

Research

Changes in Salivary Flow and Oral pH Following Use of Different Mouthrinse Formulations in Addition to Brushing Versus Brushing Only

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ABSTRACT

Purpose Mouthrinses contain inactive ingredients (e.g., solvents, such as alcohol) and active ingredients (e.g., antimicrobials, such as essential oils [EOs]) that work in combination to control gingival inflammation and regulate the oral microbiome. The purpose of this one-day, examiner-blind, randomized, parallel-group-controlled clinical trial was to understand the effect of different EO-containing mouthrinses, with and without alcohol, on salivary flow and pH.

Methods Subjects aged ≥ 18 years were recruited to participate in a one-day trial conducted at an independent research center. Salivary flow and pH were measured following a regimen of brushing and rinsing with an EO-containing mouthrinse versus brushing and rinsing with a water rinse control (BW). Eligible participants were assigned 1:1:1:1 to the BW group or one of three EO-containing mouthrinse groups. Change in salivary flow and pH after a single use of an EO-containing mouthrinse compared to the BW group was assessed at 0 (salivary flow only), then at 2.5, 5, 10, 15, and 30 minutes.

Results A total of 159 subjects completed the clinical trial. At 30 minutes following the intervention, the mean salivary flow was similar across all groups. The mean salivary pH increased in all groups through 15 minutes before returning to near-baseline levels by 30 minutes (all of which were within the normal oral pH range of 6.2–7.6). No adverse events were reported.

Conclusion The inclusion of alcohol in the tested mouthrinse formulations did not affect salivary flow or pH compared with the alcohol-free mouthrinses, indicating that a single use of either an alcohol-containing or alcohol-free EO-containing mouthrinse does not contribute to oral dryness. Future trials investigating the long-term use of mouthrinses and their effect on salivary flow and pH will help to build on the current evidence base and inform clinical decision-making.

Keywords oral health prevention, periodontology, chemotherapeutics, mouthrinses, clinical research
NDHRA priority area: **Client level: Oral health care** (prevention modalities).

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INTRODUCTION

Alcohol is commonly found in mouthrinses in concentrations of zero to up to 26% alcohol by volume (ABV), as an inactive ingredient with solvent properties that help solubilize active ingredients.¹ Alcohol-free mouthrinses often contain sodium lauryl sulfate, a surfactant that increases the solubility of other ingredients, including flavorings.^{2,3} Essential oils (EOs), including thymol, eucalyptol, methyl salicylate, and menthol, are active ingredients found in certain mouthrinses which damage plaque- and gingivitis-associated bacterial cell membranes, ultimately resulting in cell death.⁴ The anti-plaque and anti-gingivitis properties of EO-containing mouthrinses have been confirmed in multiple clinical trials and systematic reviews.⁵⁻⁸

Professional dental organizations including the American Dental Association (ADA) and the Canadian Dental Hygienists Association have position statements supporting the use of a mouthrinse where it may help control plaque and/or gingivitis when included as an adjunct to brushing and interdental cleaning.^{9,10}

It has been suggested that antiseptic mouthrinses contribute to reduced salivary flow in individuals at risk of xerostomia;¹¹ however, this may stem from studies that evaluated the overall impact of generic alcohol consumption on saliva and salivary flow. One study found, for example, that long-term alcohol intake among individuals with alcohol addiction decreased salivary secretions.¹² Alcohol-based EO-containing mouthrinses have, conversely, been found to increase salivary pH immediately after rinsing, an effect linked to increased salivary flow.¹³ Saliva is essential for the maintenance of oral health by moistening and protecting oral tissues as well as buffering the oral cavity.¹⁴ A decrease in salivary flow (also known as salivary hypofunction) can negatively impact clearance of food from the mouth and increase the risk of developing xerostomia.¹⁴ Therefore, it is important that further research is conducted to evaluate the impact on salivary flow of EO-containing mouthrinses, both with and without

alcohol; and particularly alcohol-free formulations which are currently less well studied.

A previous randomized controlled trial (clinical trial registration identification number NCT05645705) evaluated the oral tissue tolerance of a 2-week regimen of brushing and rinsing with an assigned EO-containing mouthrinse (alcohol-containing or alcohol-free formulae) compared with brushing and rinsing with water (BW). The trial assessed salivary flow and pH after initial use of assigned regimen on Day 0 and found increased salivary flow in the EO-containing mouthrinse groups compared with BW, and with no effect on salivary pH, 30 minutes after use in either group¹⁵ (manuscript under review).

