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Research Paper: Comparing the Effect of Punica Granatum linn Gel and Nystatin In The Treatment of Denture Stomatitis(Double blind Clinical Trial Study)

Shamsoulmolouk Najafi[1], Hosein ali Mahgoli[2], Mehran Bahrami[3], Zahra Faraji[4], Narges Gholizadeh[5*]

[1] *Professor, Department of Oral and maxillofacial Medicine, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Assistant Professor, Department of prosthodontics , School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran*

[3] *Associate Professor,Department of prosthodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran*

[4] *School of Dentistry, Tehran University of Medical Sciences, Tehran,Iran*

[5] *Associate Professor, Department of Oral and maxillofacial Medicine, School of Dentistry, Tehran University of Medical Sciences, ehran, Iran*

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ABSTRACT

Introduction: candida-associated-denture-stomatitis (CADS) is the most common oral infection, affecting approximately 60% of denture wearers in some populations which have a high recurrence rate in spite of medical treatments . Recently, much attention has been paid to the use of natural antimicrobial compounds. The purpose of this study was to compare the effect of punica granatum linn gel(PGLG) with the use of nystatin on growth of Candida albicans.

Materials and Methods: We prepared 32 patients with CADS divided into 2 groups of 16 patients. nystatin group and PGLG -treated group. Participant PGLG-treated groups were requested to rinse for two weeks, four times a day, 20 drops for 2-3 minutes each day, and then for 30 minutes to avoid eating and drinking. Amount of inflammation and number of colony-forming units (CFU/ml) for each group was counted and compared. Analysis of covariance considering the size of the primary lesion as co-variate was used (software spss 22).

Results: Both Inflammation and number of Candida colonies in the nystatin group were less than the PGLG -treated group (p=0.049, p=0.024).

Conclusion: Although Punica granatum linn gel led to a decrease in inflammation and Candida colonies; Nystatin was more effective both in reduction of inflammation and colony counts for the treatment of CADS.

*** Corresponding Author:**
Narges Gholizadeh.
Address: Department of Oral Medicine, Faculty of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
Tel: +989128090967
E-mail: n.gholizadeh88@gmail.com

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Introduction

Denture stomatitis (DS) is a prevalent yeast infection. In some populations, it can affect up to approximately 60% of the denture wearers. It is defined as the inflammation of oral mucosa especially palatal and gingival mucosa which is in direct contact with the denture base (1,2) In most cases DS does not have any symptoms, however , erythema and hyperplasia (petechial-hemorrhages) may be presents in some patients denture plaque is a potential risk factor for systemic disease, such as aspiration pneumonia. (3) The most common opportunistic pathogen extracted both from the palate and denture base are Candida albicans followed by C. glabrata, C. tropicalis (1).

Denture removal during night time and thorough cleaning is known to be the primary preventive measure for the onset of DS. The cleaning can be performed chemically and mechanically. Chemical therapy for Candida infections has become a new challenge, limited number antifungal drugs are in clinical use such as nystatin, miconazole, amphotericin-B and fluconazole(4). Medical therapy have limitations because of needing drug-safety considerations and having a narrow spectrum of activity, effectiveness and resistance(5)

Nowadays the interest in Herbal medications with an antifungal and an anti-inflammatory activity has increased. The medicinal plants Punica granatum are the richest source for polyphenolic compounds. Punicalagin extracted from pomegranate fruit peel have anti-inflammatory antimicrobial activity against Candida albicans with least side effects and anthocyanins along with phenolic compounds, show great antioxidant activities. (6)

The pharmacological properties of various parts of pomegranate have been studied extensively (7,8,9). Based on the above mentioned, the purpose of this study was to determine the therapeutic effects of gel Punica granatum Linn in comparison to using Nystatin drop medication for the active treatment of DS.

Materials and Methods

This study is an experimental and double blind clinical Trial where neither the participants nor the experimenters knew about the nature of the drug and who was receiving the particular treatment. our study was approved by the Medical Research Ethics Committee of Tehran University of Medical Sciences; ID number IR.TUMS.VCR.REC.1395.466, and after signing the Informed consent by the patients and being informed on the details of the treatment plan, they were entered into the study.

