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Formulation and Evaluation of Medicated Tolnaftate Nail Lacquer

Farsana. P [α], Baby Shahanas [σ], Anu Sebastian [ρ] & Ashly Merin George [ω]

Abstract- The present study was aimed towards the design and formulation of medicated nail lacquer of tolnaftate to control onychomycosis condition and improve the patient compliance. The present work investigated the amount of tolnaftate released from different formulations containing different concentration of ethyl cellulose and different proportions of thioglycolic acid and dimethyl sulfoxides for treatment of onychomycosis. Then these lacquers were compared for drying time, nonvolatile content, drug content, drug diffusion and antimicrobial studies. The stability test showed that the formulation were stable at 37° ± 2°C for 1 month. The results obtained from in-vitro diffusion studies showed that formulation F3 have completed drug release of 94.48% over 24 hrs. The F3 formulation had salicylic acid as keratolytic agent and 0.5ml of 1% w/v of thioglycolic acid as penetration enhancer. From diffusion studies, it was concluded that thioglycolic acid containing formulation (F2 and F3) have better penetration enhancement as compared to DMSO containing formulation. The best formulation was evaluated for antifungal sensitivity test against the *Candida albicans*. From the above study, it can be concluded that medicated nail lacquers proved to be a better tool .In this work, the main goal is to develop medicated nail lacquer, for maximum drug release for treating onychomycosis and achieve better patient compliance.

Keywords: medicated nail lacquer, keratolytic agent penetration enhancer, DMSO, onychomycosis.

I. Introduction

The major constrains of the preungual drug delivery (drug delivery through the nail) to nail is lack of understanding about barrier property related to the nail formulations. Topical drug delivery system owes many advantages in case of antifungal drugs such as it avoids hepatotoxicity, high tissue concentration which is required for the treatment of fungal infection of nails. Most of topical formulations in form of gels, lotions etc. pose limitations such as removal by whipping, rubbing and less adherence of formulation to the affected site of nail[1]. Medicated nail lacquer is an excellent alternative for the treatment of fungal infection of nails and high efficacy of drug can be achieved. It also provides an optimized and sustained release of drug by formation of an occlusive film which acts as "depot" after the application of lacquer on the nail.[2]

The advantages of nail lacquer include it cannot be easily removed by rubbing, washing etc the effect is long lasting, depot formation. Factors affecting drug delivery include molecular size of compound or diffusing species, degree of ionization, binding of the drug to keratin and other nail constituents, nail thickness and presence of disease. Nail lacquers containing drug are fairly new formulations and have been termed transungual delivery systems. These formulations are essentially organic solutions of a film-forming polymer and contain the drug to be delivered. When applied to the nail plate, the solvent evaporates leaving a polymer film (containing drug) onto the nail plate[3]. The drug is then slowly released from the film, penetrates into the nail plate and the nail bed. The drug concentration in the film is much higher than concentration in the original nail lacquer as the solvent evaporates and a film is formed.

Here tolnaftate is used as a drug which is a synthetic antifungal agent comes under thiocarbamate derivative with antimicrobial and antifungal activity. Salicylic acid used in the formulation as a keratolytic agent and DMSO as penetration enhancer. In this work, tolnaftate loaded nail lacquer was formulated and produce a drug release over 24 hours.

II. Materials and Methods

a) Materials

Tolnaftate was purchased from Yarrow Chem Products. Ethyl Cellulose and DMSO were purchased from Nice Chemicals, Cochin. Salicylic acid, Sodium hydroxide, Potassium Di-hydrogen Phosphate were purchased from Spectrum Reagents and Chemicals Pvt. Ltd, Cochin. Glycerin was obtained from Isochem Laboratory, Palakkad and Thioglycollic Acid from SDFCL Mumbai and Nutrient Agar Medium from Himedia Labortaries. Pvt. Ltd. Distilled Water was obtained from Grace College of Pharmacy. All other reagents used are of high purity.

b) Method

i. Preparation of Tolnaftate Nail Lacquer

Tolnaftate nail lacquer was prepared by simple mixing method. Where in the formulation, the drug concentration was kept constant. The amount of ethyl cellulose, salicylic acid, glycerine were mixed till it gives the uniform distribution of component which is used as the nail lacquer.

