Original Article

MYRRH AND CHLORHEXIDINE MOUTHWASHES COMPARISON FOR PLAQUE, GINGIVITIS AND INFLAMMATION REDUCTION: A 3-ARM RANDOMIZED CONTROLLED TRIAL

Talal Mansoor Zahid[1*], *Norah Sadiq Khan*[2]

[1]*Department of Periodontology, King Abdulaziz University, Jeddah, Saudi Arabia. tzahid@kau.edu.sa*
[2]*King Abdulaziz University Dental Hospital, Jeddah, Saudi Arabia.*

https://doi.org/10.51847/ajwgutvUNV

ABSTRACT

This research was conducted to associate the effectivity of 1% myrrh mouthwash with 0.2% chlorhexidine mouthwash in terms of inhibition of the activity of plaque and gingivitis and decrease of pro-inflammatory cytokines. The clinical trial included 10 males and 9 females (myrrh group, n = 6; chlorhexidine group, n = 7; and saline group, n = 6). Participants initially refrained from daily routine oral health care practices for about two weeks to allow the growth of experimental gingivitis. After 14 days, they were directed to stop brushing and used 15 ml of the given mouthwash twice daily for 1 minute. All clinical parameters were recorded at baseline and post-intervention. The outcome measures were modified gingival index (MGI), plaque index (PI), proinflammatory interleukin (IL)-1β biomarker, and bleeding on probing (BOP). Mixed ANOVA was utilized to perform the data analysis. All treatment groups had similar clinical parameters at baseline (P >.05 for all pairwise comparisons). The post-intervention mean values of the MGI and BOP were considerably lesser in the myrrh group than the saline group (P = .016 and P <.001, respectively). The chlorhexidine group also had lower scores in these two parameters than the saline; however, its mean difference in the MGI did not reach statistical significance (P =.09). No significant difference in the mean PI and average IL-1β scores was found between the treatment groups at any time points. In conclusion, 1% myrrh mouthwash was as good as 0.2% chlorhexidine mouthwash in reducing gingival inflammation and BOP.

Key words: Commiphora myrrha, Myrrh, Mouthwash, Chlorhexidine, Randomized controlled trial.

Introduction

Dental plaque is a polymicrobial community that adheres to the tooth and other hard surfaces in the oral cavity as a sticky biofilm. These biofilms are a causative factor for diverse pathological conditions of the oral cavity such as caries, gingivitis, or periodontitis [1, 2]. Their volume increase in the gingival crevice due to poor oral hygiene practice, which may ultimately lead to chronic inflammation and the progressive destruction of the periodontal tissue supporting the teeth [3]. Hence, dental plaque control has been the most important measure to maintain good oral hygiene and keep periodontal diseases at bay. Both mechanical and chemical measures can help attain effective plaque removal. The mechanical plaque control involves using shear forces to remove the matrix-enclosed microbial biofilms from the tooth surface. This can be achieved through a deep cleaning procedure by a professional (e.g., scaling and root planing) or self-performed such as twice-daily toothbrushing or using interdental cleaning products [4].

The chemical plaque control is usually used as an adjunct for mechanical cleaning to inhibit the growth and accumulation of microbiota. Two of the common vehicles to deliver various chemical (anti-plaque) agents include toothpaste and mouthwashes. A variety of organic and inorganic chemicals are available as anti-plaque agents including phenolic compounds, quaternary ammonium compounds, delmopinol, chlorhexidine gluconate (CHX), essential oils (e.g., methyl salicylate, menthol, and thymol), and herbal extracts [5]. Of these, CHX has been the most tested and potent anti-plaque agent. Its efficacy has been proven to be the gold standard against which other anti-plaque and anti-gingivitis agents are compared [6, 7]. CHX can exert both bacteriostatic and bactericidal properties depending on the applied concentration. However, a major side effect of CHX usage is the reduction in cell migration and survival [7, 8]. In addition, its long-term use is associated with a range of other side effects including teeth discoloration and staining, mucous membrane irritation, and taste disturbance. Hence, most dentists now recommend its use only under professional supervision [7-10].