The inclusion criteria were: people with denture-induced stomatitis who have been diagnosed and approved by Oral Medicine specialist and the exclusion criteria included: Persons who could not continue cooperation with the study due to personal or social reasons until the end, Diabetic Mellitus patients , Immune Deficiency Like Acquired Immune Deficiency Syndrome(AIDS), people who received antibacterial drug therapy, corticosteroids, radiation therapy.

In order to blind the study containers with the same color and code were used, both the examiner and patient were not aware of the drug used as both were applied topically just enough to cover the lesion; in the form of gel and droplet.

In this study, 32 patients who were referred from the prosthodontics department at TUMS have been diagnosed with denture-induced stomatitis.

These participants were selected randomly based on the inclusion criteria of being clinically diagnosed for denture-induced stomatitis by an oral Medicine specialist The diagnostic criterion was the amount of swelling and inflammation due to the denture seating, and were noted before and also after treatment for every session.

They were divided into two groups of 16(groups A and B), one of which received nystatin and the other group received pomegranate gel. The treatment lasted for two weeks and the patients were required to come back for two follow up sessions after receiving the planned treatment during their first session.

From a total of 16 patients in the group receiving pomegranate extract 9 were men

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(56.25%) and 7 were women (43.75%) and in the Nystatin group they were 8 men (50%) and 8 women (50%). The Average age of patients in the pomegranate extract group is 60.56 and in the group of nystatin recipients is 60.06.

Patients were requested to rinse with the drug 4 times daily with 15-20 drops for 2-3 minutes each time for 2 weeks and avoid eating and drinking for up to 30 minutes after use. The level of inflammation was measured by the clinician with a calibrated absolang in millimetres after undergoing treatment phases . This was done during the patient's visit on the last day of the first week as well as the last day of the second week. The patient samples were sent to the lab via plates of Sabouraud dextrose agar YGC and were incubated there for 44 hours at 23 ° C. The number of colonies was also counted in the laboratory after incubation. The following two criteria were used to determine recovery status: Table 1 & Table 2 (1,2)

Table 1. Density of Mycological Culture's Rating System

Palatal mucosal inflammation	Colony count mean candida criterion
Rating	Description
1	No candidal Growth
2	1 to 9 colonies
3	10 to 100 colonies
4	> 100 colonies
5	Undetectable

Table 2. Rating System for Erythema Surface of Palatal Mucosa

Rating	Description
1	0 cm2 and less than 5 cm2
2	5 cm2 and more than 5 cm2, less than 10 cm2
3	10 cm2 and more than 10 cm2, less than 15 cm2
4	15 cm2 and more than 15 cm2, less than 20 cm2
5	20 cm2 and more than 20 cm2, less than 25 cm2
6	25 cm2 and more than 25 cm2

Nystatin 100,000 I.U./ml was obtained from Jaberaben Hayan Pharmaceutical Company.

For PGLG preparation collected pomegranate skin was air-dried in the shade for one week to be completely dried and then crushed by grinding. Finally, a homogenous powder mix was obtained from pomegranate peel.

Extraction was done using Ethanol solvent by percolation method. Semi-industrial extraction machine was used by the Pharmacognosy Lab of the Faculty of Pharmacy, Tehran University of Medical Sciences.

The solvent obtained by extraction was evaporated by rotary apparatus under vacuum at below 40 ° C to ensure that no traces of ethanol solvent remain in the extract. To make the gel, distilled water was first mixed with propylene glycol according to the constituents, then the pomegranate extract was added to 10% mass of the final mixture and so the above mixture was perfectly mixed uniform. Finally, using a mechanical stirrer, the sodium carboxymethyl cellulose powder was added slowly and blended until the obtained gel is completely uniform. Emulsion cans containing 80 grams of the sample were prepared for the number of patients studied.

Sterile cotton swabs were used to prepare the microbial specimen from the underlying oral mucosa beneath the maxillary denture. Swabs were transferred to the laboratory in RTF medium within 48 hours and Yeast content, swabs were cultured on YGC medium, After 48 hours, yeast growth was monitored and colony counts were counted.