The purpose of this Phase 4, one-day, randomized, controlled trial was to investigate the effect on salivary flow and pH of different EO-containing mouthwash formulations. The objectives were to evaluate changes in short-term salivary flow and oral pH after a single use of a regimen of brushing and rinsing with an EO-containing mouthrinse (alcohol-containing or alcohol free) versus the control BW. The EO-containing mouthrinses included in this trial differed from those assessed in a previous trial to identify whether different EO-containing mouthrinse formulations follow a similar trend for salivary flow and pH changes.

METHODS

Trial design

This one-day, single-center, examiner-blind, randomized, parallel-group-controlled clinical trial consisted of one site visit conducted between November 13 to 16, 2023, at Salus Research Center, Fort Wayne, IN, USA. The trial adhered to the principles of the International Council for Harmonization Guidelines for Good Clinical Practice (GCP E6 [R2]), and the protocol was approved by the Institutional Ethics Committee on research involving humans (Veritas Institutional Review Board, Montreal, Quebec, CAN) and was retrospectively registered on ClinicalTrials.gov (NCT06136455). Written informed consent was obtained from all participants.

Participants

Eligible participants were aged ≥ 18 years, in good general and oral health, and had a resting unstimulated salivary sample ≥ 0.3 mL/min (assessed at baseline). All inclusion and exclusion criteria are shown in the supplementary Table S1.

Baseline assessments

Participants were required not to use oral hygiene products for at least 4 (but no more than 12) hours before their scheduled baseline visit and were not to smoke, eat, or drink (including water) within 1 hour of the baseline measurements. Unstimulated saliva samples were collected from each participant following 5 minutes at rest; participants allowed their saliva to pool in their mouth, emptying it into a collection vial as needed. The final amount of saliva was weighed and flow rate determined for study inclusion. After assessing the volume and flow rate of saliva, 0.5 mL of the sample was placed onto a pH-sensitive electrode to immediately measure the baseline pH (within 30 seconds) after which baseline saliva pH measurements were taken from the same participant sample.

Randomization and blinding

Participants eligible to continue in the trial were assigned a randomization number based on the order of trial entry. This number determined the treatment assignment for the participant according to a block randomization scheme (SAS[®] 9.4; SAS Institute, Cary, NC, USA). Examiners were blinded to the treatment regimens of the participant groups and did not have access to the area where the product was dispensed or where it was used under supervision.

Interventions

Eligible participants (N=159) were randomized to one of four intervention groups as shown in Figure 1: brush with a manual toothbrush for 1 minute then rinse with 10 mL of LISTERINE[®] Clinical Solutions Teeth Strength mouthrinse (JE) (Kenvue Brands LLC, Summit, NJ, USA) for 1 minute (n=39); brush for 1 minute then rinse with 10 mL of LISTERINE[®] TOTAL CARE ZERO mouthrinse (LTCZ) (Kenvue Brands LLC, Summit, NJ,

USA) for 1 minute (n=40); brush for 1 minute then rinse with 20 mL of LISTERINE[®] COOL MINT[®] ZERO mouthrinse (LCMZ) for 30 seconds (n=40); or brush for 1 minute then rinse with 10 mL of tap water (BW) for 1 minute (n=40). All mouthrinses contained a fixed combination of EOs (eucalyptol, menthol, methyl salicylate, and thymol) and were used as directed on label. The JE and LTCZ mouthrinses contained fluoride, and both had a pH of 3.5; LCMZ did not contain fluoride and had a pH of 4.2. JE contained alcohol; LTCZ and LCMZ did not. Participants used a dentifrice containing sodium monofluorophosphate (Colgate[®] Cavity Protection Toothpaste; Colgate-Palmolive, NY, NY, USA) and a toothbrush (Colgate[®] full head/soft bristles toothbrush); paste slurry was expectorated thoroughly after 1 minute and rinsing commenced immediately after. All trial products and materials were provided by the trial sponsor. Product use was conducted at the research center under the supervision of study personnel.

