Original Research Article

Comparative Evaluation of Effectiveness of Denture Adhesive after Incorporating Antifungal Agent – An In-vitro Study

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ABSTRACT

Aims: The aim of current study is to evaluate adhesive force and qualitative mycological culture analysis of Denture adhesive (DA) after incorporating antifungal agent in various concentrations.
Study design: Experimental study.
Place and Duration of Study: The current study was conducted at the Department of Prosthodontics, Mansarovar Dental College and Hospital, Bhopal (M. P.) from September 2017 to October 2018.
Methodology: A total of 80 specimens were prepared with heat cured acrylic resin, out of which 40 were used for qualitative anti-microbiological test, and 40 were used for Adhesive force measurement test. Both test had four groups: Group A (Control group DA without MN); Group B (DA+MN 10%); Group C (DA+MN 20%); and Group D (DA+MN 30%).
Results: The mean zone of inhibition was *8.85 ± 0.28* mm for 10% w/w Miconazole Nitrate (MN), *12.95±0.30* mm for 20% w/w MN, and *22.25 ± 0.38* mm for 30% w/w MN. There was a statistically highly significant (*P< .001*) difference between the groups, with an F value of *1077.8*.
Conclusion: Within the limitation of the study qualitative *anti-microbial* property for favorable laboratory performance can be achieved only after the addition of 20% w/w Miconazole Nitrate to denture adhesive paste.

Keywords: Anti-microbial, Adhesive force, PMMA, Removable maxillary denture, Retention, Universal testing machine.

1. INTRODUCTION

Oral cavity pathogens live in a complex habitat and immunological components that maintain the mouth healthy and free of illness keep these infections in check. Denture Stomatitis (DS)

is the most frequent illness. The term "denture stomatitis" was coined by Cahn (1936). DS is an inflammation of the mouth caused by removable dentures. Symptoms include discomfort, burning, and a foul taste [1]. People with DS often have no symptoms and are unaware they have it. Traditionally, clinical symptoms were categorized by inflammation [2].

Newton [2-4] first established a scale for grading DS inflammation. Candida species, particularly *Candida albicans*, are suspected of causing denture stomatitis, affecting 40-60% of people [5-7]. These organisms colonized and infect the denture fitting surface rapidly, producing direct cytotoxicity and activating acid proteinase and phospholipase produced by these yeasts, increasing *Candida albicans* proliferation [2,4,6-9]. Other organism, such as *Candida glabrata* [9-13], may be responsible for the sickness. *Candida albicans* thrives on the nutrient-rich surface of denture tissue [6].

Arendorf and Walker claim that 40% of healthy individuals have oral commensal. Candida might be opportunistic in denture users because dentures impede the passage of oxygen and saliva to the underlying tissue, resulting in an acidic and anaerobic environment that encourages yeast growth. *C. Albicans* colonization, plaque formation, and pathogenicity rely on solid surfaces such as acrylic resin for survival. Early yeast adhesion is affected by hydrophobic [15] (van der Waal forces) and electrostatic forces [16].

Denture adhesives (DA) are often used to improve denture retention and stability [17]. These materials come in powder, strip, cream, and cushion forms. Water-soluble polymers with mucoadhesive characteristics and essential components make up DA cream [17-18]. Mucoadhesion may be used to provide sustained oral drug release [20-21]. They may remain on the mucosa longer, enhancing pharmaceutical absorption [22]. They outperform commercial gel antifungal formulations [23].

Denture wearers may maintain DA for 6–8 hours and the layer of DA is susceptible to candidal infection. To avoid this, DA may release antimicrobial compounds like Miconazole nitrate (MN) without affecting its mucoadhesive properties [24]. According to Scher EA *et. al.,*and Leite AR *et. al.,* some denture adhesives are already antimicrobial [25].

Miconazole nitrate (MN) is a first-line broad-spectrum triazole for superficial mucosal Candidiasis. Oral mycoses have been treated using MN that is commercially available in various form e.g. Gel [26], chewing gum [27], bio-adhesive films [28], buccal patches [22], buccal tablets [29], and spray-dried polymeric micro particles of MN with enhanced drug solubility & antifungal activity [30]. MN oral gel is globally sold and has a short contact time (6-8 hours) [31-32]. There were very few studies conducted on DA with antifungal agents, but optimum concentration should be established without affecting adhesive force of DA.

The aim of the present research was to evaluate and compare the adhesive force and antifungal property of denture adhesive (DA) paste after incorporating antifungal agent Miconazole Nitrate (MN) with heat polymerized denture base resin at various concentrations. The null hypothesis was there were no differences between all groups in term of antifungal property and adhesive force of DA.

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.]

2. MATERIAL AND METHODS

The current study was conducted at the Department of Prosthodontics, Mansarovar Dental College and Hospital, Bhopal (M. P.) from September 2017 to October 2018 after taking ethical committee approval (S.No./MPMSU/ Academic/2016-17/112). According to Cartagena AF *et. al.* [33] Sample size of 10 in each group was determined with 95% confidence interval, 80% power of test, with absolute precision of 4.0.

