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Original Article

Low-Molecular-Weight Chitosan Varnish for Treatment of Oral Candidosis: A Randomized Clinical Trial

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Abstract

Background: Oral candidosis is a common fungal infection among denture wearers, often associated with poor denture hygiene and the colonization of Candida species on denture surfaces.The present study aimed to investigate the antifungal effect of low molecular weight chitosan (LMWC) varnish on oral candidosis in denture wearers, compared to nystatin ointment.

Methods: This study is a single-blind (patients blinded) Parallel-group, randomized clinical trial. Eligibility criteria were denture wearers with Newton's type II denture stomatitis, no systemic diseases, and no recent antifungal use. The study was done in Dental clinics in Kerman, Iran. The 34 patients were recruited and randomly assigned to two groups: 18 (patienchitosan), 16 (nystatin). LMWC varnish (10% wt. in ethanol-rosin base) or nystatin ointment was applied 4 times daily for 2 weeks to the denture inner surface. Primary outcomes were reduction in palatal erythema (clinical) and fungal count (blastospores/mycelia per 100 epithelial cells, microscopic(. A palatal smear for microbial assay was obtained before and after treatment. Low molecular weight chitosan varnish was prepared by dissolving chitosan in ethanol (30% wt.). Data were analyzed using the Chi-square and t-test.

Results: No harms were reported in the study. The study showed that chitosan varnish has positive effects on the treatment of oral candidosis in comparison with Nystatin. It made a significant decrease in erythema in clinical view and fungi count in microscopic view. Differences were statistically significant ($P<0.05$).

Conclusion: The results of this study and other biochemical chitosan benefits showed that chitosan could be a good substitute for the treatment of oral candidosis.

Keywords: Candida albicans, Chitosan, Oral infection, Denture stomatitis, Varnish

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Introduction

Candida albicans is one of the normal human oral flora. The host's defense depression makes C. *albicans* virulent and causes candidosis. Candidosis was manifested through various clinical views, including one or more oral sites involvement, up to affecting the whole oral cavity or dissemination and invasive form. Denture stomatitis is a common inflammatory process affecting about 60-65% of denture wearers. Denture stomatitis is defined as an inflammatory and erythematous lesion under the denture covering mucosa that can be found in both jaws, but the upper jaw is mostly affected. In the majority of patients, it is an asymptomatic condition, and a minority of patients experience pain, itching, and a burning sensation. The infection has many predisposing factors, but the principal pathogen is Candida albicans.[1-4]

Candida species have two microscopic forms: yeast form (blastospore) and mycelial form (hyphae). Hayphal form is the predominant pathogen form between patients with denture stomatitis in comparison with healthy denture users without palatal inflammation.[1,5,6]

Denture stomatitis is an inflammatory process under a removable denture in the mucosa. The clinical manifestation is an erythematous area.[1-7] Denture stomatitis has a multifactorial etiology. Poor denture hygiene, continual and nighttime wearing of removable dentures, accumulation of denture plaque, and poor-fitting dentures increase the ability of C. albicans to colonize on the denture and oral mucosal surface, where these fungi cause an opportunistic inflammation. C. albicans in the mycelium phase is parasitic, and in the blastospore phase is saprophytic.[1-7]

Chitosan is a biodegradable and biocompatible cationic polymer extracted from the exoskeletons of crustaceans.

Antimicrobial activity of chitosan has been found in a wide range of fungi and bacteria. This antimicrobial activity could be attributed to the interaction of chitosan with the cell wall of the microorganism, changes in bacterial gene expression, a lack of nutrition by chitosan binding to calcium and iron, inhibition of biofilm formation, and its maturation.[8-17] This biopolymer inhibits C. *albicans* adhesion to human epithelial cells and increases retention time on the oral mucosa. Recent studies have demonstrated the use of chitosan as a vehicle or adjuvant for antifungal activity..[8,9,11,12,18-20]

Oral candidosis affects 60-65% of denture wearers, causing discomfort; new treatments are needed due to nystatin resistance/side effects. Rationale: Existing evidence shows antifungal properties of chitosan (references to systematic reviews if available). This study aimed to evaluate the benefits (reduction in erythema and fungal count) and harms (side effects) of LMWC varnish vs. nystatin in denture wearers with oral candidosis.

Materials and Methods

Trial Design

The study is randomized single blind. (Parallel-group, RCT; 1:1 allocation ratio; individual randomization).