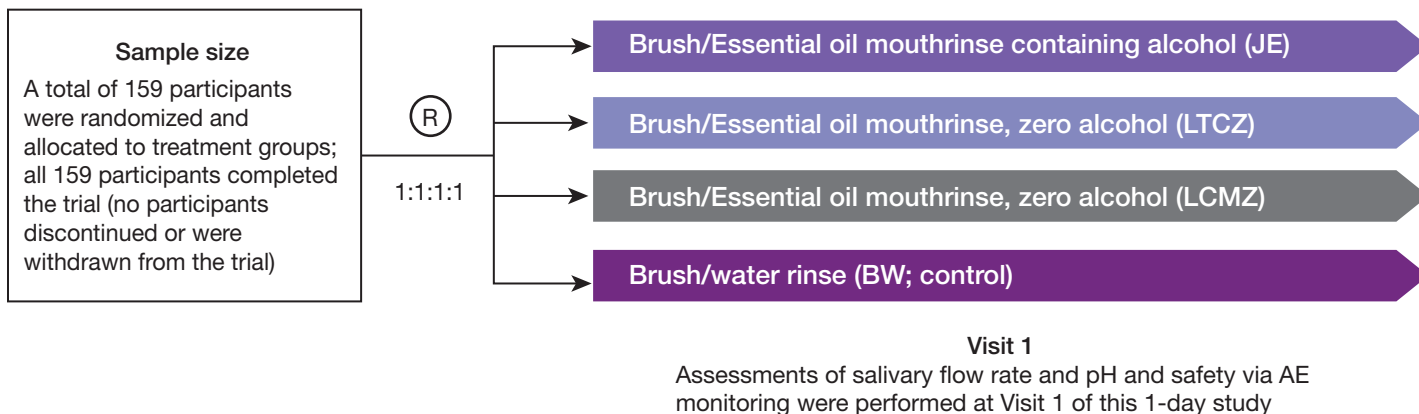
Assessments and outcomes

Primary endpoints were change in salivary flow and pH after a single use of brush plus mouthrinse intervention compared with BW at 0 minutes (for salivary flow only), and then at 2.5, 5, 10, 15, and 30 minutes. Participants assigned to LCMZ (20 mL for 30 seconds) waited 30 seconds before brushing so that all assessments were completed at the same timepoint, to maintain blinding. Timed 2-minute saliva samples were collected into separate collection vials at each assessed timepoint. The pH was measured by placing 0.5 mL of saliva from each collection timepoint (excluding timepoint 0) onto a pH-sensitive electrode. The safety profile was assessed through hard and soft tissue oral examination and recording of adverse events (AEs).

Statistical analyses

A sample size of 160 participants (40 per treatment group) was planned to provide approximately 99% power to detect a standardized effect size (defined as the difference between treatment means divided by standard deviation) of 1 for both salivary flow and

Figure 1. Trial design with intervention arms



Primary Endpoints

Salivary flow rate at baseline (for trial eligibility) and then 0 (immediately), 2.5, 5, 10, 15, and 30 minutes after a single intervention

Salivary pH at 2.5, 5, 10, 15, and 30 minutes after a single intervention

Participants brushed their teeth for 1 minute using the toothpaste and toothbrush provided. AE, adverse event; BW, brush/water rinse; JE, LISTERINE® Clinical Solutions Teeth Strength mouthrinse; LCMZ, LISTERINE® COOL MINT® Zero mouthrinse; LTCZ, LISTERINE® Total Care Zero mouthrinse; R, randomization.

pH endpoints between the EO-containing mouthrinse groups and BW group. To allow for a dropout rate of approximately 2%, 41 participants per treatment group were planned to be recruited. Demographics and baseline characteristics were compared across treatment groups using an analysis of variance, a chi-square test, or Fisher’s exact test.

Pairwise comparisons between the investigational products were based on a mixed-effects model for repeated measures analysis and included terms for investigational product and time point, and the baseline value as a covariate. Intervention-by-timepoint and baseline-by-timepoint terms were also included to compare interventions at specific timepoints. The within-participant correlation was assumed unstructured. The JE, LTCZ, and LCMZ groups were compared with the BW group.

RESULTS

Baseline characteristics

A total of 159 participants were enrolled and randomized at baseline; all participants completed the trial. Participant disposition is shown in Figure 2.