2.1 Specimens preparation –

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Stainless Steel cylinder die of size 75.0 x 12.0 mm were fabricated to create the test samples for Adhesive force measurement test. Stainless Steel disc die of size 2 mm x 0.5 mm were fabricated to create the test samples for anti-microbiological test. A total of 80 specimes were prepared with heat cured acrylic resin (Trevalon denture base material by Dentsply), forty were used for anti-microbiological test and forty were used for Adhesive force measurement test.

2.2 Denture adhesive preparation –

For present study formulations of denture adhesive (Fixon super grip cream, IPCA Health Products, Mumbai, India) and miconazole nitrate (Detrain, J.K pharmaceutical company, Chennai, India) in various concentration was obtained by weighing different concentration i.e. 10%, 20%, 30% w/w in electronic balance followed by mechanical mixing. Both tests had four groups: Group A (Control group DA without MN); Group B (DA+MN 10%); Group C (DA+MN 20%); and Group D (DA+MN 30%)

2.3 *Anti-microbial* test –

The antimicrobial evaluation for all four groups was done according to Cartagena AF *et. al.* [33] after 24 and 48 hrs.

2.4 Adhesive force measurement –

The evaluation of Adhesive force was done for all specimens described by Cartagena AF *et. al.* [33] after 6 hours and 12 hour by using Universal Testing Machine (Fuel Instruments & Engineers Pvt. Ltd., Kolhapur, Maharashtra, India)

2.5 Statistical analysis –

The obtained data were subjected to One way ANOVA and Turkey-Kramer multiple comparison test ($\alpha = 0.05$) for statistical analyses using SPSS for windows (IBM SPSS Statistics, v20; IBM Corp, Armonk, N.Y., USA).

3. RESULTS

3.1 Result of Microbiological assay –

The mean and standard deviations of zone of inhibitions in different groups was given in table 1. There was a statistically highly significant ($P < .001$) difference between the groups, with an F value of 1077.8.

Miconazole Nitrate 30% (Group - D) significantly increased antimicrobial activity. (Table 1) The one way ANOVA test was used to compare various Miconazole Nitrate concentrations. The test result indicates that there was a significant difference between the control and Miconazole Nitrate 10%, 20%, and 30% groups. This indicates that raising the proportion of Miconazole Nitrate resulted in a substantial increase in antimicrobial activity. (Table 2)

Table 1: Evaluation of Zone Of Inhibition (mm) between different groups of DA+MN

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I D no.	Group – A (Mean±SD)	Group – B (Mean±SD)	Group – C (Mean±SD)	Group – D (Mean±SD)
Am1	0±0	8.0±2.82	13.0±1.41	21.5±2.12
Am2	0±0	8.25±1.76	13.5±2.12	22.5±2.12
Am3	0±0	9.0±2.82	12.5±2.12	24.5±2.12
Am4	0±0	10.0±2.82	13.5±3.53	21.5±2.12
Am5	0±0	10.0±4.24	14.5±3.53	20.5±2.12
Am6	0±0	8.0±2.12	12.0±4.24	22.5±3.53
Am7	0±0	7.75±1.76	14.0±2.82	23.5±3.53
Am8	0±0	8.5±2.12	12.0±2.82	23.0±4.24
Am9	0±0	9.0±1.41	11.5±3.53	21.0±4.24
Am10	0±0	10.0±2.82	13.0±2.82	22.0±2.82

Table 2- One way ANOVA test for comparing the zone of inhibition (mm) between different groups of DA+MN

Source of variation	Degree of freedom	Sum of squares	Mean square	F-value	P-value
Treatment (between columns)	3	2559.9	853.29	1077.8	*<0.001**
Residual (within columns)	36	28.500	0.7917		
Total	39	2588.4			

3.2 Result of Adhesive force Measurement –

The Turkey-Kramer multiple comparison test was used to determine the differences in adhesive force between groups. The mean adhesive force in the control group was 18.10 ± 2.55 N, whereas the mean adhesive force in the Miconazole Nitrate 10% w/w group was 7.55 ± 0.98 N, the mean adhesive force in the Miconazole Nitrate 20% w/w group was 5.85 ± 0.74 N, and the mean adhesive force in the Miconazole Nitrate 30% w/w group was 4.6 ± 0.61 N. The test result indicates that a substantial difference existed between the groups. This indicates that the adhesive force was significantly reduced after the addition of Miconazole Nitrate. There was a statistically highly significant (P< .001) difference between the groups, with F = 1077.8. (Table 4)

Table 3: Evaluation of Adhesive force (N) between different groups of DA+MN

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I D no.	Group – A (Mean±SD)	Group – B (Mean±SD)	Group – C (Mean±SD)	Group – D (Mean±SD)
Ad1	15.0±0	8.0±1.41	7.0±2.82	4.5±0.70
Ad2	17.0±0	7.0±1.41	6.5±0.70	4.5±2.12
Ad3	16.0±0	8.5±2.12	5.5±2.12	4.0±2.82
Ad4	19.0±0	8.0±4.24	6.5±2.12	5.0±1.41
Ad5	18.0±0	9.0±1.41	4.5±2.12	4.5±3.53
Ad6	21.0±0	6.5±0.70	5.5±2.12	3.5±2.12
Ad7	21.0±0	8.0±1.41	5.5±3.53	5.5±3.53
Ad8	15.0±0	8.0±2.82	5.5±4.94	4.5±3.53
Ad9	17.0±0	6.0±2.82	5.5±0.70	5.5±2.12
Ad10	22.0±0	6.5±2.12	6.5±0.70	4.5±0.70

Table 4:Comparison of Adhesive force (N) between different groups of DA+MN using Turkey-Kramer multiple comparison test.

