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Clinical and Histopathological Study on Dermatophytes Infections Caused by *Trichophyton mentagrophytes* Using Animal Model

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Abstract

Background: dermatophytes are parasitic fungi that infect skin, hair and nails of both humans and animals, they are the primary causative agent of dermatophytosis, a major public health concern in some geographic regions. **Objective** : To study the pathogenesis of dermatophytes infections and the antifungal activity of essential oil extract of *Lavandula intermedia***. Methods**: Zoophilic strain of *Ttichophyton mentagrophytes* isolated from dermatophytic patients infected with tinea corporis inoculated in the back of the rabbits using the abrasion (non-occlusion) method. **Results**: Twenty animals were categorized into two groups as follows: control groups involved 10 animals, 5 animals were subjected to abrasion only, and other 5 animals were subjected to infection (after abrasion).While the other ten animals represented by study groups which involved 5 animals were exposed to infection and treated in 10^{th} day of infection with 1% terbinafine and the other 5 animals were treated with 10% lavender essential oil extract(it gave 5%and 10% MIC and MFC respectively). The treated animals showed healing in 12-14 day, while not treated animals showed spontaneous healing in 35-40 day. **Conclusion**: Rabbit model was found to be useful in the primary screening and evaluation of the anti-dermatophytic efficacy of topical formulations of antifungal agents. *T. mentagrophytes* produced infection in rabbit's skin in $2X10^6$ cells /ml. Lavender essential oil could be used as alternative antifungal agents in treatment of dermatophytosis in chronic diseases ,immunocompromised and immunosuppressed drugs therapy patients.

Key words : Dermatophytosis**,** *T. mentagrophytes* animal model

Introduction

Dermatophytoses are one of the most frequent skin diseases of human, pets and livestock [Tsang *et al.,* 1996]. The disease is widely distributed all over the world with various degrees and more common in men than in women. There are three genera of mould that cause dermatophytosis. These are *Epidermophyton*, *Trichophyton* and *Microsporum*. Contagiousness among animal communities, high cost of treatment, difficulty of control and the public health consequences explain their great importance [Chermette *et al.,* 2008].

Dermatophytes are a specialized group of fungi able to cause zoonotic superficial infections as a consequence of invading keratinized tissues of skin, hair and nails. A few antifungal compounds are available and licensed for use in human being treatment, the use of systemic drugs is limited to treat man due to their high toxicity and problems of residues in products intended for human consumption [Araujo *et al.,* 2009]. Different treatments have been recommended to control dermatophytes. In general, pharmacological treatment option includes antifungal agents , but recently the use of some natural plant products emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components are documented since the late 19th century [Martin and Ernst,2003; Aly and Bafiel, 2008].

Materials and Methods:

Patients: A total of 254 specimens were collected from patients with dermatophytosis (ring worm) and they were clinically diagnosed by Dermatologist , from November 2013 to October 2014. Out of 254 dermatophytic patients,213(83.86%) specimens of dermatophytes infections were positive in direct microscopic examination and culture , and used in phenotypic diagnosis. Tinea corporis was the highest infection in 106 (41.73%) patients and *Ttichophyton rubrum* showed 36 (16.90%) isolates followed by *Ttichophyton mentagrophytes* 31 (14.55%) , and they were much more common in males than in females.

T. mentagrophytes isolates were diagnosed using direct microscopic examination, dermatophytes test medium and physiological tests [Burns *et a.l*, 2010]. The inoculants preparations included measurement the turbidity of the supernatants and measured spectrophotometrically at a wavelength of 530 nm and transmission was adjusted in order to have the size of fungal units equal to $0.5X\ 10^4$-$5X\ 10^4$ for the *T. rubrum* and *T. mentagrophytes*. The prepared inoculates size used in the skin of the animals were ($2X\ 10^3$,$2X\ 10^4$, $2X\ 10^5$ and $2X\ 10^6$) cells/ml [Espinel-Ingroff *et al*., 1991].