Participant demographics and baseline characteristics were balanced at baseline (Table I). The mean age of participants was 50.1 years, and the majority were female (69.8%), White (88.7%), and non-smokers (96.2%).

Salivary flow after single use

Mean salivary flow in the mouthrinse and BW groups from 0 to 30 minutes is shown in Figure 3. Mean salivary flow was significantly higher at 0 minutes (JE and LCMZ), 2.5 minutes (JE, LTCZ, and LCMZ), 5 minutes (LTCZ and LCMZ), 10 minutes (LCMZ), and 15 minutes (JE) compared with BW (all $p < 0.05$). By 30 minutes, mean salivary flow in any mouthrinse group was similar (1.4 mL/min in the JE and LCMZ groups and 1.5 mL/min in the LTCZ group) and not significantly different versus the BW group (1.3 mL/min). The mean salivary flow was not numerically lower than the BW group or baseline values at any timepoint.

Salivary pH after single use

At baseline, all mean salivary pH measurements were within the normal range (pH 6.2–7.0). At all timepoints through 30 minutes, mean salivary pH was higher than or the same as levels seen in the BW group across all EO-containing mouthrinse groups (Figure

4). Mean salivary pH was significantly higher in the LTCZ group at 5 and 10 minutes and also in the JE and LCMZ groups at all time points, except for 30 minutes, versus the BW group ($p < 0.05$). Across all treatment groups, mean salivary pH was increased at 2.5, 5, 10, and 15 minutes compared with baseline. By 30 minutes, mean salivary pH was similar to baseline levels for all groups (6.7–6.8).

Figure 2. Participant randomization and disposition (N=159)

		Study groups				
		BW control	Brush/JE	Brush/LTCZ	Brush/LCMZ	Total
		n (%)	n (%)	n (%)	n (%)	n (%)
Randomized		40	39	40	40	159
Completed		40 (100.0)	39 (100.0)	40 (100.0)	40 (100.0)	159 (100.0)
Discontinued		0	0	0	0	0
Safety Analysis Set		40 (100.0)	39 (100.0)	40 (100.0)	40 (100.0)	159 (100.0)
Full Analysis Set		40 (100.0)	39 (100.0)	40 (100.0)	40 (100.0)	159 (100.0)

