Research Article

Formulation and clinical evaluation of new oxiconazole SLNs gel formulation

Rabab A. Mahmoud[(1)] , Heba F. Mansour[(2)] , Ghada A. Nasef [(3)] and Amal K. Hussein [(4)]
* Pharmacist at EL Minia health assurance hospital,
** Department of Pharmaceutics, Faculty of Pharmacy, Minia University, El Minia, Egypt
*** Department of Dermatology, Faculty of Medicin, Minia University, El Minia, Egypt.
**** Department of clinical pharmacy, Faculty of Pharmacy, Minia University, El Minia, Egypt.

Introduction

Infections that affect the keratinized tissues are of serious concerns worldwide and are increasing on a global scale. Dermatomycoses are infections of the skin, hair and nail caused as a result of colonization of the keratinized layers of the body. This colonization is brought about by the organisms belonging to the three genera namely *Trichophyton, Microsporum* and *pidermophyton (*Luilma et al., 2008).

Infection may also be caused rarely by the members of the genus *Candida* and by non-dermatophytic moulds belonging to the genera *Fusarium*, *Scopulariopsis* and *Aspergillus* (Pintotal 2006; Naved et al., 2009).

Clinical presentation

Pathogenesis

The pathogen invades the uppermost, non-living, keratinized layer of the skin namely the stratum corneum, produces exo-enzyme keratinase and induces inflammatory reaction at the site of infection (Muhsin et al., 1997). The signs of inflammatory reactions such as redness, swelling heat and alopecia (loss of hair) are seen at the infection site. Inflammation causes the pathogen to move away from the site of infection and take residence at a new site. This movement of the organism away from the infection site produces the classical ringed lesion (Dahl, 1994).

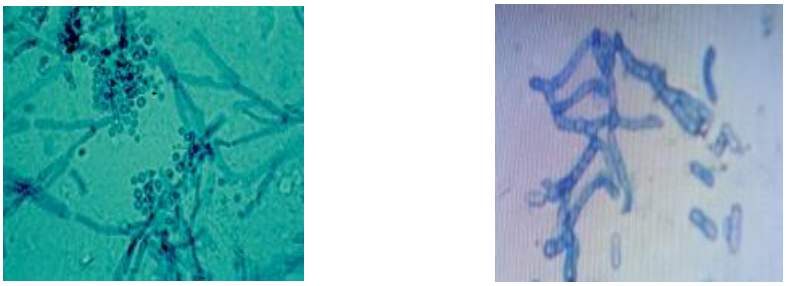
Principles of Laboratory Diagnosis of Superficial Fungal Infections

Clinical diagnosis is usually good enough for the routine management of patients. If laboratory confirmation is required, The laboratory diagnostic approach will involve a wet mount of KOH examination and a culture for proper species identification. The scales from active lesions produced by skin scraping can be collected and wrapped in color paper (and put in a properly sealed container) and sent to the supporting laboratory by mail. Cleansing of the site may be required in those grossly contaminated sites such as from a "dirty" foot before performing skin scraping. Diseased hairs should be plucked (not cut) in those cases of suspected tinea capitis (Kingman and Tinsik, 2010).

Histopathology

The purpose of histologic examination of tissue for fungi is twofold: (1) to alert clinicians to the presence of fungi and (2) to guide clinicians in the choice of antifungal therapy through provision of an inclusive but targeted differential diagnosis. This study highlights these points while also underscoring the importance of communication among pathologists, microbiologists, and clinicians in the care of immune-compromised pediatric patients (Dekio et al., 2015).

