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Original Research

Antifungal efficacy of Tea Tree Oil mixed with Denture Soft Liner on Denture stomatitis

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ABSTRACT:
Introduction: Colonization of candida on denture soft liners is the utmost significant causative factor in development of denture stomatitis. **Objectives:** This in vitro study is done to evaluate the efficacy of tea tree oil against candida albicans when incorporated into denture soft liners. **Materials and Methods:** Each 24 specimen disks were prepared and divided into 2 groups (test with tree oil and control) with 12 samples in each. Test group was mixed with tea tree oil (TTO) into soft liners (St) and control group without tea tree oil (S) were prepared. These disks were mixed with candida albicans suspension for valuation of fungal growth and were rinsed with sterile water to eliminate loosely attached superficial organisms. The attached yeasts were calculated by inoculating them on saboraud's agar. Treated and control disks were stored in distilled water for 1, 30, 60 days and washed daily with wet cotton. Data between treated and control disks were compared using t-test. **Results:** The mean colony forming units (CFU) per mm^2 for specimens without tea tree oil after water storage and wash with wet cotton for 1, 30 and 60 days was 6.8×10^6, 6.2×10^6, 6.7×10^6, respectively and for specimens with tea tree oil CFU decreased significantly to 1.8×10^6, 2.6×10^6, 32.9×10^6 after 1, 30 and 60 days. Treated disks were effective in controlling the growth of C.albicans for two months following water storage. **Conclusion:** Mixture of tea tree oil to denture soft liner expressively reduces growth of C.albicans signifying an effective antifungal management for denture stomatitis.
Keywords: Candida albicans, Colony forming units, Inoculum, soft liner

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INTRODUCTION

Denture soft liners are mostly used for therapeutic aspect in patients who are not able to bear denture induced stresses [1]. Soft liner materials, though being used extensively as dynamic impression materials and also as additions in prosthodontics for management of traumatized oral mucosa, have some microbiological and physical disadvantages [2]. One such problem is colonization of denture surface by Candida albicans and other micro-organisms, thereby producing denture stomatitis [2].

The candida related denture stomatitis is a regular condition in complete denture wearers, considered by generalized inflammation of the palatal mucosa covered by the denture [3]. It is projected to affect about 72% of this population [4]. Denture encouraged stomatitis can be managed by either denture repair or replacement, prophylactic actions adopted by the patients and prescribing antifungal drugs [5–6]. Biofilms of candida on mucosal and inert surfaces such as dentures may contribute to therapeutic failure by modifying the susceptibility to antifungal agents [7]. This treatment is complicated further in early and institutionalized patients with limitation of motor skills and special needs due to factors like loss of memory, difficulty in proper cleaning of the denture and following strict routine application of topical antifungal agent [8].

These short comings have inspired the development of other methods of drug elution, such as the addition of antifungal or antimicrobial agents with denture acrylic resin or with soft liners. A method of treatment by mixture of tissue conditioner and antifungal agents was recommended initially [9]. After that several attempts have been made to incorporate different antifungal agents such as propolis [10], zeolite [11,12], chlorhexidine [3], Fluconazole [3], punica granatum [13], Nystatin [6,14], Itraconazole [6], Miconazole [15], Ketoconazole [15], Clotrimazole [1] in the resilient liners with varying degree of success.

The current trend in natural health has paid to the growing interest in commercially available natural remedies. Medicinal plants extracts have been used in developing countries as alternate treatments to health problems. The essential oil of Melaleuca alternifolia, also known as tea tree oil (TTO) is a new multi-purpose herb that can be obtained from its leaves by steam distillation [16]. TTO has been shown to be promising as a topical antifungal agent, with recent clinical data indicating efficacy in the treatment of dandruff and oral candidiasis [17]. The major advantages of natural medicinal plant extracts as antimicrobial agents include enhanced safety and stability without any side effects, which lack with both organic and inorganic antimicrobial agents. This in vitro study was done to evaluate the efficacy of tea tree oil against candida albicans when added into denture soft liners

MATERIALS AND METHOD

The study was conducted in the department of Prosthodontics after obtaining approval from the institutional ethics committee.

