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In Vitro Susceptibility Testing of Dermatophytes on Urea

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ABSTRACT

Objective: Urea is commonly used in many anti fungal drugs to improve the penetration of drug in tinea infections. This study was organized to study the susceptibility of dermatophyte species on urea in laboratory. Method: Minimum inhibitory concentration (MIC) of urea was determined for 34 samples of dermatophytes cultured in Sabouraud dextrose broth and agar which had different concentrations of urea (5%- 30%) added. Minimum inhibitory concentration was checked by absence of fungal growth. Results: Majority of the isolates showed significant growth on 10% concentration, followed by weak growth on 20% concentration. 11 isolates of Trichophyton rubrum and 9 isolates of Trichophyton mentagrophytes required above 30% concentration for complete inhibition. No growth was recorded on 30% concentration of urea. Results showed that only in higher concentration of urea may kill the dermatophytic strains. Conclusion: Results reveal inhibitory potential of urea on dermatophyte ssuggests it to be used as an adjuvant in topical ointments and sprays.

Keywords: *Tinea. Dermatophytosis. Urea. Trichophyton rubrum, Trichophyton mentagrophytes.*

INTRODUCTION*

Dermatophytosis is a skin infection caused by invasion of keratinized tissues of skin, nail and hair of both animals and humans. Among all the human fungal infections up to 30% are dermatophytic infections [1]. Trichophyton rubrum has been found to be most prevalent specie of dermatophytes in human beings causing various tinea infections e.g. tinea pedis, tinea corporis, tinea capitis, tinea manuum, tinea unguium etc. Usually dermatophytosis is treated with topical azoles(clotrimazole, econazole, ketoconazole, miconazole, oxiconazole and sulconazole) and allylamine (Naftifine and terbinafine) classes of antifungal drugs [2-3].Some polyenes like amphotericin B and nystatin also are least effective in dermatophytosis. Also some antifungal compounds are not categorized in these two classes such as tolnaftate (tinactin), haloprogin (halotex), butenafine (mentax) and ciclopirox(loprox) [4].These drugs, however affect liver and digestion[5].Usually these drugs are available over the counter and concentration of each dose depends upon the stage of infection, site of lesion and etiology. Tinea pedis and onychomycosis takes more than 3 weeks for treatment. To make the anti fungal drug more effective in less time, many associated chemicals are incorporated. Bifonazole with added urea has proved to be helpful in treatment [6].Urea along with butenafine and lanoconazole has showed early healing of the lesions too[7].Researchers have proved urea to be a good adjuvant in anti fungal drugs for better penetration and quick healing but anti fungal potential of urea itself is not much explained. This study was aimed to check the anti fungal potential of urea on clinical samples of dermatophytes. The aim of this study was to evaluate in vitro the inhibitory activity of urea on dermatophytes samples.

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Sampling

34 clinical samples of dermatophytes were obtained from patients with tinea infections, confirmed by the consultant dermatologist.

Identification and confirmation

Initial examination of all the samples was performed with 20% KOH solution and microscopy. Positive samples were than confirmed by culture testing on Sabroud dextrose gar (SDA) and dermatophyte test medium at 25°C. Macroscopic study of the cultures confirmed samples in 2-3 weeks followed by microscopic examination of the slides stained with lactophenol cotton blue.

Urea susceptibility

To test the in vitro urea susceptibility, only the confirmed positive samples were cultured in 18 x 180mm tubes with 10 mL of Sabouraud-dextrose broth and petri dishes with SDA agar incorporated with different concentrations of urea e.g. 10%,20% and 30%. Incubated on 30°C. Tubes and petri dishes without added urea were used as control.

Table 1. Growth index of Dermatophyte isolates grown in different concentrations of urea