Histological features of dermatophytoses are often various, nonspecific, and unusual, for the histological diagnosis of dermatophytoses including neutrophils in the stratum corneum of the skin, compact orthokeratosis, and the presence of fungal hyphae between 2 zones of cornified cells(the so-called sandwich sign) (Hoss et al., 2010).



a b

Figure (1):
a- Confirmational Testing and Diagnosis,Oval yeast form and short curved hyphae (2.5-4 um wide) are demonstrated by 10% KOH.
b- 10% KOH preparation found septate hyphae (Venari et al., 1997)

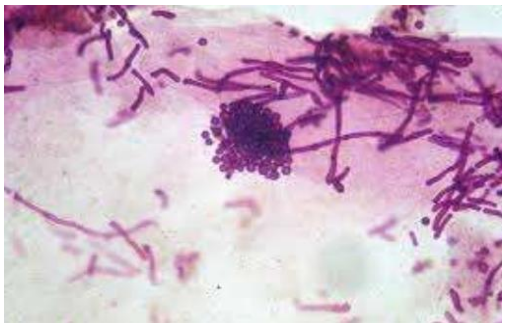


Figure (2): Dimorphic feature of tinea vercicolour (Julitron , 1997)

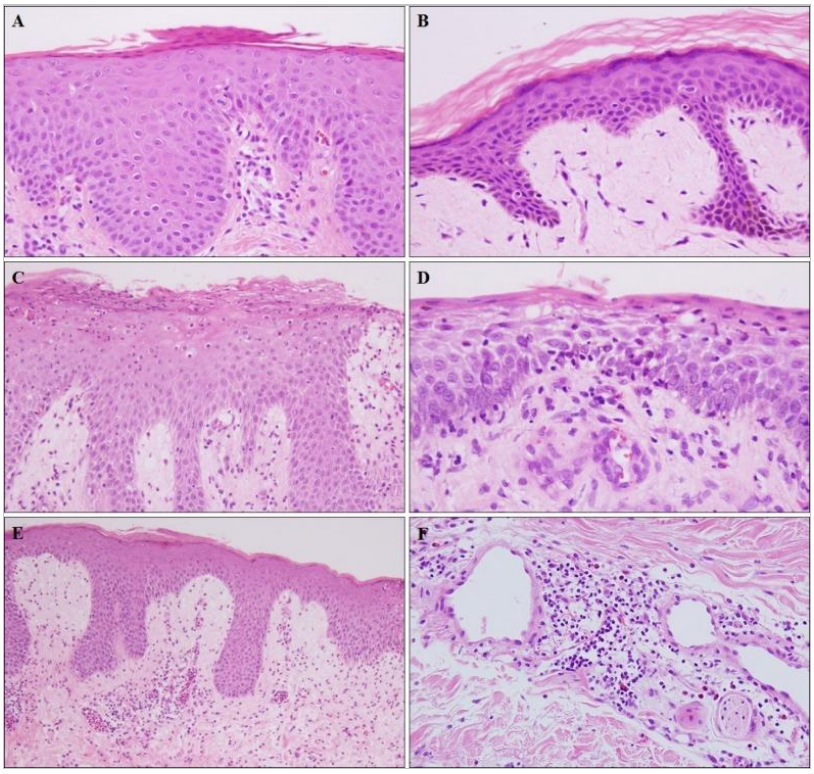


Figure (3)Skin biopsy specimens diagnosed as tinea corporis and tinea faciei showed various histological findings on H&E stained sections, including (A) parakeratosis, (B) basket weave keratin layer, (C) neutrophils in the stratum malpighii, (D) spongosis, (E) papillary dermal edema, and (F) eosinophils in the dermis (A~D: ×200; E: ×100; F: ×400) (Young et al., 2011)

The treatment is chosen according to the infection site, etiological agent and penetration ability of the drug. The penetration ability and retention in the site of infection of the agent determines its efficacy and frequency of utility. Since dermatophytes reside in the stratum corneum especially within the keratino-cytes, the antifungal agents should have a good penetrating ability. The duration of treatment mainly depends on the type of infection and symptom. Generally a two-three week treatment is required for skin lesions whereas four-six weeks of treatment for feet inflammation (Elwesiki and Hazen, 1989).

Patient and method

The present study was conducted on 28 patients with tinea of different types attending the Dermatology Department outpatient clinic of Minia University Hospital, their age ranged from 12 to 50 years.

Studied groups

Two groups were included in this study:

Group 1 included 14 patient treated with tinox 1%. Which is the cream of oxicona-zole marketed preparation.

Group 2 included 14 patients who were treated with the new gel SLNs oxiconazole formulation.

