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To Evaluate and Compare the Growth of Candida Albicans After Incorporation of Aloe Vera Extract of Different Concentrations in Soft Liner: An in Vitro Study

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KEYWORDS
Soft denture liners, Adherence test, Candida albicans, Aloe vera, Denture

ABSTRACT:

AIM: To evaluate the growth inhibition of Candida albicans, in the soft-liner material modified by aloe vera incorporation.

METHODS: consistent with a pilot study, two percentages (3% and 10%) of aloe vera powder were used. Candida adherence test was performed, the effect was evaluated on 45 samples and divided into three main groups A, B, and C of 15 each. These three groups based on time interval of 1 day, 3 day and 5 day was further divided into three subgroups each.

STATICAL ANALYSIS: Data was analysed using Statistical Package for Social Sciences (SPSS) version 21, One-way anova was used for comparison of two or more groups. Post hoc comparison was done using Tukey test.

RESULTS: The results indicated that both concentrations of aloe vera showed a statistically highly significant decrease in Candida albicans cell count in comparison to the control group.

CONCLUSION: Incorporation of aloe vera powder with self-cure acrylic soft-liner powder helps to add an anti-candidal property to the soft liner.

1. Introduction

Alveolar bone in the human jaw continuously undergoes resorption which might cause a maladaptation of the denture prosthesis, this might lead to patient discomfort, inability to chew and trauma to the soft tissue.[1] However, this maladaptation can be solved by relining the prosthesis using suitable material like a soft liner.

Soft denture liners lead to the formation of an area that is difficult to clean and support the proliferation of fungi and bacteria. Several problems are accompanied by the use of denture soft lining materials such as the formation of porosity, failure of the bond between the soft liner and the denture base, loss of resiliency, color alterations, poor tear strength, and consequent plaque accumulation with Candida albicans colonization.[2]

Among denture wearers, the most common opportunistic infection is Denture stomatitis caused by candida albicans. Despite being a multifactorial disease, the adherence of Candida albicans to the fitting surface of

Journal of Chemical Health Risks

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dentures and host cells is known as the first step in the beginning and propagation of denture stomatitis.[3]

Different modalities of treatment are used for denture stomatitis, these include systemic or local measures. Now a days lot of attention is paid to herbal agents, among the various currently available herbal agents and the most popular is aloe vera. This study is aimed to know the anti-candidial efficacy of aloe-vera when used in combination with soft liner as a denture relining material.

Aloe vera has been the topic of many scientific studies over a previous couple of years, regarding several claimed therapeutic properties. According to a study, aloe vera leaf consists of phytochemicals bioactivity, as aloe vera contains 75 potential active substance-vitamins, enzymes, minerals, sugars, amino acids, salicylic acids, and anthraquinones.[4] Aloe vera contains 8 enzymes. They are alkaline phosphatase, amylase, bradykinin, carboxypeptidase, catalase, cellulose, peroxidase and lipase.

They help in reducing inflammation when applied topically. It has vitamins A, C, E, B12, folic acid. Minerals like calcium, chromium, copper, selenium, magnesium, manganese, potassium, sodium and zinc. It contains glucose and fructose monosaccharides, glucomannans polysaccharides.[5]

Hormones like auxins and gibberellins help in wound healing. Recently a glycoprotein named alprogen with antiallergic properties and anti-inflammatory compound, C-glucosyl chromone has been found in aloe vera gel. Anti-inflammatory agents present in aloe vera can help in reducing inflammation and pain. Aloe vera keeps our body healthy with proper essential nutrients of proteins, vitamins, minerals, essential fatty acids, fiber, and water. Aloe vera is analgesic, antibacterial, antiviral, antifungal, antioxidant immune-modulating, antiseptic, anti-inflammatory.[6]

Antifungal properties of aloe vera will help in the treatment of denture stomatitis. Its antiviral properties help in the management of shingles (Herpes Zoster) and cold sores (Herpes Simplex).

