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Original Article

Comparative Evaluation of Efficacy of Antimicrobials Incorporated into Denture Adhesives: An *In Vitro* Study

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Abstract

Introduction: Denture adhesives are water-soluble and non-toxic substances placed between the denture and the tissues to enhance the normal physiological forces that hold dentures in place. They absorb water and swell to many times their original volume, and form anions that interact with the cations of the oral mucous membrane. They contain various flavoring agents such as oil of peppermint and wintergreen oil, which tend to facilitate microbial growth.

Purpose: This study aims to compare and evaluate the efficacy of four different antimicrobials incorporated into denture adhesives.

Materials and Methods: Two denture adhesive brands (FIXON and SECURE) in powder and cream formulations were used. The efficacy of four antimicrobials (nystatin, cephalexin, chlorhexidine, and silver nanoparticles) in denture adhesives was evaluated by assessing *Candida albicans* and *Streptococcus mutans* growth.

Results: Chlorhexidine digluconate and silver nanoparticles showed the highest antimicrobial effect when incorporated into denture adhesives.

Conclusion: Within this study's limitations, it can be concluded that chlorhexidine digluconate and silver nanoparticles can be viable antimicrobial therapies in denture adhesives.

Key words: Cephalexin, Chlorhexidine digluconate, Denture adhesives, Nystatin, Silver nanoparticles

INTRODUCTION

Fabricating a successful complete denture requires scientific knowledge and expertise. Even the most proficient clinicians sometimes may not be able to meet the patient's satisfaction. The psychological status of geriatric patients undergoing complete denture therapy is crucial in determining treatment success. Denture adhesives are water-soluble and non-toxic substances placed between the denture and the tissues to enhance the normal physiological forces that hold dentures in place. Denture adhesives have been used since the late 18th century and became popular in the early 19th century. The first reference by the American Dental Association to denture adhesives came from the Accepted Dental Remedies of 1935. The Council of Dental Materials, Instruments, and Equipment admitted that these products were nonmedical.[1] Denture adhesives are also known as fixatives and adherents and are supplied as powders, pastes, creams (soluble forms) and pads, and synthetic foams (insoluble forms). They absorb water and swell to many times their original volume and form anions that interact with the cations of the oral mucous membrane. They contain various flavoring agents such as oil of peppermint and wintergreen oil, which tend to facilitate microbial growth.[2-4] The purpose of this *in vitro* study is to compare and evaluate the efficacy of four antimicrobials incorporated into denture adhesives.

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MATERIALS AND METHODS

This study was conducted on two commercially available denture adhesives (FIXON and SECURE) in powder and cream formulations. Four antimicrobials were used: Cephalexin (bactericidal), nystatin (antifungal), silver nanoparticles, and 2% chlorhexidine digluconate. The culture media used were Sabouraud's dextrose agar media for *Candida albicans* and nutrient agar media for *Streptococcus mutans*. The total sample size taken was 320 and was divided into the following groups [Figure 1]:

The antimicrobials' efficacy was tested against two microorganisms' strains – *C. albicans* and *S. mutans*. Antimicrobials were added to the denture adhesives at an equal concentration of 1% (0.03 g of each of the antimicrobials in 3 g of denture adhesives). Denture adhesives without any antimicrobial incorporation were taken as controls. Vigorous spatulation was done to ensure uniform distribution. A digital weighing meter was used to measure the weights of the samples. Two methods were used to test the antimicrobial efficacy.

Lawn Culture Method

In this method, Sabouraud's dextrose agar medium was prepared. A measured quantity of Candidal strain (0.1ml) was inoculated into denture adhesive samples (controls, with nystatin, chlorhexidine digluconate, and silver nanoparticles). These inoculated samples were then streaked on the growth media with a sterile swab and were incubated for 24 h. The growth of *C. albicans* was then measured by counting the number of colony-forming units [Figure 2].

