



EVALUATION OF ANTIFUNGAL ACTIVITY OF NYSTATIN AND GRAPE SEED EXTRACT INCORPORATED INTO DENTURE ADHESIVE

Nireeksha B.N, Roseline Meshramkar, Lekha K, Raghavendra D Kulkarni and Manjunath Hosamani

SDM College of Dental Science, sattur, Dharwad

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ABSTRACT

Aim: To evaluate the efficacy of two antifungal agents incorporated into denture adhesive against *candida* infection.

Material and Methods: Forty five acrylic disks were fabricated simulating the denture. They were divided into three groups of 15 each. Group 1 Nystatin mixed with denture adhesive powder, Group 2 Grape seed extract with denture adhesive powder and an untreated group. These were kept overnight. Inoculation of *candida albicans* done and further plating of specimens done (on sabouraud's dextrose agar) to check for candidal growth.

Result: Nystatin showed very high anti candidal activity and grapeseed extract showed no anti candidal activity

Conclusion: Inclusion of Nystatin into denture adhesive powder will help prevent candidal infection in denture wearers

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INTRODUCTION

The most common Opportunistic fungal infection is and Candidiasis and Oral Candidiasis is no exception to this. It is caused by yeast formed fungal microorganisms called *Candida* and most of the species carry them naturally. This Opportunistic infection causes oral infection in higher frequency than other form of infections and the frequency has increases over the years drastically.⁽¹⁾ The mycelial form of these species, favours the adherence of fungus onto the micro pores, grooves of the constructed denture. This helps them in preventing their removal while mechanical and chemical cleansing.⁽²⁾ Denture Stomatitis is one of the most common problems faced by the denture wearers, is known to be caused by *C.albicans*. Treatment of Denture stomatitis may resolve presumption of Systemic or topical conventional antifungal agent.⁽³⁾ Denture adhesive used to improve denture retention turn into a viscous mass on contact with saliva. This in turn acts as base for candidal growth. This result in fungal colonization subsequent biofilm formation and pathogenesis development.⁽²⁰⁾

Considering that Denture stomatitis is mainly associated *C. albicans* it is important and crucial to create biocompatible antifungal therapeutic alternative with new antimicrobial active principles to which the fungus *C albicans* does not yet have resistance.⁽⁴⁾ Candidiasis is associated with multiple risk factors indicating impaired salivary gland function,

denture wearing, Oral mucosa disruption, endocrine alterations (DM, Pregnancy Hyper-Thyroidism) HIV infection⁽⁵⁻⁷⁾. The primary step in treatment of Candidiasis is the elimination of predisposing factors.

Topical antifungal agent such as nystatin, amphoterin B, miconazole and cotrimazole are first line treatment for uncomplicated cases^(8,9,10). Nystatin is a membrane active Polyene macrolide produced by *streptomyces noursei* strain and is available in various forms such as oral suspension, topical cream and oral pastiles.⁽¹¹⁻¹⁵⁾ Nystatin is not absorbed from GIT when administered orally. Therefore, Topical use of nystatin is considered most common route of administration in dentistry⁽¹⁶⁾. There is incidence of resistance of certain species to antifungal agent is a matter of concern in recent years

As reported by wide literature⁽¹⁷⁾, grapes are rich source of polyphenols, important secondary metabolite produced by higher plants which plays important role in plant Physiology and showed healthy properties in human organism mainly antioxidant, antiallergic, antihypertensive and antimicrobial⁽¹⁸⁾. The combination of denture adhesive and herbal medicines may be useful, effective and complementary to treatment of denture stomatitis.

In this study, we try to find antifungal activity of nystatin in comparison to grape seed extract when incorporated into denture adhesive. As there are not many studies conducted regarding antifungal effect of the Nystatin and grape seed extract.

*Corresponding author: Nireeksha B.N
SDM College of Dental Science, sattur, Dharwad

MATERIAL AND METHOD

A total of forty five specimens were fabricated (10mm diameter) using heat cure acrylic resin. The sides of specimen were ground and polished. Few grooves were incorporated onto specimen to simulate the surface of denture. Further all forty five specimens were sterilised (autoclaved)



Fig 1 wax pattern for fabrication of acrylic discs

These forty five specimens were divided into three groups of fifteen specimens in each

1. Denture adhesive with nystatin
2. Denture adhesive with grape seed extract
3. Denture adhesive (control group)

Group 1

- One ml of DMSO was added to a vial having 1,000,000 units of Nystatin⁽¹⁶⁾. The powder was
- Dissolved completely and 9 ml of distilled water was added to the vial. The final dilution achieved was 100,000 units per ml of Nystatin.
- Denture adhesive powder (Fixon) was mixed with 100,000 units/ml of nystatin solution to get paste of optimal viscosity. This paste was used to coat the sterile acrylic disks.
- The coated disks were kept overnight to facilitate adhesion of nystatin to acrylic pellets.



Fig 2 Acrylic disks coated with Nystatin incorporated denture adhesive

Group 2: Grape seed extract

- 750 mg of grape seed extract was mixed with 15 grams of denture adhesive powder (Fixon)⁽¹⁹⁾. It was mixed properly and sterile normal saline was used to get paste of optimal viscosity. This paste was used to coat the sterile acrylic disks.

- The coated disks were kept overnight to facilitate adhesion of Grape seed extract to acrylic pellets.



Fig 3 Grape seed incorporated denture adhesive

Group 3: Control

Mixture of denture adhesive powder (Fixon) with normal saline without any other ingredient was coated onto the acrylic pellet in sterile condition. This was kept overnight to facilitate adhesion



Fig 4 Acrylic disks coated with denture adhesive

Inoculation of *Candida albicans*

All three groups were further treated with *Candida* suspension (0.5 McFarland standard) and left to facilitate adhesion for 90 mins. The non-adherent fungal cells were removed by washing in phosphate buffered saline (PBS). The ready specimens were transferred into sterile McCartney bottles and further incubated overnight at 37° C.



