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Original Research

Comparative analysis of efficacy of Fluconazole and Nystatin incorporated into Tissue Conditioner as drug delivery method for denture stomatitis

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***ABSTRACT*:**
Background: Denture stomatitis is a pathological condition of the denture bearing mucosa caused by trauma from ill-fitting dentures. It is characterized by generalized inflammation or reddening of the palatal mucosa underneath the denture and more prevalent in complete denture wearers. The etiology of denture stomatitis can be multifactorial however, the infection by Candida species especially the Candida albicans (C. albicans), is considered to be the main etiologic factor. Treatment plan should include substitution of old prosthesis, elimination of anatomical irregularities, establishment of a nontraumatic occlusion, nutritional reconstitution, oral hygiene instructions, antifungal treatment, and systemic evaluation. The limitations have led to the development of other methods of drug elution such as incorporation of antifungal and antimicrobial agents with denture soft liners. **Aim of the study:** To compare the efficacy of Fluconazole and Nystatin incorporated into Tissue Conditioner as drug delivery method for denture stomatitis. **Materials and methods:** The present study was conducted in the department of prosthodontics of the dental institution. Candida albicans was cultured onto Sabouraud dextrose agar plate and incubated at 37ºC for 3 days. 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.**Results:** We observed that that Nystatin 5% solution was the most efficient for inhibiting attachment and colonization of C. albicans (0.22). 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 present study, we conclude that the incorporation of nystatin incorporated into tissue conditioners is more effective in treatment of chronic atrophic candidiasis in denture users as compared to Nystatin.
Keywords: Fluconazole, Nystatin, Tissue conditioners, Stomatitis.

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INTRODUCTION:
Denture stomatitis is a pathological condition of the denture bearing mucosa caused by trauma from ill-fitting dentures.[1,2] It is characterized by generalized inflammation or reddening of the palatal mucosa underneath the denture and more prevalent in complete denture wearers.[3] A significant proportion of denture wearers (72%) are affected by this condition.[4] The fungal (candidal) infections are considered to be the main contributory factor for developing the denture stomatitis hence commonly termed as Candida associated stomatitis.[5] The etiology of denture stomatitis can be multifactorial however, the infection by

Candida species especially the Candida albicans (C. albicans), is considered to be the main etiologic factor. In addition to C. albicans, other risk factors such as denture trauma, poor oral and denture hygiene, continuous and nocturnal denture wear, xerostomia, alteration in salivary pH have been reported to be associated with denture stomatitis.[6,7] Treatment plan should include substitution of old prosthesis, elimination of anatomical irregularities, establishment of a nontraumatic occlusion, nutritional reconstitution, oral hygiene instructions, antifungal treatment, and systemic evaluation. However, the success of topical application of drugs in the oral cavity may be compromised by some factors such as discomfort caused by the infection, unpleasant taste, and frequency of dosage.[7-9] These limitations have led to the development of other methods of drug elution such as incorporation of antifungal and antimicrobial agents with denture soft liners. Several attempts have been made to incorporate different antifungal agents such as propolis, zeolite, chlorhexidine, fluconazole, Punicagranatum, nystatin, itraconazole, miconazole, ketoconazole, and clotrimazole with varying degree of success.[8,9] Hence the present study was planned to compare the efficacy of Fluconazole and Nystatin incorporated into Tissue Conditioner as drug delivery method for denture stomatitis.

MATERIALS AND METHODS:

The study was conducted in the Department of General Pathology of the medical institution. The ethical clearance for study protocol was obtained from ethical committee of the institution. The study was conducted in the Department of Dentistry of Shridevi Institute of Medical science and research hospital, Tumakuru, Karnataka. 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, from the Department of molecular microbiology of ofShridevi Institute of Medical science and research hospital, 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.

