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Original ARTICLE

Comparison of Fluconazole and Nystatin incorporated into Tissue Conditioner for denture stomatitis

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***ABSTRACT*:**
Background: Denture stomatitis, also known as atrophic candidiasis, is the most common fungal infection in elder patients and in those who wear dentures. This condition is usually asymptomatic, although it can be associated with burning, bleeding, an unpleasant taste. Even though several microorganisms can cause this condition, most studies showed that Candida albicans and the ill-fitting dentures promote the development of this condition. Several studies have attempted to incorporate antifungal agents to tissue conditioner for prevention of plaque formation and treatment of denture stomatitis. **Aim of the study:** To compare Fluconazole and Nystatin incorporated into Tissue Conditioner for denture stomatitis. **Materials and methods:** The present study was conducted in the Department of Prosthodontics of the Dental institution. For the study sample, we obtained clinical isolates of Candida albicans (ATCC 10231), from the Department of molecular microbiology of medical institute, to use as test organisms for the current experimental study. Candida albicans was cultured onto Sabouraud dextrose agar plate and incubated at 37ºC for 3 days. A colony from the stock culture was then diluted in 2 ml sterile saline and a suspension of 1×106 CFU/ml was prepared. **Results:** We observed that that Nystatin 5% solution was the most efficient for inhibiting attachment and colonization of C. albicans. Fluconazole 5% solution is partially effective efficient for inhibiting attachment and colonization of C. albicans. The control solution was least effective with highest Candida density seen in control solution. **Conclusion**: Within the limitations of the present study, it can be concluded that the incorporation of Nystatin into the tissue conditioner is more efficacious than Fluconazole for denture stomatitis.
Keywords: Denture stomatitis, Nystatin, Tissue conditioners

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INTRODUCTION

Denture stomatitis, also known as atrophic candidiasis, is the most common fungal infection in elder patients and in those who wear dentures.[1] It is the result of a poor denture cleanliness, poor oral hygiene and wearing denture at night, which causes a decrease in the salivary flow below denture surface, thus facilitating the accumulation of characteristic biofilms.[2] This condition is usually asymptomatic, although it can be associated with burning, bleeding, an unpleasant taste. Even though several microorganisms can cause this condition, most studies showed that Candida albicans and the ill fitting dentures promote the development of this condition.[3, 4] Candida albicans is an oral commensally fungus found in 40% of human beings, which facilitates the formation of denture plaque, in which Candida albicans is commonly isolated as the pathogenic agents. Several studies have attempted to incorporate antifungal agents to tissue conditioner for prevention of plaque formation and treatment of denture stomatitis.[5, 6] Hence, the present study was conducted to compare Fluconazole and Nystatin incorporated into Tissue Conditioner for denture stomatitis.

MATERIALS AND METHODS:

The present study was conducted in the Department of Prosthodontics of the Dental institution. The protocol of the study was approved from the ethical committee of the institute prior to starting the study. For the study sample, we obtained clinical isolates of Candida albicans (ATCC 10231), from the Department

of molecular microbiology of medical institute, to use as test organisms for the current experimental study. Candida albicans was cultured onto Sabouraud dextrose agar plate and incubated at 37ºC for 3 days. A colony from the stock culture was then diluted in 2 ml sterile saline and a suspension of 1×106 CFU/ml was prepared. Tissue conditioner was mixed and prepared according to manufacturer's instruction. Antifungal agents, nystatin and fluconazole were mixed into tissue conditioner powder at concentrations of 5% wt/wt in a sterile plate. A sterile glass rod was used to prepare a thin film of tissue conditioner with 1mm thickness and punched as 5mm diameter disks. One specimens of pure tissue conditioner was also prepared as negative control. All disks were contaminated with 100 µl of 1 × 10 6 CFU/ml C. albicans cell suspension and the cell culture plate were incubated at 35ºC on a rotary shaker for 48 hours. The plates were incubated at 37ºC for 48 hours and the colonies were enumerated. The data was tabulated for further evaluation. The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistical significant.

