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Comparing the Effect of Green Tea Extract With Nystatin in the Treatment of Denture Stomatitis

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Abstract

Background: Denture stomatitis is a very common oral mucosal lesion, affecting approximately 50% of denture wearers in some populations. More recently there is an increased interest to use natural antimicrobial compounds, like plant extracts of medicinal plants.
Objectives: The aim of the present study was to evaluate the efficacy of green tea extract compared with nystatin drop in the treatment of denture stomatitis.
Patients and Methods: This was a clinical trial study with 30 patients in two parallel groups, 15 patients received nystatin drop and the other ones green tea extract. The patients in two weeks were recommended to use mouthwash 4 times a day each time 15 - 20 drops for 2 - 3 minutes and after that they should avoid eating and drinking for 30 minutes; patients in the two groups were suggested to use the drug at a specific time. Amount of inflammation and erythema were recorded in each session and measured with a graded blade and recorded according to a 6-point scale. At each visit, mycological samples were taken from the palatal mucosa for culture.
Results: Age and sex differences between the groups were not significant. The erythema surface of the palatal was significantly reduced in the both groups at follow-up visits compared with the pretreatment condition. No significant difference was seen between the two groups at the same visits in erythema surface and colony counts of the palatal mucosa ($P > 0.05$).
Conclusions: This study indicated that green tea extract can be effective in reducing the number of Candida colonies and erythematic area comparable to nystatin drop in the management of denture stomatitis.

Keywords: Denture Stomatitis, Oral Candidiasis, Green Tea Extract, Medical Plants

1. Background

Denture stomatitis is a very common oral mucosal lesion, affecting approximately 50% of denture wearers in some populations (1). It is the most common clinically important condition that develops in denture wearers (complete or partial dentures), but the incidence is significantly lower in those with partial dentures (2). Denture stomatitis (DS) usually does not have any symptoms. Nevertheless, classic manifestation of this situation is presence of red and swollen dots (petechialhemorrhages) under the maxillary dentures (3). Microbiological swabs from the palate and denture surface would confirm the presence of *yeastscandida*. *Candida albicans* is the most common pathogen followed by *C. glabrata*, *C. tropicalis* and others (4, 5).

The most important treatment is to remove dentures at night and clean them carefully. A clinical examination should be performed to evaluate the condition and fitness of denture. The denture may need relining, refitting or replacement (6).The cleaning options include chemical cleaning and mechanical cleaning (1). The antifungal treatments more used are antifungal suspensions based on nystatin, amphotericin-B, miconazole, and fluconazole. Studies about the use of plant extracts in treatment of different disease are performed extensively (7). Many extracts of plants and isolated essential oils have biological activities in vivo and in vitro (8).

Camellia sinensis seems to be valuable sources for antifungal agents, especially against *Candid albicans*. Green tea leaves (*Camellia sinensis* L.) has been shown to have variable antifungal activity in vitro against *Candida albicans* in different harvest times (9).

2. Objectives

The aim of the present study was to evaluate the efficacy of green tea extract compared with nystatin drop in the treatment of denture stomatitis.

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3. Patients and Methods

3.1. Patients

Thirty patients in two groups aged 45 and 60 years were selected from patients referred to the Department of Oral Medicine, Tehran University of Medical Sciences. Samples were assigned using random blocks of 6 samples in each group by Excel software (Spss 13); allocation process was performed by a person other than the person involved in the treatment. According to the results of Hirasawa and Takada (one-way ANOVA) and using Minitab software (USA) to determine the sample size considering$\alpha = 0.05$, $\beta = 0.2$ and SD = 3.1, the minimum sample size required in each group was 15 individuals.

The study was approved by the ethics committee of Tehran University of Medical Science and registered in Iranian registration Clinical Trial (IRCT) with registration number 201011052464N2. All patients gave an informed consent to participate in the study. At the first step, the palatal mucosa was examined for presence of inflammation (as a diagnostic criteria) and in the next step candidiasis confirmed by microbiologic cultures from the palatal mucosa.

3.2. Inclusion Criteria

Patients with denture stomatitis lesions in active phase (diagnostic criteria was swelling and inflammation under the denture surface).

3.3. Exclusion Criteria

Patients with diabetes mellitus, immune deficiency such as acquired immune deficiency syndrome (AIDS), as well as those who used antibacterial drug therapy, corticosteroids, radiotherapy and smokers were excluded.

3.4. Manner of Blinding

The drugs were poured in bottles with the same color and shape and differentiated based on the code and a list, provided to a third person, up to decoding. The examiner and analyzer did not know anything about drugs.

3.5. Drugs

Nystatin used as an antimycotic agent was purchased from Jaber Ebne Hayyan Pharmaceutical Mfg. Co. (Tehran, Iran).Green tea extract was prepared at the faculty of pharmacy, Tehran University of Medical Sciences.

3.6. Collection and Preparation of Tea Leaf Samples

The youngest leaves at the terminal part of the stem of the fresh green tea plant were prepared from farms of Lahijan region in Iran in 2011.

