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FORMULATION AND EVALUATION OF NAIL DELIVERY SYSTEMS OF VORICONAZOLE

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ABSTRACT

Novel nail lacquer formulations of Voriconazole (1%w/w), a second generation triazole derivative, were prepared for topical treatment of onychomycosis. The plasticized nail lacquers were prepared using nitrocellulose (RR Type) and ethyl cellulose as film formers in organic solvent system using purified papain (5%w/w) and DMSO (3%w/w) as penetration enhancers. Both the placebo and drug loaded nail lacquers were characterized for clarity, viscosity (cps), specific gravity, non-volatile content , and uniformity of coat, smoothness, gloss, water resistance and drying time of applied lacquer Moreover, the medicated lacquers were characterized for transungual permeation *(in- vitro)*, drug content, antifungal efficacy against *T. rubrum* (MTCC No.296) and *C. albicans* (NCIM No.3471).The formulation based on nitrocellulose and containing purified papain indicated good clarity, low viscosity (375-400 cps), faster drying (65-70 sec) and formed glossy, smooth films with more than 99% of VCONZ that indicated diffusion over a period of 6 hrs and remained stable with no gross change in any of the physicochemical characteristics and antifungal efficacy.

KEYWORDS: Onychomycosis, Antifungal treatment, Penetration enhancer, Medicated nail lacquers.

INTRODUCTION

Nail drug delivery systems (NDDS) are emerging nail care products for treating various fungal infections of the horny keratinized nail tissue and surrounding soft tissues including cuticle. The absorption of drugs into the nail unit, following topical application to the nail

plate, is highly desirable to treat such infections. Nail permeability is however very poor and therefore offers limited application of topical therapy especially to later stages of infection. The significance of nail permeability of drugs to topical therapeutics has been realized, primarily in the treatment of onychomycosis, which affects approximately 19% of the population. The common symptoms include discoloration, thickening and deformity of the toenails and eventual loss of nail plate.

Topical therapy is highly desirable due to its localized activity, minimal adverse systemic effects and possibly improved adherence to treatment regimen. Current research on nail permeation focuses on altering the nail plate barrier by means of chemical treatments and penetration enhancers.[6,7,10]

Recent advances in topical transungual delivery have led to the development of antifungal nail lacquers. Gladious et al (2012) investigated microemulsion (ME) based topical delivery system of VCONZ using polyoxyethylene oleyl ether (Brij 97) as the surfactant. They also investigated effect of permeation enhancers viz: sodium deoxycholate and oleic acid on the delivery of drug from formulations. They reported prolonged (4h) release of drug as well insignificant change in physical or rheological properties of the formulation based on Jojoba oil. They cited transdermal route for permeation in presence of permeation enhancers and claimed greater efficacy of sodium deoxycholate over oleic acid. They claimed that, the microemulsions possessed greater antifungal activity against *C. albicans* than supersaturated solutions of the drug. Kuchekar et al (2016) developed a topical carbomer (Grade 940) gel formulation of VCONZ for the treatment of candidiasis, aspergillosis and certain other emerging fungal infections.

Hence, the work was undertaken with the objective of enhancing permeation of VCONZ by formulating it as nail lacquers using purified papain and DMSO as penetration enhancers and check their antifungal efficacy against fungal cultures of *C. albicans and T, rubrum*.[4,9]

MATERIALS AND METHODS

MATERIALS

The drug Voriconazole (VCONZ) was a generous gift from MSN-Lab, TN (India) while nitrocellulose (NC) and ethyl cellulose (EC) were purchased from Research-Lab, Mumbai. Purified papain was procured from Loba Chemie while the fungal cultures Trychophyton rubrum (MTCC 296) and *Candida albicans* (NCIM 3471) were purchased from Microbial

Type Culture Collection and Gene Bank, Chandigarh, India and NCIM, Pune respectively. The culture media; Sabouraud dextrose agar and Potato dextrose agar were purchased from Hi-Media Laboratories Pvt. Ltd, Mumbai. All other reagents and solvents were of analytical grade.

