Original Article

Efficacy of 0.1% Chitosan-Curcuminoids Mouthwash in Treatment of Oral Lichen Planus and Prevention of Disease Relapse

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Abstract

Introduction and Objective: The aim of this study was to determine the therapeutic efficacy of 0.1% alcohol-free chitosan-curcuminoids mouthwash (CHI-CUR) which exerts anti-inflammatory and antifungal activity in management of oral lichen planus (OLP) in comparison to a standard 0.1% triamcinolone acetonide mouthwash (TA) to provide its clinical application as an alternative therapeutic agent for OLP.

Methods: A pilot single-blinded randomized controlled trial was conducted at the Faculty of Dentistry, Prince of Songkla University, Thailand between February 2019 - April 2021. Participants were aged 18 years or older with a confirmed diagnosis of OLP by an oral medicine practioner. Patients were randomly assigned to CHI-CUR or TA mouthwash at a dose of five milliliters for two min, four times a day for four weeks. Primary outcome measures include a complete relief of erythematous lesions, a reduction in the number of *C. albicans* colonies present in the oral cavity and the disease relapse.

Results: The result showed within the 4-week treatment course, that from the patients in the mild/marked erythema group, three of six patients (50%) using TA mouthwash and two of eight patients (20%) using CHI-CUR mouthwash had a complete relief of erythematous lesions, whereas patients in the ulceration group (where 3 patients used TA mouthwash and one patient used CHI-CUR mouthwash) had a decrease in site activity score level from 16 and 11 to 5 and 6 (mild/marked erythema), respectively. Both treatment groups provided comparable efficacy in relief of pain or dryness of the oral cavity within the 2-week treatment course. For inhibitory efficacy against candida colonization in the oral cavity, it was found at the fourth week after the treatment that

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all seven of the nine patients (77.8%) with candida infection in the CHI-CUR mouthwash treatment group had a complete anti-candida response and eight of the ten patients (80%) with candida infection in the TA mouthwash treatment group were found candidiasis in six of the ten patients (60%) with two of the ten (20%) having candida superinfection at 4-weeks during the treatment course. Disease relapse was not observed after 6-months follow-up time in either intervention group.

Discussion: TA mouthwash exerted a high anti-inflammatory efficacy, but it has no antifungal activity. In the present study, an alcohol free 0.1% CHI-CUR mouthwash was found to be as effective as 0.1% TA mouthwash in managing the signs and symptoms of OLP with a comparable time to remission state and a comparable efficacy in relief of pain or dryness of the oral cavity. On the contrary, a complete anticandidal response was found only in patients using CHI-CUR mouthwash. In addition, CHI-CUR mouthwash could be effective in decreasing the rate of symptom recurrence.

Conclusion and Recommendation: 0.1% alcohol-free CHI-CUR mouthwash may serve as a therapeutic alternative in treating candida-associated OLP or OLP patients who have candida superinfection undergoing topical corticosteroids therapy.

Key words: oral lichen planus, oral candidiasis, curcuminoids, chitosan, mouthwash

ประสิทธิภาพของน้ำยาบ้วนปาก 0.1% ไคโตซาน-เคอร์คูมินอยด์ในการรักษาโรคไลเคน แพลนัส ในช่องปากและการป้องกันการกลับเป็นซ้ำของโรค

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บทคัดย่อ

บทนำและวัตถุประสงค์: การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพการรักษาของน้ำยาบ้วนปาก 0.1% ไคโตซาน-เคอร์คูมินอยด์ ที่ปราศจากแอลกอฮอล์ ซึ่งมีฤทธิ์ลดการอักเสบและฤทธิ์ต้านเชื้อรา ในการจัดการโรคไลเคน แพลนัส ในช่องปาก เปรียบเทียบกับน้ำยาบ้วนปากมาตรฐาน 0.1% ไตรแอมซิโนโลน อะเซโทไนด์ ซึ่งมีฤทธิ์ลดการอักเสบ เพื่อเป็นข้อมูลการใช้ยาทางคลินิกในการใช้เป็นยาทางเลือกรักษาโรคไลเคน แพลนัส ในช่องปาก

วิธีการศึกษา: ทำการศึกษานำร่องในรูปแบบสุ่มและมีกลุ่มควบคุมแบบปกปิดทางเดียว ณ โรงพยาบาลทันตกรรม คณะทันตแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ช่วงเดือนกุมภาพันธ์ 2019 – เดือนเมษายน 2021 ผู้เข้าร่วมวิจัยมีอายุ 18 ปีขึ้นไป ที่ได้รับการตรวจวินิจฉัยแล้วว่าเป็นโรคไลเคน แพลนัส จากทันตแพทย์สาขาอายุรศาสตร์ช่องปาก ผู้ป่วยทั้งหมด 20 คน ถูกสุ่มแบ่งเป็น 2 กลุ่มให้ได้รับตำรับน้ำยาบ้วนปากไคโตซาน-เคอร์คูมินอยด์ หรือน้ำยาบ้วนปากไตรแอมซิโนโลน อะเซโทไนด์ โดยใช้บ้วนปากครั้งละ 5 มิลลิลิตร เป็นเวลา 2 นาที วันละ 4 ครั้ง ติดต่อกันเป็นเวลา 4 สัปดาห์ วัดผลลัพธ์หลักทางการวิจัยในด้านการลดระดับความรุนแรงของการเกิดรอยโรค การลดจำนวนของการเกิดโคโลนีของเชื้อราในช่องปาก และการเกิดการกลับเป็นซ้ำของโรค