Lavender oils extract (Prepared by the manufacture- Brazil),then the following dilutions were prepared :

(2.5%, 5%, 10%, 20% and 40%) in 10% dimethyl sulphoxide [Darwish *et al.* ,2010]. Antifungal activity of essential oil of lavender was determined using the CLSI broth dilution method (M38-A) [Espinel-Ingroff *et al.,* 1991].1% terbinafine spray (Novartis) gave fungicidal effect in this concentration.The abraded areas of skin in the study groups were inoculated by applying a volume of 50–100 µL of $2X10^6$ cells/ml zoophilic strain of *T. mentagrophytes*.

RESULTS AND DISCUSSION:

Experimental mycotic infections were induced in domestic rabbits and the first clinical manifestations of mycosis infections were found on the 4^{th}-5^{th} day after inoculation. In the examined infected tissue and hair follicles, we found in the shed layers of the stratum corneum PAS positive septate fibers. In the corium a mixed inflammatory infiltration was found a predominance of histiocytes and polymorphonuclear leukocytes. Domestic rabbits are useful animals for inducing experimental infection with the dermatophytes.

Infected animals treated with 1% terbinafine and 10%lavender essential oil (it gave 5%and 10% MIC and MFC respectively), once daily for 12-14 days, starting from10^{th} day of infection (with erosion of epidermis) have shown recovery from infection which manifested by well-developed epithelial tissue similar to the control group. The main histopathological recovery manifestation was disappearing of the erosion in epidermis and no recognizable morphological changes were found after passaging the dermatophyte via skin of the animals after treatment , except mild lymphocytes infiltration. The spontaneous healing of infected skin left without treatment in control group was manifested by disappearing of the erosion with mild lymphocytes infiltration within 35-40 day .

Figure-1-(normal keratinocytes layer) refers to normal rabbit skin without infection and used as control to compare the histopathological changes in infected tissues with and without treatment. All infected animals with *T. mentagrophytes* showed pathological changes with adherence of fungus to keratinocytes, through the stratum granulosum of the epidermis. In this period of infection there was a hyperkeratosis ,thickening of epidermis with hair follicle plugging in addition to keratinized squamous epithelial lining with underlying moderate periappendageal tissue and perivascular chronic inflammatory cells infiltration (lymphocytes) as in figure 2. There are fibroblastic dermal stroma with moderate periappendageal chronic inflammatory cells infiltration (lymphocytes) (Figure 3).

In 8-10 days of induced infection there is keratinized squamous epithelial lining with focal area of surface erosion and underlying moderate periappendageal tissue chronic inflammatory cells infiltration (lymphocytes),as in figure 4. The epidermis infiltrated with variable fungal septate hyphae in size in the surface of the squamous epithelium(Figure 5)**.**

Dermatophytes gain entry and establish themselves in the cornified layers of traumatized or macerated skin and its integument and multiply by producing keratinase to metabolize the insoluble, tough fibrous protein. The reason why these agents spread no deeper is not known, but it has been speculated that factors such as cell-mediated immunity and the presence of transferrin in serum inhibit fungal propagation to the deeper tissue layers and systemic disease does not occur. Some dermatophytes have evolved a commensal relationship with the host and are isolated from skin in the absence of disease. Dermatophytes do not cause systemic disease [Fujita, 1997 ; Nir *et al*. 2003].

Despite the widespread use of EO by humans in recent studies and the large evidence of their potential as complementary or alternative options for prophylaxis and treatments of dermatophytosis, their exact mechanism of action, remains poorly understood. The antidermatophytic activity of various medicinal plants may be due to the presence of secondary metabolites such as coumarin, quinones, flavonoids, phenols and tannins and their glucosides [Lima *et al*. 2011;Rios & Recio , 2005].