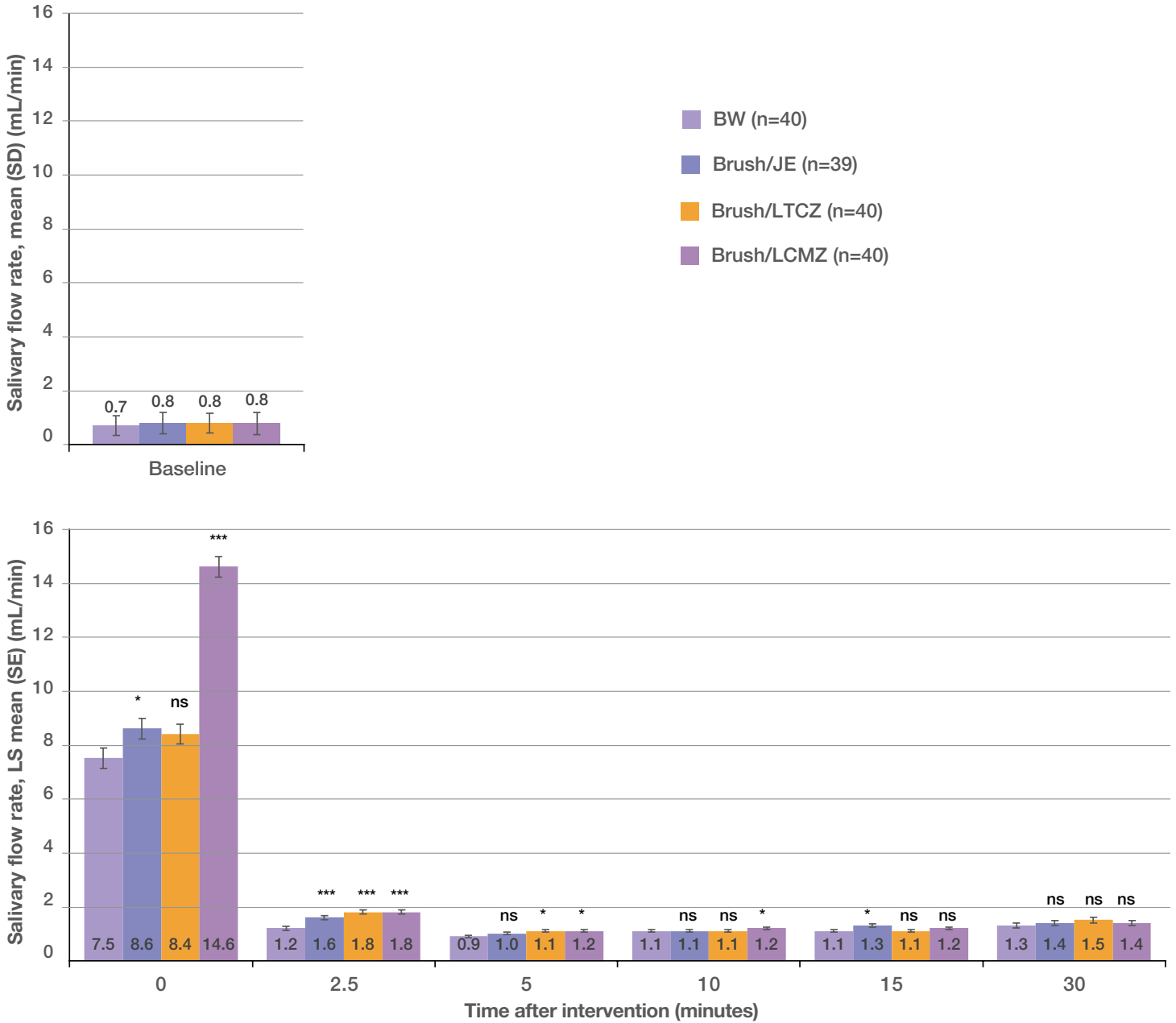
BW, brush/water rinse; JE, LISTERINE® Clinical Solutions Teeth Strength mouthrinse; LCMZ, LISTERINE® COOL MINT® Zero mouthrinse, LTCZ, LISTERINE® Total Care Zero mouthrinse.

Table I. Demographics and Baseline Characteristics (N=159)

Demographics and Baseline Characteristics	BW (n=40)	JE (n=39)	LTCZ (n=40)	LCMZ (n=40)
Mean age, years (SD)	51.5 (16.6)	47.3 (14.2)	51.0 (13.8)	50.6 (12.8)
Sex, n (%)				
Female	28 (70.0)	28 (71.8)	27 (67.5)	28 (70.0)
Male	12 (30.0)	11 (28.2)	13 (32.5)	12 (30.0)
Race, n (%)				
White	38 (95.0)	35 (89.7)	33 (82.5)	35 (87.5)
Black or African American	1 (2.5)	3 (7.7)	7 (17.5)	4 (10.0)
Asian	—	—	—	1 (2.5)
Native Hawaiian or other Pacific Islander	—	—	—	—
American Indian or Alaska Native	1 (2.5)	—	—	—
Other	—	1 (2.6)	—	—
Ethnicity, n (%)				
Not Hispanic or Latino	39 (97.5)	38 (97.4)	40 (100.0)	40 (100.0)
Smoker, n (%)				
Yes	—	2 (5.1)	3 (7.5)	1 (2.5)
No	40 (100)	37 (94.9)	37 (92.5)	39 (97.5)

BW, brush/water rinse (control); JE, brush/LISTERINE® Clinical Solutions Teeth Strength mouthrinse; LCMZ, brush/LISTERINE® COOL MINT® ZERO mouthrinse; LTCZ, brush/LISTERINE® TOTAL CARE ZERO mouthrinse; SD, standard deviation.