ผลการศึกษา: ผลการศึกษาแสดงให้เห็นว่า ภายในสัปดาห์ที่ 4 ของการรักษาในกลุ่มผู้ป่วยที่มีลักษณะรอยโรคเป็นรอยถลอก ผู้ป่วยจำนวน 3 คนใน 6 คน (50%) ในกลุ่มที่ได้รับน้ำยาบ้วนปากไตรแอมซิโนโลน อะเซโทไนด์ และผู้ป่วยจำนวน 2 คนใน 8 คน (25%) ในกลุ่มที่ได้รับน้ำยาบ้วนปากไคโตซาน-เคอร์คูมินอยด์ ตรวจไม่พบรอยโรคและไม่มีอาการของโรค ในขณะที่ในกลุ่มผู้ป่วยที่มีแผลอักเสบในช่องปาก ผู้ป่วยทั้ง 3 คน ในกลุ่มที่ได้รับน้ำยาบ้วนปากไตรแอมซิโนโลน อะเซโทไนด์ และผู้ป่วย 1 คน ในกลุ่มที่ได้รับน้ำยาบ้วนปากไคโตซาน-เคอร์คูมินอยด์ มีคะแนนความรุนแรงของแผลอักเสบลดลงจาก 16 และ 11 เหลือ 5 และ 6 ตามลำดับ ลักษณะเป็นเพียงรอยถลอก ผู้ป่วยทั้งสองกลุ่มการศึกษาไม่มีอาการปวดและปากแห้งภายในสัปดาห์ที่ 2 ของการรักษา ผลต้านการเกิดโคโลนีของเชื้อราแคนดิดาในช่องปาก พบว่า ที่สัปดาห์ที่ 4 ของการรักษา ในกลุ่มที่ได้รับน้ำยาบ้วนปากไคโตซาน-เคอร์คูมินอยด์ มีจำนวนผู้ป่วยที่ตรวจพบเชื้อราในช่องปากลดลงจาก 7 ใน 9 คน (ร้อยละ 77.8) เป็นตรวจไม่พบเชื้อราทั้ง 7 คน และในกลุ่มที่ได้รับน้ำยาบ้วนปากไตรแอมซิโนโลน อะเซโทไนด์ มีจำนวนผู้ป่วยที่ตรวจพบเชื้อราแคนดิดาในช่องปากลดลงจาก 8 คนใน 10 คน (ร้อยละ 80) เป็น 6 คนใน 10 คน (ร้อยละ 60) โดยมีผู้ป่วย 2 คนเป็นผู้ที่ตรวจไม่พบการเกิดเชื้อราแคนดิดาในช่องปากก่อนการรักษาแต่กลับพบการติดเชื้อราแคนดิดาซ้ำซ้อนในสัปดาห์ที่ 4 ของการรักษา ผู้ป่วยทั้งสองกลุ่มการศึกษาไม่มีการกลับเป็นซ้ำของโรคเมื่อทำการติดตามผลภายหลังการรักษานาน 6 เดือน

อภิปรายผล: น้ำยาบ้วนปากไตรแอมซิโนโลน อะเซโทไนด์ มีประสิทธิภาพสูงในการต้านอักเสบแต่ไม่มีฤทธิ์ต้านเชื้อรา ในการศึกษานี้พบว่าน้ำยาบ้วนปาก 0.1% ไคโตซาน-เคอร์คูมินอยด์ที่ปราศจากแอลกอฮอล์มีประสิทธิภาพเทียบเคียงกับน้ำยาบ้วนปาก 0.1% ไตรแอมซิโนโลน อะเซโทไนด์ในการจัดการอาการแสดงและอาการของโรคไลเคน แพลนัส ในช่องปากโดยมีระยะเวลาที่ไม่มีอาการของโรคและประสิทธิภาพในการลดอาการปวดแผลหรือความแห้งในช่องปากเทียบเคียงกัน ในทางตรงกันข้าม มีเพียงผู้ป่วยกลุ่มที่ได้รับน้ำยาบ้วนปากไคโตซาน-เคอร์คูมินอยด์เท่านั้นที่หายจากการติดเชื้อราแคนดิดาอย่างสมบูรณ์ นอกจากนี้น้ำยาบ้วนปากไคโตซาน-เคอร์คูมินอยด์อาจมีประสิทธิภาพในการลดอัตราการเกิดการกลับเป็นซ้ำของอาการโรคได้

ข้อสรุปและข้อเสนอแนะ: น้ำยาบ้วนปาก 0.1% ไคโตซาน-เคอร์คูมินอยด์ที่ปราศจากแอลกอฮอล์มีศักยภาพในการใช้เป็นยารักษาทางเลือกในผู้ป่วยโรคไลเคน แพลนัส ในช่องปากที่มีการติดเชื้อราแคนดิดาร่วม หรือในผู้ป่วยที่เกิดการติดเชื้อราแคนดิดาซ้ำซ้อนระหว่างการใช้น้ำยาบ้วนปากไตรแอมซิโนโลน อะเซโทไนด์รักษาโรค

คำสำคัญ: โรคไลเคน แพลนัส ในช่องปาก, โรคราแคนดิดาในช่องปาก, เคอร์คูมินอยด์, ไคโตซาน, น้ำยาบ้วนปาก