METHODS

Preformulation studies

A stock solution (100µg/ml) of VCONZ was prepared by dissolving accurately weighed 10mg quantity using MeOH: DW (1:9) system. The λ max and linearity range values of appropriately diluted solutions were noted (Shimadzu-1800)**.**

Compatibility studies

The compatibility of mixtures of VCONZ with cellulose polymers was ascertained by exposing them to the ambient environmental conditions over 15 days. The detection of changes in any of the physical or physicochemical characteristics of blends was carried out by visual inspection and FT-IR spectrophotometry (Shimadzu-8400).

Preparation of nail lacquers

The nail lacquers were prepared by dissolving the required quantities of cellulosic film forming polymers into blends of ethyl and butyl acetate (5:4) followed by addition of plasticizer over a thermostatically controlled magnetic stirrer. The methanolic solution of VCONZ and eutectic mixture (EU) of Camphor: Thymol (1:1) was subsequently added into the polymeric solutions followed by addition of either of purified papain or DMSO as penetration enhancer[1] (Table 1).

Table 1: Formulation of nail lacquers using different film forming polymers.

Formulation code	NC	EC	DEP
NLNCMDP1	10%	-	10%
NLNCMDP2	10%	-	15%
NLNCMDP3	10%	-	20%
NLECMDP1	-	10%	10%
NLECMDP2	-	10%	15%
NLECMDP3	-	10%	20%

All medicated formulations contained VCONZ (1%w/w), EU (1% w/w) along-with either, purified papain (5%w/w) or DMSO (3%w/w).

EVALUATION OF NAIL LACQUERS

The placebo and medicated nail lacquer solutions were assessed for clarity, specific gravity, viscosity, smoothness to flow and non volatile contents while their films were characterized for uniformity, smoothness of applied coat, gloss, water resistance and drying time using artificial nail holder. The medicated nail lacquers were evaluated for contents of VCONZ, antifungal efficacy, and transungual permeation (*in vitro*).

Clarity

For this, 10 ml of nail lacquer sample contained in the transparent glass container was observed visually and through magnifying glass for presence of any particulate matter, against white and dark background following standard procedure.

Specific gravity

Specific gravity of lacquers was determined using specific gravity bottle following standard procedure.[2]

Viscosity (cps)

The viscosity of nail lacquers was noted (25°C-28°C) using a Brookfield viscometer (Model LVDV, Spindle No. S64) following standard procedure.[5]

Non-volatile content

For this, 10 ml of each of sample nail lacquer formulation was transferred into separate petri dish and initial weights were recorded (W1). The individual dish was placed in the hot air oven at 105^0C for 1hr. The individual plate was re-weighed (W2) after cooling to room temperature and the difference in initial and final weights was recorded. Average of triplicate readings was noted.[5]

Smoothness to flow

For this, 2 ml of lacquer sample was poured from a height of 1.5 inches into a glass plate, spreaded it and made to flow vertically down and visually observed for smoothness of flow of the stream.[3]

Evaluation of coated films of placebo nail lacquers

Uniformity and smoothness of applied coat

For this, the lacquer formula was applied to artificial nail holder as single coat and the uniformity and smoothness of the coat was visually observed after drying.[2,5]

Gloss

Gloss of the applied coats of experimental nail lacquer was compared visually with that of a commercially available glossy nail lacquer base coat formula.[2,3]

Water resistance

For this, single uniform coat of the experimental lacquers was applied to three different sets of artificial nails of both the hands that were appropriately coded and weighed prior to application of the lacquer coat. The individual nails of each set were reweighed after air drying for 3 minutes. The nails were soaked in purified water maintained at $37^0 \pm 2\ ^0$C for 22-24 hours. The nails were air dried followed by absorption of residual water using tissue paper and were reweighed individually to note down the increase in weight if any.[1]

Drying time (min)

For this, a single coat of sample nail lacquer was applied on to a artificial nail holder with the help of nail paint brush. The time to form a dry-to-touch film coat was noted using a stopwatch for single as well as double coat.[3]

Moreover, the experimental medicated nail lacquers were characterized for following.