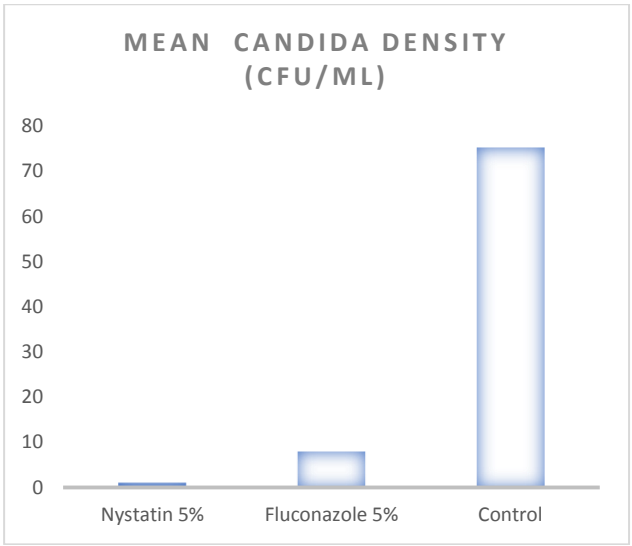
RESULTS:

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 (0.22). 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].**

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%	1.15	0.002
Fluconazole 5%	7.98	
Control	75.21	

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



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. Chow CK et al conducted an in vitro study incorporated antifungal agents into tissue conditioners to investigate the effectiveness of this method of drug delivery. Combinations of nystatin, fluconazole, itraconazole and Coe Soft, Viscogel, Fitt were tested at 1, 3, 5, 7, 9 and 11 wt/wt%, with and without sterilized saliva. 6 mm diameter cores were punched in Sabouraud plates pre-grown with standardized C. albicans. Antifungal agents plus tissue conditioner mixtures were injected into each core. Inhibition diameters were measured for 14 days. Cores with only tissue conditioners acted as negative control and showed no significant inhibition activity. Peak activity was between 65 to 89 hours; followed by a plateau. Itraconazole had greater fungicidal activity than fluconazole; while nystatin was found to have the least fungicidal activity. The most effective concentration for nearly all combinations was 5% wt/wt. Specimens with saliva showed greater antifungal activity than those without. Itraconazole altered the physical properties of Viscogel hence this combination is not recommended for clinical use. It was concluded that the treatment of chronic atrophic candidiasis by incorporation of antifungal drugs into tissue conditioners is efficacious. 5% wt/wt itraconazole mixed with Coe Soft or Fitt is recommended for clinical study where compliance of patient or care giver cannot be relied upon. Peak antifungal activity at 3 days suggests that mixtures prepared for clinical study may be replaced soon after this time for maximum effectiveness.[10,11]

Vankadara SK et al evaluated the Colonization & Inhibition of Candida albicans in selected commercially available denture lining materials material by mixing them with varying concentrations and doses of tea tree oil. Five test discs of 10mm diameter and 1.5mm thickness were prepared using commercially available soft denture lining materials (Viscogel and GC-soft). Tea tree oil of varying concentrations (10%, 20%, 30%, and 40%) and doses (0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml) were added during manipulation. Test discs kept in sterile artificial saliva were inoculated with Candida albicans (ATCC-2091 strain) and incubated for 6 weeks. These discs were fixed, dehydrated air dried and stained using 0.03% acridine orange stain and observed under Fluorescent microscope to count the colonies on the surface of each disc to evaluate the colonization. To evaluate inhibition, test discs were placed on the top of Sabouraud's dextrose agar inoculated with Candida albicans (ATCC-2091 strain). After incubation at 370C for 48 hours, the zone of Inhibition formed around the samples was measured. The GC soft liner had higher mean colonization and lesser zone of inhibition of C.albicans when compared to Visco-gel soft liner and highest zone of inhibition observed with 2 ml volume and 40% vol/vol concentration of melaleuca alternifolia. They concluded that by the addition of Tea Tree oil, Viscogel had good acquired good antifungal properties than GC-soft lining materials. 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.[12,13]

CONCLUSION:

Within the limitations of present study, we conclude that the incorporation of nystatin incorporated into tissue conditioners is more effective in treatment of chronic atrophic candidiasis in denture users as compared to Nystatin.

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