Introduction

Oral lichen planus (OLP) is a chronic immunological inflammatory mucocutaneous disease that is relatively common when compared to other oral lesions. An erosive OLP is classified as a potentially malignant disorder of the oral mucosa in which an erosive type, female gender and plaques form on the back of the tongue site are considered as risk factors for OLP transformation.[1-2] Treatment for OLP is focused mainly on eliminating mucosal ulcerations, alleviating pain during periods of activity and prolonging the remission period. Corticosteroids have a highly potent anti-inflammatory efficacy by completely inhibiting the arachidonic acid-dependent inflammatory pathway via inhibiting phospholipase A_2 activity, thereby blocking the synthesis of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes

including an inhibition on leukocyte (neutrophils, lymphocytes and macrophages) infiltration, which leads to a suppression of the production and the release of pro-inflammatory mediators.[3] Accordingly, corticosteroids is widely accepted as the most effective treatment of erosive OLP with a success rate of up to 75% in eliminating mucosal erythema or ulcerations and alleviating pain. Topical mid-potent corticosteroids preparations [such as triamcinolone acetonide (TA) oral paste/solution, 0.1% fluocinolone acetonide oral paste/gel/solution or 0.05% dexamethasone elixir] are the standard treatment in treating mild to moderate erosive OLP as side effects are fewer than with systemic administration. However, oral candidiasis is a very real considerations for prolonged use.[4] Systemic antifungals with nystatin suspension of 1:100,000 units are usually used to treat candidiasis in OLP patients, which present many economic and health related challenges in terms of high cost of treatment and potential side effects respectively, especially in cases of long-term use. Besides, cultures or histologic sections from 46% of OLP patients were found positive for fungi and more than 80% of yeast found in those patients was *Candida albicans*.[5] In addition, a disease recurrence rate of 20% in patients undergoing topical 0.1% TA ointment was observed during the follow up period of 6 months.[6] Currently, management of erosive OLP is challenging for most clinicians as it is difficult to achieve complete resolution and disease reoccurrence is also very common. Time is needed to explore other options, probably with better clinical safety and therapeutic efficacy.

Curcuminoids (a group of polyphenolic pigment extract from the rhizome of *Curcuma longa*) or curcumin (a major constituent contained in curcuminoids powder) is one of the most promising of the new treatment modalities. Curcuminoids or pure curcumin has been known widely as a potent anti-oxidant and anti-inflammatory agent as well as being a powerful enhancer of wound healing.[7-10] It was also found to possess anti-bacterial activity against oropharyngeal and upper respiratory tract bacterial pathogens and antifungal activity against *C. albicans*.[11-12] Accordingly, topical curcuminoids or curcumin oral paste or gel has been suggested to be a potential alternative to topical corticosteroids oral paste in treating OLP due to its safety and potential therapeutic benefits.[13-14] Nevertheless, no studies have been undertaken to evaluate the clinical efficacy of topical curcuminoids or curcumin preparation on candida-associated OLP and the recurrence of the disease. Our previous *in vitro* study found that the addition of chitosan in the curcumin mouthwash caused the mouthwash to have comparable anti-candida efficacy to a standard

0.2% chlorhexidine mouthwash in complete eradication of both free-floating forms and biofilms of *C. albicans* and also enhanced anti-inflammatory and ulcer healing activity of curcumin.[15] Additionally, an alcohol free 0.1% chitosan-0.5% curcuminoids mouthwash (CHI-CUR) has been found to be better than 2% chlorhexidine mouthwash in management of generalized or candida-associated denture stomatitis with high safety and high patient compliance and satisfaction.[16]

Therefore, the aim of this study was to determine the therapeutic efficacy of 0.1% CHI-CUR mouthwash that exerts anti-inflammatory and antifungal activity in management of oral lichen planus (OLP) in comparison to a standard 0.1% TA mouthwash to provide its clinical application as an alternative therapeutic agent for OLP.

Methodology

1. Material

1.1 Preparation of an alcohol-free 0.1% chitosan-0.5% curcuminoids (CHI-CUR) mouthwash by a pharmacist in the Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences[16]

0.1 g of commercial curcuminoids powder purified from turmeric (*Curcuma longa* Linn.) rhizome (Thai-China Flavors and Fragrances Industry Co. LTD) containing 79:19:2 curcumin: demethoxycurcumin and bisdemethoxycurcumin, respectively, was dissolved in a solvent solution prepared by mixing 40 ml of polyethylene glycol (PEG), 25 ml of 2% low molecular weight chitosan (Sigma Aldrich, USA) solution (chitosan 2 g in 100 ml of 1% acetic acid water), 15 ml of sorbitol, 1 ml of paraben concentration, and 100 μl of 10% NaOH (the remaining volume was made up of water until reaching 100 ml).

1.2 Study participants

Volunteers agreeing to participate in the study signed the informed consent form. Protocol and consent forms were approved by the Research Ethics Committee (REC) of the Faculty of Dentistry, Prince of Songkla University (EC6112-48-J-HR). The trial number was TCTR20200910005. The name of the registry is the Thai Clinical Trials Registry (TCTR).

The inclusion criteria were 18 years aged or older adults of both genders with pathological evaluations confirming symptomatic OLP by experienced oral medicine specialists, having the ability to use mouthwash correctly and were treated at the Dentistry hospital, Prince of Songkla University, Thailand.

The exclusion criteria were pregnant and lactating women; patients with open mouth sores because of any other diseases; patients with oral candidiasis, HIV infections or chronic systemic disease (e.g. hyperthyroidism, dia-

betes) at the study entry; patients who had used any other prophylactic mouthwashes within the last month, patients who required use of any form of treatment/medicaments (such as antibiotics, analgesics, etc.); patients who were recently treated with antifungal, antibacterial, chemotherapeutic and immunosuppressive agents within the last month; patients who had dryness of the oral cavity with a score of 4 or more (assessed by Challacombe scale) or patients who were allergic to or should not take anything with curcumin, chitosan or triamcinolone acetonide.