Specimen Preparation

The study consisted of 6 groups of soft liner specimens (each group 12 no.s), among which 3 groups (S^1, S^{30}, S^{60}) were with silicone soft liner alone and 3 groups ($ST^{1,}$ ST^{30}, and ST^{60}) were with adding of TTO into liner material as displayed in [Table-1]. The soft liners were handled conferring to manufacturer's instructions. The soft liner material was added in supplied automix cartridges and the mix was directly placed into the ring form of mould with a diameter of 5mm and 1mm thickness. Thus, all the specimens were equipped to a uniform size with smooth surfaces by placing polyester film over them. ST specimens were prepared by adding 15% concentration of TTO by weight to silicone samples and processed as above. A total of 72 specimens (36 with liner and 36 with TTO added liner) were organized and permissible for autopolymerization for 20 minutes at room temperature. Later the specimens were stored in distilled water for day 1 (S^1, ST^1); 30 days (S^{30}, ST^{30}); and 60 days (S^{60}, ST^{60}) and were cleaned with wet cotton gently for one minute each day.

Table-1: Indicating test groups of silicone soft liners wit n=12

S^1 without TTO, stored in distilled water for 1 day
ST^1 with TTO, stored in distilled water for 1 day
S^{30} without TTO, stored in distilled water for 30 days
ST^{30} with TTO, stored in distilled water for 30 days
S^{60} without TTO, stored in distilled water for 60 days
ST^{60} with TTO, stored in distilled water for 60 days

Fungal growth Valuation

Standard ATCC (10231) permitted C.albicans strains were collected. Sabouraud's dextrose agar medium was prepared. Five ml sabouraud's broth was poured into each test tube and was autoclaved. The broth was inoculated with full loop of C.albicans 24 hours before placing the discs, so that the organisms were in active growth phase when broth was added to disks. The discs were placed on a membrane in a well of Transwell plate with two disks per well after 24 hours.

An inoculum of 10^7CFU / ml was prepared, and sabouraud's broth was inoculated into each well with adjusted yeast suspension. Such plates were incubated for 24 hours at room temperature. Growth controls consisting of 1ml of SDB were inoculated for each test. The broth was removed with a sterile pipette after incubation. The disks were rinsed with sterile water to remove the loosely attached C.albicans. Surface organisms were removed from the disks by placing it in sterile test tubes containing sterile saline and sonicating for 5 minutes. Serial dilution (10x) was prepared of the eluate and 100 µl of each eluate was placed on duplicate plates with sabouraud's agar. The plates were incubated at 37^0c for 24 hours and the colonies were counted. C.albicans growth assay was carried out for the 6 groups of specimens on day 1(S1, ST1), day 30 (S30, ST30) and day 60 (S60, ST60). Student's t-test was applied to analyse the data using SPSS software.

Table-2: The mean colony forming units (CFU) per mm^2 with tea tree oil after and control group

Specimens	n	Mean ± SD	95% confidence interval	p-value
S^1	12	6.8 ± 3.9	6.6 – 7.2	0.001
ST^1	12	1.8 ± 3.4	4.1 – 5.3	
S^{30}	12	6.2 ± 5.1	5.2 – 6.3	0.001
ST^{30}	12	2.6 ± 2.3	1.7 – 2.8	
S^{60}	12	6.7 ± 5.1	3.2 – 4.3	0.001
ST^{60}	12	2.9 ± 2.1	2.6 – 3.1	
Total	72	4.8± 1.5	4.3 – 5.1	

RESULTS

Results recommended that there was a substantial difference among the mean CFU per mm^2 for soft liner with TTO and untreated control liner at each time interval at 1, 30 and 60 days. Colonization was lower in TTO combined disks in comparison to control disks ($p = 0.001$). Statistically no significant difference was found in CFU of control disks following water storage up to 60 days. Growth of C. albicans was meaningfully inhibited up to 60 days in treated disks following storage in distilled water and washing with wet cotton daily for one minute. The mean colony forming units (CFU) per mm^2 for specimens without tea tree oil after water storage and wash with wet cotton for 1, 30 and 60 days was 6.8×10^6, 6.2×10^6, 6.7×10^6, respectively and for specimens with tea tree oil CFU decreased significantly to 1.8×10^6, 2.6×10^6, 32.9×10^6 after 1, 30 and 60 days. Treated disks were effective in controlling the growth of C.albicans for two months following water storage (Table-2).

DISCUSSION

Removable prosthesis, when placed in the oral cavity produces numerous changes in the oral environment, which may adversely affect integrity of oral tissues, denture stomatitis being one of the important clinical presentations of oral candidiasis [9]. Though the aetiology is multifactorial [4], denture bio-film components, such as C.albicans play a basic role in development of candidiasis [18]. Novel agents from natural resources are essential, which can inhibit the growth of microorganisms in the biofilm, and would enhance the effective alternative therapeutic modalities, as the action of antifungal agents may be limited by their penetration and chemical reaction into biofilm matrix, the extracellular polymeric material [19]. Recently, incorporating extracts of medicinal plants into biomaterials have been in practice and found to be a natural alternative with excellent antifungal effects [16]. TTO, the volatile essential oil from Australian native plant Melaleuca alternifolia, have been largely employed primarily for its antimicrobial and anti inflammatory properties, and shows promise as a topical antifungal agent [20]. This present study incorporated TTO into silicone soft liner and evaluated its efficacy against growth of C. albicans.