All patients have been subjected to the following:

A -an informed consent has been obtained from all patients enrolled in the study for photography and treatment.

B- history, general local examination, laboratory investigation baseline.

C- Photographs were taken before starting treatment and one month later.

Treatment protocol

Group 1 was treated with tinox 1% cream (marketed oxiconazole preparation).

Group 2 was treated with SLNs oxiconazole gel (the new formulation).

Patients are subjected to skin irritation test in which was performed on human volunteers. 28 volunteers were chosen for single formulation and study was performed after taking their informed consent. It was performed by applying gel on an area of2 square inch to the back of hand. Then the examination for the presence of lesion or irritation was done.

Clinical histological changes were assessed in each group before and after treatment, during the period of follow up . The clinical improvement was rated by the patient and the physician as excellent, good, fair and poor according to the following criteria:

1- Excellent: both the patient and the physician agreed that the result was satisfactory.

2- Good: the result although acceptable was not quite up to expectation of the patient, but the physicians were pleased with the outcome.

3- Fair: the improvement was evaluated by both the patient and the physician to be less than expected but still with same improvement.

4- Poor: unsatisfactory results to the patient and /or the physician.

Technique

To advise the patient to rub the cream or the gel twice daily, without exposure to sun for two/ four weeks according to different types

Clinical assessment

High resolution digital photographs were using identical camera folder setting (Nikon-COOL Pix L 26) at the base line, and four weeks after final treatment.

Clinical improvement was evaluated by two blind study physicians by comparing photographs.

The criteria for evaluations, using a quartile grading scale was as follows:

0=no improvement.

1=mild (percent improvement, less than 25%).

2=moderate (percent improvement 25-49%).

3=good (percent improvement 50-74%).

4=excellent (percent improvement more than 75%).

Statistical analysis

Clinical data and results of laboratory tests were analyzed using statistical package for social science (SPSS) for windows version 16.

A- Descriptive method

mean standard deviation (SD), frequency distribution and cross tabulation (Wing, 2014).

B- Significant test

Chi-square test was used to compare the qualitative data members of different groups, two or more groups to be compared qualitatively .

Results

This study was conducted on 28 patient of different tinea infection selected from the outpatient clinic of Dermatology Department of Minia university. Their age ranged from 12 to 50 years. Group one was composed of 14 patients, the age ranged from 12 to 43 years old. All of them were males. Cases types were 5 tinea pedis, 9 cases were tinea vercicolor fungal infection.
Group 2 was composed of 14 patients their age ranged from17 to 50 years old,
13 patients of this group were males and one patient was a female
Cases fungal types were 3 tinea pedis, 8 cases were tinea vercicolor , 3 cases were tinea cercinata fungal infection.

Evaluation of Clinical improvement

* Group 1 was composed of 14 patients who were treated with marketed oxiconazle cream for 4weeks for tinea pedis and 2 weeks for other types of fungal tinea infections

None of patients treated with tinox assessed excellent evaluation (0%). One patient assessed good result (7.14%). Four patients assessed moderate result (28.57%). Nine cases assessed mild result (64.2%).

** Group 2 was composed of 14 patients who were treated with the new oxiconazole SLNs gel formulation; they were treated for 4 weeks for tinea pedis infection and two weeks for other types of tinea fungal infection. Nine patients treated with our formula assessed excellent result (64.2%). Four cases assessed good result (28.57%). None assessed moderate result (0%). One assessed mild result (7.14%).

Evaluation of group 2 patient satisfaction

Results proved that (64.2%) rated themselves as satisfied. (28.57%) rated themselves as somewhat satisfied while (7.14%) were not satisfied.

