

Possible Role of Superoxidised Water in the Treatment of Trichophyton Infections: An *In vitro* Study

Munesh K. Gupta, Soni Sinha, Nidhi Pandey, Pradyot Prakash and Ragini Tilak*

Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221 005, India.

Abstract

Superficial fungal infections are the common health seeking problem. These fungal infections are caused by three genera of Dermatophytes, naming *Trichophyton*, *Microsporum*, *Epidermophyton*. Among these three, *Trichophyton* spp. causes most of these lesions. *Trichophyton* spp. primarily infect the moist areas of human bodies. Different groups of antifungal agents are used in the treatment of these fungal infections. Despite availability of antifungal treatment, an increasing number of recurrent superficial fungal infections have been reported. We assessed the antidermatophytic activity of superoxidised water; a newer disinfectant, against *Trichophyton* spp. isolated from the cutaneous fungal infections. A total of 79 isolates of *Trichophyton* species including *mentagrophytes* (n=43) and *rubrum* (n=32) were exposed to freshly generated superoxidised water. A 50µl of 0.1 OD of the *Trichophyton* conidia were exposed to 500 µl of undiluted, 5 & 10 times diluted superoxidised water for one minute. A 10µl of these exposed conidia were inoculated on SDA. A 10µl of conidia (no exposure to superoxidised water) was also inoculated on SDA tube. Tubes were incubated at 28°C in BOD incubator. No growth was observed in tubes having exposed conidia only to undiluted Superoxidised water, even after the 28 days of continuous incubation at 28°C. Growth within 7days was observed in the tubes having unexposed conidia and exposed to dilute Superoxidised water. Undiluted superoxidised water showed the *In vitro* inhibition on *Trichophyton mentagrophytes* and *Trichophyton rubrum* which might be useful in future in the treatment of these infections.

Keyword: Dermatophyte, Superoxidised water, Trichophyton, Disinfection, Skin, Sterisol.

*Correspondence: raginijain29@rediffmail.com; 91-9415812317

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INTRODUCTION

Dermatophyte infections of the skin, hair, and nail are the common cutaneous fungal infection¹. These dermatophytes primarily infect the moist areas of the human body such as groin, armpits, scalp, sub-mammary area where they result in a different clinical manifestation as *Tinea cruris*, *Tinea capitis*, *Tinea pedis* etc^{2,3}. Three genera of dermatophyte; *Trichophyton*, *Microsporum*, and *Epidermophyton*, cause these fungal infections. *Trichophyton* spp. infects the skin, hair, and nail whereas *Microsporum* spp. infect the skin & hair. *Epidermophyton* sp. infects the skin and nail. Most of the human dermatophyte infections have been reported due to *Trichophyton mentagrophytes* and *T.rubrum*².

A different group of the antifungal agents is used in the treatment of these dermatophyte infections. These antifungal drugs either inhibit the ergosterol synthesis, the major component of the fungal cell wall or inhibit the functional activity of the microtubules^{4,5,6}. Although these antifungal drugs are quite effective in the treatment of these fungal infections but in the present decade, a large number of non-responding dermatophyte infections has been reported from different regions of India. Thus there is a need for a newer effective treatment for these infections.

Superoxidised water (SOW) is a newly disinfectant which is generated by the electro-chemical activation. It is a mixture of different oxidizing agents containing hypochlorous acid and sodium hypochlorite with pH of 5.0-6.5. Superoxidised water is bactericidal, fungicidal, virucidal and sporicidal solution⁷. Free radical, produced during the oxidation-reduction process,

denature the protein of bacterial cell wall. It also impairs the solute transport as well as the salt balance, resulting in the cell lysis⁸. Potent disinfectant role of superoxidised water against bacteria and molds has been reported by different authors but, Best of our knowledge, no in vitro study has been conducted against the dermatophytes⁹⁻¹¹. Against this background, we assessed the in-vitro activity of superoxidised water (SOW) against the dermatophytes isolated from skin lesions.

MATERIALS AND METHODS

Disinfectant property of freshly generated SOW (sterisol), generated by Steri-Gen® disinfectant generating system, was assessed against 79 *Trichophyton* species strains isolated from cutaneous lesions. These *Trichophyton* spp. were subcultured on potato dextrose agar and kept at 35°C for 7 days. The conidia were harvested with the tween 20 & normal saline and 0.1OD conidial suspension was prepared. 50µl of this conidial suspension was exposed to the 500µl of the freshly generated SOW, and 5 & 10 times diluted SOW. After exposure of one minute, a 10µl suspension was inoculated on SDA tube. A 10µl of the unexposed conidial suspension was also inoculated on the SDA tube as a growth control. These tubes were incubated at 28°C and observed for the growth.