Fig 5 Incubation in McCartneys bottles

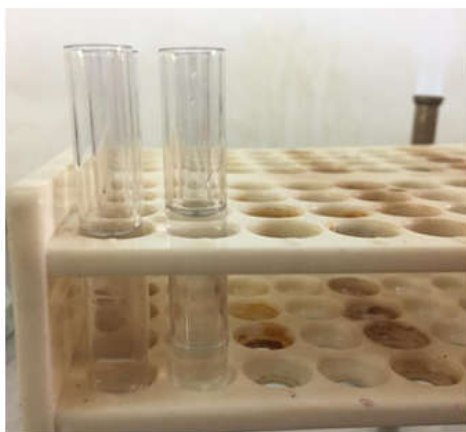


Fig 6 Candida suspension

Colony formation

After 24 hours, all the surfaces of the treated disks from each group were firmly impressed on sterile Sabouraud’s Dextrose Agar (SDA) plates and the plates were incubated for 48 hours at 37°C. Colony counting was done at the end of 48 hours of incubation.

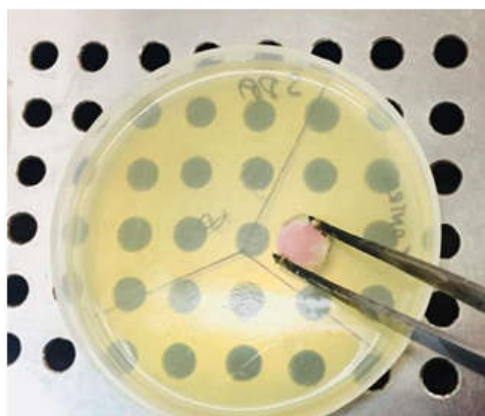


Fig 7 acrylic disks firmly pressed over SDA

RESULT

Forty five samples used in study worked effectively. All three groups were analysed for their antifungal effect and compared with each other. The controls worked satisfactorily. Nystatin was effective in preventing growth of candida *albicans* in all the disks when used in concentration of 100,000 units per ml to prepare denture adhesive paste. Grape seed extract was completely ineffective in preventing the growth of Candida *albicans* at the concentration used.

Antifungal activity of nystatin is seen best among the 3 groups

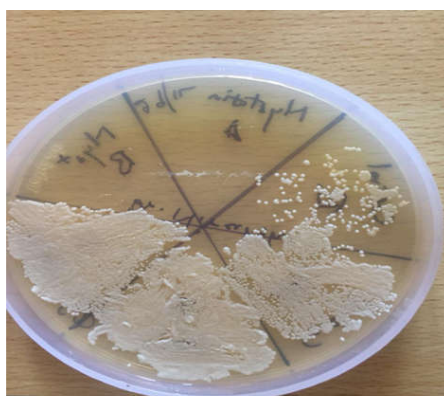


Fig 8 Candidal growth seen on SDA

Colony counts of all the three groups and for all the 15 disks per group

Table 1 Depicts the result obtained at end of this study

SL. No	Nystatin (Group I)	Grape Seed (Group II)	Control (Group III)
1	Nil	Uncountable	Uncountable
2	Nil	Uncountable	Uncountable
3	Nil	Uncountable	Uncountable
4	Nil	Uncountable	Uncountable
5	Nil	Uncountable	Uncountable
6	Nil	Uncountable	Uncountable
7	Nil	Uncountable	Uncountable
8	Nil	Uncountable	Uncountable
9	Nil	Uncountable	Uncountable
10	Nil	Uncountable	Uncountable
11	Nil	Uncountable	Uncountable
12	Nil	Uncountable	Uncountable
13	Nil	Uncountable	Uncountable
14	Nil	Uncountable	Uncountable
15	Nil	Uncountable	Uncountable

CONCLUSION

When nystatin was incorporated into denture adhesive, it interfered with development of *albicans* Biofilm on surface of acrylic disk. However incorporation of grape seed extract did not change much of growth of candidiasis. Therefore we can conclude with synergistic action of Nystatin when incorporated with denture adhesive and its role in treatment of denture stomatitis.

DISCUSSION

Denture adhesive is used widely by elderly individual to improve the quality of maxillary and mandibular denture. Although it’s believed an ideal denture does not require any adhesive but there are few unavoidable situation where adhesive will have to advised.

Denture stomatitis is one of common problems that denture wearers face. This could be due to failure to maintain proper hygiene. This leads to growth of *C. albicans* which causes denture stomatitis. It is seen that denture adhesive markedly increases growth of candida⁽²⁰⁾ this could be due to patient’s inability to maintain satisfactory hygiene.

However in this study we checked the efficiency of two antifungal agent incorporated into denture adhesive. Similar study conducted by Giovanna Simonetti, Anna Rita¹⁹ et al has proven the efficiency of grape seed extract in antifungal activity. Also, HoseinEslami, HosseinBabaeiet al study has shown antifungal activity of grape seed extract to be advantageous againts *C. Glabrata* and *C.krusei*²¹. Regarding efficacy of nystatin, meta-analysis done by Xinlyuchen Zhao Zhi-min Yan hong hua¹⁶has showed that nystatin pastille was significantly superior to placebo in treating denture stomatitis. In this study, Nystatin has proven to be very effective when incorporated into adhesive. However, grape seed extract was directly taken and there is no information regarding its growth and type of grape seed extract taken, this could have affected the result if the study and might have resulted in its failure to show antifungal activity. This is one of the drawbacks of this study. Therefore we can conclude nystatin is effective in its antifungal activity while grapesed extract has proven not effective.

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