Tea Tree Oil: Anew Antifungal Agents Against Candida Albicans Cells on Heat-Cured Acrylic Resin Denture Base Material. An in vitro study.

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ABSTRACT

Aims: In this research, the antifungal effect of tea tree oil on Candida that colonizes the heat cured acrylic resin denture base material was evaluated. Materials and Methods: In this clinical trial, 24 patients wearing upper complete dentures for more than one year were selected randomly from patients who were attending private dental clinics at Mosul city. Swabs from inner surface of upper complete dentures were taken for each patient then Candida albican were isolated, after that these dentures were immersed in the antifungal drugs (tea tree oil solution 15% and fluconazole solution 64 µg/ml) at 24 h and 48 hr. After that another swap was taken for each sample of complete denture to determine the antifungal effects of these agents. Results: The results of this study revealed that tea tree oil solution 15% and fluconazole solution 64 µg/ml had a significant antifungal effects, although tea tree solution effect was significantly less than fluconazole effects compared to control group [Candida albican culture (before immersion)]. Conclusions: The use of therapeutically active compounds extracted from plants like tea tree oil will offers a natural alternative with excellent antifungal effect and can be employed to reduce number of Candida albicans cells including those adhered to acrylic denture base material. This approach to fungal control is believed to be a practical for home care of dentures. **Key words:** Tea tree oil, Fluconazole, complete dentures, Candida albicans.

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INTRODUCTION

Candidiasis is by far the most common type of oral fungal infection⁽¹⁾ Which can be associated with the formation of biofilms on bioprosthetic surfaces (2) and Candida albicans colonization on denture material is widely recognized as the main cause for the developing of denture stomatitis in denture user. (3,4) The use of antifungal agents has been showen to play an important role in the control of denture plaque and prevent the Candida-associated denture stomatitis. (5)

Tea tree oil (Melaleuca alternifolia) Figure (1) is a new multi-purpose herb and its properties are contained in the oils of its leaves which contain 1.8% oil that can be obtained by steam distillation. This oil contain more than 48 compounds (6) and the one that is related to its effectiveness as antifungal agent is terpinen-4-ol which is the main active ingredient ⁽⁷⁾that exert its antifungal actions by altering membrane properties and compromising membrane associated functions. (8)



Figure (1): Tea tree oil Solution

The aims of this study are to evaluate the antifungal effect of tea tree oil on surface of heat-cured acrylic resin denture base material and to compare its effect with the effect of fluconazole solution which is effective antifungal drug especially in persistent or locally invasive

candida albicans infections. (1)

MATERIAL AND METHODS

The methodology in this study was carried out by isolation of *Candida albicans* this is done by taking swabs from the inner surface of upper dentures for 24 patients selected randomly, those were wearing their dentures for more than one year⁽⁹⁾ and they were attending private dental clinics at Mosul city.

Swabs were cultured on sabouraud dextrose medium at 37 °C for 24–48 hr ⁽¹⁰⁾ and identified according to culture characteristics, microscopic appearance and germ tube formation. ⁽¹¹⁾

The antifungal drugs solutions were prepared according to their concentration, the tea tree oil solution was purchased from local market in a concentration of 15% from the Australian tea tree industry association which is the source of this material in the market, while the fluconazole solution in a concentration of 64 μ g/ml was prepared from fluconazole tablet (LABORATE pharmaceuticals).

The weighted amounts of fluconazole tablet were allowed to dissolve in distilled water (DW) immediately at the time of work. The solutions (Tea tree oil solution and fluconazol solution) were kept at 25±1°C, this temperature was chosen as parameter in this study. The solution are the solution of the solution and fluconazol solution were kept at 25±1°C, this temperature was chosen as parameter in this study.

After these steps ,the acrylic resin denture base material were immersed in

the antifungal drugs (tea tree oil solution 15% and fluconazole 64 µg/ml) for different times of immersion (24 hrs and 48hrs), then another swap was taken for each sample of complete dentures to determine the antifungal effects of these agents. The antifungal assay was carried out using broth microdilution method. (8) The optical density value was measured using spectrophotometer (CECIL CE 1021, England) at 590nm wave length. Swabs were inserted immediately inside test tubes containing sterilized Brain Heart infusion broth as transporting medium until they were cultured on Sabouraud's Dextrose medium at 37°C. (7,12)

Regarding the statistical analysis, one way analysis of variance at level of significance 0.05 was used, the means were compared using Duncan New Multiple Range Test to determine the difference between the studied groups.