Results of present study suggested that TTO treated disks presented significant antifungal efficacy against C. albicans compared to untreated disks upto 60 days, and this was in agreement with Al-Mashhadane et al., [16] showed that 15% TTO had significant antifungal effect against C.albicans on the surface of heat cure acrylic denture base material. This study immersed the denture in TTO for 24–48 hours instead of adding it in the denture itself. Our study has added TTO into the soft liner so that there is continuous sustained release of TTO exhibiting antifungal activity up to 60 days, avoiding other alternative mechanical and chemical denture cleansing methods [21].

This study also supports the results of Hammer et al., [17] suggested that the treatment of C.albicans with TTO exert antifungal action by altering membrane properties of fungal cells, which may alter their permeability and affect the membranes ability to osmo regulate the cells adequately or to exclude toxic materials. Our study results are also in agreement with Emira et al., [22] suggested that plants essential oils significantly prevent the formation of biofilm at low concentrations and the potential bio active compounds in TTO has distinct influence on candida cell growth, function and biofilm formation by interfering any of the steps involved in bio film development and has a potential anti-adhesive effect of candida strains on PMMA.

Pachava et al evaluated Antifungal effect of Denture Soft Liner mixed with Tea Tree Oil and observed that adding of tea tree oil to denture soft liner significantly reduced growth of C.albicans suggesting a new form of intra oral effective antifungal management for denture stomatitis.[23] Chincholikar et al evaluated Two Antifungal Agents added in Auto Polymerising Denture Base Resin, Heat Polymerising Denture Base Resin and concluded that 1) Fluconazole was recognized to be further effective than herbal neem extract against Candida albicans; 2) Permanent silicone soft liner was established to be the most effective polymeric system for sustained release of antifungal agents up to 21 days.[24]

Baygar et al assessed Functional denture soft liner with antimicrobial and antibiofilm properties and found that Carvacrol-incorporation visibly decreased the colonization and plaque formation of oral pathogens, particularly C. albicans accumulation. Carvacrol may be useful as a hopeful agent for antibacterial and antifungal management for denture soft lining materials.[25] Vankadara et al evaluated the Colonization and Inhibition of Candida albicans in selected commercially available denture lining materials material by mixing them with varying concentrations and doses of tea tree oil. The GC soft liner had advanced varage colonization and lesser zone of inhibition of C.albicans when compared to Visco-gel soft liner . the addition of Tea Tree oil, Viscogel had good acquired good antifungal properties than GC-soft lining materials.[26]

CONCLUSION

Soft liners mixed with TTO have resulted better antifungal efficacy up to 60 days indicating the opportunity of this essential oil for therapeutic use against denture stomatitis.