Side effects:

Regarding for group 1 patients who were treated with oxiconazoele market preparation:
No patient suffered from pruritus, dermatitis, folliculitis, maceration or nodules while 3 patients suffered from stinging sensation, 10 patients suffered from iritation, redness and rash. .
Regarding for group 2 patients who were treated with SLNs oxiconaozle gel formulation:
No patient reported pruritus, stinging, macerations, folliculitis, or nodules. While 3 patient reported irritation, rash and redness

Comparison between results of SLNs oxiconazole gel and tinox market preparation:

On comparing the 2groups regarding clinical improvement it has been found that:
Excellent degree treatment by tinox was zero. Good was 7.14% Moderate was 28.57% while Mild was 64.2%, While scores of clinical improvement of SLN oxiconazole preparation were as follows:
Excellent was 64.2 % Good was 28.57% Moderate was zero % Mild was 7.14%

From the previous results:-

The score excellent, the score good& the score mild were higher in the group treated by the SLNs gel formulation under study. While the score moderate was higher in the group treated by marketed tinox. As regard to patient satisfaction, the total of highly satisfied and somewhat satisfied was 35.714 % for tinox (five cases) and 92.77% for the SLN gel formulation (13 cases). Not satisfied was64.2% for tinox marketed preparation (9 cases) and 7.14%for SLNs gel (one case). It is worth to mention that the patient satisfaction was parallel with the clinical improvement.

Cases under study

A-cases treated with oxiconazole cream of market preparation

B-cases treated with SLNs oxiconazole new gel formulation

a- Before **b-After**

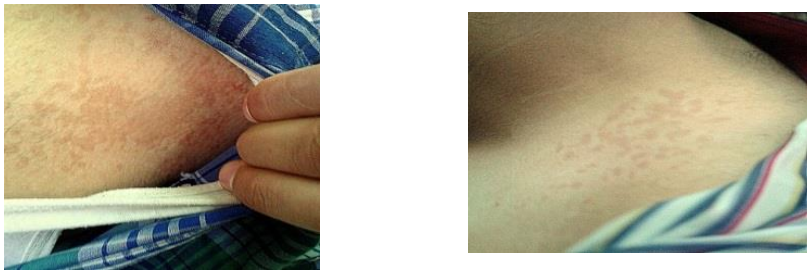
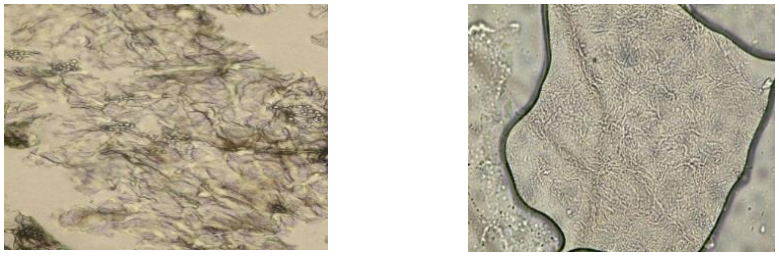


Figure (4):

a- A case of tinea vercicolor before treatment with tinox cream market preparation.

b- The case after treatment with tinox cream marketed preparation, for two weeks showing moderate improvement.



a **b**

Figure (5) a-Ascrape before treatment showing spores of tinea vercicolor b- the scrape after treatment

a- **Before** **b- After**

Figure (6): a-A case of tinea pedis before treatment with tinox., b-The case treated with tinox for four weeks showing mild improvement.

a- **Before** **b- After**

Figure (7):

a- A case of tinea vercicolor before treatment with tinox.

b- The case after treatment for 14 days showing good result.

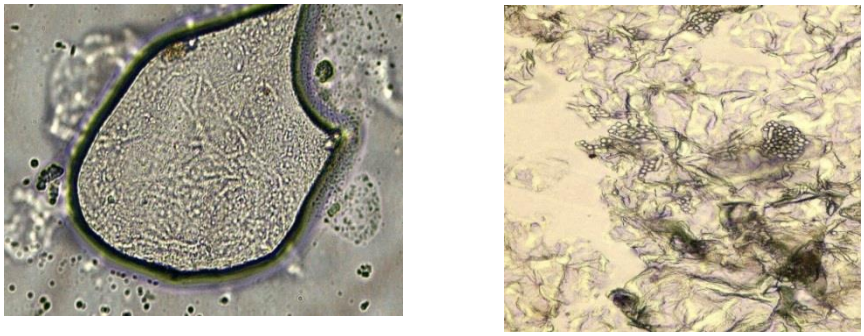


Figure (8) : a-The scrape before treatment, b- The scrape after treatment.

a- Before **b-After**

Figure (9):

a- A case of tinea vercicolor before treatment with tinox

b- The case treated for 14 days with tinox showing mild improvement.