Ethics

The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee in research, Kerman University of Medical Sciences (Ethical code: IR.kmu.REC.1394.237), and the protocol was registered in the Iranian Registry of Clinical Trials (Registration code: Irct2015071223169n1).

Eligibility Criteria

Patients who have worn dentures for many years and without systemic diseases were selected for the research program. Patients with a history of using antifungal drugs, antibiotics, and corticosteroids within the previous month, Alzheimer's disease, immunodeficiency, chewing muscle disorder, and psychological disorders were excluded from the study. Forty patients participated; six of whom were excluded because of improper using drug. All included patients had Newton's type II denture stomatitis. (Eligibility: Inclusion - Long-term denture wearers with Newton's type II denture stomatitis, no systemic diseases; Exclusion - Recent antifungal/antibiotic/corticosteroid use, Alzheimer's, immunodeficiency, muscle/psychological disorders. Recruitment: Referral to clinics. No site/provider eligibility, as interventions delivered by patients).

Intervention and Comparator: They were divided into two groups: chitosan varnish and nystatin ointment. Treatment was planned for two weeks. Patients were randomly divided into two groups. The first examination was done on the chair and under the light of the denture unit. The size and location of the erythematous plaque were measured using a gauge (Iran) and recorded on the first, seventh, and 14th day. Patients were instructed to lubricate the inner surface of the denture with Nystatin ointment (group one) or chitosan varnish (group two) after cleansing it. The frequency of using the drug was four times a day after a meal and before sleep for two weeks. Patients in both groups must put the denture in disinfectant liquid during the night. Low molecular weight chitosan varnish was prepared by dissolving chitosan in ethanol (30% wt.). Materials: Low molecular weight chitosan (5000 Da) was purchased from Hangzhou New Asia International Co., Ltd. (China). First, Rosin solution in ethanol (30% wt.) was prepared. Chitosan (5000 Da) was added. This solution was stirred for 8 hours until chitosan dissolved completely. Alcohol-based Chitosan varnish (10% wt.) was finally prepared.

Intervention

LMWC varnish (10% wt. chitosan 5000 Da in rosin-ethanol base) was applied to the denture inner surface 4x/day for 2 weeks. Comparator: Nystatin ointment, same application. Both self-administered after denture cleansing; dentures were placed in disinfectant overnight. No tailoring/modifications were performed. Allowed care: Standard denture hygiene. Fidelity/adherence: Not assessed. Materials access: Contact the author for the preparation manual.

Outcomes

Primary - Erythema size (gauge measurement, days 0,7,14); Fungal count (blastospores/mycelia per 100 cells, days 0,14). Secondary - None specified.

Sample Size

The sample size for the clinical trial was determined a priori using a power analysis for a two-tailed t-test comparing the difference between two independent means (LMWC varnish vs. Nystatin groups). With an anticipated effect size Cohen's d of 0.9 (indicating a large treatment effect), an alpha level of 0.05, and a desired statistical power of 0.80 ($1 - \beta = 0.80$), the calculation assumed equal allocation ($N2/N1 = 1$). The analysis yielded a noncentrality parameter δ of 2.916, a critical t-value of 2.021, and degrees of freedom of 40, resulting in a required sample size of 21 participants per group, for a total of 42 subjects. This ensures adequate power to detect a clinically meaningful difference in treatment outcomes between the two groups.

Randomization

Participants were randomized to the LMWC varnish or Nystatin groups using block randomization with a block size of 7 to ensure balanced allocation throughout the trial.

Allocation Concealment

Allocation concealment was achieved using sequentially numbered, opaque, sealed envelopes prepared by the statistician, who also implemented the randomization. The trial employed single-blind masking, with both

patients and outcome assessors blinded to treatment allocation.

Case Selection

This is a randomized and single-blind study. Patients who referred to dental clinics were selected. First, they were aware of the study design and then signed the standard questionnaire and consent. Demographic data, including age, gender, and medical history, were obtained. Patients with a history of using antifungal drugs, antibiotics, and corticosteroids within the previous month, Alzheimer's disease, immunodeficiency, chewing muscle disorder, and psychological disorders were excluded from the study.[21] Forty patients finally participated, six out of whom were excluded because of improper use of the drug.

Clinical Evaluation and Treatment

Patients were randomly divided into two groups. The first examination was done on a chair and under the light of the denture unit. All included patients had Newton's type II denture stomatitis. The size and location of the erythematous plaque were measured using a gauge (Iran) on the first, seventh, and 14th day and recorded.