Contents of VCONZ

For this, **t**he quantity of experimental lacquer formulations equivalent to 10 mg of VCONZ was dissolved using 100 ml acetone and was diluted appropriately (1 into 10) with acetone to estimate the contents of drug spectrophotometrically at a wavelength of 256.0 nm.[1,3]

Antifungal efficacy

Antifungal efficacy of both the methanolic solution of VCONZ and its plasticized nail lacquer was estimated by cup plate method using separate sets of sterile petri plates of Potato dextrose agar (PDA) and Emmons's modified sabouraud agar (EMSA) that were inoculated with 0.2 ml of suspension of *C. albicans and T. rubrum* respectively. The plates were kept undisturbed at room temperature for 30 min. for proper setting of media and then were incubated at 22- 27°C for 72hrs. The antifungal efficacy was calculated in terms of diameter of zone of inhibition produced by the drug or lacquer formulation.[1,3]

Transungual permeation (*in vitro*)

For this, Franz diffusion cell with receptor volume of 25 ml was used and the studies were carried out using phosphate buffer (pH 7.4, 37 ± 1°C) as receiving fluid. The solution of

VCONZ (equivalent to 100 µg) in phosphate buffer was placed in the donor compartment and was allowed to permeate through an active diffusion area of 0.25 cm^2. The contents of the cell were stirred using magnetic stirrer and samples of 2 ml were drawn from the receiver at the interval 1 hr over a period of 6 hrs, and the amount of VCONZ transported was estimated spectrophotometrically at 256 nm.[1,3,8]

RESULS AND DISCUSSION

The organoleptic, physicochemical and solubility characteristics of VCONZ supported its identity and purity (Table 2). Moreover the spectral characteristics viz. wavelength maxima (256 nm) and linearity of concentration and absorbance range (2-10 µg/ml) further supported the quality and purity of VCONZ "Fig 1".

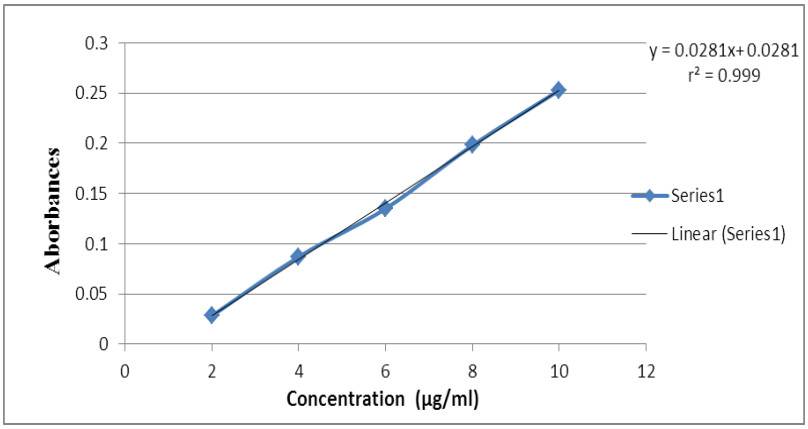


Fig. 1: Calibration curve of VCONZ in MeOH: DW (1:9).

Compatibility of VCONZ and formulation excipients

The IR spectra of physical mixture of VCONZ and formulations ingredients did not indicate any gross change in structure of any of them suggesting no effect on their mutual compatibility "Fig. 2". "Fig. 3".