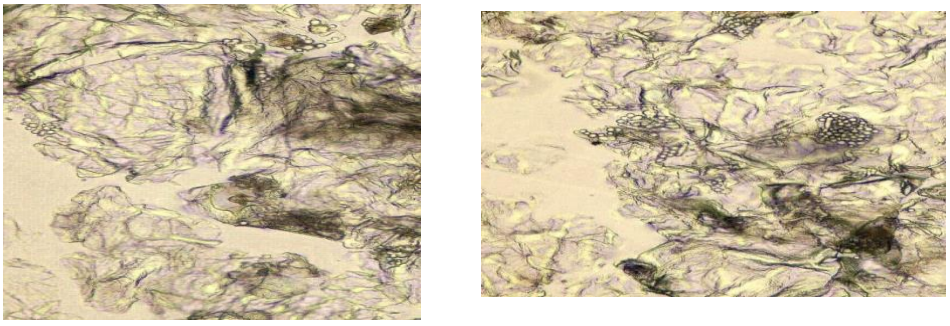


Figure (10): Scrapes of the case before treatment showing spores of tinea vercicolor.

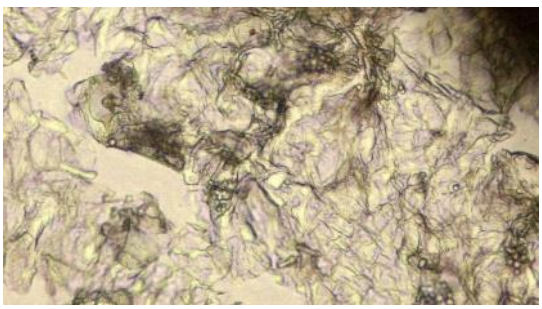
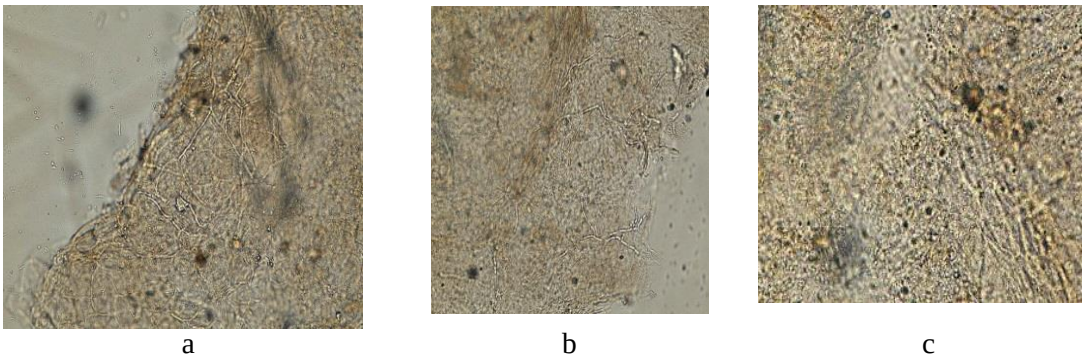


Figure (11): A scrape after treatment cases treated with new gel formulation SLNs oxiconazole

a-Before b-After

Figure (12)
a- A case of tinea cercinata before treatment with the new formulation.
b- The case after treatment with the SLNs formulation for 14 days showing good improvement