The present study aimed to get used from the anti-candida property of aloe vera to be incorporated with the powder of soft liner in different concentrations. An evaluation of this effect through the Candida adherence test.

Materials and method

This is in-vitro research that was conducted in the Department of Prosthodontics Crown and Bridge, NIMS Dental College and Hospital. This in-vitro study was conducted in order to evaluate and compare the growth of candida albicans after the incorporation of aloe vera extract at different concentrations in a soft liner.

Selection of material:

- Aloe vera powder [(Organic, United Kingdom) Batch no.-OABP/2019/03]
- GC soft liner tissue conditioner [(Tokyo, Japan) Batch no.-1905081]

Fabrication of the metal Mold:

A metal mold was fabricated. A disk-shaped metallic die (8 mm in diameter and 3 mm in cross- section).

Equipment used:

- Electronic weighing balance
- Vacuum forming machine
- Compound Microscope

Preparation of samples & grouping of samples:

Subsequently retrieving a sample from the aluminium die (Figure1). A thermoplastic sheet was heat compressed over the samples (Figure 2&3). The thermoplastic sheet fabricated on samples was retrieved and subsequently used as a mold to prepare the samples for microbiological testing (Figure 4).

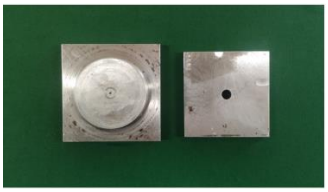

Figure 1. MASTER DIE

Journal of Chemical Health Risks

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JCHR (2024) 14(2), 149-154 | ISSN:2251-6727

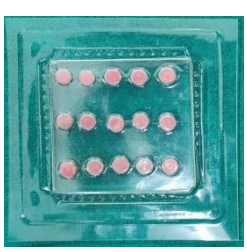
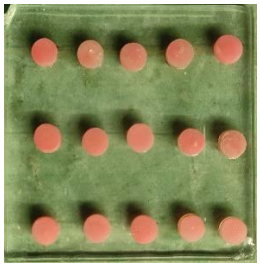

Figure 2&3. SAMPLES

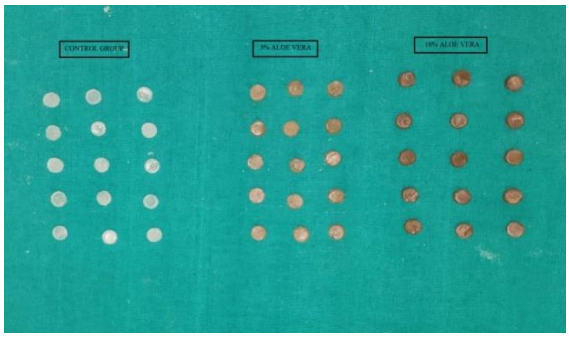

Figure 4: SAMPLES GROUPING

Mixing aloe vera with soft liner:

3% & 10% aloe vera powder was hand mixed with soft liner according to the manufacturer's instructions in a plastic mixing cup. With the help of a mixing spatula, the soft-liner mixture was applied over the thermoplastic sheet mold and allowed to gel. The set samples were placed into 2 ml cryovials. A total number of 45 samples was fabricated and divided into three main groups A, B and C of 15 each. These three groups based on time interval was further divided into three subgroups each.

Preparation of inoculums:

A standard strain of ATCC 24433 candida albicans was inoculated into Sabouraud Dextrose broth and incubated at 37°C. After 8 h, Candida albicans suspension was standardized by dilution with sterile broth to a density that is visually equivalent to barium sulfate standard 0.5 McFarland.

Inoculation of Samples with Candida albicans:

In 2 ml cryovials, 1 ml of Sabouraud dextrose agar broth was filled using the P1000 micropipette, and 10 µl of diluted Candida albicans was dropped into each vial using a P10 micropipette. These vials were then stored in an incubator under 37°C for 1, 3, and 5 days.