Herbal mouthwashes have long been thought to be a suitable alternative to CHX for dental plaque and gingivitis reduction owing to their low side effect profile. Clinical trials conducted to determine the effectiveness of these mouthwashes have also demonstrated their efficacy as an adjunctive treatment [11]. In traditional medicine, myrrh (*Commiphora myrrha*), an oleo-gum-resin native to Middle Eastern and North African countries, was employed in the treatment of a wide range of inflammatory situations for

hundreds of years [12]. Several studies have also found it promising in the management of various oral-related disorders, including inflamed gingiva, aphthous ulcers, and intramucosal wounds [13, 14]. However, of the various herbs, tested up to now as a mouthwash formulation, the potential for a myrrh-based mouthwash is comparatively less explored. In our previous pilot study, we compared the efficacy of myrrh-based mouthwash with CHX and found it to be slightly more effective than CHX in reducing plaque accumulation and gingival inflammation [15]. Similar findings were reported in earlier studies, where the efficacy of myrrh was found to be comparable to that of CHX [16, 17]. These studies, however, had several limitations in study design and sample selection.

Therefore, this study aims to establish the earlier findings on the efficacy of myrrh mouthwash in comparison to CHX using further laboratory tests, including the pro-inflammatory interleukin (IL)-1β biomarker, bleeding on probing (BOP), modified gingival index (MGI), and plaque index (PI). We hypothesize that there is no difference between 1% myrrh mouthwash and commercially available CHX 0.2% mouth rinse in relation to decreasing load of plaque, gingival inflammation control, and pro-inflammatory mediator (IL-1β) inhibition.

Materials and Methods

Study design

The study was a randomized controlled clinical trial done at the King Abdulaziz University, Faculty of Dentistry (KAUFD), Jeddah, Saudi Arabia. We adhered to the declaration of Helsinki for Bio-medical research which involves human subjects and the CONSORT 2010 Statement for reporting multi-arm trials. The ethical approval of the study was obtained from the Research Ethics Committee at KAUFD (protocol number: 058-15). The protocol for this trial is publicly available at ClinicalTrials.gov (NCT04723732). Each participant signed a written informed consent before inclusion. The study was conducted between August 2017 and April 2018.

Patient selection

Participants were selected from patients seeking treatment at the dental clinic of KAUFD. A poster was hung in the waiting room area inviting patients to take part in the study as volunteers. Interested patients were included in this research sticking to the inclusion/exclusion criteria. Inclusion criteria were: good periodontal health (i.e., absence of clinical attachment loss and less than ten percent BOP); the presence of more than 20 teeth with at least five teeth per quadrant; no history of systemic disease; absence of oral prophylaxis in the past six months. exclusion criteria were: more than 3mm pocket depth; severe malocclusion; the presence of braces or orthodontic wires; use of antibiotic and/or anti-inflammatory prescriptions in the last 6 months; tobacco consumption; lack of compliance with the study agenda; pregnant or women who breast-feed.

Due to the lack of previous studies, we calculated the sample size based on a pilot study conducted in our center on 12 participants [15]. The study showed a mean difference of 0.29 ± 0.17 between the test and the reference group in post-intervention values. We entered these pilot results in a statistical tool [18] named "Sample Size Calculator for Comparing Two Independent Means." The tool estimated a sample size of 6 patients for each group based on 80% power and a 5% level of significance ($P < 0.05$, two-sided). We recruited 8 patients in each group considering possible loss to follow-up during the study. A total of 24 eligible patients, 12 male, and 12 female, with ages between eighteen and fifty-five years, were recruited.

Procedure

Patients were asked to complete a medical history questionnaire after the initial dental screening to confirm eligibility. The first visit included oral hygiene instructions and professional mouth cleaning processes such as oral prophylaxis or supra-gingival scaling–if needed. This was done 14 days before the study. At the second session, the examination of the periodontium was performed to confirm the good health of the gum and periodontium. Participants were then taught to refrain from cleaning teeth and any oral hygiene measures for two weeks to grow experimental gingivitis.

At the third session (experimental period, day 0), a periodontal examination was done to record the baseline values of gingival, plaque, bleeding, and inflammatory parameters. Participants were placed randomly into 1 of the 3 groups [1:1:1] through simple randomization technique (i.e., computer-generated random numbers): (a) normal saline, (b) 0.2% chlorhexidine gluconate mouthwash, and (c) 1% Commiphora myrrh mouthwash. Each group had eight participants. The myrrh mouthwash (1% g) was prepared following the same procedure described in the pilot study.15 Chlorhexidine gluconate 0.2% (Avalon Pharma, Riyadh) and normal saline 0.9% NaCl solution, 500 ml (Pharmaceutical solutions industry, Jeddah) were used as positive and negative controls, respectively.