Tolnaftate was mixed properly and then the thioglycolic acid added in F2, F3 and DMSO in F4 and F5 and mixed the solution in the magnetic stirrer. Then the solvent mixed and volume made up to fix quantity and mixed properly.

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Table 1: Composition of Tolnaftate Nail Lacquer

Sl. No.	Ingredients	F1	F2	F3	F4	F5
1	Tolnaftate (mg)	20	20	20	20	20
2	Salicylic Acid (mg)	40	40	40	40	40
3	Ethyl Cellulose (g)	2	2	3	2	3
4	Glycerine (ml)	2	2	2	2	2
5	Thioglycolic Acid (%)	--	0.2	0.5	--	--
6	DMSO (ml)	--	--	--	0.2	0.5
7	Ethanol (ml)	20	20	20	20	20

20 mg of Tolnaftate was loaded in this formulations and various combination were tried to get effective nail lacquers. Tolnaftate was added to the ethanol which containing salicylic acid which is to improve the drug permeation and 2 g of ethyl cellulose as one of the polymer are added which is also act as film former. Glycerin was mixed till it gives the uniform distribution of the component which is used in the nail lacquer. To enhance the penetration level of the formulation, thioglycolic acid and dimethyl sulfoxide were added. Now the solution was kept on the magnetic stirrer till tolnaftate get completely mixed and volume made upto fix quantity and mixed properly. After preparation of nail lacquer, stored and further used for evaluation studies.

III. Evaluation Study

a) Drying Time

A film of sample was applied on a glass petri dish with the help of brush. The time to form a dry to touch film was noted using a stopwatch.

b) Nonvolatile Content

8 ml of sample was taken in a glass petri dish of about 8cm in diameter. Samples were spread equally. The dish was placed in the oven at 105^0C for 1 hr. The petri dish was removed, cooled, and weighed. The difference in weight of sample after drying was determined that gives the volatile content present. The difference in weights was recorded.

c) Water Resistance

This is the measure of the resistance towards water permeability of the film. This was done by applying a continuous film on a surface and drying, then immersing it in water. The weight before and after immersion was noted and increase in weight was calculated. Higher the increase in weight, lower the water resistance.

d) Stability Study[4]

Stability study was conducted by storing the optimized formulation at 40°C and 37±20°C for 1 month. The formulation was then evaluated for drying time, non-volatile content, in-vitro adhesion, water resistance and drug content.

e) Smoothness to Flow

The sample was poured on a glass slide on an area of 1.5 square inches and spread on a glass plate by making glass slide to rise vertically. And smoothness of flow was determined by comparing with standard marketed nail lacquer.

f) Drug Content Estimation[5]

Nail lacquer equivalent to 200 mg was dissolved in 50 ml phosphate buffer solution of pH 7.4. Then the solution was ultra sonicated for 15 mints. The resulting solution was filtered, made up to 100 ml with phosphate buffer solution of pH 7.4. From the above solution take 10ml and made up to100ml with PBS of pH 7.4. Then the diluted solution was estimated spectrophotometrically at wavelength of 254 nm and determined the drug content.

g) Diffusion Studies Across Artificial Membrane[6]

Diffusion studies were performed using artificial membrane (cellophane).The membrane was soaked for 1hr in solvent system (phosphate buffer, pH 7.4), and the receptor compartment was filled with solvent. Test vehicle equivalent to 4 mg was applied evenly on the surface of the membrane. The prepared membrane was mounted on the cell carefully to avoid entrapment of air bubbles under the membrane. The whole assembly was maintained at 37°C, and the speed of stirring was kept constant (600 rpm) for 24 hrs. The 2 ml aliquot of drug sample was taken after a time interval of 2 hrs and was replaced by the fresh solvent. Each experiment was replicated at least thrice. The drug analysis was done using UV spectrophotometer at 254 nm.

h) In-Vitro Transungual Permeation Studies[7]

