COMPARATIVE EVALUATION OF ANTIFUNGAL ACTIVITY AND FLEXURAL STRENGTH OF HEAT CURED ACRYLIC DENTURE BASE RESIN INCORPORATED WITH LEMONGRASS OIL, LAVENDER OIL AND CHLORHEXIDINE GLUCONATE – AN INVITRO STUDY

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Abstract-

Background:

Denture stomatitis is inflammation of oral mucosa due to various factors like oral and denturehygiene, saliva, denture age and degree of colonization with C.albicans. Denture cleanliness is essential to prevent malodor, poor aesthetics and accumulation of plaque/calculus. Denturecare products should be easy to handle, effective in removing inorganic/organic deposits, bactericidal and fungicidal, non-toxic and inexpensive.

Aim:

The aim of this study was to to evaluate and compare the antifungal effectiveness of three antifungal agents on heat-polymerised acrylic denture base resin when used as a denture soaking agent along with evaluation and comparison of the effects of the said three agents onits Flexural strength.

Materials and Methods:

A total of twenty eight acrylic specimens were divided into 4 groups (n=7); G1: Distilled water; G2: 2% Chlorhexidine gluconate; G3: Lemongrass oil; G4: Lavender oil and immersed in 1ml of each agent for 24h at 37^0C after previous inoculation with C.albicans suspension. Colony counting tests were performed using Sabouraud dextrose agar plates and compared for the antifungal property. They were also subjected to flexural strength tests.

Statistical analysis used:

One-way ANOVA followed by Post hoc Tukey's analysis test for antifungal activity andflexural strength were performed.

Results:

Results showed significant difference in antifungal activity and flexural strengths of testgroups under study.

Conclusion:

It was concluded that Chlorhexidine gluconate, Lemongrass and Lavender oil are effectiveantifungal agents and decreased the flexural strength of acrylic denture base resin.

***Keywords*: Acrylic resin, Denture cleanser, Denture hygiene, Antifungal denture cleansers**

Introduction

Denture stomatitis, also known as chronic atrophic candidiasis or denture sore mouth, refersto a visible chronic inflammation with a characteristic redness of the mucosa in the oral cavity that is covered by a complete or partial removable dental appliance[1]. It is associated with the formation of biofilms on the bioprosthetic surfaces[2].

Candida albicans is an innocuous commensal of the microbial communities of the human oralcavity[3]. Dentures may produce a micro environment conducive to the growth of Candida by enhancing the adherence of candida to acrylic, reduced salivary flow under the surface of the denture and poor oral hygiene[4]. Other risk factors such as Diabetes mellitus, immunosuppression, medications (e.g. antibiotics and corticosteroids), Vitamin A and folate deficiency can disturb the balance of the oral flora leading to an increase in Candida as an opportunistic infection.

Many studies have evaluated the effect of denture cleansers and disinfectant solutions on the initial Candida adherence to denture base materials. However, little attention has been paid tothe effect of these denture cleansing agents on Candida-associated mature biofilm, the cells ofwhich are known to be more resistant to antimicrobial compounds and chemical cleansing[5].

Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions. Lemongrass oil is characterized for montrene compounds and citral is the major component (65-85%), in addition to that it also contains, geraniol, geranylacetate and myrcene[6]. Lavender oil mainly consists of linalool and linalyl acetate (75%)[7].

The mechanism of action of essential oils appears to be predominantly on the fungal cell membrane, disrupting its structure causing leakage and cell death; blocking the membrane synthesis; inhibition of the spore germination, fungal proliferation and cellular respiration[6]. The use of chlorhexidine has been well established [8,9]. Chlorhexidine gluconate is used as a 0.2 % mouth rinse and 2% suspension as an overnight denture disinfectant[10].

The literature documents several reports on the use of Chlorhexidine gluconate[11], lemongrassoil[6], lavender oil[7] against the growth of Candida species. In the pertinent literature, no comparisons of the antifungal effect of the above mentioned three agents against

the growth of Candida species on the acrylic resin surface were found.
Thus, the aim of this in vitro study was to evaluate and compare the antifungal effectivenessof lemongrass oil, lavender oil and chlorhexidine gluconate on heat-polymerised acrylic denture base resin when used as a denture soaking agent along with evaluation and comparison of the effects of the said three agents on its Flexural strength.

Materials and Methods
Materials: Modeling wax (DPI)
Heat cure acrylic resin polymer and monomer (Trevalon)Dental plaster and stone
Candida albicans strain (ATCC 24433)Saline
Distilled water
Lemongrass oil (Pure Source India)Lavender Oil (pure source India) Chlorhexidine Gluconate (Prevest) Dimethyl Sulphoxide
Sabouraud dextrose agar
Armamentarium: Kavo flasksTest tubes
Screw capped bottlesTweezers
Pipette Incubator Cotton swabs
Universal testing machine (Tinius Olsen)

Preparation of samples to test antifungal effect
Preparation of Candida Suspension
Standard strain of ATCC 24433 *C.albicans* was inoculated into saline solution and incubatedat 37^0C for 24h. The suspension was then used for soaking the acrylic specimens at 37^0C for 24h in order to contaminate the samples.