RESULTS

The results of this study that had been done on swabs of *Candida albicans* taken from the inner of fitting surfaces of complete denture bases showed that there was significant antifungal effect of tea tree oil $(0.25 \pm 0.1 \text{nm})$ compared to control group (before immersion) (Table 1) although it was significantly less than floconazole $(0.09 \pm 0.003 \text{nm})$.

Table (1): Duncan's New Range Test for the anti–fungal effect of the control, tea tree oil and Fluconazole on candida albicans cells

Drugs	No.	Absorbance mean(nm)±SD	Duncan's Group*	Significancey
Control (<i>Candida albicans</i> culture, before immersion)	24	0.71 ± 0.3	A	Significant
Tea tree oil (15%)	12	0.25 <u>+</u> 0.1	В	Significant
Fluconazole (64 µg/ml)	12	0.09 <u>+</u> 0.003	C	Significant

^{*}Different letters mean significant difference exist; Nm: Nanometer; SD : Standard deviation; μg : Microgram

The statistical analysis demonstrated that both times of immersion (24 hours, 48 hours) in tea tree oil solution (5.1 \pm 0.4, 4.98 \pm 0.5) (Table 2) and floconazole solution (2.9 \pm 0.7, 2.7 \pm 0.5) (Table 3) were

effective in reducing the number of *Candida albicans* cells and there was no significant differences between two times of immersion.

Table (2): The antifungal effect of Tea tree oil on *Candida albicans* cells at different times of immersion

Time of Immersion (Hour)	Number of <i>Candida albicans</i> cells mm ² ± SD	Duncan's Grouping *
Control time 0 Hour	28.9 ± 2.9	A
24 Hours	5.1 ± 0.4	В
48 Hours	4.98 ± 0.5	В

^{*}Different letters mean significant difference exist;SD: Standard deviation

Table (3) antifungal effect of fluconazole on Candida albicans cells at different times of immersion.

Time of Immersion (Hour)	Number of Candida albi- cans cells mm ² ± SD	Duncan's Grouping *
Control time 0 Hour	29.4 ± 2.4	A
24 Hours	2.9 ± 0.7	В
48 Hours	2.7 ± 0.5	В

^{*}Different letters mean significant difference exist;SD: Standard deviation.

DISCUSSION

Denture stomatitis is one of the important oral diseases caused by Candida albicans, it is which is the most common intraoral infection among individuals who wear dentures. (14) In the this study, the isolation of Candida albicans was achieved more frequently from the inner or fitting surfaces of denture base rather than from corresponding mucosa because Candida albicans colonization on the fitting denture surface is heavier than on the palatal mucosa due to the fact that microorganisms are partly eliminated with shedding of the epithelial cells. (15) The results of this study showed that tea tree oil solution 15% had a significant antifungal effect against Candida albicans on surface of heat cured acrylic denture base material and this was in agreement with Hammer etal., (8) showed that the treatment of Candida albicans with tea tree oil exert antifungal action by altering membranes properties of fungal cells which may alter their permeability and affect the membranes ability to osmoregulate the cells adequately or to exclude toxic materials $^{(8,16)}$ and also study of Francesca M⁽¹⁶⁾ showed that tea tree oil was highly effective in accelerating Candida albicans clearance from experimentally infected rat vagina(17), but in disagreement with results of Tong M (17) evaluated the efficacy of 10% tea tree oil cream compared with 1% tolnaftate and placebo creams in the treatment of tinea

pedis which showed that tea tree oil cream 10% was no more effective than placebo in achieving a mycological cure. (18)

Although there are many antifungal agents on the markets, tea tree oil offers a natural alternative and reports indicate that using this agent in dental hygiene show it to be an extremely effective antimicrobial agent. (19)

CONCLUSIONS

The essential oil of *Melaleuca alternifolia* (tea tree) has the antifungal effect and its ability to reduce number of fungal cells especially *Candida albicans* cells that adhered to acrylic resin denture base material surface.