Subject withdrawal criteria included patients who had a site activity score of 7 or more (received artificial saliva), patients who had an increased severity of site activity score at week 2 after the treatment (received 0.1% fluocinolone acetonide paste or 0.1% dexamethasone solution) or patients who had *C. albicans* colonies in the oral cavity of over 400 CFU/ml at week 2 after the treatment (received 0.1% nystatin suspension)

2. Method

2.1 Study design

The trial design was a single-centre, randomized controlled, parallel arm trial. The study took place at the Faculty of Dentistry, Prince of Songkla University, Hat-Yai, Thailand between February 2019 and April 2021. The sample size was calculated according to the formula described by Kelsey et al (1996) for clinical trial studies[17] as shown below and a study by Laxmi et al (2015)[6] which found that 0.1% TA caused a disease relapse of 20% whereas a low-level laser therapy caused a disease relapse of 5% within 6 months.

$$N = \frac{(Z_{\alpha/2}\sqrt{2P(1-P)} + Z_{\beta}\sqrt{P_0(1-P_0)+P_1(1-P_1)})^2}{(P_1-P_0)^2}$$

$$= \frac{(1.96\sqrt{2(0.125)(1-0.125)} + 0.84\sqrt{0.05(1-0.05)+0.2(1-0.2)})^2}{(0.2-0.05)^2} = 75$$

Level of significance = 5%, Power = 80%, P = (P0=P1)/2 = (0.05+0.2)/2 = 0.125

Thus 10 patients per group were enrolled allowing for a dropout rate of 20% for the present pilot study.

A total of 20 patients were randomized and allocated to two different interventions including 0.1% CHI-CUR or 0.1% TA mouthwash using online software from http://www.graphpad.com/quickcalcs, GraphPad Software, Inc. The block randomization size was 4 and the allocation ratio between the two groups was 1:1. Five milliliters of each intervention was given to the patient to be used for 2 min, four times a day at 8 am, 12 pm, 4 pm and before bed for four weeks.

2.2 Efficacy and safety evaluation

2.2.1 Outcome Measures:

Primary outcome : Complete relief of erythematous lesions, pain and dryness of the oral cavity

: Reduction in the number of *C. albicans* colonies present in the oral cavity

: Disease relapse

Secondary outcome: Patient safety

Demographic data and medical history were collected through anamnesis on the first day. The diagnosis of OLP was performed through clinical evaluation by an oral medicine specialist on the first day (W0), two weeks after the first examination (W2) and 4 weeks after the first examination (W4). Erythematous lesions (site activity score) and the dryness of the oral cavity were assessed on W0, W2 and W4 using the Challacombe scale. A site activity score was obtained according to the Challacombe scale by multiplication of two scores; the site score and activity score. The site and activity scores were calculated by the following criteria.

Site score: 0 = no lesion at the site, 1= less than 50% of area affected, and 2 = greater than 50% of area affected.

Activity score: 0 = reticular, 1 = mild erythema, 2 = marked erythema, and 3 = ulceration.

The dryness of the oral cavity was assessed using the Challacombe dryness scale of 0-3.

Pain was assessed before the treatment (W0) and at W2 and W4 after the treatment using a Visual Analog Pain scale (VAS; patient reporting scale of 0-10).

An evaluation form was given to the family or the patient to record all aspects and possible side effects associated with the study drug. Detailed interviews and evaluations were conducted to analyze safety and any possible side effects of the drug.

Disease relapse was evaluated by telephone interviews at the 6-month follow-up time

2.3 Candida infection investigation[18]

Patients were asked to rinse 10 ml of sterile phosphate buffer saline 0.01 M pH 7.2 for 1 min and then spit out the saliva in the sampling bottle (whole mouth method). The saliva samples were collected before the treatment (W0) and at W2 and W4 after the treatment (Figure 1). Samples were taken three times for every patient. After inoculation on sabouraud dextrose agar (SDA) and tryticase soy agar (TSA) medium, respectively, the cultures were incubated at 35°C for 2 to 3 days. Biochemical reactions were used to identify different fungi.

2.4 Statistical analysis

Wilcoxon's matched-pairs signed-ranks test was used for the comparison between the

site activity score before and after intervention. Mann Whitney's U test was used to compare the clinical outcome (between the treatment groups. Values of $p < 0.05$ were considered to be statistically significant.

Results

1. Clinical efficacy evaluation of the CHI-CUR mouthwash in treating OLP

The participant's flow diagram is shown in Figure 1.

The basic characteristics of the patients, the lesion classification and the level of pain and dryness of the oral cavity are provided in Table 1. Before the treatment (W0), a total of 20 patients were randomized and allocated to two different interventions including 0.1% CHI-CUR (n = 10) or 0.1% TA mouthwash (n = 10). However, one patient in the CHI-CUR-treated group was lost to follow up at two weeks after the treatment and outcome status was unknown. Therefore, a per protocol analysis