Patients were instructed to lubricate the inner surface of the denture with Nystatin ointment (group one) or chitosan varnish (group two) after cleansing it. The frequency of using the drug was four times a day after a meal and before sleep for two weeks. Patients in both groups must put the denture in disinfectant liquid during the night.

Microbiologic Assessment

Palatal smears were obtained by sterile cotton swabs on the first day before the onset of treatment and on the 14th day after finishing treatment.

Swabs were rolled onto a glass slide (Germany), which was fixed with absolute ethyl alcohol 99.7% (Iranian brand). Samples were stained by the Gram method, and the number of blastospores and mycelia was counted per 100 epithelial cells.

Materials

Low-molecular-weight chitosan (5000 Da) was purchased from Hangzhou New Asia International Co., Ltd. (China). First, Rosin solution in ethanol (30% wt.) was prepared. Chitosan (5000 Da) was added. This solution was stirred for 8 hours until chitosan dissolved completely. Alcohol-based Chitosan varnish (10% wt.) was finally prepared.

Trial Protocol

No changes to protocol after commencement (e.g., no alterations in eligibility, outcomes, or analyses). Six participants were excluded post-randomization for improper drug use, but this was per protocol.

Data Analysis

The data were analyzed using an independent sample t-test, a paired t-test, and a Chi-square test, and the per-protocol analysis method was used. The statistical significance was set at 0.05.

Results

Clinical Findings

Totally, 34 participants were analyzed in the study (18 chitosan, 16 nystatin). The flow of participants through the trial is illustrated in the CONSORT diagram (Figure 1).

No harms were reported. The study showed that chitosan varnish has positive effects on the treatment of oral candidosis in comparison with Nystatin. It significantly decreased erythema in clinical view. Differences were not statistically significant ($P=0.994$) (Figure 2).

Microscopic Findings

Blastospore count in the chitosan group showed statistically significant decrease before and after the treatment ($P=0.003$), which is similar to the nystatin group ($P=0.009$). LMWC varnish decreased the blastospore form of C. *albicans*, which is similar to Nystatin. This finding was statistically not significant ($P=0.518$). After the treatment, the reduction in mycelium number in LMWC varnish and Nystatin was statistically significant ($P=0.015$ and <0.001, respectively), and the difference between the groups after the treatment was not significant ($P=0.973$) (Tables 1 and 2).

Discussion

Denture stomatitis is an inflammatory process under a removable denture in the mucosa. The clinical manifestation is an erythematous area.[2,3]

Denture stomatitis has a multifactorial etiology. Poor denture hygiene, continual and nighttime wearing of removable dentures, accumulation of denture plaque, and poor-fitting dentures increase the ability of C. *albicans* to colonize on the denture and oral mucosal surface, where these fungi cause an opportunistic inflammation. C. *albicans* in the mycelium phase is parasitic, and in the blastospore phase is saprophytic.[1,5,22] In this study, LMWC varnish significantly improved this erythematous area ($P<0.001$), which is similar to Nystatin ($P<0.001$). The study revealed a significant decrease in blastospore and mycelium score/count by LMWC varnish ($P=0.004$ and $P=0.014$, respectively).

The present study demonstrated a good therapeutic effect of LMWC varnish on treating Candida-associated denture stomatitis.

Various mechanisms have been reported for the antimicrobial activity of chitosan. Large-molecular-weight chitosan could not penetrate the cell wall of microorganisms. Its amino groups interact with the anionic component of the cell wall, so the leakage of intracellular constituents causes cell death. While low-molecular-weight chitosan enters the intracellular parts of the cell, it binds to the DNA, restrains RNA combining to protein, and results in destroying the DNA transcription. Furthermore, chitosan binds to calcium and iron, causing a lack of nutrients for fungi. Moreover,