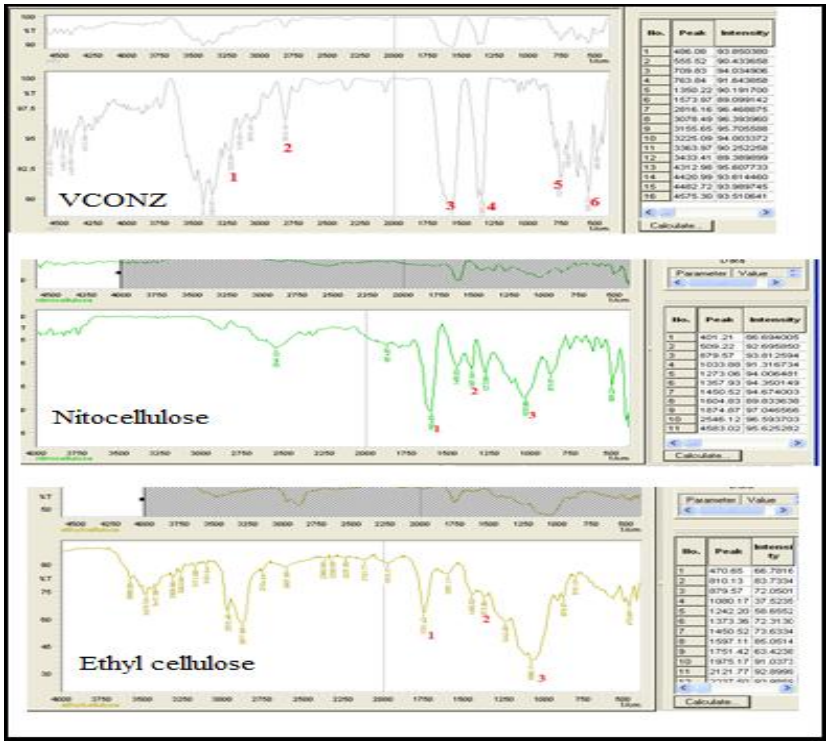


Fig. 2: IR Spectra of VCONZ, individual film forming polymer.

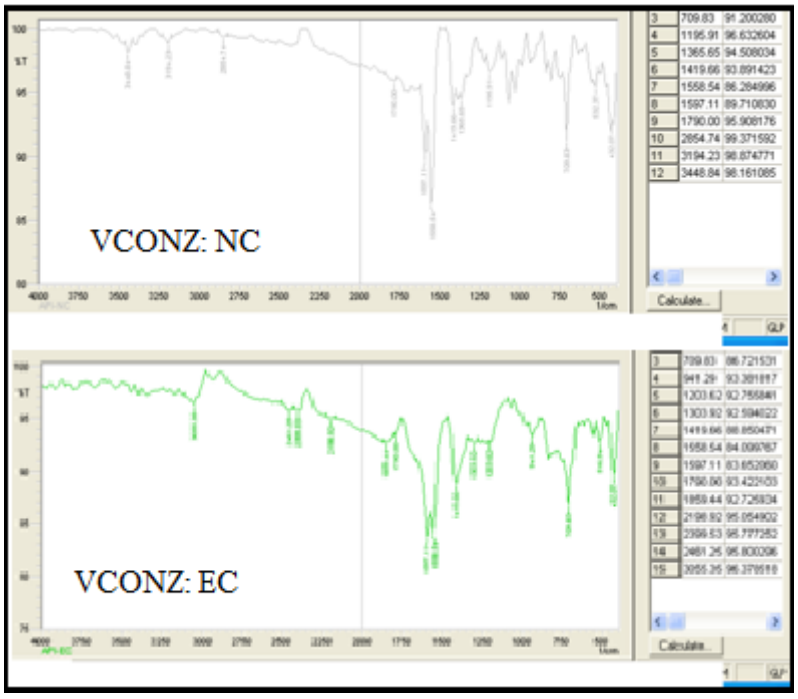


Fig. 3: IR Spectra of blends of VCONZ, with film forming polymer.

Characteristics of placebo nail lacquer formulations

All the lacquers were clear solutions and possessed smooth flow indicating absence of suspended particles. Moreover, the applied coats of lacquers indicated smooth surface with very minimal or no gain in weight due to high water resistance.

Table 2: Physicochemical characteristics of medicated nail lacquers containing different film formers.