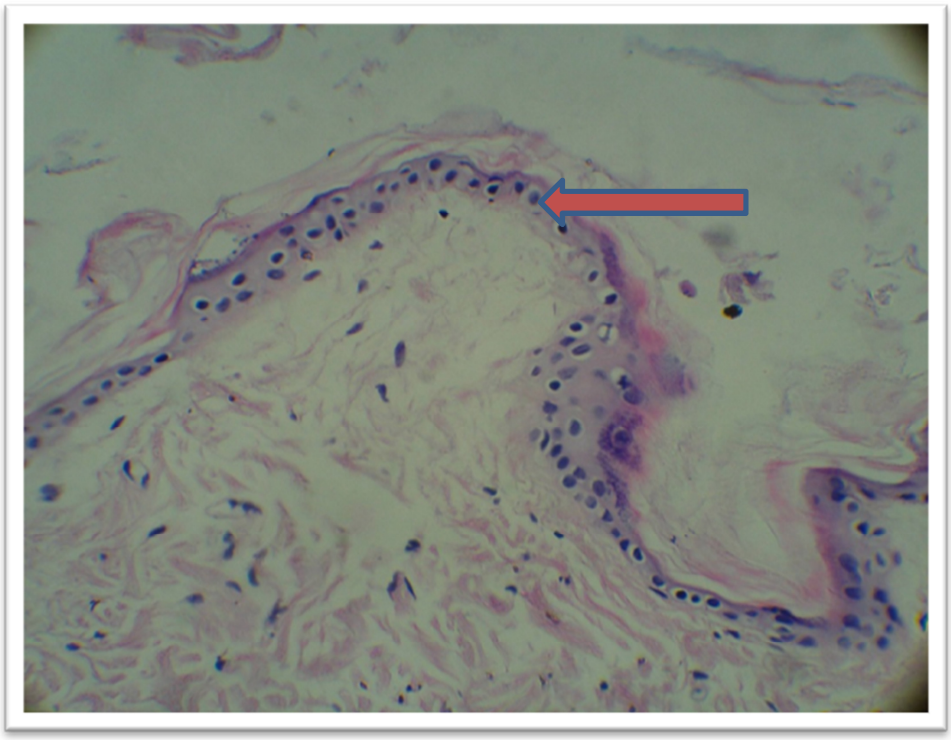

Figure-1- The normal rabbit skin-keratinocyte layer (stained with H&E, 40X)

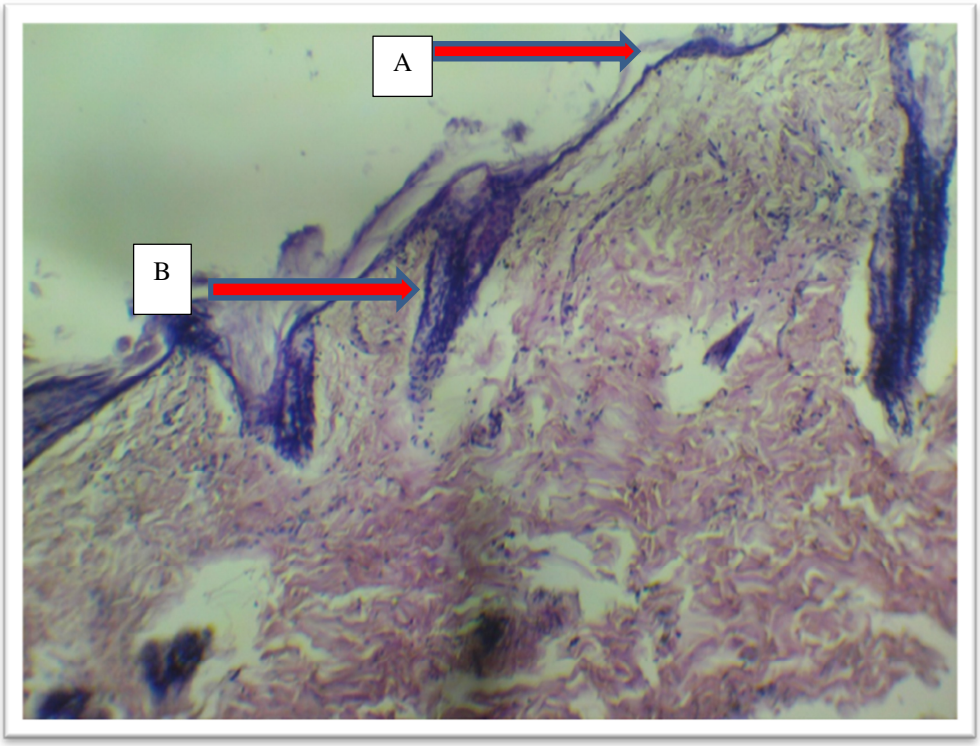

Figure-2- The bland looking (A) Hyperkeratosis , thickening of epidermis with (B) hair follicle plugging in 4-5 days (stained with H&E,10 X)