3.7. Preparation of Tea Extraction

Fresh tea leaves after gathering were transferred immediately to the laboratory and dried in laboratory environment. The dried leaves were milled and the powder was screed to become uniform and extracted by percolator and hydro-alcoholic solution in 70°C, for 24, 48 and 72 hours. The extracts were concentrated by rotary device up to 4.5% of the dry remaining after filtration. The extraction, called “mother extraction” was kept in a refrigerator. At the time of prescription for patients, every milliliter of the “mother extraction” was fined by 5 milliliter of distilled water and prepared in 30 milliliter glasses with dropper for one week of usage by patients.

3.8. Implementation of Survey

This was a randomized, controlled, experimental double blind clinical trial study with 30 patients in two parallel groups, 15 patients received nystatin drop and the other ones green tea extract (0.58%). The patients were recommended to use mouthwash 4 times a day each time 15 - 20 drops for 2 - 3 min and after that they should avoid eating and drinking for 30 minutes; they were suggested to use the drug in two weeks at a specific time. Amount of inflammation and erythema recorded before and after the treatment in each session. To determine improvement, we used two criteria of rating system for density of mycological culture and erythema surface of palatal mucosa on days 0, 7, 14 after commencement of therapy (Tables 1 and 2). After selection of patients, at each visit (7), mycological samples were taken from the palatal mucosa by rubbing the swab on it; swabs after sampling were placed in RTF (reduced transport fluid) environment and transported to the laboratory within 48hours. To assess the culture of yeast, swabs were cultured on YGC medium (yeast extract glucose chloramphenicol agar), then incubated at 37°C and after 48 hours colonies were counted and expressed in terms of densities. At each visit, the erythema of the palatal mucosa was examined with a graded blade and recorded according to a 6-point scale.

3.9. Statistics Analysis

Data was analyzed using the Minitab software (USA) considering $\alpha = 0.05$ and $\beta = 0.2$.

Mann-Whitney U (between groups), Wilcoxon sign rank and Friedman test were used to compare the groups.

4. Results

Thirty patients with denture stomatitis referred to the Department of Oral Medicine, School of Dentistry Tehran University of Medical Sciences entered the trial and 27 patients completed the study (three person due to sensitivity to nystatin drops and hospitalization excluded); 20 patients were female (74.1%) and 7 male (25.9%).The green tea extract group (group A) consisted of 11 females and 4 males with a mean age of 62.13 years (SD = 9.724). The nystatin group (group B) contained 9

females and 3 males with a mean age of 64.83 years (SD = 7.590). No statistically significant differences were observed between the groups regarding gender in the groups (P = 0.58).The number of candidal colonies cultured from the palatal mucosa was statistically similar between the two groups at all visits and the difference between the two groups was not significant (on days 0, 7, and 14 P = 0.786, P = 0.980, and P = 0.612, respectively).

However, in both nystatin and green tea groups, colony count difference was considerable and at all visits colony counts significantly reduced compared with starting the treatment (P = 0.000). Comparing colony count at each time in both groups is as follows; the difference between the two groups was not significant except one of them(on days 0, 7, P = 0.000, days 7, 14, P = 0.074 and days 0, 14, P = 0.006).There was a significant reduction in palatal mucosal colonies in the both groups expect on 7thand 14th days, in which the reduction was not considerable (Figure 1).

The erythema surface of the palatal mucosa was significantly reduced in both groups at follow-up visits compared with the pretreatment situation and at different follow-up, there was no significant difference between the two groups at the same visits (on days 0, 7, 14, P = 0.858, 0.535, 0.498, respectively (Figure 2). Nevertheless, in both nystatin and green tea groups the degree of inflammation was different (P = 000.0). Comparing each two times regarding inflammation is as follows; in green tea group on days 0 and 7 P = 0.001, on days 0 and 14 P = 0.001 and on days 7 and 14, P = 0.040.

Table 1. Rating System for Density of Mycological Culture

Rating	Description
1	No candidal growth
2	1 - 9 colonies
3	10 - 100 colonies
4	More than 100 colonies
5	Uncountable