In order to compare colonies after their exposure to materials, analysis of co-variance was used given the Initial colony was used as co-variate. And to compare the size of the lesion after exposure to the substance Analysis of covariance considering the size of the primary lesion as co-variate was used (software spss 22)

Results

Covariance analysis test was used to measure the secondary colonies in the groups, Considering the initial colony count as co-variate. The test showed that there was a significant value achieved between the two groups at different times and at different follow up times.(p=0.049)(Fig 1 and 2)

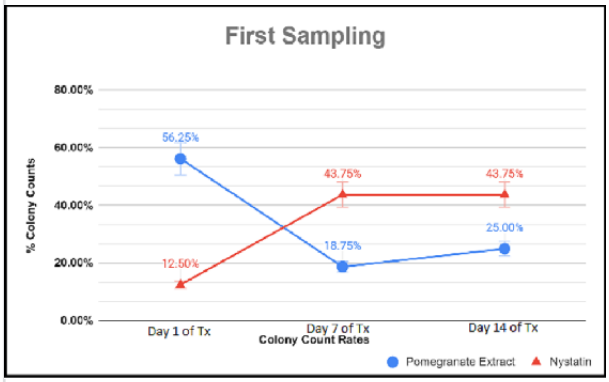

Figure 1. Colony Count Comparison for Both Groups at Different rates(First session)

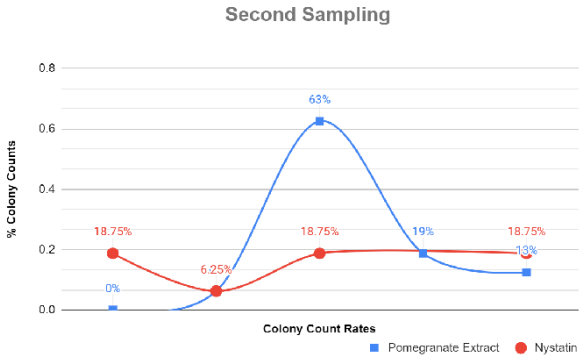

Figure 2. Colony Count Comparison for Both Groups at Different rates(Second session)

And also to compare the size of the secondary lesion in the groups Analysis of variance was performed by considering the size of the primary lesion as co-variate. This test showed that there were significant differences in lesion size at the first and second sessions at different times and different follow up times(Pv=0.024). (Fig 3 and 4)

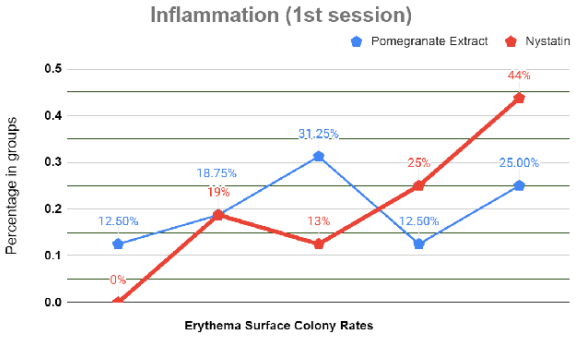

Figure 3. Comparison of Erythema Surface of Both Groups at Different Rates(1st session)

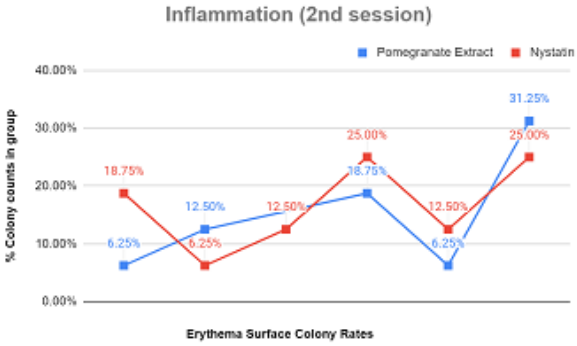

Figure 4.Erythema Surface Comparison for Both Groups at Different Rates(2nd session)

Discussion

Currently there is a great interest in plant-based natural drug products that are derived from herbal sources due to better tolerance and decreased adverse drug reaction. Several investigations have been done on the effect of pomegranate extract on different microorganisms in vitro and suggest clinical assessment of its effect. According to studies, o adverse effects from using pomegranate have been reported on the mucosa.

This study is the first double blind randomized clinical trial comparing the effectiveness of using pomegranate extract with nystatin for the treatment of DS. Therefore, we have decided to compare the effectiveness of pomegranate and nystatin antifungal medication on DS .

The scientific name of this plant is based on modern botanical sources known as punica granatum and in this study the pharma-

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ceutical form of the mucoadhesive pastet and Oral Mucosal Gel were prepared from Pomegranate Fruit Hydroalcoholic Extract and so has become standardized.

