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Antimicrobial effects of Thyme Essential Oil (*Thymus vulgaris*) in combination with Sodium Hypochlorite (NaOCl)

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INTRODUCTION

Gram-positive bacteria *Streptococcus mutans* and *Staphylococcus aureus* are important pathogens responsible for infections associated with dental caries and other medical implants. In according to Italian Society of Endodontics (SIE) protocol, the use of a 5% NaOCl and 80% ethanol disinfectant solution is recommended in root canal during the endodontic treatments and for root canal disinfection. The use of this solution could cause complications and hypersensitivity reactions (1, 2). Several studies indicate that essential oils (EOs) could be used as novel endodontic materials (e.g. irrigants and medicaments) (3).

The aim of this work was to evaluate the use of Thyme Essential Oil (TEO, *Thymus vulgaris*) in synergistic combinations with NaOCl in order to reduce the concentrations of NaOCl in oral therapies, to improve the antiseptic efficacy, and to reduce side effects.

MATERIALS AND METHODS

The experiment was performed using the microbroth dilution method (4) (Figure 1) to determine the antimicrobial activity of the solution (9,28mg/mL TEO and 1% NaOCl), in the presence of organic material (6% sheep blood) against reference strains, *S. mutans* (ATCC70061) and *S. aureus* (ATCC43300). The antimicrobial activity was evaluated after 1min, 3min, 5min of contact with both bacterial strains at room temperature conditions, as previously reported (5). Aliquots of each suspension were diluted (ten-fold dilutions), cultured into Plate Count Agar (PCA) plates and incubated at 37°C for 24h and 48h to monitor bacterial growth. Each test was performed in triplicate. The tests conducted on *S. mutans* were carried out by incubating the strain at 37°C in an atmosphere with % CO_2.
Statistical analyses were performed using GraphPad Prism v8.1.2 (Dotmatics, Boston, USA). A significance level of $p < 0.05$ was chosen to determine statistical significance.

Figure 1. Microbroth dilution method for valuation of the antibacterial activity.

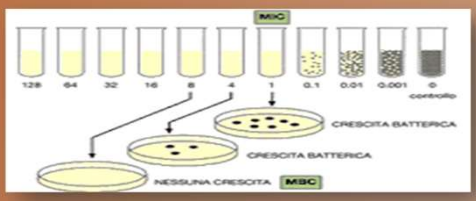

Figure 2. Antibacterial activity of TEO and NaOCl in the presence of 6% erythrocytes against ATCC bacterial strains.

RESULTS

The combination of TEO and NaOCl has demonstrated total effectiveness in inhibiting microbial growth even in the presence of organic material. The solution of NaOCl in combination with TEO would allow to reduce the concentration of NaOCl currently used for dental procedures by at least 4 times (CFU= 0.00 for all tested strains, $p < 0.05$). The results obtained at 24h are comparable to those obtained at 48h in all tests.

DISCUSSION AND CONCLUSION

Our data confirm the efficacy of TEO as antimicrobial compound with possible applications as irrigant in endodontic treatments. TEO showed also a high biocompatibility and no interference when mixed with NaOCl was observed. In addition, the combination of TEO and NaOCl at low concentrations totally inhibited microbial growth even in the presence of organic material. The combination with TEO could allow reduced NaOCl concentrations, with the possibility for clinicians to increase safety in irrigation protocols avoiding or reducing the side effect of accidental NaOCl extrusion on the periapical tissues.

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