Kirby-Bauer Method

In this method, the nutrient agar medium was prepared and 24 h after the preparation, the *S. mutans* strain was streaked on the growth media. Then, bores were made in this media with an 8 mm sterile cork borer. Samples were placed in the prepared bores and the culture plates were incubated for 24 h. The antimicrobial susceptibility was tested by measuring the zones of inhibition around each sample. The zone of inhibition is the diameter of the area around each sample where there is no streptococcal growth and is measured with vernier calipers [Figure 3].

RESULTS

The growth of *C. albicans* was assessed by measuring the number of colony-forming units. The antibiotic susceptibility of *S. mutans* was estimated by measuring the zones of inhibition (in mm). The numbers were presented that were the mean and standard deviation of the number of colony-forming units and zones of inhibition (in mm) [Table 1]. Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 15.0, the statistical analysis software. The data were analyzed by analysis of variance test.

The number of colony-forming units of *Candida* decreased from control groups (164.3, 29.5, 168.8, and 29.5) to nystatin groups (34.2, 20.1, 48.4, and 18.2), followed by chlorhexidine groups (14.7, 11.4, 23.2, and 11.4) and silver nanoparticles groups (5.2, 9.1, 1, and 7.1). There was high statistical significance ($P < 0.00001$) in the growth of *C. albicans* between the control groups and the groups incorporated with antimicrobials. The values of zones of inhibition increased

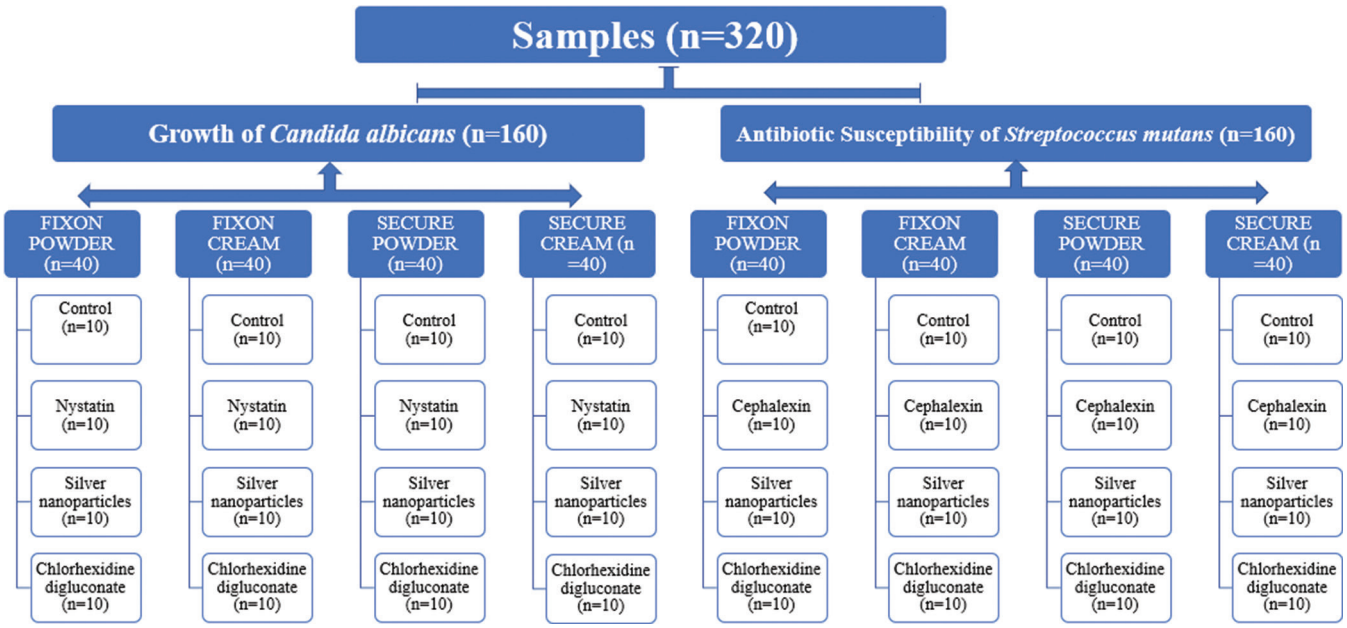

Figure 1: Schematic representation of distribution of samples

Table 1: Comparison of growth of *Candida albicans* (Colony Forming Units) and antibiotic susceptibility of *Streptococcus mutans* (Zones of Inhibition in mm) by one-way ANOVA analysis