In Hooves from freshly slaughtered cattle, free of adhering connective and cartilaginous tissue, were soaked in distilled water for 24 hrs. Membranes of about 1-mm thickness were then cut from the distal part of hooves. In-vitro permeation studies were carried out by using Franz diffusion cell (respective volume, 100 ml) the hoof membrane was placed carefully on the cell, and the surface area available for permeation was 1.4 cm^2. Then the test vehicle equivalent to 4 mg was applied evenly on the surface of the nail membrane. The receptor compartment was filled with solvent A (phosphate buffer, pH 7.4), and the whole assembly

was maintained at 37°C with constant stirring for 30 h. The 5 ml aliquot of drug sample was taken after a time interval of 2 h and was replaced by the fresh solvent A. The drug analysis was done by using double-beam UV spectrophotometer at 254 nm.

i) Determination of Zone of Inhibition[8]

Agar cup-plate method was used to determine in vitro antifungal activity against Candida albicans. Nutrient agar plates were prepared and sterilized by autoclaving at 120°C, 15 pounds pressure for 15 min. 70 ml nutrient agar media was then inoculated with fungal strain i.e. C. albicans (2 mL of inoculum to 100mL of nutrient agar media). The mixture was then poured in two sterilized petri plates and five wells of 5 mm diameters were prepared via sterile cork borer in each petri plate. 0.2 ml each of optimized formulation, control formulation were transferred to the cups aseptically and labelled accordingly as optimized and control formulation. Negative and positive controls were also prepared which consist of un-inoculated media and media seeded with test organism but deprived of antifungal agent, respectively. The prepared petri plates were maintained at room temperature for 2 h to allow the diffusion of the solutions in to the medium and then incubated at 28°C for 48 hrs. The diameter of zone of inhibition surrounding each of the well was recorded.

IV. Results

The prime objective of the work was to formulate tolnaftate nail lacquer containing two different penetration enhancers and different concentrations of polymer and to find out which polymer concentration and concentration of penetration enhancers gave better release as well as to carry out the antifungal testing on the best formulation obtained.

a) Identification by FTIR Spectroscopy

IR spectrum of tolnaftate was compared with the standard spectrum and the sample spectrum (Fig. 1) showed all the characteristic peaks in the relevant region. So IR spectra verified the authenticity of the procured sample. The IR spectrum of tolnaftate, ethyl cellulose and salicylic acid combination does not show deviation as compared to standard spectrum of tolnaftate is shown in (Fig. 2).

