Evaluation of different treatment methods against denture stomatitis: a randomized clinical study

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**Objective.** The aim of this clinical study was to determine the efficacy of *Uncaria tomentosa* (cat’s claw) against denture stomatitis (DS).

**Study Design.** Fifty patients with DS were randomly assigned into 3 groups to receive 2% miconazole, placebo, or 2% *U. tomentosa* gel. DS level was recorded immediately, after 1 week of treatment, and 1 week after treatment. The clinical effectiveness of each treatment was measured using Newton’s criteria. Mycologic samples from palatal mucosa and prosthesis were obtained to determine colony forming units per milliliter (CFU/mL) and fungal identification at each evaluation period.

**Results.** *Candida* species were identified with HiCrome Candida and API 20C AUX biochemical test. DS severity decreased in all groups (*P < .05*). A significant reduction in number of CFU/mL after 1 week (*P < .05*) was observed for all groups and remained after 14 days (*P > .05*). *C. albicans* was the most prevalent microorganism before treatment, followed by *C. tropicalis*, *C. glabrata*, and *C. krusei*, regardless of the group and time evaluated. *U. tomentosa* gel had the same effect as 2% miconazole gel.

**Conclusions.** *U. tomentosa* gel is an effective topical adjuvant treatment for denture stomatitis. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;118:72-77)

Although they are often present as benign commensal organisms in healthy individuals, *Candida* spp produce a broad range of serious illnesses in compromised hosts. Treatment has become increasingly difficult and expensive. Oral candidiasis is the most common fungal infection in humans, primarily among the elderly and denture users. It can affect up to 65% of elderly people who use total dentures. Denture stomatitis (DS) is a term describing this infection when related to the use of a dental prosthesis.

Species of *Candida* are the principal causative agent of DS, an inflammatory reaction of the supporting tissues of total and partial removable prostheses. DS is characterized by hyperemia and edema, sometimes accompanied by hemorrhagic petechiae. Clinical symptoms of the disease may include pain, irritation, and disturbance of salivation; however, many patients with this disease have no symptoms.

Various treatments have been proposed against DS, and topical or systemic antifungal medications are the most common. Nystatin, amphotericin B, clotrimazole, and miconazole are antifungal agents often used against oral candidiasis. Treatment also includes cleaning the prosthesis and verifying the need for change in the denture. In addition, suspension of the use of the prosthesis can reduce the inflammatory component. However, factors such as fungal resistance to the medicine and drug toxicity have led to the search for alternative treatments.

Phytotherapeutic agents are prepared exclusively with plants or parts of medicinal plants. A great deal of recent research has focused on their effectiveness. They are less costly, are less toxic, and have fewer side effects than many synthetics.

*Uncaria tomentosa* (of the family Rubiaceae), known as cat’s claw, is usually used in Peruvian medicine. In vitro and in vivo studies in animals and humans have confirmed its effectiveness. It is a good anti-inflammatory arthritis treatment. Its properties are due to the combined activity of its different components rather than any one isolated compound. In addition, it is an important source of bioactive substances, such as monoterpenoid oxindole alkaloids, which have immunomodulatory and antitumoral properties.

**Statement of Clinical Relevance**

*Uncaria tomentosa* gel was clinically evaluated for the treatment of denture stomatitis. It showed similar antimicrobial activity as 2% miconazole gel. However, its use must be associated with hygiene methods for a successful treatment.
Few studies, with contradictory results, were found in the literature regarding the effect of *U. tomentosa* on opportunistic fungi. Ccahuana-Vasquez et al. observed in vitro that tested concentrations of *U. tomentosa* did not inhibit *Candida albicans*. On the other hand, Herrera et al. indicated that 2% cat’s claw gel is effective in vitro against microorganisms frequently found in the oral cavity. However, clinical studies have not tested its effect on DS. Thus, the purpose of this clinical study was to determine the efficacy of *U. tomentosa* gel on DS. The hypothesis was that *U. tomentosa* gel could reduce the infection of dentures’ supporting tissues.

**MATERIALS AND METHODS**

**Participants**

For determining sample size, a power analysis (significance level, 5%; power, 80%) was used that was in agreement with previous studies. Calculation resulted in a minimal sample size of 15 patients in each group.