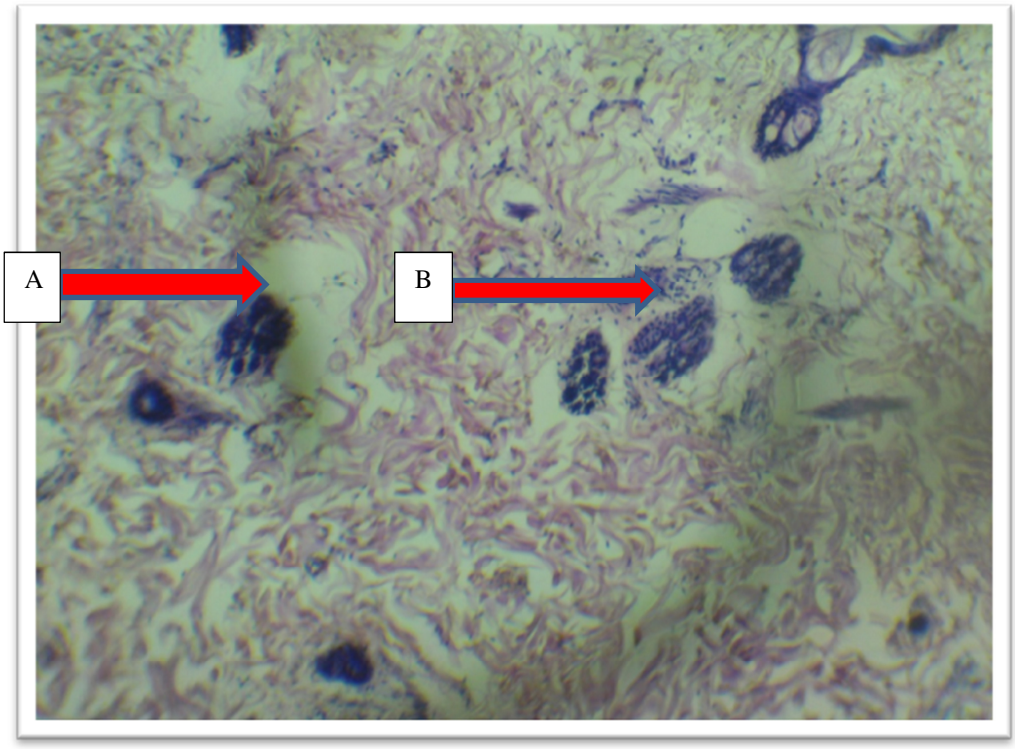

Figure-3- The bland looking fibroblastic dermal stroma(A) with lymphocytes infiltration(B) in 4-5 day (stained with H&E,40 X)