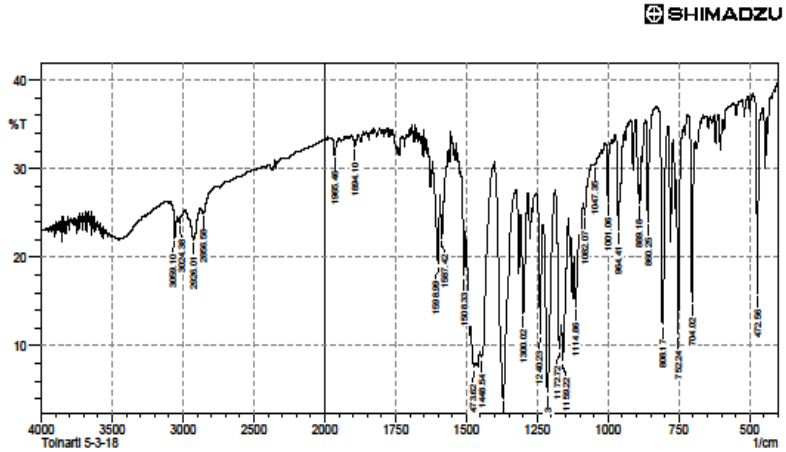

Fig. 1: IR Spectrum of Tolnaftate

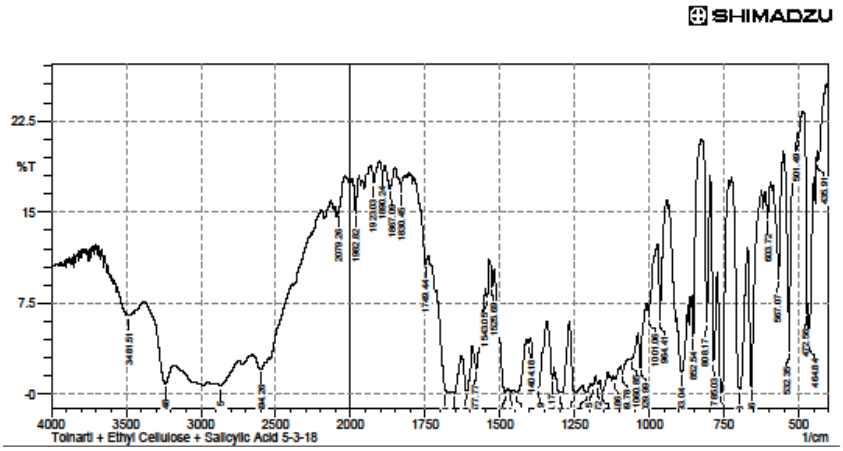

Fig. 2: IR Spectrum of Tolnaftate Ethyl Cellulose Salicylic Acid Combination

V. Formulation of Tolnaftate Nail Lacquer using Different Penetration Enhancers

Tolnaftate nail lacquers with different penetration enhancers combinations were prepared by simple mixing method. For that 20 mg of tolnaftate was loaded in various solutions, so as to get a concentration of 1mg/ml. Different penetration enhancers such as thioglycolic acid and dimethyl sulfoxides were used in the F1, F2, F3, F4, F5. Different formulations containing different concentration of ethyl cellulose and different proportions of thioglycolic acid (F2 and F3) and dimethyl sulfoxides (F4 and F5) combinations were prepared.

VI. Evaluation of Nail Lacquer

Formulated nail lacquers were subjected to preliminary evaluation tests. Nail lacquers with any imperfection in smoothness of flow, Water resistance, Drying time and in stability were excluded.

a) Drying Time

Drying time for formulations F1 to F5 was found between 61 seconds to 70 seconds. It was found that as the polymer concentration increases from 2% w/v to 3% w/v the drying time increases respectively. The time required for the solvent to evaporate from the more viscous solution is more than the less viscous solution.

b) Non Volatile Content of Tolnaftate Nail Lacquer

It was seen that as the polymer concentration increases from 1% w/v to 2% w/v the non-volatile content increases. The formulation which had higher concentration of polymer showed higher non-volatile content as the amount of polymer present in the sample for determination of nonvolatile content was more as compared to the formulation which contained lower concentrations of polymer. Non-volatile content depends and varyupon the concentration of polymer used.

c) Water Resistance Test

Table 2: Water Resistance Test

Sl. No.	Formulation Code	W1(g)	W2(g)	Difference In Weight (g)
1	F1	6.00	6.24	0.24
2	F2	6.00	6.22	0.22
3	F3	6.00	6.22	0.22
4	F4	6.00	6.51	0.51
5	F5	6.00	6.50	0.50

W1 and W2 - Weight of glass slide along with nail lacquer before and after dipping in water.

From the water resistance test, it can be seen as the polymer concentration increases the water resistance increases, as the concentration of polymer decreases the water resistance decreases. Formulations F1, F2, and F4 showed lower water resistance as compared to F3 and F5.

d) Stability Study

The stability study data indicated that the medicated nail lacquer, showed good stability for 1 month when it was stored at temperature of 37±2ºC. There is no significant change is observed in color, non volatile content, viscosity, drying time and smoothness.

e) Smoothness to Flow

Smoothness of flow for formulations F1, F2, F3, F4 and F5 showed satisfactory flow property compared to marketed product.

f) Drug Content Estimation

Table 3: Drug Content

Formulation	Drug Content (%)
F1	90.00± 0.209
F2	92.50±0.167
F3	94.28±0.006
F4	91.25±0.474
F5	92.76±0.178