Fifty individuals who ranged between 45 and 85 years of age, had general good health, were denture wearers for at least 1 year, and had denture stomatitis type I, II, or III according to Newton’s criteria were selected from the Department of Dentistry of the State University of Ponta Grossa. The selection of these patients included a general health questionnaire and initial clinical examination. The procedures carried out in the study complied with Resolution 196/96 of the Brazilian Health Ministry, and the protocol was approved by the Ethics Committee of the Ponta Grossa State University (Protocol 17572/10). Each participant voluntarily signed an informed consent form before enrolling.

Exclusion criteria were based on medical histories. Excluded were women of childbearing age; patients with impaired hepatic or renal function, diabetes, xerostomia, hypoparathyroidism, immune alterations, chemotherapy, or radiation therapy; patients who had received any recent treatment with antibiotics, antifungals, or steroidal agents within 4 weeks before the study; and those with poorly fitting dentures.

The selected participants were examined for oral lesions, oral hygiene, and denture conditions. Newton’s classifications of type I (bleeding spots), type II (diffuse erythematous areas), and type III (granular inflammation) were used. Candidiasis was identified based on the results of microbiologic cultures collected from the palatal mucosa and from the denture surface. One colony or more from the palatal mucosa and more than 100 colonies from the denture were considered as positive results.

**Study design**

The study was double-blind. According to the stratified randomization list, 50 patients were randomly assigned to 1 of the 3 experimental groups as follows: group M (positive control), 2% miconazole gel (20 mg/g, Daktarin gel; Janssen-Cilag, São Paulo, São Paulo, Brazil); group P (placebo group), hydroxyethyl cellulose (1.5% Natrosol; Fleming Manipulações, Ponta Grossa, Paraná, Brazil); and group UT (experimental group), 2% *U. tomentosa* gel (Fleming Manipulações). The patients did not know which treatment they would receive.

All patients (from all groups) received treatment on admission (day 0) and were told to apply the gel on the base of the denture after meals, 3 times a day, for 1 week. Each application had 2.5 mL (1 teaspoonful) of gel on the denture, with surplus gel kept in the mouth until the next application. The patients cleaned their dentures before each application with a toothbrush and water; they suspended the use of the denture at night, leaving it in a glass of regular water. At each visit, the palatal mucosa was observed for local adverse reactions, and the patients were asked whether they experienced any irritation, burning, nausea, vomiting, or diarrhea.

The effectiveness of treatment was clinically assessed using Newton’s criteria (1962) for scoring the severity of DS, with 0 for a healthy palate. It was tested on day 0, day 7 (end of treatment), and day 14 (7 days after treatment). At follow-up visits, visual observations were made, and color photographs of the mucosa were taken using a digital camera (EOS Rebel T2i; Canon, Tokyo, Japan). The operator was always the same (L.Y.T.), as were the conditions, such as place, light, angle, and patient position. Two independent calibrated dentists blindly analyzed the photographs taken of each patient.

**Mycologic examination**

For each patient, *Candida* was recovered every week by rubbing oral swabs along the palatal mucosa and the tissue surface of the upper denture. Each swab was placed into a test tube containing 5 mL of brain-heart infusion medium (Himedia Laboratories, Mumbai, India). It was then vortexed for 1 minute to suspend the organisms from the swab. An aliquot of 50 μL from this suspension was plated on Sabouraud dextrose agar (SDA) plates (Himedia Laboratories) with 5 μg/mL chloramphenicol, another 50 μL was plated on HiCrome Candida Agar (Himedia Laboratories), and both plates were incubated at 37°C for 48 hours. A digital colony counter (CP 600 Plus; Phoenix Ind Com Equipamentos Científicos, Araraquara, São Paulo, Brazil) quantified the colonies on SDA and determined the number of colony-forming units per milliliter (CFU/mL). Values of CFU/mL from samples of the palatal mucosa were correlated with those of the tissue surface of the upper denture.
The colonies on HiCrome Candida were presumptively identified by color and confirmed by biochemical tests. First, 1 colony of each color type grown on HiCrome Candida was inoculated onto a fresh SDA plate. After 48 hours at 37°C, API 20C AUX (Bio-Mérieux SA, Marcy-l’Etoile, France) was used to identify the isolates, using Apiweb software (Bio-Mérieux SA).