Test strains (Dermatophytes	Concentration of Urea				
	Control (C)	10%	20%	30%	TOTAL
Trichophyton rubrum (S.4)	+++	+++	+	-	
Trichophyton rubrum (S.11)	+++	+++	-	-	
Trichophyton rubrum (S.12)	+++	+++	-	-	
Trichophyton rubrum (S.38)	+++	+++	++	-	
Trichophyton rubrum (S.45)	+++	+++	-	-	
Trichophyton rubrum (S.46)	+++	+++	++	-	11
Trichophyton rubrum (S.57)	+++	+++	-	-	
Trichophyton rubrum (S. 69)	+++	+++	+	-	
Trichophyton rubrum (S.73)	+++	+++	-	-	
Trichophyton rubrum (S.95)	+++	+++	+	-	
Trichophyton rubrum (S. 109)	+++	+++	+	-	
Trichophyton mentagrophytes (S.15)	+++	++	+	-	
Trichophyton mentagrophytes (S.26)	+++	+	-	-	
Trichophyton mentagrophytes (S.37)	+++	+++	+	-	
Trichophyton mentagrophytes (S.47)	+++	+	+	-	
Trichophyton mentagrophytes (S.55)	+++	+	-	-	09
Trichophyton mentagrophytes (S.96)	+++	+	-	-	
Trichophyton mentagrophytes (S.97)	+++	++	+	-	
Trichophyton mentagrophytes (S.103)	+++	+	-	-	
Trichophyton mentagrophytes (S.110)	+++	+	+	-	

Test strains (Dermatophytes	Concentration of Urea				
	Control (C)	10%	20%	30%	TOTAL
Trichophyton tonsurans (S.51)	+++	+	-	-	05
Trichophyton tonsurans (S.51)	+++	-	-	-	
Trichophyton tonsurans (S.53)	+++	-	-	-	
Trichophyton tonsurans (S.70)	+++	-	-	-	
Trichophyton tonsurans (S.114)	+++	-	-	-	
Trichophytonerinacei (S.22)	+++	+	-	-	02
Trichophytonerinacei (S.100)	+++	+	-	-	*03*
Microsporum canis (S.5)	+++	++	+	-	07
Microsporum canis (S.7)	+++	+	+	-	
Microsporum canis (S.36)	+++	++	-	-	
Microsporum canis (S.64)	+++	++	-	-	
Microsporum canis (S.90)	+++	+++	+	-	
Microsporum canis (S.101)	+++	++	-	-	
Microsporum canis (S.114)	+++	+++	+	-	

**S- Strain number

Minimal inhibitory concentration (MIC) determination

After 1 week of culture, control tube and petri dish developed growth while absence of growth was declared as negative result. The MIC was determined on the basis of growth rate which are as follows: C = Control (plentiful growth); (-) = no growth; (+) = weak growth; (++) = average growth; (+++) = plentiful growth;

RESULTS AND DISCUSSION

Among 34 positive samples, strains were identified as *Trichophyton rubrum*(11), *Trichophyton tonsurans* (5), *Trichophyton mentagrophytes* (9), *Trichophytonerinacei* (2) and *Microsporum canis* (7) (Table 1). 10% concentration didn't affect any culture, however only weak growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes* was observed on 20% urea concentration. 30% urea showed absolutely no growth.

To treat dermatophytosis, usually topical anti fungal drugs are prescribed by the physician which could be in the form of ointment, spray, gel, lotion or cream. However the choice and concentration of drug depends upon etiology and extent of lesions. In some cases, previous medical and family history also helps to determine the right choice of drug because these infections are highly contagious. In earlier day griseofulvine were used to treat tinea infections but with time and advanced research, many other drugs such as terbinafine, ketoconazole and itraconazole have been used widely for effective disease management. In comparison to previously used drugs, these antifungal agents have less risk of side effects and affiliated secondary infections (hepatic and gastric). One of the many reasons behind this is the use of urea and other penetrating agents which aid in quick healing of the lesions especially in onychomycosis. A previous study on 23 individuals showed that propyleneglycol with added urea and lactic acid shows good results in 21 patients for onychomycosis treatment[8]. In another study, 12 patients showed 100% effective treatment by using ciclopirox cream

with 40% urea. [9].Trichophyton rubrum was observed as the most common specie followed by *T. mentagrophytes*, *T tonsurans*, *T erinacei* and *Microsporum canis*. No epidermophyton specie was found. The current study showed that mostly species were not sensitive to urea on 10% concentration. Some species of *T rubrum* and *T. tonsurans* showed weak growth on 20% concentration which was completely absent in 30% concentration. However a previous study antimycotic activity of 5% urea concentration against *T. rubrum* isolates. No growth on higher concentration (30%) was observed. These results show the inhibitory potential of urea against dermatophytes signifying the addition of urea into topical ointments. Further studies can specify the exact concentration to inhibit each specie separately [10].

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