Comparison	Mean Difference	q¥	P-value
Group A Vs Group B	10.550	22.954	***P< .001******
Group A Vs Group C	12.250	26.652	***P< .001******
Group A Vs Group D	13.500	29.372	***P< .001******
Group B Vs Group C	1.700	3.699	*P> .05 NS*
Group B Vs Group D	2.950	6.418	***P< .001******
Group C Vs Group D	1.250	2.720	*P> .05 NS*

Note: ¥If the value of q is greater than 3.813 then P value is less than .05; ***= highly significant; NS = Not significant.

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4. DISCUSSION

Due to its ease of manipulation and cheap cost, polymethyl methacrylate (PMMA) has been the most commonly used denture base material. Despite its widespread use, PMMA's characteristics remain insufficient. The durability of removable dentures is significantly impacted by fractures or microbial growth. Microorganism colonization and subsequent biofilm formation on the denture surface are significant contributors to the development of denture stomatitis (DS), which is potentially a public health issue.

Since post-prosthesis care is often overlooked during the manufacture of removable dentures, it has been recommended that patient instructions on the usage of adjunctive devices such as denture adhesives should be included in post-placement care [34]. The preparation and usage of two agents were chosen for this study: denture adhesive paste and MN antifungal agent. In this study, we have attempted incorporating various formulations and concentration of MN-micro particles on DA to achieve efficient antifungal activity, without impairment on adhesive force.

The current research demonstrates the comparison of various Miconazole nitrate concentrations using the one way ANOVA test. The test result indicates that there was a substantial increase in antimicrobial activity after the addition of Miconazole nitrate and that the activity rises when the concentration of MN is increased that is highly significant. The results of present study are in accordance with results reported by previous study [33] for MN in gel form.

The tukey-kramer multiple comparison test was used to compare various Miconazole nitrate concentrations for adhesive force measurement. The test result indicates that there was highly significant difference between the control and Miconazole nitrate 10%, 20%, and 30% groups. This indicates that the adhesive force was significantly reduced after the addition of Miconazole nitrate. The results of present study are in contradiction with results reported by previous study [33] for MN in gel form. It may be due to use of polymer particles in previous study, which increase viscosity of DA. In current study as the MN concentration increases, the adhesive forces decreased. It may be due to reduced viscosity of DA.

The zone of inhibition agar disc diffusion test was used to determine antimicrobial property in this research. This test has been developed as a fast, low-cost, and easy technique for predicting dental materials antifungal properties. Several researchers have been conducted to determine the antimicrobial potential of dental materials using this technique [35-36].

Candida albicans can potentially contribution to other morbidities e.g. Cancer [36], cardiac risk [1]. Potential *C. albicans* mechanisms of contributing to disease include potent induction of IL-17 signaling, breach of gut epithelial barriers and activation of multiple cancer-associated factors [39].

Although ISO 10873 recommends a standard test, several authors have evaluated the adhesive strength of DA [40-42], and mucoadhesive drug delivery systems using alternative techniques [42]. The test employed here, as suggested by Zhao *et. al.,*[34], has the benefit of being easy to administer and needing no specialized equipment. Acrylic resin cylinders are easy to produce and simple to place in the testing equipment. Nonetheless, this technique, like the one prescribed by ISO 10873, it does not take into account variables that may affect the findings, such as the presence of natural saliva, keratinized mucosa, and intaglio surface. Other limitations are: use of a single species of oral biofilm, qualitative analysis, not considered toxicity, and a single heat-polymerized denture foundation. Future

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study should be carried out with large sample size, qualitative analysis of mycology test, and considering toxicity of the intervention with oral environment simulation that will give more accurate results regarding antimicrobial efficacy without affecting adhesive force of DA.

5. CONCLUSION

Within the limitation of study, anti-microbial property of denture adhesive paste increases with the addition of Miconazole Nitrate. Qualitative anti-microbial property for favorable laboratory performance can be achieved only after the addition of 20% w/w Miconazole Nitrate to denture adhesive paste.

CONSENT (WHERE EVER APPLICABLE)

Since the present study was in-vitro, so there was no need of patient consent.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Ethical approval had been taken from institutional ethical committee before starting the study. (S.NO./MPMSU/ ACADEMIC/2016-17/112)
COMPETING INTERESTS DISCLAIMER: No competing interest

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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ACRONYMS & ABBREVIATIONS

PMMA = Polymethyl methacrylate

DA = Denture adhesive

MN = Miconazole nitrate

DS = Denture stomatitis

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