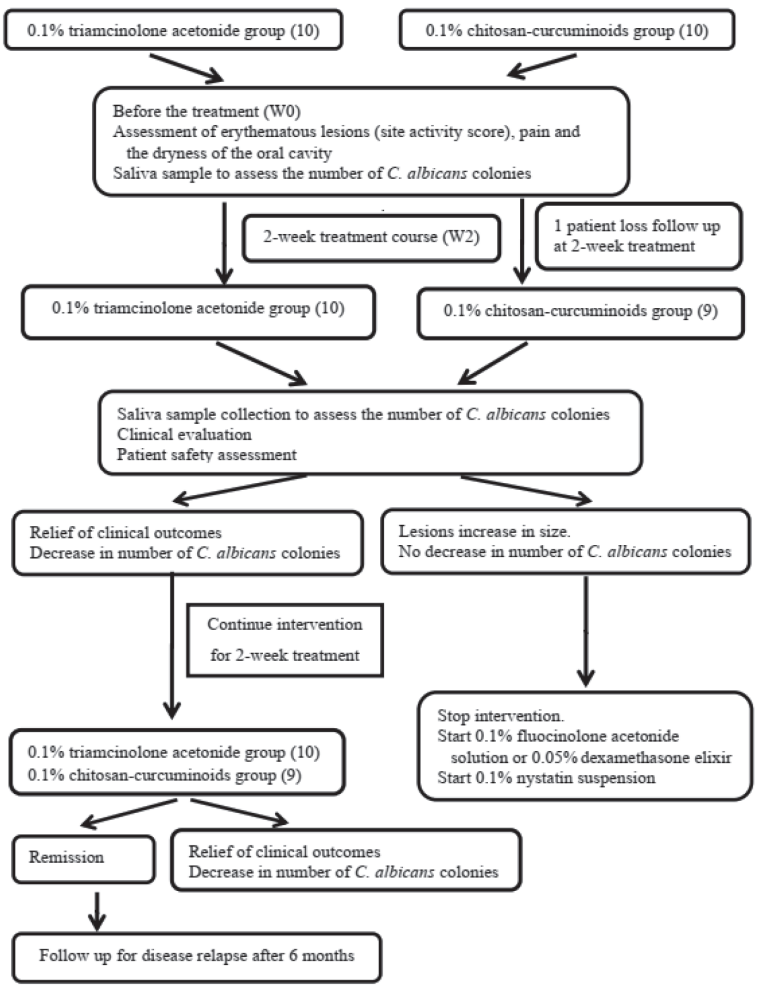

Figure 1 Flow chart of study

Table 1 Basic characteristics of patients

Characteristics	TA group (n = 10)	CHI-CUR group (n = 9)	*P*
Age (Year)(mean ± SD)	56.3 ± 6.4	49.1 ± 13.7	0.16
Sex (Female/Male)	8/2	9/0	0.47
Underlying diseases (Yes/No)	5/5	4/5	1.00
Hyperlipidemia	1	4	
Hypertension	3	0	
Diabetes mellitus	1	0	
History of OLP (Yes/No)	6/4	5/4	1.00
Smoking (Yes/No)	0/10	0/9	-
Drinking (Yes/No)	1/9	1/8	1.00
Type of OLP (n)			
Mild/marked erythema	6	8	0.09
Ulceration	3	1	0.58
Non-erosive reticular	1	-	-
Pain (median)	2(0-9)	3(0-7)	0.60
Dryness (median)	1(0-2)	1(0-4)	0.69
No of *C. albicans* colonies (CFU/ml)(mean ± SE)	$0.7 \times 10^3 \pm 0.4 \times 10^3$ (n = 8/10)	$1.2 \times 10^3 \pm 1.2 \times 10^3$ (n = 7/9)	0.69

SD = Standard deviation SE = Standard error of mean

TA = 0.1% triamcinolone acetonide mouthwash

CHI-CUR = 0.1% alcohol-free chitosan-curcuminoids mouthwash

$p < 0.05$ comparison of different mouthwash groups

of the CHI-CUR-treated group included only those patients who adhered to the protocol (n = 9). The remaining 9 patients in the 0.1% CHI-CUR-treated group were female. Only 2 patients included in the clinical study were male and both were in the TA-treated group. Patient ages ranged from 35-63 years old. The base line characteristics on underlying disease, history of having OLP, smoking, drinking, activity score, pain level and the dryness of the oral cavity for both groups were not significantly different from each other. Most of the patients in both treatment groups had mild/marked erythema. High concentrations of *C. albicans* colonies were found before the treatment in 8 out of 10 (80%) patients receiving TA mouthwash and 7 out of 9 (77.8%) patients receiving CHI-CUR mouthwash. The number of *C. albicans* colonies was not significantly different from each other.

Both treatment groups had a comparable time to remission state within the 4-week treatment course and a comparable efficacy in relief of pain or dryness of the oral cavity within the 2-week treatment course as shown in Table 2. Seven out of 9 (77.8%) patients using CHI-

Table 2 Treatment efficacy of OLP and anti-candida efficacy

Outcomes	Week 0			Week 4		
	TA (n = 10)	CHI-CUR (n = 9)	*p*	TA (n = 10)	CHI-CUR (n = 9)	*p*
Site activity score						
Non erosive reticular						
Median (min-max)	0 (n = 1)	-	-	remission (n = 1)	-	-
Mild/marked erythema						
Median (min-max)	4 (1-8) (n = 6)	6.5 (3-12) (n = 8)	0.15	remission (n = 3)	remission (n = 2)	0.58
				3 (0-11) (n = 3)	3 (0-7) (n = 6)	0.16
Ulceration						
Median (min-max)	11 (3-35) (n = 3)	16 (n = 1)	0.99	5 (1-36) (n = 3)	6 (n = 1)	0.75
Pain (VAS: 0-10)	2 (0-9)	3 (0-7)	0.60	0	0	-
Dryness	1 (0-2)	1 (0-4)	0.69	0 (0-4)	0 (0-2)	0.64
No of *C. albicans* Colonies (CFU/ml) (Mean ± SE)	$0.7 \times 10^3 \pm 0.4 \times 10^3$ (n = 8)	$1.2 \times 10^3 \pm 1.2 \times 10^3$ (n = 7)	0.69	$0.6 \times 10^3 \pm 0.2 \times 10^3$ (n = 6)	0 (n = 7)	0.04

TA = 0.1% triamcinolone acetonide mouthwash

CHI-CUR = 0.1% alcohol-free chitosan-curcuminoids mouthwash

Site activity score = Site score X Activity score.