Tea tree oil offers a natural alternative with excellent antifungal effects and it can be used for home care of dentures.

REFERENCES

- 1. John A, Frank J. Pharmacology and Therapeutics for Dentistry.5th ed. An Affiliate of Elsevier, USA.2004; Pp: 665–801.
- 2. Christophe C, Gyslaine D. Antimetabolic activity of caspofungin against *Candida albicans* and *Candida parapsilosis* biofilms. *J Antimicrob chemother*.2005; 56(3):507–512.
- 3. Santarpia R, Brant E, Brasseur M, Hong A, Pollock J. A comparison of the inhibition of blastospora viability and germ tube development in *Candida albi*-

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cans by Histidine peptides and keto-

conazole. Archs Oral Biol. 1998; 33(8):

567–573.

4. Waters M, Williams D, Jagger R, Lewis M. Adherence of *Candida albicans* to experimental denture soft lining materials. *J Prosthet Dent* .1997; 77 (3): 306–312.

- Web B, Thomas C, Willcox M, Harty D, Knox K. *Candida* associated denture stomatitis. Etiology and management. A review part 3: Treatment of oral candidiasis. *Aust Dent J.* 1998; 43(4): 244–249.
- 6. Murray M. The healing power of herbs, Tea tree oil .Rocklin CA: Prima publishing .1996: Pp 321–326.
- 7. Watsh L. The antimicrobial effects of an essential oil on selected oral pathogens. *J Periodontology* .1987; 8:11–15.
- 8. Hammer K, Carson C, Riley T .Antifungal effects of Melaleuca alternifolia (tea tree)oil and its components on *Candida albicans*, *Candida glabrata* and *Sccharomyces cerevisiae*. *J Antimicrob Chemother*.2004; 53: 1081–1085.
- 9. Archilla A, Urquia M, Cutando A .Denture stomatitis: Quantification of interleukin-2- production by mononuclear blood cells cultured with *Candida albicans*. *J Prosthet Dent* .1996; 75:426-431
- 10. Prescott L, Harley J, Klein D. Microbiology. 3rd ed. Wmc Brown Communication Inc, USA .1996; Pp: 507,782–789.
- Jawetz E, Mel nick J, Adelberg E. Medical Microbiology. Alange Medical Book .21st ed. Appleton and Lange, Norwalk, Connecticut, Sain Meoat, Cali-

- fornia .USA.1998;Pp:603-605,611-614.
- 12. Nagham H, Ghada Y, Tariq Y. The effect of some antifungal agents and chlorhexidine on *Candida albicans* adherence on acrylic resin denture base surface (In vitro study). *Al–Rafidain Dent J.* 2004;4(1):65–72.
- 13. Sheen S, Harrison A. Assessment of plaque prevention on dentures using an experimental cleanser. *J Prosthet Dent*.2000; 48(6):594–601.
- 14. Kulak Y, Arikan A, Delibalta N. Comparison of three different treatment methods for generalized denture stomatitis. *J Prosthet Dent* . 1994;72(3):283–288.
- 15. Budtz–Jorgensen E. Ecology of *Candida* associated of denture stomatitis .Review article. *Microbiol Ecol Health Dis.* 2000; 12:170–185.
- Christine F, Brain J, Thomas V. Mechanism of Action of *Melaleuca alternifolia* (Tea tree) Oil on *staphylococcus aureus* Determined by Time–Kill, Lysis, Leakage, and salt Tolerance assays and electron microscopy. *Antimicrob Agents Chemother*. 2002; 46 (6): 1914 1920.
- 17. Franscesca M. In vitro and in vivo activity of tea tree oil against azole—susceptible and resistant human pathogenic yeasts. *J Antimicrob Chemother*. 2003;51:1223–1229.
- 18. Tong M.Tea tree oil in the treatment of tinea pedis. *Australas J Dermatol*. 1992;33(3):145–149.
- 19. Cynthia Olsen. The benefits of tea tree. *Australian tea tree guide*. 1998; 1147(888); P:5254.

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