Clinical efficacy of a 1% *Matricaria chamomile* L. mouthwash and 0.12% chlorhexidine for gingivitis control in patients undergoing orthodontic treatment with fixed appliances

Paula Goes1,2), Caio S. Dutra3), Mário R. P. Lisboa2), Delane V. Gondim2,4), Renata Leitão2,4), Gerly A. C. Brito2,4), and Rodrigo O. Rego5)

1) Department of Pathology and Legal Medicine, Federal University of Ceará, Fortaleza, CE, Brazil
2) Morphofunctional Science Post-Graduation Program, Federal University of Ceará, Fortaleza, CE, Brazil
3) Private Practice, Fortaleza, CE, Brazil
4) Department of Morphology, Federal University of Ceará, Fortaleza, CE, Brazil
5) Dental School, Federal University of Ceará, Sobral, CE, Brazil

(Received April 6, 2016; Accepted June 16, 2016)

Abstract: This pilot study evaluated the clinical efficacy of a mouthwash containing 1% *Matricaria chamomilla* L. (MTC) extract in reducing gingival inflammation and plaque formation in patients undergoing orthodontic treatment with fixed appliances. This randomized, double-blind, placebo-controlled study enrolled a total of 30 males and females (age, 10-40 years) with fixed orthodontic appliances and a minimum of 20 natural teeth. The participants were allocated to three groups (n = 10 each) and asked to rinse with 15 mL of a placebo, 0.12% chlorhexidine (CHX), or 1% MTC mouthwash, immediately after brushing for 1 min, in the morning and evening, for 15 days. Data (mean ± SD) on visible plaque index (VPI) and gingival bleeding index (GBI) were recorded on days 1 and 15. The placebo group exhibited increases in VPI and GBI (10.2% and 23.1%, respectively) from day 1 to day 15. As compared with placebo, VPI and GBI significantly decreased in the MTC group (~25.6% and ~29.9%, respectively) and the CHX group (~39.9% and ~32.0%, respectively).

In summary, MTC reduced biofilm accumulation and gingival bleeding in patients with gingivitis, probably because of its antimicrobial and anti-inflammatory activities. (J Oral Sci 58, 569-574, 2016)

Keywords: chamomile; gingivitis; mouthwash.

Introduction

The oral environment is greatly altered by orthodontic treatment with fixed appliances. Marked accumulation of dental biofilm around orthodontic bands and brackets frequently results in poor oral hygiene that leads to gingival inflammation around fixed appliances, which is characterized by edema, redness, and bleeding upon probing (1). Prevention programs are needed in order to address the potential adverse effects of fixed orthodontic appliances on gingival tissue (2).

Chlorhexidine (CHX) in the form of a varnish, mouthwash, or dentifrice gel is the most widely used antiseptic and has yielded beneficial results as a preventive strategy. It also improves symptoms of periodontal disease, an important undesirable outcome of treatment with fixed orthodontic appliances (2).

CHX is effective in treating gingivitis (3). However, despite its effectiveness in reducing levels of microorganisms in the oral cavity, long-term use of CHX products is associated with local side effects such as impaired sense of taste, tooth staining, increased formation of...
supragingival calculus, and occasional irritation and desquamation of mucous membranes (3). To overcome these adverse effects, the World Health Organization (WHO) recommended that researchers investigate the therapeutic benefits of natural products, herbs, and plant extracts for gingival and gum tissues.

Matricaria chamomilla L. (MTC), also known as chamomile (family Asteraceae), is widely used, well-known, and one of the oldest medicinal plants in the world (4). The main components of the plant include several phenolic compounds (primarily terpenoids, chamazulene, and sesquiterpenes). MTC also contains the flavonoids apigenin, quercetin, patuletin, and luteolin (5). The anti-inflammatory and antioxidant effects of apigenin and quercetin have been extensively described (6,7).

Previous studies evaluated the neuroprotective (8), anti-allergic (9), antioxidant (10), anti-inflammatory, antiseptic, and spasmyloytic properties of MTC (11). An MTC mouthwash was effective in treating recurrent aphthous stomatitis (12) and oral mucositis (13). In addition, an MTC mouthwash reduced plaque accumulation and gingival inflammation, without significant effects on tooth staining, when used twice a day for 4 weeks (14). Therefore, this study evaluated the effects of a mouthwash containing MTC extract for patients with gingivitis associated with fixed orthodontic appliances.

**Materials and Methods**

**Study design**

The present study was designed as a randomized, double-blind, placebo-controlled pilot study and adhered to the Consolidated Standards of Reporting Trials (CONSORT) checklist. The study design was approved by the Ethics Committee for Human Research at the Federal University of Ceará and was conducted in accordance with both resolution 196/96 of the Brazilian Ministry of Health, under protocol number 53/10, and the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.