Preparation of Specimens
Twenty-eight square wax patterns (10mm*10mm*2mm)[12] were fabricated using modeling wax (Hindustan Modeling wax No 2, The Hindustan Dental Products, Hyderabad, India). The patterns were then invested in a denture flask using compression moulding technique. The wax was eliminated and heat polymerising acrylic resin (denture base material, Trevalon, Dentsply India Pvt Ltd) was packed in the mould in the ratio of three parts of polymethyl methacrylate (PMMA) powder and one part of PMMA monomer. All the samples were polymerised with the same batch of acrylic resin using a standard heat-curing resin cycle, which involves processing the resin at 74^0C for approximately 2h and increasing the temperature of the water bath to 100^0C and processing for 1h.
The specimens were then removed from the flasks. No finishing and polishing procedures were performed to simulate the inner surface of a complete denture. However, the excess wasremoved and samples were sandpapered with 320-grit sandpaper.
The specimens were divided into four groups as followed:

Group 1: 7 contaminated specimens for soaking in Distilled water

Group 2: 7 contaminated specimens for soaking in 2% Chlorhexidine GluconateGroup 3: 7 contaminated specimens for soaking in Lemongrass oil
Group 4: 7 contaminated specimens for soaking in Lavender oil

Procedure for soaking the Specimens
1ml of the prepared Candida suspension was dispensed into sterilized screw capped bottles followed by immersion of acrylic specimen and stored for 24h at 37^0C. The above process was repeated for all the 28 specimens. After that each acrylic specimen was removed from itsscrew capped bottle using sterile tweezer and placed in screw capped bottles containing 1ml of the tested oils, chlorhexidine gluconate and distilled water which was again stored for 24h at 37^0C.

Inoculation of the agar plates and colony counting tests
The colony counting tests were done in two stages. In the first stage. 0.01ml of the Candida suspension was taken from the screw capped bottles containing the acrylic specimen, plated on Sabouraud dextrose agar and incubated at 37^0C for 48h followed by counting of candida colonies (as CFU/ml). In the second stage, 0.01ml of each of the tested oils, chlorhexidine gluconate and distilled water were taken from the respective screw capped bottles, plated on Sabouraud agar and incubated at 37^0C for 48h followed by counting of candida colonies (asCFU/ml).
The agar plates were further compared for the effective antifungal property of each agentagainst Candida albicans.

Preparation of samples to test flexural strength
Preparation of Specimens
Thirty-five wax patterns (65mm*10mm*2.4mm) length, width and depth respectively according to the ADA Specification were fabricated using the same procedure as described for preparation of specimens for antifungal tests. They were divided into five groups asfollowed:
Group 1: 7 specimens as Control group (Kept dry after retrieval from flasks)Group 2: 7 specimens to be soaked in Distilled water
Group 3: 7 specimens to be soaked in 2% Chlorhexidine GluconateGroup 4: 7 specimens to be soaked in Lemongrass Oil
Group 5: 7 specimens to be soaked in Lavender oil

Flexural strength test equipment and Procedure
All the Samples barring group 1 (control) were soaked in Distilled water for 48h and stored at 37^0C for conditioning. After that seven samples per group were soaked in tested oils and chlorhexidine gluconate for 24h at 37^0C. The specimens were then subjected to flexural strength tests using Tinius Olsen's Universal testing machine (1kN Capacity – Model H1K-S) at a rate of 5mm/min. The load used to cause the fracture of specimen was noted as seen on LCD Display attached to the machine.

RESULTS

I. DESCRIPTIVE STATISTICS

Evaluation of Antifungal Effect
The antifungal effect of chlorhexidine gluconate, lemongrass and lavender oil on four different groups of heat polymerized acrylic denture base was evaluated. Number of samples, mean, range and standard deviation is shown in Table 1.

Evaluation of Flexural strength test
The effect of distilled water, chlorhexidine gluconate, lemongrass oil and lavender oil on flexural strength of five different groups of heat polymerizing acrylic denture base was evaluated. Number of samples, mean, range and standard deviation is shown in Table 2.