Sr. No.	Formulation code	SG (gm/cm^3)	VIS (cps)	NVC (%)	SMT (Grade)	GLC (Grade)	DT (sec)
NC without penetration enhancer							
1.	MNLNC1	0.950-1.1	379-400	20.1±0.57	Good	Good	64 -72
2.	MNLNC2	0.950-1.00	375-398	20.3±0.51	Good	Satisfactory	64 - 68
NC with papain							
3.	MNLNCPPN1	0.990-1.10	365-400	20.3±0.59	Good	Good	64 – 70
4.	MNLNCPPN2	0.950-1.10	375-400	20.3±0.57	Good	Good	64 – 74
NC with DMSO							
5.	MNLNCDS1	0.950-1.10	375-400	20.3±0.57	Satisfactory	Good	64 - 74
6.	MNLNCDS2	0.950-1.10	375-400	20.3±0.57	Good	Satisfactory	64 - 74
EC without penetration enhancer							
7.	MNLEC1	0.850-1.10	345-398	21.5±0.56	Good	Satisfactory	70 - 72
8.	MNLEC2	0.960-1.13	355-402	20.2±0.57	Satisfactory	Satisfactory	64 - 73
EC with Papain							
9.	MNLECPPN1	0.950-1.10	375-400	21.1±0.47	Satisfactory	Good	64 - 74
10.	MNLECPPN2	0.850-1.10	385-400	20.9±0.57	Good	Satisfactory	64 - 74
EC with DMSO							
11.	MNLECDS1	0.950-1.10	395-400	22.3±0.67	Good	Satisfactory	61 – 74
12.	MNLECDS2	0.960-1.13	374-400	21.3±0.46	Satisfactory	Good	64 - 74

Contents of VCONZ

All the selected nail lacquer formulations containing NC (10%) with or without penetration enhancers (purified 5%w/w and DMSO 3%w/w) reported ≥99 % of VCONZ (Table 3).

Table 3: Content of VCONZ in nail lacquers.

Sr. No.	Formulation code	Drug content %
1.	MNLNC1	99.43±0.472
2.	MNLNCPPN1	98.5±0.173
3.	MNLNCDS1	99.5±0.264

Antifungal efficacy of sample of VCONZ against causative fungal species

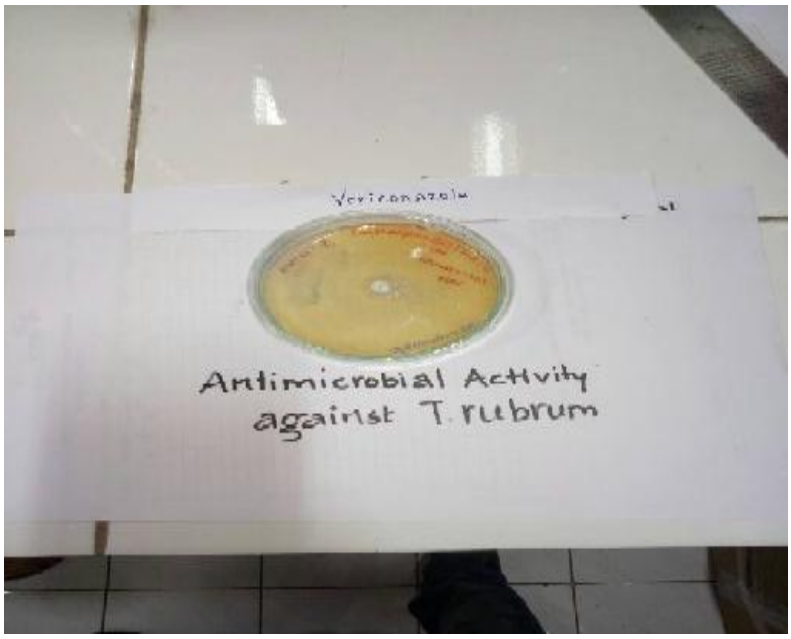
The zone of inhibition of VCONZ measured 26mm and 90mm when tested against *C. albicans* and *T.rubrum* respectively. Similarly, the zone of inhibition of EUM measured 90 mm for both *C. albicans* and *T. rubrum.* Therefore, the cultures are found to be sensitive to both VCONZ and EUM. The formulations based on NC: EUM lacked sensitivity against *C. albicans* but were moderately sensitive to *T. rubrum*. The zone of inhibition of EC: EUM against *C. albicans* measured only 10mm. Therefore, the culture of *C. albicans* was found to be resistant for EC: EUM. Moreover, The zone of inhibition of VCONZ were distinctly greater than the standard. Hence, both the fungal cultures were sensitive to VCONZ with susceptibility of *T. rubrum* substantially greater than that of *C. albicans* (Table 4, "Fig.4" and "Fig. 5").