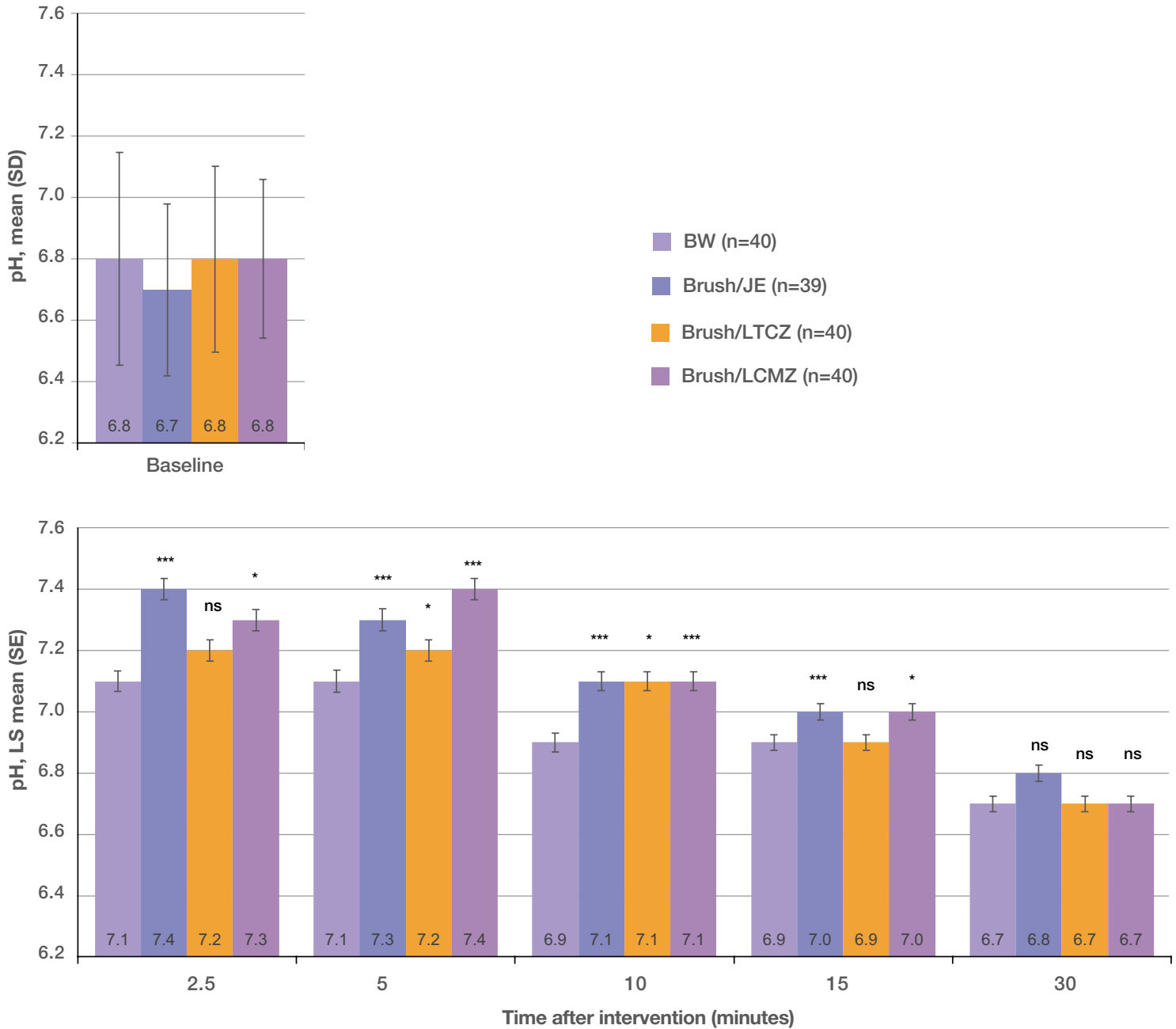
Figure 3. Salivary flow at baseline, 0, 2.5, 5, 10, 15, and 30 minutes following intervention



p-values were versus the BW control: * $p < 0.05$; *** $p < 0.001$.

Participants in the JE, LTCZ and BW groups rinsed with 10 mL for 1 minute; those in the LCMZ group rinsed with 20 mL for 30 seconds. BW, brush/water rinse (control); JE, brush/LISTERINE® Clinical Solutions Teeth Strength mouthrinse; LCMZ, brush/LISTERINE® COOL MINT® Zero mouthrinse; LS, least squares; LTCZ, brush/LISTERINE® Total Care Zero mouthrinse; ns, not significant; SD, standard deviation; SE, standard error.

Figure 4. Salivary pH at baseline, 2.5, 5, 10, 15, and 30 minutes following intervention



p-values were versus the BW control: **p*<0.05; ****p*<0.001.