A 1-way analysis of variance and the Tukey multiple comparison test compared age, gender, and time of prosthesis use for each participant. The Friedman test and the Dunn multiple comparison test ($\alpha = .05$) analyzed the effectiveness of each treatment. The Kruskal-Wallis test and the Dunn multiple comparison test ($\alpha = .05$) also compared clinical and microbiologic results of the 3 groups. The Spearman r test ($\alpha = .05$) analyzed correlation between CFU/mL of the palatal mucosa and CFU/mL of the prosthesis. Data were analyzed using GraphPad Prism 6 (GraphPad Software Inc).

RESULTS

Of the 50 patients, 2 were withdrawn from the study for not returning to the controls. The remaining 48, who had a mean age of $63.83 \pm 9.3$ years, entered the trial; this group was 89.58% women and 10.41% men. The miconazole group (group M) consisted of 13 women and 2 men with a mean age of 65.8 years; the mean time of use of the prosthesis was 11.4 years. The placebo group (group P) consisted of 16 women with a mean age of 63.18 years; the mean time of use of the prosthesis was 19.5 years. The $U$ tomentosa group (group UT) consisted of 14 women and 3 men with a mean age of 62.7 years; the mean time of use of the prosthesis was 14.76 years. There was not a significant difference in gender between the groups ($P = .59$) or in the time of use of the prosthesis ($P = .21$).

Figure 1 shows the severity of DS. Before treatment (day 0), type II was observed in all groups, and the severity diminished over the evaluation periods (7 and 14 days), with no significant differences between the treatments ($P > .05$) (Figure 2).

Candida spp were detected from each patient rubbing oral swabs along the palatal mucosa and the surface of the upper denture. The CFU/mL values were logarithm-transformed to achieve a normal distribution for statistical analyses.

The CFU/mL values of samples taken from the palatal mucosa were significantly lower than those taken from the prosthesis, but a direct correlation between them was observed ($r = 0.452; P < .0001$). Figure 3 shows the mean of CFU/mL in log based on the total number of Candida cells from the palatal mucosa and from the surface of the upper denture. Similarly to the clinical results, the concentration of colonies was highest on day 0 and diminished in subsequent evaluation periods (7 and 14 days), with no significant differences between the groups ($P > .05$).

Table I shows the identification of Candida spp in the patients evaluated. It can be seen that $C$ albicans was the most prevalent species, followed by $C$ tropicalis, $C$ glabrata, and $C$ krusei, regardless of the group evaluated. All microorganisms reduced in numbers throughout the trial, except $C$ tropicalis in group UT and $C$ glabrata in group M, whose numbers increased from day 7 to day 14.

DISCUSSION

The purpose of this clinical study was to determine the efficacy of $U$ tomentosa gel against DS. The hypothesis of this study was that $U$ tomentosa gel could eliminate the infection of the supporting tissues of the dentures. We found a reduction of DS severity after 7 days of treatment, from grade 2 to grade 1, that remained after 14 days (see Figure 1). However, there were no differences among the 3 groups. Thus, it is not possible to affirm that the $U$ tomentosa gel was solely responsible for this reduction. The reduction of fungal load from dentures and palates after treatments, associated with patients’ compliance with the oral and denture hygiene (including denture removal during sleep), might be responsible for the observed effect. In this study, treatment started with meticulous denture hygiene and after removal of other local factors. Although not sufficient to treat DS, optimal denture and oral hygiene are essential to maintain low levels of microorganisms on dentures and within the mouth. Consistent with this, we observed a statistically significant relationship between DS, yeast presence, and denture cleanliness.
We found that *Uncaria tomentosa* could be an alternative treatment for denture stomatitis, agreeing with the findings of Paiva et al.\(^{28}\) They evaluated the clinical effectiveness of *Uncaria tomentosa* on oral candidiasis and found that it was as effective as miconazole. This may be explained by the 2 chemotypes, with different patterns of tetracyclic or pentacyclic oxindole alkaloids, present in *Uncaria tomentosa*. The pentacyclic oxindole alkaloids have immune modulatory effects antagonistically inhibited by tetracyclic oxindole alkaloids.\(^{11,29,30}\) Additional research is required to identify the plant’s therapeutic agents.