Site score: (0) no lesion at site; (1) less than 50% of area affected; (2) if greater than 50%, not defined anatomically

Activity score: (0) normal; (1) mild erythema; (2) marked erythema; (3) ulceration

$p < 0.05$ comparison of different mouthwash groups (Mann-whitney U test)

CUR mouthwash had a complete anti-candida response at 4 weeks of the treatment course whereas 6 out of 10 (60%) patients using TA mouthwash still found a candida infection at 4 weeks of the treatment course. Moreover, 2 out of 10 patients using TA mouthwash who had no detectable fungal colonization before using the intervention had a superinfection of *C. albicans* at 4 weeks of the treatment course. Disease relapse was not observed at the 6-month follow-up time in either intervention group.

2. Safety analysis

Patients were asked to list any specific side effects and their response are recorded in Table 3. No serious side effects were noticed in either patient group and none of the patients had to leave the study because of oral or systemic adverse events. The major adverse effects reported in patients-treated with TA mouthwash were oral dryness/thirsty, hypersalivation, decreased tasting and a burning sensation. The oral dryness/thirsty and burning sensation were also reported mainly in patients-treated with CHI-CUR mouthwash. A few patients-treated with CHI-CUR mouthwash could feel mild to moderate nausea. Yellow staining on the tongue was found after rinsing the CHI-CUR mouthwash, however, the staining disappeared after drinking or eating.

Table 3 Patient safety reported during the course of study

Patient's safety	TA (n = 10)	CHI-CUR (n = 9)
Oral dryness/thirsty	5	3
Difficulty in speaking	1	2
Difficulty in drinking/eating	2	0
Nausea/vomiting		
Mild to moderate	1	3
Severe	0	0
Decreased food consumption	2	1
Difficulty in sleeping	1	1
Cramping/diarrhea	1	1
Staining on the tongue	0	0
Decreased tasting	3	1
Bitter/dull/astringent taste	3	3
Hypersalivation	3	1
Burning sensation	4	3

TA = 0.1% triamcinolone acetonide mouthwash
CHI-CUR = 0.1% alcohol-free chitosan-curcuminoids mouthwash

Discussion

The design of the present study needed to be a single-blinded randomized controlled trial. Both CHI-CUR and TA mouthwash were delivered in an amber glass bottle to the pharmacist and the investigator who were blind as to which mouthwash it was. The preparation of CHI-CUR formulation was naturally clear but yellow color solution whereas the TA formulation was a clear and colorless solution so that the participants especially in whom who had ever use TA mouthwash may be able to iden-

tify which intervention they received. Nevertheless, it needs to be in keeping with the TA formulations as a clear, colorless solution to avoid any impact on the therapeutic efficacy and safety of the mouthwash as the US FDA has approved that a yellow color (tartarzine and sunset yellow) has antimicrobial activity.[19] In addition, tartarzine has been reported to exert an antioxidant property[20] whereas sunset yellow has been found to exhibit an immunomodulatory property.[21] Moreover, both dyes may cause an allergy.[22]

Only 2 of the 20 patients included in the clinical study were male which reflected similar results presented in previous studies that women were more prone to OLP than men.[23] The age range of patients included in the study and the severity of the disease corroborated with the literature showing a higher prevalence of OLP in the age groups between 30-60 years and that erosive reticular (mild/marked erythema) type OLP was most commonly found. In the present study, cultures from 80% of patients in each intervention group were found positive for *C. albicans*.

Either curcuminoids or pure curcumin has been known widely as a potent antioxidant agent due to its free radicals scavenging activity and activation of the Nrf2-ARE pathway which prevent the decline of antioxidant enzyme activities.[7-8] It has also been shown to exhibit potent anti-inflammatory efficacy through an inhibition on the activity of various pro-inflammatory enzymes including inflammatory mediators involved in inflammation[7] and a modulation of sirtuins activity which further activates transcription and expression of inflammatory markers.[9] In addition, it has been claimed as a powerful enhancer of wound healing by modulating cell proliferation, collagen synthesis and decreasing reactive oxygen species.[10] As oxidative stress and inflammatory factors play important roles in the pathogenesis of OLP, curcuminoids or curcumin may offer potential benefits in treatment of OLP. A previous randomized, double-blinded, placebo-controlled trial of oral curcuminoids at a dose of 2,000-6,000 mg/day in OLP patients for 2-4 weeks had shown a controversial result of no detectable effect and significant efficacy in controlling signs and symptoms of OLP which may be related to an extremely poor bioavailability of curcuminoids that need a high-dose of oral administration.[24-25] Consequently, the idea of using topical curcuminoids is more interesting and viable in management of OLP. A randomized controlled trial in evaluating the efficacy of a topical 5% curcuminoids oral paste in treating OLP by applying it three times a day for four weeks compared with that of 0.1% TA oral paste showed that no statistically significant difference was noted between the two intervention groups.[13] Notwithstanding, patients preferred TA paste to curcuminoids due to the undesirable yellowish color of curcuminoids particularly on exposed areas, and it had a burning sensation that might have affected

the pain sensation during the treatment. It was also noticed in patients who had previously experience with TA mouthwash that the pain resolving efficacy of TA in the oral paste preparation seemed to be less than that of the mouthwash preparation. Another intervention study with a smaller dose of curcuminoids was conducted to compare the efficacy of 1% curcuminoids gel with 0.1% TA oral paste in managing the signs and symptoms of OLP.[14] The results revealed that the application of 1% curcuminoids oral gel six times daily for 12 weeks was almost as effective as application of 0.1% TA oral paste thrice daily.