Participants in the JE, LTCZ and BW groups rinsed with 10 mL for 1 minute; those in the LCMZ group rinsed with 20 mL for 30 seconds. BW, brush/water rinse (control); JE, brush/LISTERINE® Clinical Solutions Teeth Strength mouthrinse; LCMZ, brush/LISTERINE® COOL MINT® Zero mouthrinse; LS, least squares; LTCZ, brush/LISTERINE® Total Care Zero mouthrinse; ns, not significant; SD, standard deviation; SE, standard error.

Safety

There were no treatment-emergent AEs and investigational product-related AEs observed or reported.

DISCUSSION

The aim of this randomized controlled clinical trial was to evaluate the effect of EO-containing mouthrinses, with and without alcohol, compared with a BW group on salivary flow and pH after a single use. Changes in the EO-containing mouthrinse groups followed a similar trend to that seen in the BW group, with increased salivary flow and pH from 0 to 15 minutes following the intervention but returning to similar levels to baseline at 30 minutes. Following a single intervention with the investigational products, salivary flow was significantly increased for up to 15 minutes compared with the BW group, but by 30 minutes salivary flow had returned to similar levels to baseline and the BW group. Throughout the 30-minute observation period, mean salivary flow remained numerically higher than the respective baseline measurements across all groups. The difference in salivary flow at 0 minutes between the LCMZ group (14.6 mL/min) and the other trial arms (BW, 7.5 mL/min; JE, 8.6 mL/min; LTCZ, 8.4 mL/min) can be explained by the increased volume of mouthrinse (20 mL vs. 10 mL) used for LCMZ (Figure 3). It was also observed that the mean salivary flow for the alcohol-containing versus alcohol-free mouthrinse groups were similar through 30 minutes.

In this trial, pH increased at 0 minutes following intervention but returned to levels similar to baseline by 30 minutes. For all mouthrinse groups, mean salivary pH did not drop below the values observed in the BW group or the respective pH levels reported at baseline and was similar between alcohol-containing and alcohol-free mouthrinse formulae through 30 minutes (Figure 4). These findings are in alignment with the results of a previous trial of EO-containing mouthrinses¹⁵ and with other studies that indicate that EO mouthrinses, whether alcohol-containing or alcohol-free, do not cause salivary hypofunction in individuals with good general and oral health.¹⁶⁻¹⁸ Many mouthrinses

have a low pH to improve the chemical stability of the ingredients, but the impact of low pH remains an area in which research is ongoing.¹⁹

The focus of this trial was salivary flow and oral pH after a single use of mouthrinse. The findings of this study suggest that adjunctive use of an EO-containing mouthrinse, either alcohol-containing or zero-alcohol, does not cause salivary hypofunction and therefore is unlikely to impact the health of oral soft tissues, at least in short-term use. Furthermore, there is no evidence that use of alcohol-based or zero-alcohol formulations of EO mouthrinses have an immediate acidifying effect on the saliva, in fact, the converse appears to be the case, and would therefore not be expected to impact tooth enamel. Future studies should assess the impact of long-term mouthrinse use on salivary flow and pH and expand the investigations to look at the impact of mouthrinses on the constituents of saliva. Furthermore, salivary pH was not assessed at 0 minutes as this sample would have been a mixture of mouthrinse and saliva and would not provide an accurate reading. The timepoint at 0 minutes should be included in future studies to investigate the immediate impact of mouthrinse formulations on salivary pH. The difference in mouthrinse volume used for the alcohol-containing mouthrinse versus the alcohol-free formulations (which reflected per-label instructions) in this study may have been a confounding factor. Additional outcomes that could be included in future trials include sensory questionnaires, intraoral pH, absorption rate of active mouthrinse ingredients, and changes in salivary flow and pH in pediatric populations.

CONCLUSION

A single intervention of an EO-containing mouthrinse in addition to toothbrushing in this trial resulted in increased salivary flow from 0 to 30 minutes versus BW and the pH levels were within the normal range across all groups throughout the trial. These findings may support the hypothesis that EO-containing mouthrinses do not appear to cause salivary hypofunction or lower salivary pH, despite the low

pH of the products. Changes in salivary flow for alcohol-containing and alcohol-free mouthrinses were similar versus a water rinse, indicating that the inclusion of alcohol in mouthrinse may not contribute to a reduction in salivary flow. No new safety signals were identified. Based on these findings, a longitudinal study examining the effects of long-term EO-containing mouthrinse use, with and without alcohol, on salivary flow and pH is warranted.