Inoculation of agar plate using spot inoculation method 24 hours inoculation:

The agar plate was divided into a total of 15 boxes, i.e, 5columns (One for each sample) and 3 rows (One for each group of antifungal agents). 10 µl of candida albicans Sabouraud Dextrose Agar broth solution was pipetted out from the cryovials using a P10 micropipette and spot inoculated into each box. The agar plate was sealed using Parafilm M sealing tape to prevent cross contamination and stored in an incubator at 37°C for 24 hours. The plate was examined for growth of Candida albicans after 24 hours.

A similar procedure was carried out for the 3rd and 5th day of inoculation by obtaining the inoculum from the labeled 3rd day and 5th day cryovials, respectively. Both the agar plates were examined after 24 hours interval to evaluate the growth of Candida albicans.

The 24 hours, 3rd day, and 5th day agar plates was further compared for the effective antifungal property of each agent against Candida albicans.

Technique:

Compound Microscope was used to evaluate the antifungal growth.

Statistical analysis:

Data was analysed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive data was reported for each variable. Summarized data was presented using Tables and Graphs. Data was normally distributed as tested using Shapiro Wilk test ($P>0.05$). Hence non parametric test, one-way anova was used for comparison of two or more groups. Post hoc comparison was done using Tukey test. A level of $p<0.05$ was considered statistically significant.

Result:

The results of incorporation of aloe vera with the powder of self-cure acrylic soft liner with different concentrations revealed that aloe vera incorporation in

Journal of Chemical Health Risks

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JCHR (2024) 14(2), 149-154 | ISSN:2251-6727

3% and 10% caused a statistically significant decrease in the mean values of candida albicans count compared to control (Figure 5)

		N	Mean	Std. Deviation	F value	p^a value	p^b value
1st day	Group I	15.000	6.0894	.01758	2.812	0.171, ns	NA
	Group II	15.000	6.0861	.01313			
	Group III	15.000	6.0764	.01569			
3rd day	Group I	15.000	6.1073	.01642	122.957	0.001*, sig III<II<I	I & II: 0.001*, SIG
	Group II	15.000	6.0431	.01401			I & III: 0.001*, SIG
	Group III	15.000	6.0195	.01700			II & III: 0.001*, SIG
5th day	Group I	15.000	6.1074	.01445	95.72	0.001*, sig III<II<I	I & II: 0.001*,NS
	Group II	15.000	6.0024	.01570			I & III: 0.001*, SIG
	Group III	15.000	5.9332	.05623			II & III: 0.001*, SIG

One way anova test[a],

Post hoc comparison

Level of significance set at $p < 0.05$, *statistically significant

Figure 5: COMPARISON OF MEAN OF TOTAL VIABLE COUNT AMOUNG THREE GROUPS AT DIFFERENT FOLLOW UP

Discussion

The increase in the number of candida albicans adherent cells, which might refer to the fact that when soft lining materials are soaked in water or an aqueous cleaning solution undergo two responses: leaching out of the plasticizers and other soluble components, and water or saliva are absorbed inside voids, which favor the colonization of yeasts and candida which may lead to inflammatory process.[7]

Denture stomatitis is an opportunistic infection related to an inflammatory process that comprises the mucosal surface that is present beneath the dentures. Although the etiology is multifactorial, C. albicans is that the only microorganism with a longtime pathogenic role.

Tissue conditioners are incorporated with antifungal agents and have been used as an antifungal drug delivery system to treat the inflamed tissue under the denture bearing area. To overcome the obstacles of other therapies this can be a promising method of drug delivery system.

In literature, Nystatin and Viscogel are the most commonly used combination of antifungal agents and tissue conditioners. Nystatin when incorporated into tissue conditioners shows antifungal and antimicrobial activity, but its effect was comparatively less as compared with the azole groups.[8, 9, 10, 11]

Amphotericin b is another commonly used antifungal agent. Although it is a good and potent antifungal agent when incorporated into tissue conditioners, it is completely ineffective this could be due to the inability of the compound to be stable or change in its chemical structure when incorporated into tissue conditioners.[12,13] Among the organic antimicrobial agents, azole group derivatives were found to be the most effective and potent against c. Albicans.[8,9,10] Although they are found to be effective but prolonged usage may cause drug resistance, hepatotoxicity, and renal toxicity.[14] Topical application of the same can result in unpleasant taste.