RESULTS

A total of 79 dermatophyte strains, including 43 strains of *T. mentagrophytes*, 32 strains of *T. rubrum*, and 4 *Trichophyton* spp. were included in the present study. Both the SDA

Table1. Effect of SOW on different *Trichophyton* species isolated from superficial fungal infections

Dermatophytes	Freshly generated SOW			
	Control	Exposure to undiluted SOW	Exposure to 5 times diluted SOW	Exposure to 10 times diluted SOW
<i>T. mentagrophytes</i> (n=43)	+	-	+	+
<i>T.rubrum</i> (n=32)	+	-	+	+
<i>Trichophyton</i> spp. (n=4)	+	-	+	+

Note: Growth inhibition (-); No Growth inhibition (+)

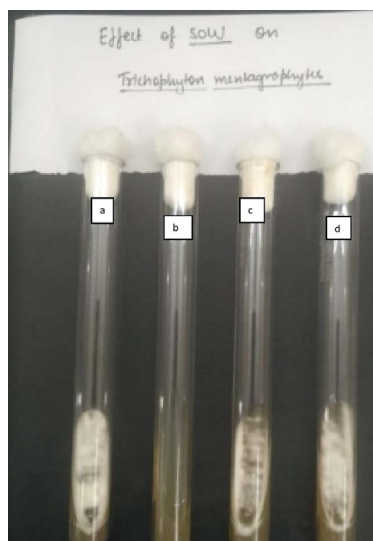


Fig. 1. Effect of different dilution of SOW on *T.mentagrophyte*. (a): control tube; no exposure to SOW, (b) no growth after exposure to undiluted SOW, (c) growth after exposure to 5 times diluted SOW, (d) growth after exposure to 10 times diluted SOW

tubes containing 10 μ l of exposed and non exposed conidia were examined for the growth. The tubes were read daily in the 1st week, on every 2nd day in the second week, every 3rd day in the third week and final results were observed at 28 days. In control tubes, growth was observed between 5-10 days whereas no growth was observed in SDA tubes, containing exposed conidia to undiluted superoxidised water, even after 28 days of continuous incubation. Complete inhibition of all *Trichophyton* spp. was only observed in the tube containing conidia suspension, exposed to undiluted SOW whereas in tubes having diluted SOW exposed conidia, growth was observed. (Table 1, Fig. 1)

DISCUSSION

Trichophyton infections are the major superficial fungal infection to which a dermatologist encounters most frequently in their OPDs. These *Trichophyton* spp. primarily infect the moist areas of the human body where moisture provides the favorable environment for the growth of these dermatophytes². These *Trichophyton* spp. infect most superficial layers of skin, hair, and nail where they release the keratinase enzyme which helps in its invasion.¹² In skin lesion, caused by *Trichophyton* spp., a red erythematous lesion

with central clearing is observed which constantly increase in size. Itching at the margin of the lesion is a cardinal feature of such fungal infections. Infected hair becomes dry, atrophied and may result in alopecia. The infected nail becomes brittle, dried and discolored.

Treatment of these dermatophyte infections is very difficult and time taking. For the treatment of these dermatophyte infections, oral or topical antifungal agents either in the form of a cream or an ointment are used. Most of these antifungal agents are used topically whereas other drugs as itraconazole, griseofulvin and terbinafine are used orally as these drugs concentrate in the skin^{13,14}.

Superoxidised water (SOW) is a newer disinfectant which has the ability to kill the microbes within the seconds of exposure. In previous studies, its efficacy has been assessed against the different bacterial and fungal pathogens including yeast and filamentous fungi⁹. In this present study, we assessed the efficacy of the freshly generated SOW against the *Trichophyton* spp. isolated from different clinical samples where complete growth inhibition was observed after the exposure to the only undiluted SOW.

Superoxidised water (SOW), FDA approved product, is generated by oxidation-reduction reaction where normal saline is passed over the titanium coated electrode at a current of 6-9amp. This SOW contains a mixture of oxidizing substance including hypochlorous and sodium hypochlorite in equilibrium. This solution with a pH of 5-6, is non-hazardous to the skin, non-irritating to the eyes. These all feature favor its use in the treatment of cutaneous fungal infections^{7,15}.

SOW is being frequently used in the dressing of the wound where it has been shown to enhance the granulation of the infected wound. In a study, published by Kapur et al, reported that use of the SOW resulted in early healing with reduced inflammation⁸. Similar results on wound healing have been reported by Sharma et al¹⁶. A meta-analytic study by Eftekharizadeh et al also reported the SOW as a safe, effective, cost-effective irrigation and cleaning agent¹⁷. Although this study provides a satisfactory in-vitro activity but also the results should be evaluated in vivo which necessitate a clinical trial.

CONCLUSION

In vitro complete inhibition of the *Trichophyton* spp., by the freshly generated superoxidised water, might open a new era in the treatment of superficial fungal infection as it is economical, safe, non-irritating, non-allergic to the skin.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All the generated/analyzed dataset has been included in manuscript.

ETHICAL STATEMENT

Not applicable.

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