Table 4: Susceptibility of *C. albicans* and *T. rubrum* (zone of inhibition) against VCONZ, EUM, NC: EUM, EC: EUM.

Sensitivity of *C. albicans* to VCONZ	**Diameter of zone of inhibition (mm)**	**Sensitivity of *T. rubrum* to VCONZ**	**Diameter of zone of inhibition (mm)**
Sensitive	26.	Sensitive	90
Sensitivity of *C. albicans* to EUM	90	Sensitivity of *T. rubrum* to EUM	90
Sensitive		Sensitive	Yes
Sensitivity of *C. albicans* to NC:EUM	10	Sensitivity of *T. rubrum* toNC:EUM	12
Resistant	Yes	Moderate	Yes
Sensitivity of *C. albicans* to EC:EUM	10	Sensitivity of *T. rubrum* to EC: EUM	14.8
Resistant	Yes	Sensitive	Yes

The values of zone of inhibition produced by VCONZ and EUM and those produced by blends of NC, EC with EUM were compared with those for ampicillin standard (Dynmicro, Thane) which correlated the sensitivity /resistance of *Candida albicans* cultures (Resistant: Zone of inhibition <11mm, Moderately sensitive: 12- 13mm., Sensitive: > 14 mm). The values of zone of inhibition for T.rubrum produced by VCONZ were compared with following standards for correlating the sensitivity /resistance of culture (Resistant: Zone of inhibition <10mm, Moderately sensitive: 11-15mm. Sensitive: > 16 mm).

Fig. 4: Sensitivity of *C. albicans* against VCONZ, EUM, and blends of NC, EC with EU.