To avoid the complications of organic antimicrobial agents, researchers started incorporating inorganic compounds such as SNPs (Silver nanoparticles), magnesium oxide, photocatalysts, and silver zeolite into tissue conditioners. [15] However, the disadvantages of using SNPs include their high cost, discoloration of tissue conditioner when incorporated with the same, and metallic taste.

In addition to these, several studies have been carried out which have investigated the effect of natural and herbal antimicrobial agents against c. Albicans by incorporating them into tissue conditioners, which have shown good antifungal and antimicrobial activity.[16, 17, 18] The main advantage of using natural and herbal medicaments over organic and inorganic compounds is its ready availability, minimal or no side effects, and price effectiveness.

One such herb is aloe vera that has antifungal and antimicrobial property but has not been incorporated into tissue conditioner to check its effectiveness against candida associated denture stomatitis. In this study, aloe vera (Aloe barbadensis) powder was used.

The present study aimed to get used from the anti-candida property of aloe vera to be incorporated with the powder of soft liner in different concentrations Candida adherence test were performed to evaluate the growth of

Journal of Chemical Health Risks

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candida albicans after incorporation of aloe vera at different concentrations in the soft liner.

The results of incorporation of aloe vera with the powder of self-cure acrylic soft liner with different concentrations revealed that aloe vera incorporation in 3% and 10% caused a statistically significant decrease in the mean values of candida albicans count compared to control (Aloe vera 0%).

This might be due to the presence of a novel protein of molecular weight 14 kda within the constituents of aloe vera, which has antifungal and anti-inflammatory properties. The action of this protein is through inhibition of trypsin which shows protease inhibitory function.[19] Furthermore, aloe vera 10% has the lowest mean value and there was a statistically significant difference between aloe vera 3% and 10% and this might be due to the increased concentration of aloe vera and increasing its effectiveness. This was in agreement with Abduljabbar, et al. In 2016 who mentioned that aloe vera extract in alcoholic solvents induce significant inhibition in the growth of candida albicans, and this inhibitory effect increase as the concentration of aloe vera increase.[20]

Conclusion

With the limitation of the study following conclusion was made:

Aloe vera can be used as an effective natural herb against *Candida albicans* when incorporated with soft lining material.

The incorporation of *aloe vera* powder into acrylic soft lining material powder results in a statistically significant reduction of *Candida albicans* adherent cells after data analysis it was found that:

1. There was no significant change on day first comparing candida albicans count among all three groups

2. Least grown seen in group III (Incorporated 10% aloe vera), followed by group II (incorporated 3% aloe vera) and maximum in group I (Control group) on day third.

3. There was significant decrease in candida albicans count on fifth day. Least grown seen in group III (Incorporated 10% aloe vera), followed by group II (incorporated 3% aloe vera) and maximum in group I (Control group)

Increase with concentration incorporation of aloe vera powder into acrylic soft lining material powder results in a statistically significant reduction of Candida albicans adherent cells.

For future prospects Aloe vera is a miracle plant with a vast number of benefits and medicinal advantages of aloe vera including the analgesic, anti-inflammatory, antiseptic, antibacterial, antifungal, antiviral, antioxidant and immune-modulating properties which are well-documented. Incorporation of aloe vera in into acrylic soft lining material can help in reducing inflammation and pain of traumatized/ abused tissue due to anti-inflammatory agents present in aloe vera. Also, much of the clinical applications of aloe vera in dentistry are yet to be recognized which mandates further clinical research in this regard so that the whole mankind can be benefitted from its wide range of properties and application

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Journal of Chemical Health Risks

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