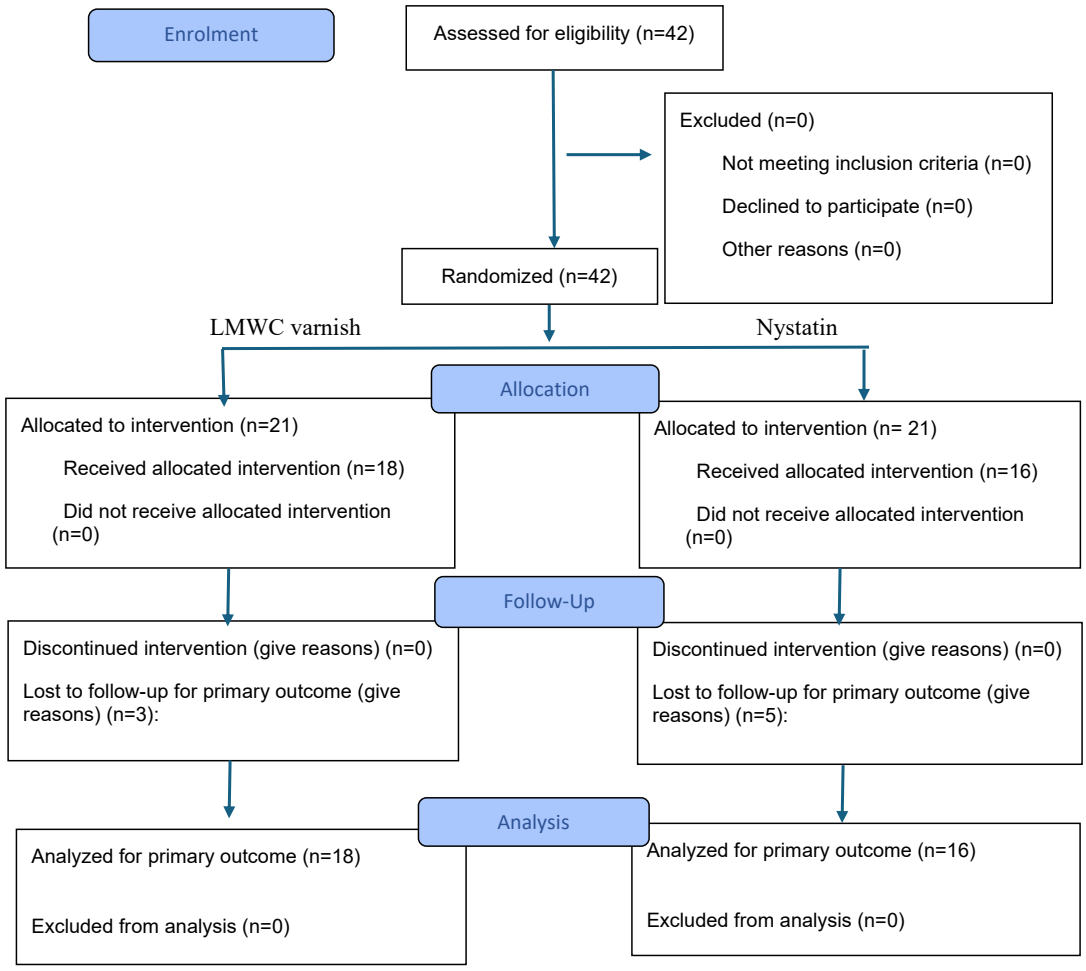

Figure 1. Flow diagram of the progress through the phases of a randomized trial of two groups (that is, enrolment, intervention allocation, follow-up, and data analysis)

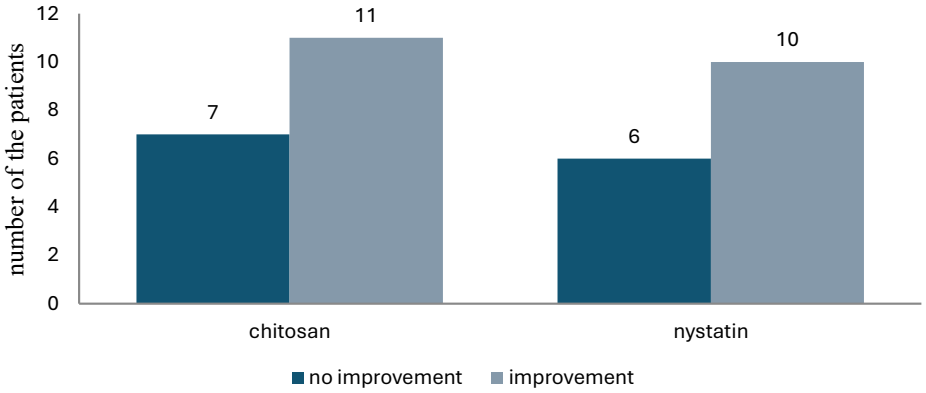

Figure 2. Clinical improvement in the two groups

Table 1. Mean number of blastospores before and after the treatment in different groups