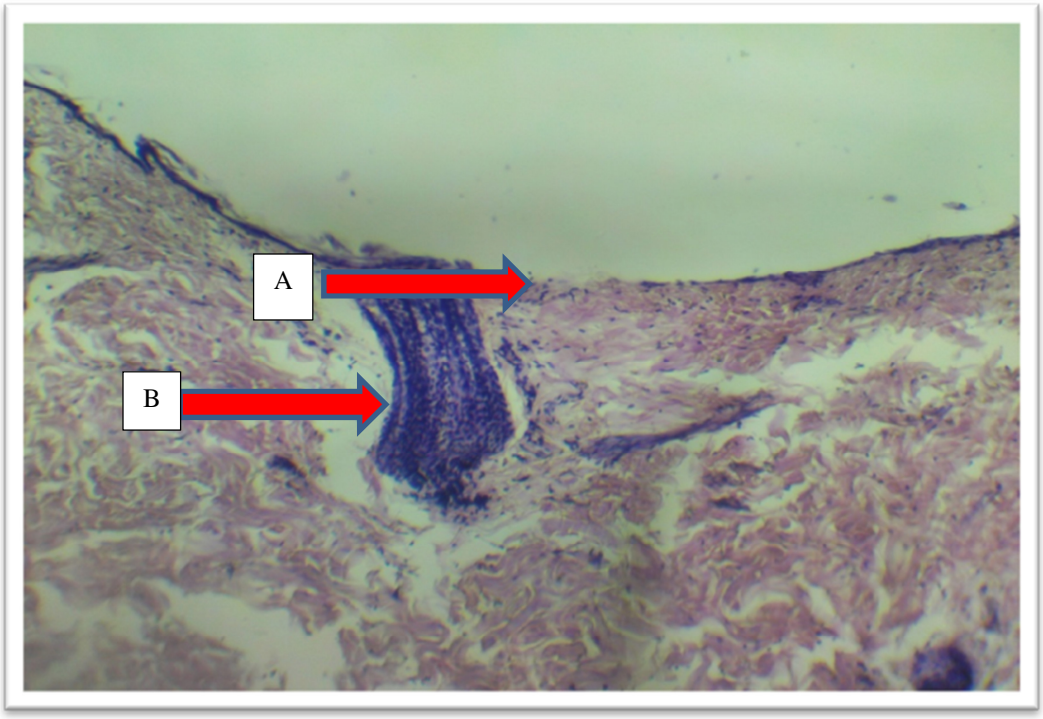

Figure-4- The bland looking with A- surface erosion and B- lymphocytes infiltration in 8-10 days (stained with H&E,10 X)

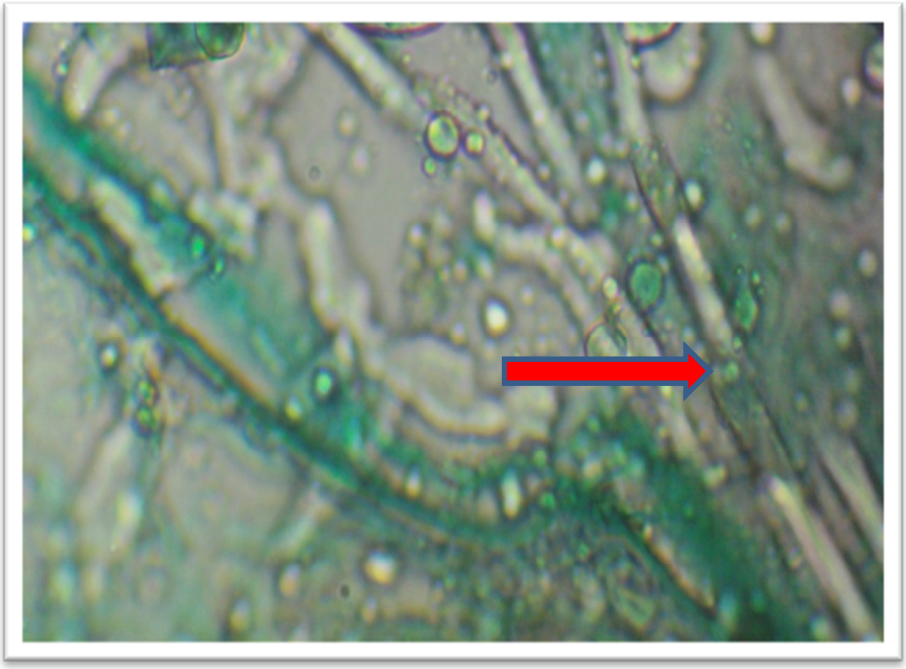

Figure -5- Proliferation of septate hyphae of *T. mentagrophytes* in epidermis in 8-10 days (stained with PAS,40X).

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