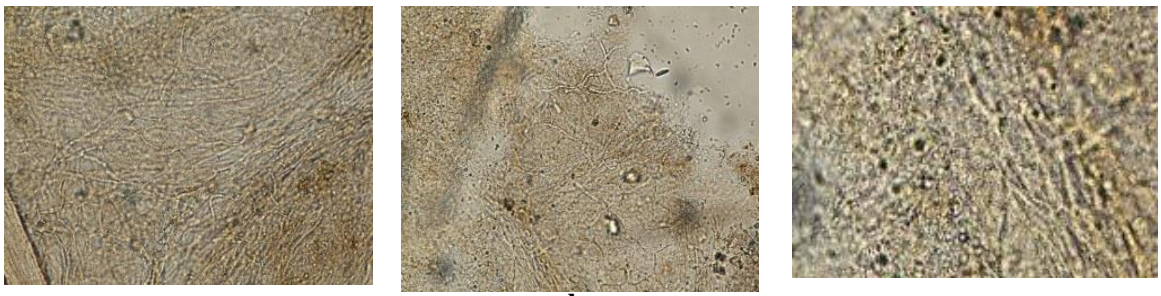


a b c

Figure (13): a and b the Scrapes before treatment showing Hyphea of tinea circinata
c- After treatment with the SLNs formulation showing no Hyphea

a-Before b-After

Figure (14):
a- A case of tinea cercinata before treatment with the SLNs formulation.
b- The case after treatment for 14 days showing excellent improvement.

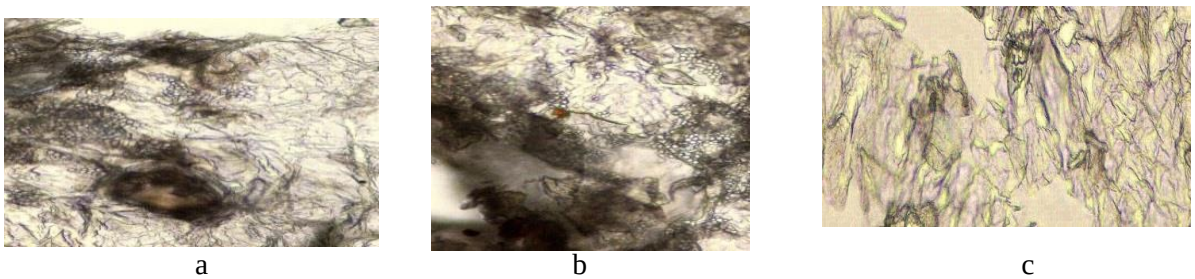


a b c

Figure (15): a and b- Scrapes before treatment showing Hyphae of tinea circinata c- scrape after treatment

a- Before **b- After**

Figure (16)
a- A case of tinea vercicolor before treatment with the SLNs formulation.
b- The case after treatment with SLNs gel formulation for 14 days showing excellent improvement.



a b c

Figure (17) : a and b Scrapes before treatment with oxiconazole SLNs gel formulation showing spores of tinea vercicolor. C-the scrap after treatment showing no spore

a- Before **b- After**

Figure (18):
a- A case of tinea pedis before treatment with the SLNs gel formulation.
b- The case after treatment for four weeks showing excellent result.

Discussion

SLNs have been used for topical application for various drugs such as imidazole antifungals (Souto et al., 2006). SLNs penetrated stratum corneum along with skin surface targeting epidermis (Chen et al., 2006) , this was useful for the improvement of the sustaining release action of oxiconazole (Jenning et al., 2000) leaded to good accumulative uptake of the drug, better delivering to the site of action, and produced higher tissue concentrations (Liu et al., 2014).

The present study has been conducted to study the clinical and histopathological changes after treatment of different cases of tinea and then comparing changes. between best formulation of the SLNs gel and the market preparation.

Cases were enrolled in this study:
Their age ranged from 12 to 50 all of them were males except one case 50% of them treated with market tinox preparation & other 50% treated with our SLNs oxiconazole new gel formulation.

The present study demonstrated 64.2% improvement of the treated cases using evaluation of by two dermatologists. Evaluating the before and after photos by quartile grading scale. The total number of excellent cases was 9(64.2%), total number of good cases was 5(35.71%). Total number of moderate cases was 4(28.57%). The number of mild cases was 10(71.34%).

Histopathological changes were performed before and after treatment. These results show that the prospected formulation (SLNs oxiconazole loaded gel) is better than the results of the tinox cream market oxiconazole . It is also worth of to mention that the patient satisfaction paroled with the clinical evaluation. To the best of our knowledge this study was the first study formulated new oxiconazole SLNs gel dosage form treating dermatological tinea species , then proved the obtained results clinically. Providing an ideal formulation for local delivery, a good retention at the application site and controlled release of the drug, application of bio-adhesive gels provide long stay adequate drug *penetration.*

Summary and conclusion
Tinea is a fungal infection with multiple causes. Several drugs can be used to treat tinea, our study proved that oxiconazole SLNs gel caused better results improvement than the market cream dosage form in the market.