IMPLICATIONS FOR DENTAL HYGIENE PRACTICE

- Single use of EO-containing mouthrinses produces only a brief increase in salivary flow during the first 15 minutes, returning to baseline thereafter, indicating no sustained effect on salivary flow.
- Despite their low product pH, single use of EO-containing mouthrinses does not reduce salivary pH, as values remain comparable to those observed after using a water rinse.
- Alcohol-containing and alcohol-free EO mouthrinses show similar effects on salivary flow and pH, indicating that alcohol content does not impair salivary function and allowing recommendations to be based on patient preference and sensitivity rather than salivary concerns.

DISCLOSURES

This research was funded by Kenvue Brands LLC and was conducted by its research team in partnership with Salus Research, Inc. (Fort Wayne, IN, USA), a third-party independent clinical research site qualified by the American Dental Association. Kimberly Milleman and Jeffery Milleman are Directors at Salus Research, Inc., and declare no conflicts of interest with respect to the research, authorship, or publication of this article. Mary Lynn Bosma and Patricia Gorecki are former employees of Johnson & Johnson Consumer, Inc. and Kenvue Brands LLC, respectively. Yang Ding, Urmila Lanka, and Alicia DelSasso are employees of

Kenvue Brands LLC. The authors declare no conflict of interest or any direct involvement with study execution that would influence its outcome.

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Table SI. Participant Eligibility Criteria

Inclusion criteria

- Able to comprehend and follow the requirements and restrictions of the clinical trial based upon research site personnel's assessment
- Signed, informed consent to participate in the trial
- Males and females aged 18 years and older, in good general and oral health, without known allergies to commercial dental products
- Participants aged ≥ 60 years must show evidence of being fully vaccinated against COVID-19
- Women of childbearing potential must have a negative urine pregnancy test and be using a medically acceptable method of birth control for ≥ 1 month before baseline and during participation in the trial
- Participants must have ≥ 20 teeth with scorable facial and lingual surfaces
- Resting baseline unstimulated salivary sample must be ≥ 0.3 mL/min
- Absence of significant oral soft tissue pathology (excluding plaque-induced gingivitis) or advanced periodontitis
- Absence of a fixed or removable orthodontic appliance or removable partial dentures

Exclusion criteria

- A history of significant adverse events, including sensitivities or suspected allergies, following the use of oral hygiene products such as toothpastes, mouthrinses and red food dye
- Dental prophylaxis within 4 weeks before baseline visit
- Requirement for prophylactic antibiotic coverage prior to invasive dental procedures
- Use of antibiotics, anti-inflammatory or anti-coagulant therapies, phenytoin sodium or diphenylhydantoin, cyclosporin A, immunostimulants or immunomodulators within the past month prior to baseline visit. Intermittent use of ibuprofen, aspirin, and use of oral steroids and calcium channel blockers are acceptable at the discretion of the investigator
- Use of chemotherapeutic anti-plaque or anti-gingivitis oral care products within the past month
- Known allergy or sensitivity to any of the investigational product and/or product ingredients (cinnamyl alcohol, benzyl alcohol, citral, citronellol, linalool, limonene)
- Self-reported pregnancy or lactation (oral tissue changes related to pregnancy/lactation may affect interpretation of clinical trial results)
- Self-reported smokeless tobacco product use
- Males with pregnant partner or a partner currently trying to become pregnant
- Suspected alcohol or substance abuse
- Any other medical or psychiatric conditions that would make the participant unsuitable for the trial based on the discretion of the Principal Investigator
- Significant medical or oral conditions which may interfere with an individual's participation in the clinical trial, including cancer, chronic kidney disease, chronic obstructive pulmonary disease, immunocompromised state from solid organ transplant, serious heart conditions, (such as heart failure, coronary artery disease, or cardiomyopathies), sickle cell disease, type 2 diabetes mellitus at the discretion of the investigator
- Participation in a clinical trial within 30 days of baseline visit
- Diagnosed temporo-mandibular joint dysfunction/disorder
- Wearing of bruxing devices, dental aligners or retainers
- Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the subject inappropriate for entry into this clinical trial