II. INFERENTIAL STATISTICS

One-way ANOVA followed by Post hoc Tukey's analysis test for antifungal activity [Table 3] and flexural strength [Tables 4] were carried out between the groups for assessing the significant difference between any of the five groups. The significant level was set at $P <$ 0.05. There was highly significant difference in antifungal activity and flexural strengths of test groups under study.
The microbiological study carried out to determine the antifungal activity of Heat cure PMMA resin against C. albicans when incorporated with 2% chlorhexidine gluconate, lemongrass and lavender oil displayed decrease in the growth of C. albicans when incubated for 24h at 37^0C. [Figures]
The study carried out to determine the flexural strength of Heat cure PMMA resin showed decrease in strength when incorporated with 2% chlorhexidine gluconate, lemongrass and lavender oil respectively

DISCUSSION

Conventional Dentures are the most common alternatives in restoration of lost teeth enabling individuals in improving oral function, enhancing phonetics, facilitating social engagement and in leading an aesthetically acceptable life[13]. The current rates of edentulism have been estimated to be between 7% and 69% of the adult population internationally[14]. Candida albicans is a commensal in the oral cavity of 45-65% of healthy individuals with a higher prevalence found in children and young adults. In denture wearers, the prevalence of candida increases to 60-100%. Denture stomatitis is an opportunistic infection related to an inflammatory process which compromises the mucosal surface beneath the dentures[15].
The diagnosis of denture stomatitis depends on clinical findings, such as, the presence of erythema and edema on the palatal mucosa and gingiva covered by the denture base[16].
Maintaining hygiene of the dental tissues and dentures is a significant challenge for dentistry. The two major approaches used for the denture cleaning are mechanical and chemical methods. Mechanical methods include brushing (using water, soap, toothpaste, or abrasives) and ultrasonic treatment. Chemical methods include immersion in cleansing solutions.
Chemical cleansers are inexpensive, easy, comfortable, reach areas difficult to clean mechanically thus resulting in efficient cleansing[17].
It was shown that most Candida species are susceptible to topical antifungal drugs like amphotericin B, nystatin, Miconazole, Flucanazole and Clotrimazole.
Nystatin has shown antifungal and antimicrobial activity but its effect was comparatively less as compared with the azole groups [18,19,20]. Miconazole 2% is the antifungal that has been most successful in its application. However, the azole groups have certain side effects such as unpleasant taste, poor patient compliance, hypersensitivity, drug resistance, hepatoxicity and renal toxicity.

Among the new therapies proposed for the treatment of denture stomatitis photodynamic therapy and the use of nanoparticles deserve special mention. Photo dynamic therapy includes use of methylene blue, toluidine blue and porphyrin derivatives as photo sensitizers. Nanomaterials include use of polymethyl methacrylate silver nanoparticle discs to reduce the adherence of candida albicans.
There is an immense interest in the search for new antimicrobial agents of plant origin due to increase of antibiotic resistance of synthetic drugs[21]. Medicinal plants have been used to treat different diseases because of its advantages such as cost efficiency, easy availability and relatively fewer side effects.
This study evaluates and compares the antifungal properties of Lemongrass oil, lavender oil and chlorhexidine gluconate.
For evaluation of antifungal activity against C.albicans inoculation of the agar plates was done using the testing agent followed by candida colony counting after 48h which showed that control group had highest CFU/ml with decrease in distilled water group followed by chlorhexidine, lemongrass (100% and 10%) and lavender oil (100% and 10%) which showed absence of any CFU/ml suggesting that these are effective agents against C.albicans. This was found to be in compliance with the results of the study conducted by Spiechowicz et al., according to which chlorhexidine is effective in preventing C. albicans attachment to, and growth on the acrylic resin.[22] According to a study conducted by Amornvit P et al., lemongrass oil proved to be an effective

antifungal at concentration of 0.25% (v/v) or at higher level[23] while lavender oil proved to be an effective antifungal agent at a concentrationof 0.29% for oropharyngeal strains as reported by D'Auria F.D. et al.[24]

Furthermore, results of the flexural strength evaluation showed that when heat cure PMMA was incorporated with chlorhexidine showed increase in flexural strength as compared to distilled water however, a decrease in flexural strength was observed when it was incorporated with lemongrass oil and the lowest was found when incorporated with lavenderoil.

Limitations of the study**:** This study evaluates the antifungal effect of lemongrass oil and lavender oil at 100% & 10% and the tested oils proved to be completely effective at both theconcentrations. Further studies can be conducted to evaluate the minimum inhibitory concentration of the said oils when incorporated into heat cure PMMA.

Conclusions**:** Within the limitations of this in-vitro study, the following conclusions weredrawn:

- Chlorhexidine gluconate (2%), Lemongrass oil, Lavender oil proved to be effectiveantifungal agents.
- Chlorhexidine gluconate (2%), Lemongrass oil and Lavender oil are equally effective ininhibiting candida albicans growth on heat polymerised acrylic resin.
- Incorporation of distilled water, chlorhexidine gluconate, lemongrass oil and lavender oildecreased the flexural strength of heat polymerised acrylic denture base resin.
- Incorporation of Chlorhexidine gluconate showed higher flexural strength whencompared to lemongrass and lavender oil.
- Incorporation of lemongrass and lavender oil showed comparable flexural strengths.

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