The present work conducted to study the clinical improvement on volunteers of outpatient in EL-Minia dermatology hospital department. The new formulation proved that it had much better result s after evaluation by two dermatologists.

It worth mentioning to say that satisfaction of the group of patients treated with our developed formulation, was more than that of the group of patients treated with market cream oxiconazole . Anyhow side effects were tolerable in both groups, but less side effects obtained in the group treated with SLNs oxiconazole loaded gel. It can be concluded that:
The newly developed formulation treated effectively different types of tinea, with better results than marketed oxiconazole cream (Tinox).

References

1- MuhsinTM, Aubaid AH, Al-Duboon AH. Extracellular enzyme activities of dermatophytes and yeast isolates on solid media. Mycoses. 1997 Dec 1;40 (11-12):465-9.
2- Dahl, M.V. (1994) Dermatophytosis and immune response. Journal of the American Academy of Dermatology, 31(3), S34-S41.
3- Hoss D, Berke A, Kerr P, Grant-Kels J, Murphy M. Prominent papillary dermal edema in dermatophytosis (tinea corporis). J Cutan Pathol 2010; 37:237-242.
4- Vinrai Kumar et al. Robbins and Cotran: Pathologic Basis of Disease 7th eds. (1997): Julintorn Somran, MD. Pathology of Fungal Infection.
5- Dekio F, Bhatti TR, Zhang SX, et al. Positive impact of fungal histopathology on immune compromised pediatric patients with histology-proven invasive fungal infection. Am J Clin Pathol. 2015; 144:61-67.
6- Elewski, B.E. and Hazen, P.G. (1989) The superficial mycoses and the dermatophytes. Journal of the American Academy of Derma-tology, 21(4), 655-673.
7- Souto EB, Muller RH. The use of SLN and NLC as topical particulate carriers for imidazole antifungalagents. Harmazie 2006;61:431-37
8- Chen H, Chang X, Du D, Liu W, Liu J. Weng T, et al. Podophyllotoxin-loaded solid lipid nanoparticles for epidermal targeting. J Control Release 2006; 110:296-306
9- Jenning V, Schafer-Korting M, Gohla S. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release

properties. J Control Release 2000; 66:115-26

10- 11-Li RY, Wing AP, Xu JH, Xi LY, Fu MH, Zhu M, Xu ML, Li XQ, Lai W, Liu WD, Lu XY. Efficacy and safety of 1% terbinafine film-forming solution in Chinese patients with tinea pedis: A randomized, double-blind, placebo-controlled, multicenter, parallel-group study. Clinical drug investigation.2014 Mar1;34(3):223-30.

11- Hoss D, Berke A, Kerr P, Grant-Kels J, Murphy M. Prominent papillary dermal edema in dermatophytosis (tinea corporis). J Cutan Pathol 2010; 37:237-242.

12- King-man HO and Tin-sik CHENG Common Superficial fungal Infections VOL.15 NO.11 NOVEMBER 2010

13- Luilma, A.G., Sidrimb, J.J.C., Domingos, T.M., Cechinel,V.F. and Vietla, S.R. (2008) In vitro antifungal activity of dragon's blood from Croton urucurana against dermatophytes. Journal of Ethnopharmacology, 97(2), 409-412.

14- Young F (2012) Vaginal health. Nursing Standard. 16, 23, 47-52.

15- Naveed, A.M., Naeem, R. and Nasiruddin (2009) Nondermatophyte moulds and yeasts as causative agents in Onychomycosis. Journal of Pakistan Association of Dermatologists, 19(2), 74-78.

16- Pinto, E., Pina-Vaz, C., Salgueiro, L., Goncalves, M.J.,Costa-de-Oliveira, S., Carlos, C., Palmeira, A., Rodrigues, A. and Martinez-de-Oliveira, J. (2006) Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species. Journal of Medical Microbiology, 55(10), 1367-1373.