RESULTS

Table 1 shows the mean Candida density (CFU/ml) for Nystatin 5%, Fluconazole 5% and control solution. We observed that that Nystatin 5% solution was the most efficient for inhibiting attachment and colonization of C. albicans. Fluconazole 5% solution is partially effective efficient for inhibiting attachment and colonization of C. albicans. The control solution was least effective with highest Candida density seen in control solution. The results were statistically significant ($p<0.05$) [Fig 1].

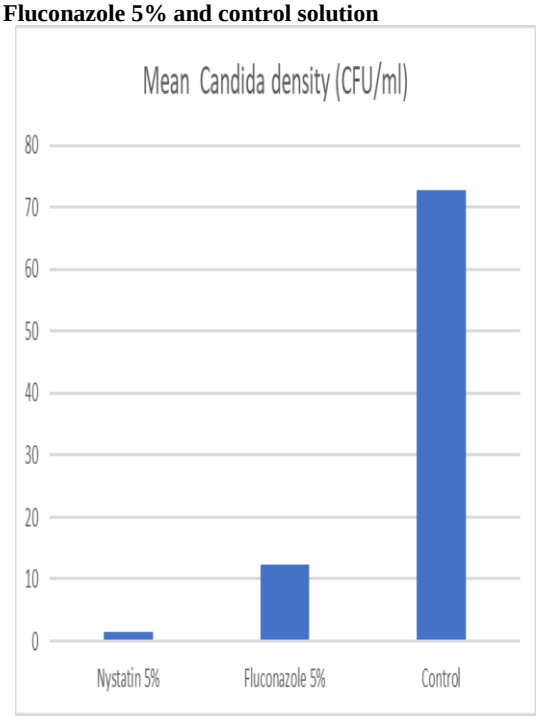
DISCUSSION

In the present study nystatin showed higher inhibitory effects than fluconazole as it almost completely inhibited the production of *C. albicans* in tissue conditioner disks, however fluconazole could partially prevent the growth and adhesion of *Candida*. The results were compared with studies from literature. Falah-Tafti A et al evaluated the efficacy of the two common antifungal agents mixed with tissue conditioner against Candida albicans. Tissue conditioner disks (Acrosoft) with 5mm diameter and 1mm thickness containing different concentrations of nystatin and fluconazole (1%, 3%, 5%, 10% wt/wt) as well as disks with no antifungal agents (8 disks for each group) were prepared for experimental biofilm formation by inoculation with Candida albicans cell suspensions. The specimens were incubated in cell culture microtiter plate wells containing Sabouraud's broth in a rotator shaker at 30°C for 48 hours. Then, the specimens were rinsed and sonicated in sterile water to remove surface organisms. The attached yeasts were enumerated by inoculation of the yeast suspension on Sabouraud's agar. The data was compared using Kruskal-Wallis and Dunn's tests using prism software. P value less than 0.05 was considered significant. The 1% to 10% mixture of nystatin and tissue conditioner completely inhibited the attachment and colonization of Candida albicans, although for fluconazole only a 10% concentration caused complete inhibition. Nystatin showed a potentially higher effect in inhibition of candida attachment and colonization compared to that of fluconazole and a statistically significant difference was seen between 5% and 1% fluconazole. They concluded that tissue conditioner with 1% to 10% nystatin or 10% fluconazole can completely inhibit the adhesion and colonization of Candida albicans. Amin WM et al monitored the release of the antifungal drugs Fluconazole, Chlorhexidine and a combination of the two from an auto-polymerized poly (methyl methacrylate) denture base resin; and investigated the effect of the released drugs upon the growth of Candida albicans. A high performance liquid chromatography-Ultra violet (HPLC-UV) method was used in the analysis of the released drugs into distilled water from PMMA discs doped with the antifungal drugs Fluconazole (10%), Chlorhexidine (10%) and a combination of the two drugs (5% each).