Compared to other delivery systems, mouthwash preparation is a common commercially available dosage form being used for a number of specific problems in the oral cavity, especially in patients with widespread symptomatic erosions since the direct application of oral paste, cream or gel preparation is uncomfortable and adheres poorly to the moist mucous membrane. In patients with widespread symptomatic lesions, TA mouthwash is commonly used for controlling the symptoms of the disease. Nevertheless, about 11.4% of the OLP patients treated with 0.3% or 0.5% TA mouthwash had been found to have fungal superinfection.[26] It had also been reported that 20% of OLP patients treated with 0.1% TA ointment for 4 weeks had recurrence of the disease during the follow up period of 6 months.[6] In the present study, an alcohol free 0.1% CHI-CUR mouthwash was found to be as effective as 0.1% TA mouthwash in managing the signs and symptoms of OLP with a comparable time to remission state and a comparable efficacy in relief of pain or dryness of the oral cavity. Nevertheless, a complete anticandidal response was found only in patients using CHI-CUR mouthwash. It was also found from the present study that 20% of patients treated with 0.1% TA had a superinfection of *C. albicans* at 4-weeks of the treatment. However, a disease relapse was not observed at the 6 month-follow-up time in either intervention group in the present study. The anti-inflammatory and anti-candida efficacies of CHI-CUR mouthwash obtained from the present study was consistent with our previous clinical study in patients with denture stomatitis[16] and confirmed its potential as a potent preventive and therapeutic agent against oral candidiasis. Due to the bioadhesive, anti-candida, anti-inflammatory and wound healing properties of chitosan[27-29], it can be extremely beneficial to maintain the concentration of curcuminoids in an oral cavity and enhance the therapeutic efficacy of curcuminoids. Avoiding the use of alcohol to dissolve curcuminoids in the mouthwash formulation is also crucial for a better clinical response and patient compliance. Considering the chronic nature of OLP, risk of oral candidiasis upon usage of topical corticosteroids, and potential therapeutic benefits with the safety profile of CHI-CUR mouthwash; a randomized controlled trial with larger number of patients

and a longer follow-up time is highly recommended for further insight to the efficacy of CHI-CUR mouthwash for treating OLP. Since antifungal activity in TA doesn't exist and this study has a small group of volunteers (as a pilot study), future trial in larger population and longer treatment times to compare this 0.1% chitosan-curcuminoid formulation and TA and nystatin (when candidiasis was found) is also suggested.

Conclusion

The obtained clinical findings indicate that CHI-CUR mouthwash may serve as a safe and potential topical therapeutic alternative in treating patients with candida-associated OLP or OLP patients who suffer from candida superinfection undergoing topical corticosteroids therapy.

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Conflict of interest

The authors declare no conflicts of interests.

References

1. Giuliani M, Troiano G, Cordaro M, Corsalini M, Gioco G, Muzio LL, Pignatelli P, Lajolo C. Rate of malignant transformation of oral lichen planus: A systematic review. Oral Dis. 2019;25(3):693-709. https://doi.org/10.1111/odi.12885
2. Tsushima F, Sakurai J, Uesugi A, Oikawa Y, Ohsako T, Mochizuki Y, Hirai H, Kayamori K, Harada H. Malignant transformation of oral lichen planus: a retrospective study of 565 Japanese patients. BMC Oral Health. 2021;21:298. https://doi.org/10.1186/s12903-021-01652-7
3. Lodi G, Manfredi M, Mercadante V, Murphy R, Carrozzo M. Interventions for treating oral lichen planus: corticosteroid therapies. Cochrane Database of Sys Rev. 2020;2:CD001168. https://doi.org/10.1002/14651858.CD001168.pub3
4. Alqahtqani SS, Alabeedi FM. Association of oral candidiasis with oral lichen planus in patients using corticosteroid therapy-meta-analysis. J Popul Ther Clin Pharmacol. 2023;30(1):e1-13. https://doi.org/10.47750/jptcp.2023.1020.
5. Hooman E, Keyvan P, Sara P, Zomorodian K, Saki M, Saki N, Valizadeh M, Kardeh S. Prevalence of common Candida species in oral lichen planus patients: a cross-sectional study in south of Iran. Galen Med J. 2014;3:252-5. https://doi.org/10.31661/gmj.v3i4.181
6. Laxmi GD, Khan M, Johar K, Vijaylakshmi KR, Nikita G. A comparative study on use of diode laser and topical triamcinolone acetonide 0.1% in the management of oral lichen planus. Int J Dent Med Res. 2015;1(5):12-9.
7. Menon P, Sudheer R. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol. 2007;595:105-25. https://doi.org/10.1007/978-0-387-46401-5_3
8. Dai W, Wang H, Fang J, Zhu Y, Zhou J, Wang X, Zhou Y, Zhou M. Curcumin provides neuroprotection in model of traumatic brain injury *via* the Nrf2-ARE signaling pathway. Brain Res Bull. 2018;140:65-71. https://doi.org/10.1016/j.brainresbull.2018.03.020
9. Ungurianu A, Zanfirescu A, Margină D. Regulation of gene expression through food-curcumin as a sirtuin activity modulator. Plants. 2022;11(13):1741. https://doi.org/10.3390/plants11131741
10. Panchatcharam M, Miriyala S, Gayathri VS, Lonchin Suguna. Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol Cell Biochem. 2006;290:87-96. https://doi.org/10.1007/