The positive results in the placebo group could be explained by the fact that simply letting patients know about their disease can raise their concern and compliance to improve oral hygiene.\(^{31}\) In addition, the application of placebo gel could have prevented retention of yeasts on the denture surface.\(^{23}\) Because the gel formulation keeps the active substance in contact with the lesion for a longer time, it has shown more success on its application than other oral antifungals such as nystatin. The product is applied directly to the previously cleaned prosthesis, which acts as a tray, allowing longer contact time with the lesion, potentially causing a better response and faster regression of symptoms.

On day 7, there were fewer CFU/mL in group M than in groups P and UT. Thus, miconazole seems to be more efficacious in reducing the number of colonies than *Uncaria tomentosa* (experimental group) and Natrosol (placebo group). Miconazole is a scientifically proven antifungal.\(^{35}\) Azoles have a cytostatic or cytotoxic effect, inhibiting the metabolism of fungal cell membrane components.\(^{32,33}\) In addition, the azole stimulates intracellular reactive oxygen species that are inducers of apoptosis in yeasts.\(^{33,34}\) Despite miconazole’s proven antifungal action, some clinical isolates have shown resistance,\(^{34}\) probably owing to the protective effect of antioxidants during miconazole treatment.\(^{33}\) Thus, the use of alternative treatments such as phytotherapeutic agents is promising.

There are a variety of methods for identifying yeasts from clinical samples. These include traditional methods, such as the germ-tube test, morphology studies, and carbohydrate utilization; rapid methods, such as enzymatic and fluorogenic tests; commercially available methods; automated systems; and molecular

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**Fig. 2.** Palate of patients from 3 experimental groups. **A**, Patient 1 at baseline. **B**, Patient 1 after treatment with miconazole. **C**, Patient 2 at baseline. **D**, Patient 2 after treatment with placebo. **E**, Patient 3 at baseline. **F**, Patient 3 after treatment with *Uncaria tomentosa*.

**Fig. 3.** Mean and SD of colony-forming units for each group (M, miconazole; P, placebo; UT, *Uncaria tomentosa*) in different time points. Considering time points: (*) \(P < .05\) with P and UT groups at day 7. Considering groups in different time points: (**) \(P < .05\) with 7 and 14 days. Kruskal-Wallis and Dunn post hoc test.
typing techniques. This study used HiCrome Candida agar and biochemical test API 20C AUX. C albicans was the most prevalent species, followed by C tropicalis, C krusei, and C glabrata, regardless of the group evaluated. These results are in agreement with several studies. A large variation in species frequency has been reported in different regions of the world. Although several investigators found C tropicalis one of the most common non-albicans species isolated in Brazil and South America, C glabrata is found more frequently in North America.

The week-to-week reduction of microorganisms had 2 exceptions, C tropicalis in group C and C glabrata in group A, which grew back by day 14 after decreasing by day 7, suggesting that treatments were not effective for this species. C glabrata exhibits quite clinically significant cross-resistance to older azole drugs (fluconazole and itraconazole) and to voriconazole.

On the other hand, C tropicalis cells obtained from the oral cavity of denture wearers with denture stomatitis are more adherent to buccal epithelial cells than those obtained from patients without signs of disease. The ability of C tropicalis strains to form biofilm on different surfaces is another potential virulence trait, which may increase their resistance to antifungal treatment. Although these factors may explain the resistance by C glabrata and C tropicalis in the present study, further investigations are required to examine and better understand the mechanism of pathogenicity of these species of Candida.

The literature reports several alternative treatments and different times of treatment against DS with optimal results ranging between 1 and 4 weeks, with the effectiveness depending on medical therapy or prosthesis treatment. In the present study, 1 treatment week was sufficient to be effective. However, further studies increasing the time of treatment with U tomentosa gel are needed.

**CONCLUSIONS**

*U tomentosa* gel was an effective topical adjuvant treatment (i.e., it had the same effect as 2% miconazole gel); however, the influence of the prescribed hygiene methods should be evaluated in future studies. In addition, *U tomentosa*, as an herbal treatment, might be more economical than other treatments for patients.

**Table 1.** Number and percentage of patients in whom *Candida* species were found

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