Percentage drug content for all the lacquers was found to be satisfactory and in between 90% to 94% which is reported in table 3. Highest % of drug content was found to be 94.28 % (F3) and the lowest % of drug content was 90.00 % (F1). Drug content more than 90% in the formulation shows the high amount of drug present in the formulation, ensuring that the methods of formulation and the ingredients selected are not affecting the stability of drug. High drug content also gives the assurance that, a good therapeutic outcome can be expected.

g) In vitro Diffusion Studies Across Artificial Membrane

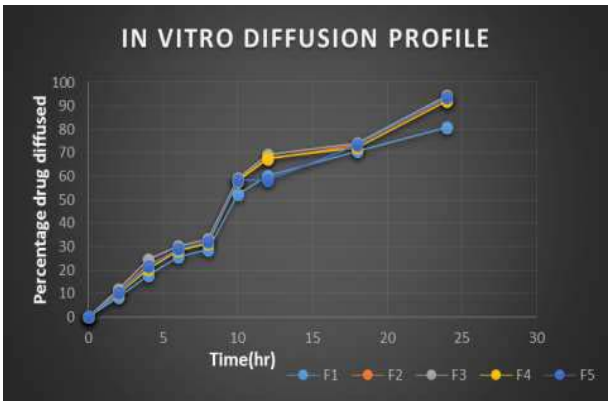

Fig. 3: In Vitro Diffusion Profile of Tolnaftate

In vitro diffusion studies were conducted using diffusing cell for 24 hours. Formulation F3, F5 containing highest concentration of penetration enhancer (thioglycolic acid and DMSO) showed the highest release of 94.48 % and 93.58 %. It was found that as the penetration enhancer concentration increases, the release of drug increases. From the data obtained by evaluation of nail lacquer, formulation F3 was found to be best formulation among all the four formulations.

h) In vitro Transungual Permeation Studies

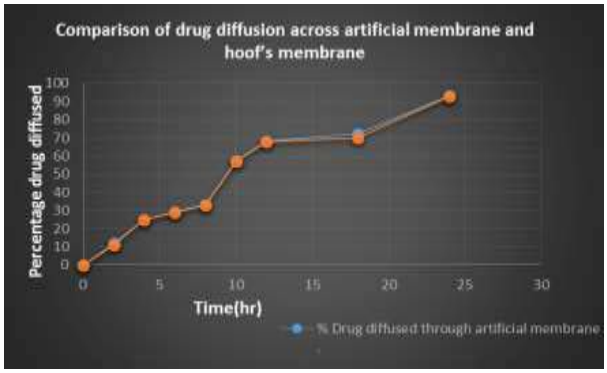

Fig. 4: In-Vitro Permeation Studies of Formulations F1 to F5

In vitro permeation studies, it was found that formulation F3 showed release of 92.78% at the end of 24 hours. From in vitro diffusion studies and in-vitro permeation studies it was found that thioglycolic acid was proved to a better penetration enhancer as compared to dimethyl sulfoxide. The effect of thioglycolic acid was attributed to its small molecular weight and damage caused on the keratin network and decrease in lipid content in the dorsal nail layer; this act which loosened the nail structure, allowing tolnaftate to penetrate easier.

i) Determination of Zone of Inhibition

Formulation prepared with ethyl cellulose, salicylic acid, and glycerine respectively having 1mg/ml dose of tolnaftate were subjected to antifungal activity. 20 mg tolnaftate was loaded in various combinations and compared the obtained zone diameter as that of zone of inhibition of tolnaftate in ethanol. The zone of inhibition obtained were determined in Candida albican organism and compared with tolnaftate standard. From the analysis, formulation showed comparable zone of inhibition with that of tolnaftate standard solution.

Fig. 5: Antifungal Activity of Various Tolnaftate Loaded Formulations

F1-Tolnaftate nail lacquer without penetration enhancer.
F2-Tolnaftate nail lacquer with 0.3ml of thioglycolic acid.
F3-Tolnaftate nail lacquer with 0.5ml of thioglycolic acid.
F4-Tolnaftate nail lacquer with 0.3ml of DMSO.
F5-Tolnaftate nail lacquer with 0.5ml of DMSO.