This standardization was based on the amount of total phenolic compounds. Among the 122 phytochemicals were found in pomegranate fruit skin ,the amounts of ellagic acid, gallic acid, and ponicalagine were more noticeable in the skin and so these compounds have more therapeutic effects than other compounds, all of which are from the tannins family(10).

Results of different studies indicate the inhibitory activity of gel Punica granatum on the adhesion of different bacterial species and the fungi on the oral mucosal surface therefore this gel can be used in the treatment of oral cavity infections such as periodontal diseases and oral candidiasis. (9)

The anti-inflammatory effect of punicalagin is due to decrease in production of nitric oxide and PGE2 by inhibition of pro- inflammatory proteins production(11).

Pomegranate extract exerts its anti-inflammatory activity by inhibiting NF - κB (nuclear factor kappa - B) activity and preventing ERK - 1 or ERK - 2 (Mitogen activated protein) activity. Inhibition of NF - κB and inflammatory cell pathway inhibits the production of bone resorption factors and eventually cures periodontal diseases(12).

Antioxidant properties of pomegranate peel extract can absorb free radicals caused as a result of wounds and enhance cellular activity. The appearance of the wound as well as the histological examination of the wound healing showed that the ointment containing 5% pomegranate peel extract can be very effective and can be introduced as a suitable medication.(13)

In a study in 2010, Mr. Paula Cristina et al. emphasised the urge to develop new therapeutic agents against opportunistic yeast such as Candida Albicans as well as other microorganisms, can be resistant to antimicrobials during long-term treatment, especially in immunocompromised patients(14). Many researchers have been studying Plants that contain antimicrobial compounds, but still many of these compounds are still unknown.

Gavanji et al. carried out a study using gel containing 65% of pomegranate extract in aphthous patients showed that application of this gel compared to placebo gel can reduce the pain duration of lesions up to 3 days and the recovery time up to 5 days.(15)

In a clinical trial study done at Islamic Azad University of Khorasgan, 210 patients with aphthous lesions were treated with mouthwash containing 10% aqueous extract or 10% hydroalcoholic extract of pomegranate. Treatment period of these lesions was reduced by up to 4 days in patients compared with control group. (16)

Pomegranate flower extracts both in aqueous and hydroalcoholic form have high levels of antioxidant and phenol. However, the percentage of these substances are higher within the hydroalcoholic extract.

These substances have been able to give anti-inflammatory, antibacterial, and wound healing properties to the extract. Many studies have been done on the whole pomegranate plant and so far, it has been concluded that parts of its leaf, pomegranate flower, pomegranate seeds and pomegranate fruit peels have high levels of phenols. Moreover, pomegranate peel is at higher rank. Pomegranate fruit peel has higher levels of tannins, antioxidants, polyphenols compared to pomegranate's flower.

As initially explained, the compounds of ellagic acid and gallic acid, which are very effective in wound healing procedure, are part of the tannins of this extract. Pomegranate fruit peel extract has less radical adsorbent than pomegranate flower when warm and it can show more anti-inflammatory properties at a lower dose. Although anthocyanins and flavonoids in the peel extract are lower than pomegranate flowers however they are very significant and high compared to other parts of the pomegranate that cannot be ignored.(5,16,17)

Derakhshan and colleagues have done a study on 'The effects of pomegranate peel extract on

recurrent aphthous stomatitis' where they concluded that PPE gel was significantly effective in reducing the pain, ulcer size, and healing process of ulcers over a period of one week. (18)

The use of herbal based products for treatment purposes such as Punica granatum L have become one of the most popular cultivated productions in Iran with a wide range of uses for different therapeutic purposes. The pomegranate peel extract revealed great antifungal activity against C. albicans, which was comparable with the nystatin drug used as a standard routine treatment for oral Candida infections. (19)

The group which used the Nystatin drug during the treatment time achieved a more significant value for the reduction of Candida colonies and the rate of mucosal inflammation in this study. Furthermore, the pomegranate peel extract with all of its palliative and curative properties has shown to be less effective in reducing the number of Candida colonies and inflammation thereof.

Conclusion

In this study, we have compared the effects of both pomegranate peel extract and nystatin in treatment of CADS, where it can be concluded that Nystatin showed greater effects in the reduction of candidal colony count and mucosal inflammation in comparison to the pomegranate extract drug used. However, for a more detailed study on the effects of pomegranate peel extract and its inflammatory processes on the candida colony counts, more extensive studies with larger sample sizes are recommended.

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