Table 2. Rating System for Erythema Surface of Palatal Mucosa

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

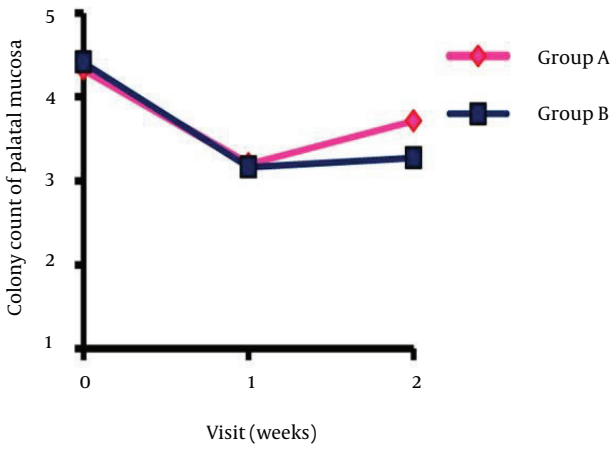

Figure 1. Comparison of Colony Count of Both Groups at Different Times

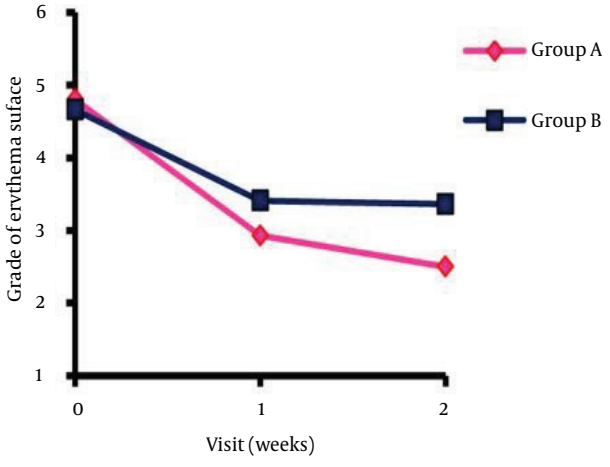

Figure 2. Comparison of Erythema Surface of Both Groups at Different Times

5. Discussion

Candida albicans is a dimorphic yeast strongly Gram-positive able to live as usual commensal organism in the oral cavity of healthful people. It is the most common yeast isolated from the oral cavity (10).

The pathogenesis of candida-associated denture stomatitis is multifactorial. It includes local and systemic factors related to host and to candida capability to adhere and proliferate in the host epithelial tissues (11). Candida-associated denture stomatitis occurs when the conditions of micro oral environment are favorable for the growth and adhesion of yeast or when the host immune system defense is disrupted.

Increased tendency for candidiasis infections occurs in susceptible people such as HIV^+ patients, diabetic patients, broad-spectrum antibiotics consumers and people under chemotherapy. Due to better influence and fewer adverse effects of herbal drugs, the researchers have interest to study in the field of herbal medicine (12, 13). A literature survey revealed that only a few studies performed to support the indication of medicinal plant preparations in the management of oral stomatitis (14, 15).

Green tea is mainly produced from *Camellia sinensis*. The Assan type (*Camellia sinensis assamica*) has the highest content of catechins. They are contributed to taste (which make it excessively bitter), aroma, astringency and color in tea (16).

Over the last years, numerous epidemiological and clinical studies have revealed several physiological activities of green tea, which may improve the health and prevention or treatment of some chronic diseases.

Polyphenols are strong antioxidants and have important biological properties and constitute the most interesting group of green tea leaf components, and in consequence, green tea can be considered an important dietary source of polyphenols, particularly flavonoids. The main flavonoids present in green tea include catechins (flavan-3-ols).

The four major catechins are epigallocatechin-3-gallate (EGCG), which accounts for approximately 59% of the total catechins, epigallocatechin (EGC) (19%), epicatechin-3-gallate (ECG) (13.6%) and epicatechin (EC) (6.4%) (17). Many studies demonstrated that the extract of green tea polyphenols has antimutagenic, antidiabetic, antibacterial, anti-inflammatory and hypocholesterolemic properties (18-21). Beneficial effects in oral diseases such as protection against dental caries, periodontal disease and tooth loss have been also described (22). Catechin from tea has also been reported to have an antimicrobial effect against oral (23, 24), intestinal (25, 26) and food-borne bacteria (26). Hara et al. also showed antimicrobial activity of tea catechins including EGC, EC, EGCG and ECG against the growth of *Clostridium botulinum* (27).

This study was a randomized double-blind clinical trial research comparing the effects of 0.58% green tea extract and nystatin in the treatment of denture stomatitis.

Antifungal effects of green tea catechins have been studied experimentally in several studies. Anibal et al. indicated the necessity to develop new therapies against opportunistic fungi of the Candida genera (12).

Hirasawa and Takada and Sitheeque et al. showed antifungal activity of both green and black tea catechins against *Candida albicans* (13, 28). Previously, Aladag et al. showed in vitro antifungal activity of methanol extracts of green tea leaves (*Camellia sinensis* L.) in different harvest times (9).

Various studies have investigated the effect of green tea on candida species in vitro and suggested to assess its effect clinically. Green tea is a common drink and no adverse effects reported on the mucosa. In addition, according to studies, no clinical studies have been performed about the effect of tea extract on candida. Therefore, we decided to compare the efficacy of green tea extract and nystatin on denture stomatitis.

In this study, we compared nystatin and green tea extract and determined that the effects of both in reducing inflammation and candidal colony count have not statistical difference, whichis consistent with other studies.

The results of this study showed that green tea extract is dramatically effective in improving denture stomatitis compared with nystatin; their effectiveness on *Candida albicans* was not significantly different and both were almost equally effective on denture stomatitis.

Tea is a popular beverage and is a potent antioxidant. In addition to antifungal properties, it has other benefits. As an herbal drug it is acceptable for patients. Therefore, it is recommended to use this herb in the treatment of fungal diseases. According to wide anti-fungal and anti-inflammatory activities of green tea, it is necessary to study its mechanisms as well.

Footnote

Authors' Contribution: Samira hajheidary and Shamsolmoulouk najafi were performer, Farideh Siavoshi perform performed tasks related to microbiology. Gholamreza Amin performed. Narges Gholizadeh read the article, Samira hajheidary extract of green tea.

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