Groups	Mean ± SD		*P*-value
	Before treatment	After treatment	
LMWC varnish	13.22 ± 13.70	1.78 ± 3.24	0.003
Nystatin	11.88 ± 13.47	2.38 ± 2.84	0.009
P-value	0.711	0.518	

Table 2. Mean number of mycelia before and after the treatment in the two groups

Groups	Mean ± SD		*P*-value
	Before treatment	After treatment	
LMWC varnish	3.06 ± 3.97	0.50 ± 0.61	0.015
Nystatin	3.51 ± 2.88	0.49 ± 1.25	<0.001
P-value	0.598	0.973	

chitosan inhibits biofilm formation and maturation by reducing the adhesion of yeast and metabolic activity of the biofilm.[8-14,16,17] The present study had two main differences with other studies, as mentioned: For the first time, rosin-based varnish of chitosan was used in this study. Most of the varnishes are rosin-based. Rosin

derivatives' characteristics, including film-forming and coating properties, make them good materials for use in sustained release and drug delivery systems. It could be especially used in local treatment of the oral lesions, where the presence of saliva makes it difficult.[10,23,24]

In contrast, low-molecular-weight chitosan (LMWC) demonstrates enhanced permeability across the fungal cell wall; therefore, it can enter intracellular compartments. Once internalized, it binds to nucleic acids, interferes with transcriptional processes, and disrupts protein synthesis, thereby impairing fungal replication and survival. This intracellular mechanism has been supported by molecular studies demonstrating that chitosan alters gene expression related to cell surface integrity and virulence pathways, including inhibition of SAGA complex component expression in *Candida albicans*.[25] Furthermore, chitosan has been shown to affect efflux pump activity, which may enhance antifungal susceptibility and reduce resistance mechanisms.[26]

The molecular weight of chitosan is another substantial factor in its antifungal activity. In this study, low-molecular-weight chitosan (5000 Da) was used. It has been shown that the reduction of the molecular weight leads to an increase in antimicrobial activity of chitosan.[27] So, based on the above-mentioned findings in the present study, we have used two forms of chitosan (varnish and low-molecular-weight) simultaneously. Our results are consistent with the results of many studies.[9,10,28-30] Nanoparticle-based chitosan formulations encapsulating farnesol have shown potent antifungal and antibiofilm activity against *Candida albicans* biofilms both in vitro and in vivo.[31,32] Similarly, chitosan-based cationic hydrogels have demonstrated strong antifungal and antibiofilm effects against clinical isolates of *Candida auris*.[2]

Recent advances in nanotechnology have further enhanced the therapeutic potential of chitosan. Chitosan nanoparticles encapsulating bioactive compounds such as farnesol have demonstrated enhanced disruption of quorum-sensing pathways and biofilm architecture.[32,32] Chitosan nanoparticle-incorporated probiotics have also shown promising antifungal activity in experimental models of oral candidiasis.[33] Moreover, clinical translation of these findings has been demonstrated in a randomized controlled trial where miconazole-loaded chitosan nanoparticle gels showed superior antifungal efficacy compared to conventional formulations in diabetic patients with oral candidiasis.[34] These findings highlight the importance of formulation strategies in maximizing antifungal activity.

This study has several limitations, including a small sample size, lack of assessor blinding, absence of long-term follow-up, and being conducted at a single site. Generalizability is limited to Iranian denture wearers. Despite these constraints, the results support the interpretation that low-molecular-weight chitosan (LMWC) varnish is a safe and effective alternative to nystatin for treating Candida-associated denture stomatitis.

Conclusion

LMWC varnish developed in this study showed satisfactory antifungal activity against C. *albicans* in clinical and mycological assays. Considering the lack of side effects of LMWC varnish, its biocompatibility, and Nystatin-associated complications, it seems that LMWC varnish could be a good substitute for Nystatin in denture stomatitis treatment.

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Authors' Contribution

Conceptualization: Zahra Atai, Mohammad Atai, Zahra Khodadadi Bohlouli, Paniz Amini
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Writing–review & editing: Zahra Atai, Mohammad Atai, Zahra Khodadadi Bohlouli, Paniz Amini

Competing Interests

Authors declare that there is no conflict of interest.

Ethical Approval

The study was approved by the Ethics Committee in Research, Kerman University of Medical Sciences (Ethical code: IR.kmu.REC.1394.256).

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