s11010-006-9170-2

11. Luer S, Troller R, Jetter M. Topical curcumin can inhibit deleterious effects of upper respiratory tract bacteria on human oropharyngeal cells *in vitro*: potential role for patients with cancer therapy induced mucositis. Support Care Cancer. 2011;19:799-806. https://doi.org/10.1007/s00520-010-0894-x
12. Khan N, Shreaz S, Bhatia R, Ahmad SI, Muralidhar S, Manzoor N, Ahmad Khan L. Anticandidal activity of curcumin and methyl cinnamaldehyde. Fitoterapia. 2013;83:434-40. https://doi.org/10.1016/j.fitote.2011.12.003
13. Kia SJ, Shirazian S, Mansourian A, Fard LK, Sajjad Ashnagar S. Comparative efficacy of topical curcumin and triamcinolone for oral lichen planus: a randomized, controlled clinical trial. J Dent (Tehran). 2015;12(11):789-96.
14. Thomas AE, Varma B, Kurup S, Jose R, Chandy ML, Kumar SP, Aravind MS, Aruna Ramadas A. Evaluation of efficacy of 1% curcuminoids as local application in management of oral lichen planus-interventional study. J Clin Diagn Res. 2017;11(4):ZC89-93. https://doi.org/10.7860/JCDR/2017/20898.9715
15. Mahattanadul S, Mustafa MW, Kuadkaew S, Patthrarachayakul S, Ungphaiboon S, Sawanyawisuth K. Oral ulcer healing and anti-Candida efficacy of an alcohol-free chitosan-curcumin mouthwash. Eur Rev Med Pharmacol Sci. 2018;22:7020-3. https://doi.org/10.26355/eurrev_201810_16173
16. Mustafa MW, Ungphaiboon S, Phadoongsombut N, Pangsomboon K, Chelae S, Mahattanadul S. Effectiveness of an alcohol-free chitosan-curcuminoid mouthwash compared with chlorhexidine mouthwash in denture stomatitis treatment: a randomized trial. J Altern Complement Med. 2019;25(5):552-8. https://doi.org/10.1089/acm.2018.0459
17. Kelsey JL, Whittemore AS, Evans AS, Thompson WD. Methods in observational epidemiology. 2nd ed. New York: Oxford University Press; 1996.
18. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH. Manual of clinical microbiology. 7th ed. Washington DC: American society for microbiology; 1999.
19. Kofie W, Dzidzoramengor C, Adosraku R. Synthesis and evaluation of antimicrobial properties of azo dyes. Int J Pharm Pharm Sci. 2015;7(4):398-401.
20. Leulescu M, Rotaru A, Pălărie I, Moantă A¸ Cioateră N, Popescu M, Morîntale E, Bubulică MV, Florian G, Hărăbor A, Rotaru P. Tartrazine: physical, thermal and biophysical properties of the most widely employed synthetic yellow food-colouring azo dye. J Therm Anal Calorim. 2018;134:209-31. https://doi.org/10.3390/molecules25235757
21. Verma N, Gupta S, Das M, Triphathi A. Integrated approaches to find the therapeutic molecular targets of sunset yellow implicated as anti-inflammatory agent. 34th Annual Conference of Society of Toxicology (India) STOX-2014:92-3.
22. Leonard BJ. Toxicological aspects of food safety. Proceedings of the European Society of Toxicology Meeting held in Copenhagen. 1st ed. California: Springer Science & Business Media; 2013.
23. Li C, Tang X, Zheng X, Ge S, Wen H, Lin X, Chen Z, Lu L. Global prevalence and incidence estimates of oral lichen planus: A systematic review and meta-analysis. JAMA Dermatol. 2020;156(2):172-81. https://doi.org/10.1001/jamadermatol.2019.3797
24. Chainani-Wu N, Madden E, Lozada-Nur F, Silverman S Jr. High-dose curcuminoids are efficacious in the reduction in symptoms and signs of oral lichen planus. J Am Acad Dermatol. 2012;66:752-60. https://doi.org/10.1016/j.jaad.2011.04.022
25. Amirchaghmaghi M, Pakfetrat A, Delavarian Z, Ghalavani H, Ghazi A. Evaluation of the efficacy of curcumin in the treatment of oral lichen planus: a randomized controlled trial. J Clin Diagnostic Res. 2016;5:134-7. https://doi.org/10.7860/JCDR/2016/16338.7870
26. Gonzalez-Garcia A, Diniz-Freitas M, Gandara-Vila P, Blanco-Carriôn A, García-García A, Gândara-Rey JM. Triamcinolone acetonide mouth rinses for treatment of erosive oral lichen planus: efficacy and risk of fungal over-infection. Oral Dis. 2006;12(6):559-65. https://doi.org/10.1111/j.1601-0825.2006.01238.x
27. Costa EM, Silva S, Pina C, Tavaria FK, Pintado MM. Evaluation and insights into chitosan antimicrobial activity against anaerobic oral pathogens. Anaerobe. 2012;18:305-9. https://doi.org/10.1016/j.anaerobe.2012.04.009
28. Gavhane N, Gurav S, Yadav V. Chitosan and its applications: a review of literature. IJRPBS. 2013;4:312-31.
29. Miguel SP, Moreira AF, Correia IJ. Chitosan based-asymmetric membranes for wound healing: a review. Int J Biol. Macromol. 2019;127:460-75. https://doi.org/10.1016/j.ijbiomac.2019.01.072