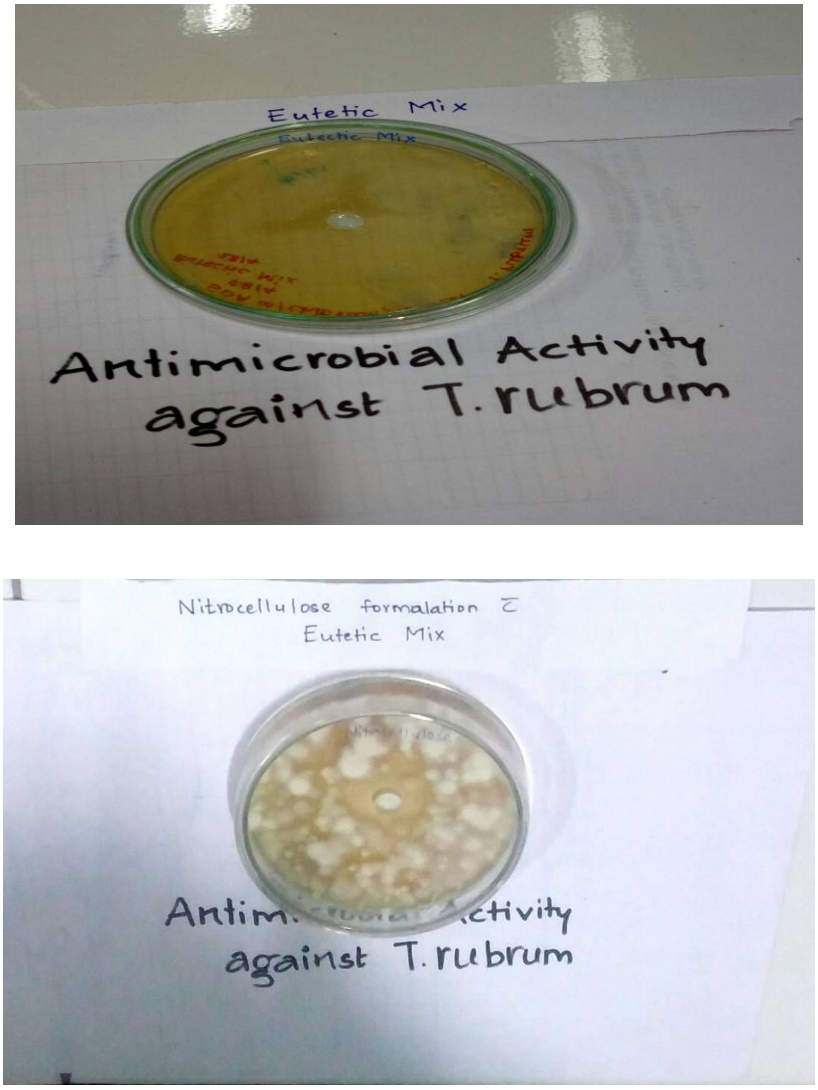


Fig 5: Sensitivity of *T.rubrum* against VCONZ, EUM, and blend of NC: EUM.

Transungual permeation (*in vitro*)

The diffusion profiles of VCONZ from nail lacquers of NC containing purified papain and DMSO demonstrated higher release than that from formulations containing no penetration enhancer over 6 hrs. The order of extent of diffusion of VCONZ from the nail lacquers was in the order MNLNCPPN > MNLNCDS > MNLNC **(“**Fig.6”**).**

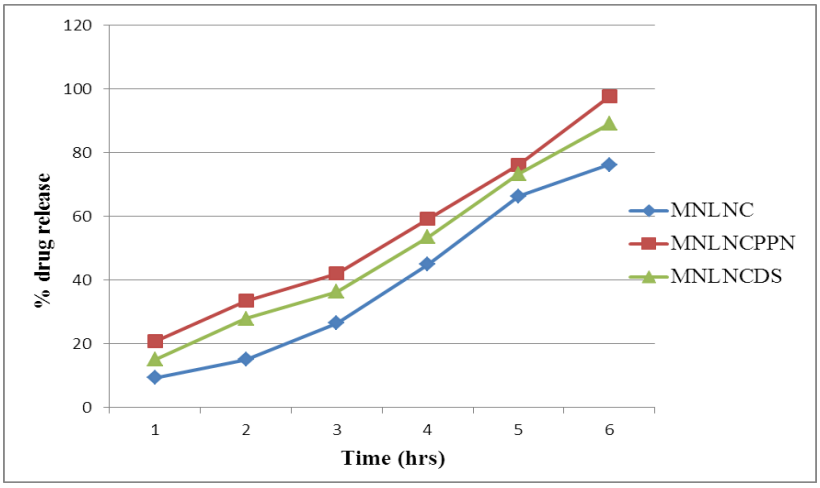


Fig 6: Comparative transungual diffusion (*in vitro*) of VCONZ from nail lacquer formulations with and without penetration enhancers.

CONCLUSION

The Voriconazole nail lacquers with nitrocellulose and ethyl cellulose film formers were found to be effective in inhibiting the growth of *C.* albicans and *T. rubrum*. The permeation enhancers purified papain, di-methyl sulphoxide; facilitated the Voriconazole's diffusion through artificial nail holder over prolonged periods. Hence, it can be concluded that, medicated nail lacquers of VCONZ can be used as promising alternative for its currently available topical delivery systems in the treatment of onychomycosis. Apart from controlling the growth of infecting fungi, these medicated nail lacquers also offer advantages of superior cosmetic appeal and freedom from staining and greasiness, the features essential for improved patient compliance and acceptability.

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