Sample	Growth of *Candida albicans*					Antibiotic Susceptibility of *Streptococcus mutans*				
	Group	Mean	SD	f-ratio value	*P*-value	Group	Mean	SD	f-ratio value	*P*-value
Fixon powder	Control	164.3	45.988	99.365	1.00E-05	Control	5.3	4.922	13.209	5.70E-06
	Nystatin	34.2	4.661			Cephalexin	23.4	5.947		
	Chlorhexidine	14.7	5.922			Chlorhexidine	24.8	2.859		
	Silver nanoparticles	5.2	6.321			Silver nanoparticles	23.9	8.478		
Fixon cream	Control	29.5	9.095	15.257	1.44E-06	Control	12.3	2.945	23.621	1.26E-08
	Nystatin	20.1	6.196			Cephalexin	24.4	2.836		
	Chlorhexidine	11.4	7.933			Chlorhexidine	22.1	5.384		
	Silver nanoparticles	9.1	6.505			Silver nanoparticles	25.2	3.765		
Secure powder	Control	168.8	66.446	49.267	7.93E-13	Control	5.3	4.522	25.924	4.13E-09
	Nystatin	48.4	10.782			Cephalexin	23.4	5.947		
	Chlorhexidine	23.2	3.521			Chlorhexidine	24.8	2.859		
	Silver nanoparticles	1	2.16			Silver nanoparticles	23.9	8.478		
Secure cream	Control	29.5	9.095	17.859	2.88E-07	Control	9.5	2.838	42.492	6.45E-12
	Nystatin	18.2	7.099			Cephalexin	30.5	5.986		
	Chlorhexidine	11.4	7.933			Chlorhexidine	29	5.517		
	Silver nanoparticles	7.1	4.201			Silver nanoparticles	31.3	5.292		

from the control group (5.3, 12.3, 5.3, and 9.5) to the cephalexin group (23.4, 24.4, 23.4, and 30.5), silver nanoparticles group (23.9, 25.2, 23.9, and 31.3), and chlorhexidine group (24.8, 22.1, 24.8, and 29). There is a high statistical significance ($P < 0.00001$) in antibacterial susceptibility between the control groups and the groups incorporated with antimicrobials. Control groups showed the lowest zones of inhibition (more streptococcal growth), and silver nanoparticles showed the highest zones of inhibitions [Table 1].

There was decrease in *C. albicans* growth from powder to cream formulations in both the brands. Similarly, when powder formulations were compared with cream formulations, denture adhesive creams showed increased antimicrobial activity (higher zones of inhibition) than powders. This difference was statistically significant with SECURE powder and cream forms [Table 2]. The antimicrobial efficacy among the two denture adhesive brands (FIXON and SECURE) was heterogeneous [Table 2].

The efficacy of each antimicrobial is assessed by subjecting the values to the *post hoc* Turkey HSD test. There was a statistical significance ($P < 0.05$) between the nystatin and silver nanoparticles group. In contrast, no such significance was seen between nystatin and chlorhexidine groups and chlorhexidine and silver nanoparticles group. There was no statistical significance between cephalexin, 2% chlorhexidine digluconate, and silver nanoparticles in inhibiting the growth of *S. mutans* [Table 3].