Table 1: Mean Candida density (CFU/ml) for Nystatin 5%, Fluconazole 5% and control solution

Specimens	Mean Candida density (CFU/ml)	p-value
Nystatin 5%	1.39	0.005
Fluconazole 5%	12.36	
Control	72.65	

Fig 1: Showing mean Candida density (CFU/ml) for Nystatin 5%, Fluconazole 5% and control solution



The antifungal efficacy of the released drugs was monitored, microbiologically, employing "well" technique on a Saborauds culture medium inoculated with a resistant strain of Candida albicans. It was shown that Fluconazole, Chlorhexidine and the combination of the two drugs can be successfully incorporated with PMMA. It was found that the drugs leach steadily out of the PMMA resin into distilled water at mouth temperature and that sustained drug release continued throughout the 28 days test period. It was also shown that the released drugs demonstrated an antifungal activity against the resistant Candida albicans and this was most remarkable in the combined drugs samples. They concluded that the sustained release of anti-fungal drugs from the

PMMA resin clearly constitutes a new dosage form of these drugs via the poly (methyl methacrylate) delivery system. [7, 8]

Barua DR et al compared the efficacy of neem leaf extract and three other antimicrobial agents incorporated in a tissue conditioner against both Candida albicans and Streptococcus mutans. Standard strain of Candida albicans and Streptococcus mutans were inoculated into Sabouraud Dextrose broth and Mitis-Salivarius-Bacitracin broth respectively incubated at 37°C. Tissue conditioner (Viscogel) mixed with two different concentrations of ketoconazole, nystatin and chlorhexidine diacetate (5%, 10% w/w) and neem leaf extract (7.5% w/w and 15% w/w) and control group (plain tissue conditioner) were placed into punch hole (6 mm diameter) agar plate inoculated with Candida albicans and Streptococcus mutans. A total of 216 samples were prepared for both Candida albicans and Streptococcus mutans. Mean Inhibition Diameter (MID) across each punch holes were measured in millimetres at 24 hours and seven days and data were statistically analysed using Kruskal Wallis test followed by Mann-Whitney U test. Both ketoconazole and nystatin (10% w/w) showed maximum inhibition of 32 mm and mean of 31.75 followed by 15% w/w neem leaf extract with an inhibition of 21 mm and mean of 20.67 after 24 hours against Candida albicans whereas chlorhexidine diacetate (10% w/w) showed mean of 25.67 followed by chlorhexidine diacetate (5% w/w) and neem extract (15% w/w) which showed mean of 24.17 and 23.67 respectively against Streptococcus mutans. It was concluded that neem leaf extract exhibited considerable potential to be an efficacious antimicrobial agent against both Candida albicans and Streptococcus mutans. Mousavi SA et al evaluated the antibacterial and antifungal properties of a tissue conditioner after incorporation of ZnO–Ag nanoparticles into their structure. In this in vitro study, 4 microorganisms were evaluated at 6 concentrations of ZnO–Ag nanoparticles at 24- and 48-hour intervals, using 168 samples. The nanoparticles were mixed at a ratio of 50% Ag and 50% ZnO and were homogenized with the tissue conditioner at 0.625, 1.25, 2.5, 5, 10 and 20 wt% according to the MIC technique principles. After culturing the microorganisms, a spectrophotometer was used for determining proliferation of microorganisms with the use of turbidity after 24 and 48 hours of incubation at 37ºC. Complete inhibition of the proliferation of Candida albicans, Enterococcus faecalis and Pseudomonas aeruginosa was observed at 24- and 48-hour intervals at a concentration of 10%; such inhibition was observed at 20% concentration of nanoparticles with Streptococcus mutans. In addition, the most effective concentration of ZnO–Ag nanoparticles at both 24- and 48-hour intervals was 5% with C. albicans and 2.5% with E. faecalis. In addition, the most effective concentration at 24- hour interval with S. mutans was 10% and with P. aeruginosa they were 5% at 24-hour and 2.5% at 48-hour intervals. In conclusion, incorporation of ZnO–Ag nanoparticles into tissue conditioners resulted in the inhibition of bacterial proliferation. [9,10]

CONCLUSION

Within the limitations of the present study, it can be concluded that the incorporation of Nystatin into the tissue conditioner is more efficacious than Fluconazole for denture stomatitis.

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