Comparative antifungal activity of tolnaftate loaded thioglycolic acid formulation with marketed suspension.

The antifungal activity of tolnaftate loaded with various combinations were studied using nutrient agar medium. Here ketoconozole in DMSO taken as standard and compared the zone diameter obtained by tolnaftate with that of various penetration enhancers. It was found that thioglycolic acid tolnaftate loaded lacquer have similar zone diameter as those of ketoconozole drug standard. Moreover presence of thioglycolic acid in all formulations can also contribute to prevent the development of onychomycosis because it inhibits Candida albicanto nail plate.

VII. Summary and Conclusion

The present study aimed to produce a formulation for treating onychomycosis. This formulation includes antifungal agents, penetration enhancers (DMSO and thioglycolic acid) and keratolytic agent salicylic acid for additional benefits.

The nail formulations excluding polymer with omitted as the formulation showed tackiness, dullness etc. Out of 36 formulations, best 5 were chosen for further formulation and evaluations was done. FTIR studies revealed that no chemical interaction between drug and polymer. Then these lacquers were compared for drying time, non volatile content, drug content, drug diffusion and antimicrobial studies. All formulations showed good film formation and other parameters. The stability test showed that the formulation were stable at 37° ± 2° C for 1 month.

The results obtained from in-vitro diffusion studies showed that formulation F3 have completed drug release of 94.48% over 24 hrs. The F3 formulation had salicylic acid as keratolytic agent and 0.5 ml of 1 % w/v of thioglycolic acid as penetration enhancer. This indicates the combination of permeation enhancer and keratolytic agent resulted in an improved permeation and sustained drug release. The nonvolatile content of F3 was found to be 1.04. F3 formulation showed rapid drying rate. From diffusion studies, it was concluded that thioglycolic acid containing formulation (F2 and F3) have better penetration enhancement as compared to DMSO containing formulation. From the above study, it can be concluded that medicated nail lacquers proved to be a better tool as a drug delivery system for ungual drug delivery of an antifungal in the treatment of onychomycosis.

References Références Referencias

1. Patel R. P, Naik S. A, Patel N. A, Suthar A. M. Drug Delivery across human nail. International Journal of Current Pharmaceutical Research, 2009, 1(1): 1-7.
2. Sabreen J, Divyakumar B, Kiran B. Preungual drug delivery systems of terbinafine hydrochloride nail lacquer. Asian Journal of Pharmaceutical, 2008, 02: 53-06.
3. D. H. Singh, S. Roy Chowdhury, D. Sharma, V. Bhandari, M. Singh. Indo Amer. J. Pharma. Res. 2013, 3(6): 4460-4470.
4. Amrita Sawant Dessai, Shripathi D., Dr. A. R. Shabaraya. Formulation and *in vitro* characterisation of nail lacquer containing fluconazole for preungual drug delivery system. International Journal of Pharmaceutical Research and Bio-Science. 2014, 3(2): 200-214.
5. Deepa Dhiman, Sunit Kumar, Aman Mittal. Formulation & evaluation of medicated nail lacquer of fluconazole to explore the difficulties in penetration of drug across nail plate and enhancement of bioavailability of antifungal drug. European Journal of Pharmaceutical and Medical Research 2016, Jan-March: 266-270.
6. Azharuddhin M, Suresh B. H, Shabaraya A. S, Prasad N. G. Z. Preparation and *in vitro* evaluation of antifungal nail lacquer. International Journal of Universal Pharmacy and Bio Sciences July-August 2013, 2(4): 78-85.
7. R. Manavalan, Barish, E. Abraham Theodore, Aswanivm, R. Venkatanarayanan. Formulation and evaluation of a medicated nail lacquer for the treatment of onychomycosis. International Journal of Research in Pharmaceutical and Nano Sciences, 2016, 5(4): 201 - 211.
8. Jaiswal M, Kumar M, Pathak K. Zero order delivery of itraconazole via polymeric micelles incorporated in situ ocular gel for the management of fungal keratitis. Coll Surf B: Biointerf 2015, 130: 23-30.