DISCUSSION

The crucial role of denture adhesives in removable partial dentures has been documented in the literature. They are used in stabilizing trial denture bases during jaw relation and try-in procedures. They help in the patient's ability to adapt to new dentures and boost their confidence. They provide an extra sense of security to denture patients who are public speakers, teachers, business executives, and attorneys. They are also valuable adjuncts for administering drugs in prostheses designed as radiation carriers or radiation protection prostheses.[4-7] Despite the widespread use of adhesives, dentists continued to maintain a negative attitude toward these products. This was due to the misuse of adhesives and the general attitude that the dentist is incompetent or incapable of making a tight-fitting denture. The patients must be educated and instructed about the proper use of denture adhesives and should be cautioned against their misuse.

Another major drawback of these adhesives is that the flavoring and sweetening agents present in them often tend to harbor microbial growth and lead to denture candidiasis and sore mouth.[8,9] Studies conducted by Sampaio *et al.* showed that even the commercially available denture adhesives with antiseptics like p–hydroxybenzoic acid methyl ester and propylparaben showed no significant antimicrobial effect.[10] In the present study, various antimicrobials were incorporated into denture adhesives, and their efficacy against inhibiting candidal and streptococcal growth was studied.

In the present study, denture adhesive creams showed higher antimicrobial activity than powders. This difference was a statistically significant in the case of SECURE powder and cream. This may be because it was easier to obtain a homogeneous mass while mixing the antimicrobials to cream denture adhesives. This was in agreement with an earlier study which showed lower contamination among

Table 2: Comparison of growth of *Candida albicans* (Colony Forming Units) and antibiotic susceptibility of *Streptococcus mutans* (Zones of Inhibition in mm) among powder-cream, powder-powder, and cream-cream formulations of the two brands by paired "*t*" test

Treatment pairs/ samples	Fixon powder versus Fixon Cream		Secure powder versus secure cream		Fixon powder versus secure powder		Fixon cream versus secure cream	
	t-value	*P*-value	t-value	*P*-value	t-value	*P*-value	t-value	*P*-value
Paired "t" test for growth of *Candida albicans*								
Controls	9.09313	0.00000392	6.56828	0.00005149	-0.1761	Insignificant	0	Insignificant
Nystatin	5.70914	0.00014546	7.39746	0.00002057	-3.82251	0.00203714	0.67116	Insignificant
Chlorhexidine	1.05385	Insignificant	4.29921	0.00099648	-3.8966	0.00181910	0	Insignificant
Silver nanoparticles	−1.35964	Insignificant	4.08283	0.00137317	1.98826	0.03900748	0.81665	Insignificant
Paired "t" test for antibiotic susceptibility of *Streptococcus mutans*								
Controls	−1.72786	Insignificant	−2.48738	0.01728742	1.85996	0.04790975	2.16455	0.02931577
Cephalexin	1.13498	Insignificant	−2.66063	0.01301010	1.283	Insignificant	−2.91211	0.00862609
Chlorhexidine	0.38342	Insignificant	−2.13713	0.03065485	−1.26046	Insignificant	−2.83031	0.00985638
Silver nanoparticles	0.11505	Insignificant	−2.34139	0.02196332	0.40227	Insignificant	−2.96983	0.00785376

Table 3: *Post hoc* comparisons of growth of *Candida albicans* (Colony Forming Units) and antibiotic susceptibility of *Streptococcus mutans* (Zones of Inhibition in mm) among four antimicrobials