**Population and sample**

Thirty patients (4 males, 26 females; mean age, 28.8 ± 3.28 years; age range, 10-40 years) from among those referred for treatment to the Periodontology Clinic of Federal University of Ceará were recruited after a screening examination that included a full medical and dental history and intraoral examination. The subjects were enrolled from January through December 2011. The inclusion criteria were age older than 10 years, presence of at least 20 natural teeth, good general health, a mean plaque index greater than 1.5, presence of established gingivitis concurrent with fixed orthodontic appliances and associated with visible plaque, no evidence of destructive periodontal disease, and a minimum of eight sites with bleeding on probing. Exclusion criteria were presence of any systemic disease, use of drugs that could affect the periodontium, antibiotic therapy during the previous 3 months, pregnancy, breast-feeding, smoking, continuous use of mouthwashes containing chemical agents during the previous 3 months, and history of allergies to toothpaste, mouthwash, or herbal medicine. Only patients who fulfilled the inclusion criteria were invited to participate. All participants were given verbal and written information concerning the study and signed a written consent form after reviewing all information received.

**Experimental groups**

All mouthwashes were prepared by a specialized magistral pharmacy. A standard mouthwash formulation, consisting basically of quinine, was used in this study. The aqueous MTC extract (batch number PROD004257) was obtained from the flower by Mapric Cosmetic and Pharmaceutical Products, Sao Paulo, Brazil (chemical abstract service [CAS] number 64-17-5; 7732-18-5; 99-76-3). Blinding and patient allocation were controlled by one of the authors, who distributed the mouthwashes in three identical plastic bottles, labeled A, B, or C. All other investigators and the participants were unaware of the contents of each bottle. The participants were randomly allocated to one of three groups and received one bottle of the assigned mouthwash: i.e., either placebo, 0.12% CHX, or 1% MTC. All participants also received a dental hygiene set comprising a toothbrush, dental floss, and a commercial dentifrice without anti-gingivitis properties (Sorriso, Kolynos do Brazil Ltd., Osasco, SP, Brazil) and were instructed in the use of these items. They were asked to rinse with 15 mL of their assigned mouthwash, 30 min after brushing, for 1 min in the morning and evening, for 15 days. To reinforce these verbal instructions, the participants received written recommendations to follow at home.

**Clinical examinations**

Patients were assessed by interview and a periodontal examination that evaluated visible plaque index (VPI) (15) and gingival bleeding index (GBI) (15) for the canines and lateral and central incisors (a total of 12 teeth) on days 1 and 15. These teeth were selected because they offer easy access and visualization in patients with orthodontic appliances. One examiner conducted all the
examinations. VPI and GBI were evaluated at four sites per tooth (mesial, buccal, distal, and lingual) (15). The gums were examined for presence of bleeding, which was recorded 30 seconds after running a WHO probe on the gingival margin. The values for four sites per tooth were recorded to obtain the means for VPI and GBI (15). Changes in VPI and GBI for each volunteer were calculated as the difference in the respective index on day 15 and day 1.

Pre-study intra-examiner reliability was assessed by performing measurements of 10 patients. The Kappa coefficient was used to assess agreement between examinations. The mean Kappa coefficient was 0.8 for VPI and 0.83 for GBI.

**Statistical analysis**
The data are expressed as mean ± SD and were analyzed by analysis of variance (ANOVA), to identify any significant differences ($P < 0.05$), followed by the $t$-test with $\alpha$ adjusted by the number of time points (Bonferroni correction).

**Results**
This randomized controlled clinical pilot trial enrolled 30 participants with gingivitis associated with fixed orthodontic appliances (Fig. 1): 26 females and four males aged 10–40 years. Each group had 10 volunteers. Mean age was $21.6 \pm 6.9$ years in the placebo group, $18.6 \pm 3.0$ years in the CHX group, and $21.5 \pm 6.0$ years in the MTC group. The groups did significantly differ in relation to
age.

Five patients in the CHX group reported burning or change in taste after 14 days. One patient in the placebo group reported tongue numbness on the first day of use. MTC mouthwash was well tolerated and was not associated with any adverse effects.

Analysis of change in VPI from day 1 to day 15 (Table 1) showed dental biofilm accumulation in the placebo group. Patients receiving CHX or MTC mouthwash exhibited a reduction in dental biofilm ($P = 0.0001$), as compared with placebo. The CHX and MTC groups did not differ with respect to VPI index ($P > 0.05$)

Table 1 shows change in GBI from days 1 to 15. GBI increased in the placebo group, which indicates increased gingival bleeding. As compared with the placebo group, GBI decreased in participants receiving the CHX and MTC mouthwashes ($P = 0.003$). GBI did not differ between participants receiving CHX and MTC mouthwashes ($P > 0.05$)

**Discussion**

VPI and GBI significantly decreased, as compared with placebo, in participants receiving a 1% MTC mouthwash but did not differ between participants receiving the 1% MTC mouthwash and 0.12% CHX mouthwash, the gold-standard therapy. Treatment with 1% MTC did not alter values for probing pocket depth or gingival recession during the study period.