REFERENCES

1. Vojdani M, Zibaei M, Khaledi AAR, Zomorodian K, Ranjbar MA, Boshehri S. In-vitro Study of the Effect of Clotrimazole Incorporation into Silicone Soft Liner on Fungal Colonization. Shiraz Univ Dent J. 2009;9(Suppl. 1):19–23.
2. Bal BT, Yauzyilmaz H, Yucel M. A pilot study to evaluate the adhesion of oral microorganisms to temporary soft lining materials. J Oral Sci. 2008;50(1):1–8
3. Amin WM, Al – Ali MH, Salim NA, Al – Tarawneh SK. A new form of intra oral delivery of antifungal drugs for the treatment of denture induced oral candidosis. European J Dent. 2009;3:257–66.
4. Budtz-Jorgersen E. oral candidiasis in long term hospital care denture wearers with denture stomatitis. Oral Dis. 1996;2(4):285–90.
5. Muzyka BC. Oral fungal infection. Dent Clin North Am. 2005;49:49–65
6. Chow CKW, Matear DW, Lawrence HP. Efficacy of antifungal agents in tissue conditioners in treating candidiasis. Gerontology. 1999;16:110–19.
7. Ryalat S, Darwish R, Amin W. New form of administering chlorhexidine for treatment of denture – induced stomatitis. Therapeutics and Clinical Risk Management. 2011;7:219–25.
8. Casemiro LA, Martins CHG, Pires-de-Souza FCP, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver – zinc zeolite part-I. Gerodontology. 2008
9. Gupta H, Bhat A, Prasad KD, Prasad KMS, Kumar KV. An innovative method of incorporating antifungal agents into tissue conditioners: An invitro study. Trends Biomater Artif Organs. 2011;25(2):63–66.
10. Santos VR, Gomes RT, de Mesquita RA, de Moura MD, França EC, de Aguiar EG, et al. Efficacy of Brazilian propolis gel for the management of denture stomatitis: a pilot study. Phytother Res. 2008;22(11):1544–47.
11. Nikawa H, Yamamoto T, Hamada T, Rahardjo MB, Murata H, Nakanoda S. Antifungal effect of zeolite incorporated tissue conditioner against Candida albicans growth and/or acid production. J Oral Rehabil. 1997;24:350–57.
12. Jang KS. Inhibitory effect of antifungal agents incorporated in denture lining materials against candida albicans. J Korean Acad Prosthodont. 1999;37(3):293–300.
13. Vasconcelos LC, Sampaio MC, Sampaio FC, Higino JS. Use of Punica granatum as an antifungal agent against candidosis associated with denture stomatitis. Mycoses. 2003;46(56):192–96.
14. Thomas CJ, Nutt GM. The invitro fungicidal properties of Visco-gel, alone and combined with nystatin and amphotericin B. J Oral Rehabil. 1978;5:167–72.
15. Quinn DM. The effectiveness, invitro, of miconazole and ketoconazole combined with tissue conditioners in inhibiting the growth of Candida albicans. J Oral Rehabil. 1985;12:177–82.
16. Al-Mashhadane FAM. Tea tree oil: A new antifungal agents against candida albicans cells on heat cured acrylic resin denture base material. An invitro study. Al – Rafiadain Dent J. 2007;7:54–7s.
17. Hammer KA, Carson CF, Riley TV. Antifungal effects of Melaleuca alternifolia (tea tree) oil and its components on candida albicans, candida glabrata and sacchromyces cerevisiae. J Antimicrobial Chemotherapy. 2004;53:1081–85.
18. Kulak Y, Kazazoglu E. In vivo and invitro study of fungal presence and growth on the three tissue conditioning materials on implant supported complete denture wearers. J Oral Rehabil. 1998;25:135–38.
19. Kanathila H, Bhat AM, Krishna PD. The effectiveness of magnesium oxide combined with tissue conditioners in inhibiting the growth of candida albicans: An invitro study. Indian J Dent Res. 2011;22(4):613.
20. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) oil: A review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 2006;19(1):50–62.
21. Yadav R, Yadav VS, Garg S, Mittal S, Garg R. Effectiveness of different denture cleansing methods on removal of biofilms formed in vivo. J Cranio Maxillary Diseases. 2013;2(1):22–27
22. Emira N, Mejdi S, Aouni M. Invitro activity of Melaleuca alternifolia (Tea tree) and Eucalyptus globules essential oils on oral candida biofilm formation on polymethyl methacrylate. J of Medicinal Plant Research. 2013;7(20):1461–66.
23. Pachava,KR, Nadendla LK, Choudary Alluri LS, Tahseen H, Poojitha Sajja N.. Invitro Antifungal Evaluation of Denture Soft Liner Incorporated with Tea Tree Oil: A New Therapeutic Approach Towards Denture Stomatitis. J Clin Diagn Res. 2015 Jun; 9(6): ZC62–ZC64.
24. Chincholikar S , Sridevi J , Kalavathy N , Singh S , Kapoor A , Saumya S. Comparative Evaluation of Two Antifungal Agents Incorporated in Auto Polymerising Denture Base Resin, Heat Polymerising Denture Base Resin and Permanent Silicone Soft Liner-An In Vitro Study. Journal of Clinical and Diagnostic Research. 2019 Jan, Vol-13(1): ZC49-ZC54
25. Baygar T, Ugur A, Sarac N, Balci U, Ergun G. Functional denture soft liner with antimicrobial and antibiofilm properties. Journal of Dental Sciences. 2018;13:213-219
26. Vankadara SK, Hallikerimath RB, Patil V, Bhat K, Doddamani MH. Effect of Melaleuca alternifolia Mixed with Tissue Conditioners in Varying Doses on Colonization and Inhibition of Candida albicans: An In Vitro Study. Contemp Clin Dent. 2017 Jul-Sep; 8(3): 446–450.