	Growth of *Candida albicans*				
Treatment Pair	Fixon Powder (*P*-value)	Fixon Cream (*P*-value)	Secure Powder (*P*-value)	Secure Cream (*P*-value)	Inference
Post hoc Turkey HSD Tests					
Control versus Nystatin	0.0010053	0.0318712	0.0010053	0.0074187	Significant
Control versus Chlorhexidine	0.0010053	0.0010053	0.0010053	0.0010053	Significant
Control versus Silver nano	0.0010053	0.0010053	0.0010053	0.0010053	Significant
Nystatin versus Chlorhexidine	0.265644	0.0527039	0.3539318	0.1789006	Insignificant
Nystatin versus Silver nanoparticles	0.0430956	0.0091941	0.0168274	0.0087321	Significant
Chlorhexidine versus Silver nanoparticles	0.7810394	0.8900618	0.465503	0.5535168	Insignificant
Antibiotic susceptibility of *Streptococcus mutans*					
Control versus Cephalexin	0.0010053	0.0010053	0.0010053	0.0010053	Significant
Control versus Chlorhexidine	0.0010053	0.0010053	0.0010053	0.0010053	Significant
Control versus Silver nanoparticles	0.0010053	0.0010053	0.0010053	0.0010053	Significant
Cephalexin versus Chlorhexidine	0.5067427	0.5459532	0.8999947	0.8999947	Insignificant
Cephalexin versus Silver nanoparticles	0.8999947	0.8999947	0.8999947	0.8999947	Insignificant
Chlorhexidine versus Silver nanoparticles	0.8159887	0.294143	0.8999947	0.7184465	Insignificant

denture adhesive creams.[10] When the two commercial denture adhesives were compared (FIXON and SECURE), the results were heterogeneous and not favoring one. This might be because of the variations in the composition of the adhesives.

Nystatin is a polyene antifungal agent produced by *Streptomyces noursei*. It has fungicidal activity against a broad spectrum of pathogenic fungi. It is also widely used as a topical formulation in treating oral candidiasis. It inhibits fungal growth through interaction with ergosterol, leading to loss of selective membrane permeability and eventually cell death.[11] Cephalexin is a beta-lactam antibiotic that belongs to first-generation cephalosporins. It is effective against Gram-positive and some Gram-negative bacteria. It disrupts the growth and inhibits the synthesis of the peptidoglycan layer of the bacterial cell wall.[12]

Schroeder Ju investigated chlorhexidine's ability to inhibit dental biofilm in 1969. It is available in three formulations: Acetate, digluconate, and hydrochloride, at different concentrations. Chlorhexidine digluconate (or chlorhexidine gluconate) is a cationic bis-biguanide with physiologic pH. It has a wide antibacterial spectrum and is active against Gram-positive and Gram-negative bacteria.[13,14] Mozayeni *et al.* compared the antifungal activity of 2% chlorhexidine digluconate with calcium hydroxide and nanosilver gels. They concluded that 2% CHX gels had significantly higher antifungal activity than nanosilver gels.[15] Shino *et al.* compared the antimicrobial effect of chlorhexidine and ketoconazole on *C. albicans* and found that chlorhexidine showed a significant antifungal activity which is comparable with ketoconazole.[16]

Silver nanoparticles constitute a very promising approach for the development of new antimicrobial systems.[17] Their antibacterial action is driven by the oxidative dissolution process. The positively charged silver ions (Ag^{+}) react with negatively charged ions of the bacterial cell wall and cause aggregation, thereby leading to bacterial dissolution and

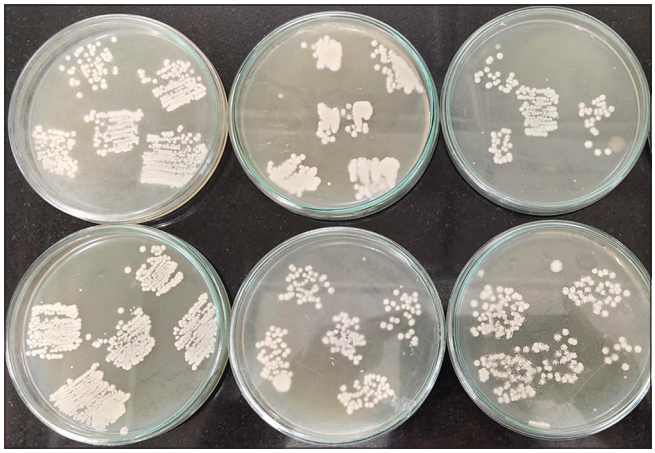

Figure 2: Growth of *Candida albicans* on Sabouraud's dextrose agar medium by Lawn culture method