Assignment of the mouthwashes to the groups was double-blind. The allocation of assigned interventions was concealed using anonymous, unlabeled opaque bottles. A general dentist (blinded at the baseline) performed the initial dental screening, oral hygiene procedures, and the distribution of bottles. Another dentist masked to the randomization list carried out the pre-and post-intervention periodontal examination. Patients were directed to persistently abstain from daily oral healthcare practices (e.g. tooth brushing or flossing) and use 15 ml of the given mouthwash two times every day for one minute. They were also instructed to use the given measuring cup, shake the bottle before using, refrain from the use other mouth rinses, and refrain from eating or drinking thirty mins following the use of the mouth rinse. We also asked them to report any complaints or side effects.

Patients' compliance to the assigned mouthwashes was primarily evaluated using a follow-up sheet given to them. They were also repeatedly reminded by phone calls to use their mouthwashes correctly. Additionally, to double-check the appropriate use of mouthwashes, they were even asked to bring back the bottles to verify the amount of solution used. Afterward (day 14 later), the same examiner reevaluate the participants and recorded the final values of all clinical parameters. Professional scaling, oral prophylaxis, and fluoride application were done at the end of the study.

Outcome measures

Primary outcomes were assessed using Trombelli *et al*. [19] MGI, O'leary *et al*. [20] PI, Ainamo and Bay [21] BOP, and Human IL-1β ELISA kit (BioVendor R&D – Laboratorni medicine a.s., Karasek, Czech Republic). Throughout this study, all participants underwent a weekly check-up to monitor PI and MGI values. BOP and IL-1β were assessed at baseline and post-intervention (day 14) to determine the presence of gingival bleeding and active inflammation. A standardized periodontal probe with a 0.6 mm tip and 1 mm marking was used to assess BOP. A skilled examiner measured bleeding on probing by exerting probing forces going beyond 0.25 N (25 g).

Two teeth were selected for IL-1β sample collection: first premolar (#12) and third molar (#16). Supragingival plaques were removed from the teeth before gingival crevicular fluid (GCF) collection. Filter paper strips of 2×8mm were used to collect GCF. The strips were placed into the gingival crevice and left in that position for 30 seconds. They were then stored in Eppendorf tubes filled with 400µl phosphate-buffered saline (PBS). The tubes were subsequently placed on ice and moved to the laboratory where they were frozen at −20°c until assayed. Distilled water was used to elute GCF from the strips. The sample was removed following the manufacturer's instructions. Human IL-1β ELISA kit by BioVendor was used to obtain the IL-1β level. Putting reagents in place, implementation of the test protocol, and calculation of results were done through abiding by the manufacturer's Product Data Sheet enclosed with the kit. The absorbance of each strip was read on a spectrophotometer at 450 nm wavelength.

Intraexaminer reliability

We conducted intraexaminer reliability of sulcular depth in some patients. The evaluation was done on 2 visits with one week difference. Gingival index and plaque index assessment were performed by employing clinical scenarios and pictures on 2 different time points. The intraexaminer reliability using the intraclass coefficients were 0.88, 0.92, and 0.82 for gingival, plaque, and sulcular depth, respectively.

Data collection and analysis

Statistical analysis was done using dedicated software (SPSS 24, IBM Corp., Armonk, NY, USA). Both pre-and -post-intervention data were collected. Mixed ANOVA was utilized to perform the data analysis. One between-subject variable (i.e., the interventions) and one within-subject variable (i.e., the time, pre-mouthwash vs. post-mouthwash) were included in the analysis. The assumption of sphericity was assessed with Mauchly's test. The violation of assumption was corrected with the Greenhouse-Geisser or Huynh-Feldt Corrections for Departure from Sphericity. Pairwise comparisons with Bonferroni adjustment were performed as a post-hoc follow-up test to determine the statistical difference between treatment groups at different time points. For all statistical analyses, $P < .05$ was considered statistically significant.

Results and Discussion

A sum of 24 individuals were initially involved in this study. 5 were later excluded for not showing up in the follow-up visits **(Figure 1)**. The final study population included 19 subjects, 10 males and 9 females (myrrh group, n = 6; CHX group, n = 7; and saline group, n = 6). The mean age of the participants were 30 (±10.55) years. No major difference between the groups at baseline was seen. **(Table 1)** shows the mean values of MGI, BOP, PI, and IL-1β across groups at baseline and after the intervention.

