to track how the skin's dermis and epidermis were changing over time. The H&E staining showed that the skin was thin, that the

sub-sebaceous layer was well-developed, and that a hair follicle and shaft had formed **(Ghannoum et al., 2016; Mensing et al., 1992).**

Table 9: Histopathology H&E staining of the skin sections of before and after treatment with ENNL

Histopathology	**ENNL**
Normal-appearing skin with a prominent epidermis, subcutaneous fat deposit, and hair follicles.	
Hematoxylin and Eosin (H & E) displaying results Congested subcutaneous blood vesicles, epidermal atrophy, exocytosis, perivascular dermatitis, and the formation of dark spots infiltrated with cells before therapy are all symptoms of Peri-glandular necrosis and cellular infiltration.	
The growth of the hair follicle and shaft after application of the cream's formulation is shown by Hematoxylin and Eosin staining (H & E), which also reveals the skin's thin epidermis and well-developed sub-sebaceous layer.	

3 Conclusion

In this study, efinaconazole-loaded niosomes were made by sonicating probes with cholesterol and different amounts of non-ionic surfactants (Span 60 and Pluronic L121). Optimized nanoemulsion (EFN2) was used to make Efinaconazole Niosomal nail lacquer (ENNL-5) with the best viscosity, spreadability, thickness, smoothness, and drying time compared to nail lacquer for topical use. In studies of in vitro release and ex vivo penetration, the enhanced Niosomal nail polish (ENNL-5) worked better than those on the market. The increased in vitro antifungal activity of the improved Niosomal Nail Lacquer (ENNL-5) shows that it may be a promising way to treat onychomycosis more quickly than with traditional treatments while also reducing its symptoms. So, Niosomal Nail Lacquer is a safe, effective, and efficient way to treat onychomycosis on the skin.

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Conflicts of interest

No conflicts of interest are declared by the authors.

Authors' contribution

Vibhavari M. Chatur & Shashikant N. Dhole were involved in the sample selection, the planning and execution of lab research, the interpretation of data, and the writing of the report. Shashikant N. Dhole's efforts include data analysis and chemical identification. The final document was interpreted and approved by each author.

4 References:

1. Jo Siu WJ, Tatsumi Y, Senda H, et al. (2013) Comparison of in vitro antifungal activities of efinaconazole and currently available antifungal agents against a variety of pathogenic fungi associated with onychomycosis. Antimicrob Agents Chemother, 57(4):1610–1616.
2. Ogura H, Kobayashi H, Nagai K, et al. (199) Synthesis and antifungal activities of (2R, 3R)-2-aryl-1-azolyl-3-(substituted amino)-2-butanol derivatives as topical antifungal agents. Chem Pharm Bull; 47(10):1417–1425.
3. Piraccini BM, Sisti A, Tosti A. (2010) Long-term follow-up of toenail onychomycosis caused by dermatophytes after successful treatment with systemic antifungal agents. J Am Acad Dermatol, 62(3):411–414.

4. Scher RK, Baran R. (2003) Onychomycosis in clinical practice: factors contributing to recurrence. Br J Dermatol, 149(Suppl 65):5–9.
5. Ghannoum MA, Hajjeh RA, Scher R, et al. (2000) A large-scale North American study of fungal isolates from nails: the frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. J Am Acad Dermatol, 43(4):641–648.
6. Kanaani L, Tabrizi MM, Khiyavi AA, Javadi I. (2017) Improving the efficacy of cisplatin using Niosome nanoparticles against human breast cancer cell line BT-20: An in vitro study. Asian Pacific Journal of Cancer Biology, 2(2):27-9.
7. Amiri B, Ahmadvand H, Farhadi A, Najmafshar A, Chiani M, Norouzian D. (2018) Delivery of vinblastine-containing niosomes results in potent in vitro/in vivo cytotoxicity on tumor cells. Drug development and industrial pharmacy; 44(8):1371-6.
8. Alemi A, Zavar Reza J, Haghiralsadat F, Zarei Jaliani H, Haghi Karamallah M, Hosseini SA, Haghi Karamallah S. (2018) Paclitaxel and curcumin coadministration in novel cationic PEGylated niosomal formulations exhibit enhanced synergistic antitumor efficacy. Journal of Nanobiotechnology, 16(1):1-20.
9. Sayed E, Karavasili C, Ruparelia K, Haj-Ahmad R, Charalambopoulou G, Steriotis T, Giasafaki D, Cox P, Singh N, Giassafaki LP, Mpenekou A. (2018) Electrosprayed mesoporous particles for improved aqueous solubility of a poorly water soluble anticancer agent: in vitro and ex vivo evaluation. Journal of Controlled Release, 278:142-55.
10. Sharma V, Anandhakumar S, Sasidharan M. (2015) Self-degrading niosomes for encapsulation of hydrophilic and hydrophobic drugs: an efficient carrier for cancer multi-drug delivery. Materials Science and Engineering: C, 56:393-400)
11. Khan DH, Bashir S, Khan MI, Figueiredo P, Santos HA, Peltonen L. (2020) Formulation optimization and in vitro characterization of rifampicin and ceftriaxone dual drug loaded niosomes with high energy probe sonication technique. Journal of Drug Delivery Science and Technology, 58:101763
12. Khan MI, Madni A, Hirvonen J, Peltonen L. (2017) Ultrasonic processing technique as a green preparation approach for diacerein-loaded niosomes. AAPS PharmSciTech, 18(5):1554-63.
13. Ravalika V, Sailaja AK. (2017) Formulation and evaluation of Etoricoxib niosomes by thin film hydration technique and ether injection method. Nano Biomed Eng ;9(3):242-8