CHX is one of the most widely used and thoroughly investigated antiseptics and is present in a large number of products used to control plaque and gingivitis. CHX digluconate is safe, stable, and effective in preventing and controlling biofilm accumulation, breaking up existing plaque, and inhibiting and reducing gingivitis development (16). The binding properties of the CHX molecule result in a broad bactericidal and bacteriostatic spectrum and high substantivity of up to 12 h within the oral cavity (17). Because CHX strongly binds to tissues, it is poorly absorbed in the gastrointestinal tract reducing its systemic toxicity (18). However, some side effects have been reported during CHX treatment. The most common side effect of long-term CHX rinse use is an extrinsic brown staining of the teeth and tongue (3). Other common side effects are increased calculus formation and change in

**Table 1** Changes in Visible Plaque Index and Gingival Bleeding Index

|                        | Placebo          | 0.12% CHX        | 1% MTC          |
|------------------------|------------------|------------------|-----------------|
| Change in Visible Plaque Index (%) | 10.16 ± 14.60 | −39.93 ± 31.19*   | −25.59 ± 17.73* |
| Change in Gingival Bleeding Index (%) | 23.15 ± 35.67 | −32.05 ± 29.35*   | −29.90 ± 44.64* |

CHX = chlorhexidine; MTC = *Matricaria chamomilla* L. Data are expressed as mean ± SD. (*) indicates significant difference vs. placebo (ANOVA and Bonferroni test; $P < 0.05$).

We found that a 1% MTC mouthwash significantly reduced VPI in patients with gingivitis associated with fixed orthodontic appliances. Previous studies also reported that an MTC dentifrice (20) and mouthwash (14) were clinically safe and reduced the plaque index and GBI after 21 and 15 days of use, respectively, which suggests that MTC has antimicrobial effects. *In vitro* studies showed that *M. chamomilla* was effective against 25 different gram-positive and gram-negative bacteria and 20 strains of *Listeria monocytogenes* (5). A study of oral pathogens found on oral biofilm reported that an MTC extract was effective against three strains of *Staphylococcus aureus* and *Candida* (21) and that it inhibited growth of *Streptococcus mutans* and *Streptococcus sanguinis*, which are important initial colonizers (22). Existing evidence suggests that the antimicrobial effects of MTC are attributable to its terpenic derivatives chamazulene, β-bisabolol, and A and B bisabolol-oxides (23).

The present study showed that a mouthwash containing 1% MTC significantly reduced GBI after 15 days of treatment, as compared with the increase in GBI seen in the placebo group. The reduction in the 1% MTC group was similar to that in the 0.12% CHX group, and the difference between groups was not significant. Past and present evidence indicates that MTC has immunomodulatory activity (24). The flavonoid apigenin, a constituent of MTC extract, has important anti-inflammatory and antioxidant activities. It inhibits nitric oxide (NO) production and the activities of hyaluronidase, collagenase, and the cyclooxygenases, enzymes that play key roles in the inflammatory process (6). The anti-inflammatory activity of apigenin was also observed in *in vitro* studies of human periodontal ligament cells stimulated with nicotine and lipopolysaccharide. Apigenin significantly inhibited the release of NO, prostaglandin E$_2$, interleukin-1β, -6, and
-12, and tumor necrosis factor-alpha (7). Chamazulene, another constituent of MTC, significantly protects against lipid peroxidation (25) and inhibits leukotriene B4 formation (by 50%) (26), cyclooxygenase-2, and paw edema (27). Bisabolol, an essential oil found in MTC extract, was found to reduce edema, leukocyte migration, protein extravasation, tumor necrosis factor-alpha release, and neutrophil degranulation (28).

Although CHX is considered the gold standard for biofilm control and gingivitis treatment, the present findings have clinical relevance, as the patients receiving 0.12% CHX and 1% MTC did not differ in relation to VPI or GBI. As we noted above, an important advantage of MTC over CHX is that patients in the 1% MTC group did not report side effects commonly associated with CHX, including ulcerations, allergic reactions, burning sensation, change in taste, supragingival calculus deposition, and tooth staining (29).

In summary, MTC reduced biofilm accumulation and gingival bleeding in patients with gingivitis, probably because of its antimicrobial and anti-inflammatory activities, and did not cause side effects associated with CHX. Large-scale studies are needed in order to confirm that this herbal product is effective for treatment of human gingivitis.

Acknowledgments

This work was supported by grants from Brazilian agencies (CNPq, Process 471407/2009-7; CAPES and FUNCAP, Process 247.01.00/09).

Conflict of interest

The authors declare no conflicts of interest.

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