(Table 2) presents mixed ANOVA tests carried out to evaluate the between and within-group differences. There was a statistically major difference in MGI and BOP scores ($P = .014$ and $p < .001$, respectively) when both intra- and inter-group variations were considered (time * treatment). No major changes in the clinical parameters of PI and IL-1β were found between treatment groups and time.

(Table 3) shows a pairwise comparison of outcome measures between all treatment groups at two-time points. No major difference in the mean MGI, BOP, PI, and average IL-1β between the treatment groups ($P > .05$ for all pairwise comparisons) at baseline (time point 1)was observed. After the intervention period (time point 2), the mean MGI and BOP scores were considerably lesser in the myrrh group in comparison to the control group (mean difference=1.121, $P=.016$ and mean difference =44.173, $P < .001$, respectively). The CHX group also had lower mean MGI and BOP scores compared to the control. However, the mean MGI difference between CHX and control was not statistically significant ($P = .09$). there was no major difference in the mean PI and average IL-1β scores between treatment groups at any of the two times ($P > .05$ for all comparisons).

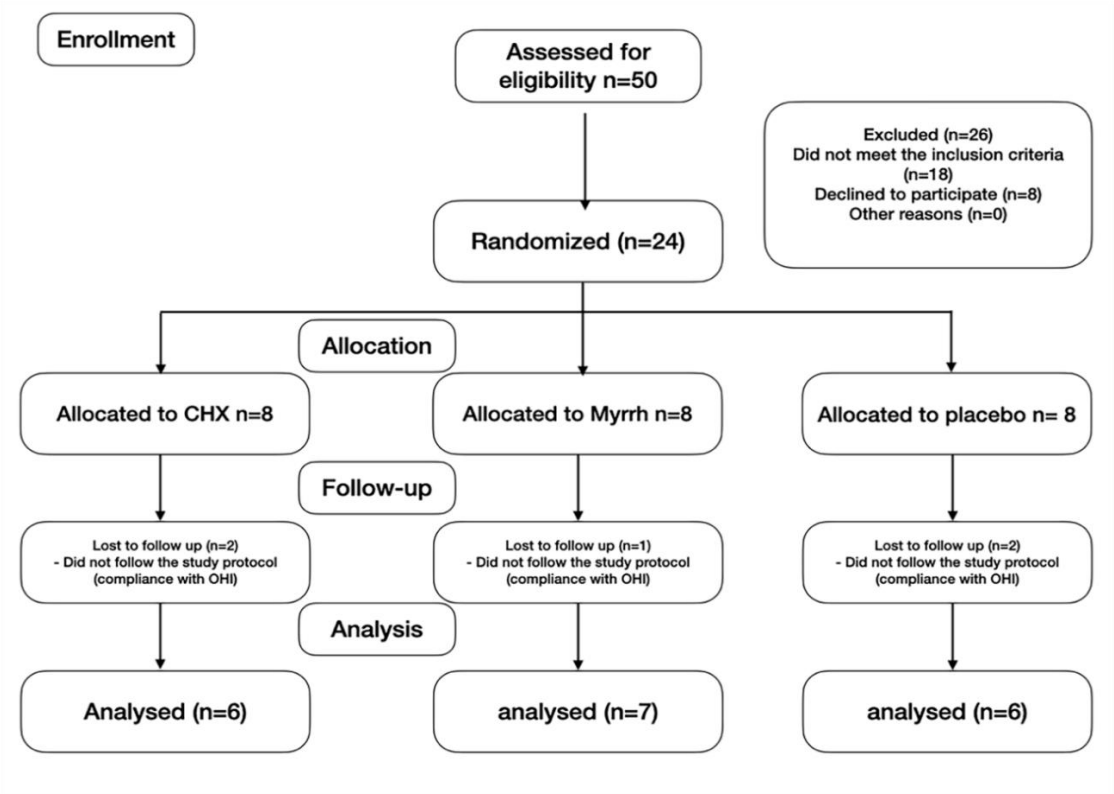

Figure 1. CONSORT 2010 flow diagram participants

Table 1. Descriptive statistics for various measurements

Groups	MGI Mean (SD)		BOP Mean (SD)		PI Mean (SD)		IL-1β Average[a] Mean (SD)	
	Baseline	14 Days	Baseline	14 Days	Baseline	14 Days	Baseline	14 Days
Control	3.45 (0.96)	3.91 (0.46)	74.99 (8.83)	83.61 (8.49)	88.23 (28.36)	67.44 (39.18)	74.9 (20.3)	124.3 (88)
CHX	3.5 (0.3)	3.05 (0.53)	71.63 (13.68)	39.44 (16.1)	73.37 (33.3)	86.56 (19.03)	80.8 (29.5)	70.3 (30.1)
Myrrh	3.68 (1.18)	2.79 (0.8)	63.05 (17.61)	40.55 (13.5)	74.46 (34.71)	65.62 (31.83)	90.9 (17.8)	98.4 (46.4)