14. Basak R, Bandyopadhyay R. (2013) Encapsulation of hydrophobic drugs in Pluronic F127 micelles: effects of drug hydrophobicity, solution temperature, and pH. Langmuir; 29(13):4350-6.
15. Essa EA. (2010) Effect of formulation and processing variables on the particle size of sorbitan monopalmitate niosomes. Asian Journal of Pharmaceutics (AJP) ;4(4)
16. Kanaani L, Tabrizi MM, Khiyavi AA, Javadi I. (2017) Improving the efficacy of cisplatin using Niosome nanoparticles against human breast cancer cell line BT-20: An in vitro study. Asian Pacific Journal of Cancer Biology; 2(2):27-9.
17. Mahtab A, Anwar M, Mallick N, Naz Z, Jain GK, Ahmad FJ. (2016) Transungual delivery of ketoconazole nanoemulgel for the effective management of onychomycosis. AAPS PharmSciTech, 1477-90.
18. Li D, Martini N, Wu Z, Chen S, Falconer JR, Locke M, Zhang Z, Wen J. (2022) Niosomal nanocarrier for enhanced dermal delivery of epigallocatechin gallate for protection against oxidative stress of the skin. Pharmaceutics; 14(4):726.
19. Chouhan SS, Farooqui N, Mishra DK. (2021) Formulation and evaluation of liposomal loaded nail lacquer containing Luliconazole an antifungal drug. Asian Journal of Pharmacy and Pharmacology, 7(3):131-7.
20. Thatai P, Sapra B. (2018) Terbinafine hydrochloride nail lacquer for the management of onychomycosis: Formulation, characterization and in vitro evaluation. Therapeutic Delivery, 9(2):99-119.
21. Akhtar N, Sharma H, and Pathak K. (2016) Onychomycosis: potential of nail lacquers in Transungual delivery of antifungals. Scientifica.
22. Kataria P, Sharma G, Thakur K, Bansal V, Dogra S, Katare OP. (2016) Emergence of nail lacquers as potential Transungual delivery system in the management of onychomycosis. Expert Opinion on Drug Delivery; 13(7):937-52.
23. Tabata Y, Takei-Masuda N, Kubota N, Takahata S, Ohyama M, Kaneda K, Iida M, Maebashi K. (2016) Characterization of antifungal activity and nail penetration of ME1111, a new antifungal agent for topical treatment of onychomycosis. Antimicrobial Agents and Chemotherapy, 60(2):1035-9.
24. Valdes BS, Serro AP, Gordo PM, Silva A, Gonçalves L, Salgado A, Marto J, Baltazar D, Dos Santos RG, Bordado JM, Ribeiro HM. (2017) New polyurethane nail lacquers for the delivery of Terbinafine: formulation and antifungal activity evaluation. Journal of Pharmaceutical Sciences; 106(6):1570-7.

25. Ghannoum M, Sevin K, Sarkany M. (2016) Amorolfine 5% nail lacquer exhibits potent antifungal activity compared to three acid-based devices indicated for the treatment of onychomycosis: an in vitro nail penetration assay. Dermatology and therapy; 6:69-75.
26. Mensing H, Polak- Wyss A, Splanemann V. (1992) Determination of the subungual antifungal activity of amorolfine after 1 month's treatment in patients with onychomycosis: comparison of two nail lacquer formulations. Clinical and Experimental Dermatology; 17(s1):29-32.