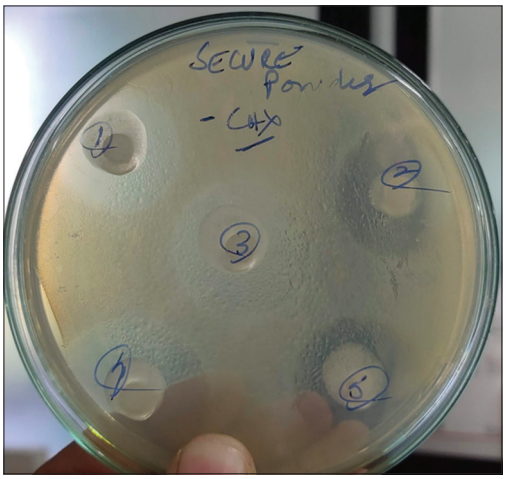

Figure 3: Antibiotic susceptibility of *Streptococcus mutans* on nutrient agar medium by Kirby-Bauer method

passivation. They are also known to have antifungal activity and act by permeating the fungal cell wall and damaging the cell wall and cellular contents.

Bates *et al.* suggested that denture adhesives may inactivate innate immune mediators in the oral cavity, increasing the risk of *C. albicans* infections. They also reported that the inclusion of antifungal antibiotics (nystatin, amphotericin B, fluconazole, chlorhexidine gluconate, and chloride) to denture adhesives aids in a significant decrease of *Candida* infections and denture stomatitis.[18] Garaicoa *et al.* reported that *C. albicans* strains were susceptible to chlorhexidine dihydrochloride and fluconazole and may be candidates for inclusion in adhesive formulations compared to amphotericin B, chlorhexidine digluconate, and nystatin.[19] Chen *et al.* evaluated the pH and effects of streptococcal growth of denture adhesives. They reported that adhesives produce a pH below the critical pH of hydroxyapatite and may not be suitable for patients with natural teeth.[20] Almeida *et al.* reported that enriched fractions of *Equisetum giganteum* and *Punica granatum* combined with denture adhesives, played a collaborative role in biofilm control, and can be considered for temporary use in the treatment and prevention of denture stomatitis.[21] Rajaram *et al.* compared the influence of three different forms of a commercially available denture adhesive material on the growth of *Candida* species. They monitored the pH and number of colonies in the growth medium at different incubation periods and found that the strip form of adhesives showed a prolonged antifungal effect.[22]

In the present study, the antifungal effect of 2% chlorhexidine was significantly higher than nystatin, but there was no such significance with that of silver nanoparticles. There were no significant differences in antibacterial activity of 2% chlorhexidine, cephalexin, and silver nanoparticles. Hence, it can be inferred that both chlorhexidine and silver nanoparticles had significantly better antifungal and antibacterial activity.

Chlorhexidine digluconate is known to have the advantage of substantivity. That is the capacity of binding to soft and hard oral tissues, resulting in a long-lasting effect after administration. After a single mouthwash, about 30% of the active component remains in the oral mucosa, while negligible amounts are ingested. The cationic property reduces its absorption, either by skin or mucosae, including gastrointestinal tract mucosae.

While on the other hand, the systemic absorption and cytotoxicity of silver nanoparticles should be further studied. Furthermore, another demerit observed while using silver nanoparticles were that it has an inherent greyish black color that might hinder the patient's acceptance.

Limitations

1. As it is an *in vitro* study, the biologic environment of the oral mucosa could not be simulated
2. The retentive ability and viscosity of denture adhesives incorporated with antimicrobials should further be studied
3. Systemic influence and cytotoxicity of silver nanoparticles should be evaluated.

CONCLUSION

Within the study's limitations, 2% chlorhexidine digluconate and silver nanoparticles can be viable antimicrobial therapies in denture adhesives.

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