MGI = Modified gingival index, BOP = Bleeding on probing, PI = Plaque index, IL-1β = interleukin-1β,
SD = Standard deviation.
[a] IL-1β averaged over teeth 12 and 16.

Table 2. Mixed ANOVA results for MGI, BOP, PI and IL-1β

Measurements	Source	Type III SS	df	Mean Square	F	*P*	Non-centrality Parameter	Observed Power
MGI	Treatment	1.50	2	0.75	0.78	0.47	1.57	0.16
	Time	0.82	1	0.82	3.01	0.10	3.01	0.37
	Time * Treatment	3.03	2	1.52	5.58	.014*	11.16	0.78
BOP	Treatment	5545.83	2	2772.91	9.71	.002*	0.55	19.43
	Time	2227.89	1	2227.89	25.96	<.001*	0.62	25.96
	Time * Treatment	2752.49	2	1376.25	16.03	<.001*	0.67	32.07
PI	Treatment	721.74	2	360.87	0.29	0.75	0.04	0.58
	Time	283.79	1	283.79	0.37	0.55	0.02	0.37

	Time * Treatment	1788.78	2	894.39	1.15	0.34	0.13	2.31
IL-1β average	Treatment	3901.64	2	1950.82	0.75	0.49	0.09	1.49
	Time	2262.23	1	2262.23	1.60	0.22	0.09	1.60
	Time * Treatment	5701.12	2	2850.56	2.02	0.17	0.20	4.04

a IL-1β averaged over teeth 12 and 16
* Statistically significant ($p < .05$)

Table 3. Pairwise Comparisons of MGI, BOP, PI and IL-1β Scores at Two Time Points

Outcome Measure	Time	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	*p*	Lower 95% CI	Upper 95% CI
MGI	1	Control	CHX	-0.05	0.53	1	-1.46	1.36
		Control	Myrrh	-0.23	0.51	1	-1.60	1.13
		CHX	Myrrh	-0.18	0.51	1	-1.55	1.18
	2	Control	CHX	0.86	0.36	0.09	-0.11	1.83
		Control	Myrrh	1.12*	0.35	.016*	0.19	2.05
		CHX	Myrrh	0.26	0.35	1	-0.67	1.19
BOP	1	Control	CHX	3.36	8.15	1	-18.42	25.14
		Control	Myrrh	11.94	7.85	0.443	-9.04	32.93
		CHX	Myrrh	8.59	7.85	0.871	-12.40	29.57
	2	Control	CHX	44.17	7.58	<.001*	23.92	64.42
		Control	Myrrh	43.06	7.30	<.001*	23.55	62.57
		CHX	Myrrh	-1.11	7.30	1	-20.63	18.40
PI	1	Control	CHX	14.86	18.70	1	-35.13	64.86
		Control	Myrrh	13.77	18.02	1	-34.41	61.95
		CHX	Myrrh	-1.09	18.02	1	-49.27	47.09
	2	Control	CHX	-19.12	18.01	0.912	-67.26	29.01
		Control	Myrrh	1.83	17.35	1	-44.56	48.21
		CHX	Myrrh	20.95	17.35	0.735	-25.44	67.33
IL-1β	1	Control	CHX	-5.92	13.16	1	-41.08	29.25
		Control	Myrrh	-15.94	12.68	0.68	-49.83	17.95
		CHX	Myrrh	-10.02	12.68	1	-43.91	23.86
	2	Control	CHX	54.00	34.21	0.402	-37.45	145.45
		Control	Myrrh	25.98	32.97	1	-62.15	114.10
		CHX	Myrrh	-28.02	32.97	1	-116.15	60.10

Based on estimated marginal means
*. The mean difference is significant at the .05 level.
b. Adjustment for multiple comparisons: Bonferroni.

This randomized, double-blinded clinical trial was conducted to compare and contrast the effects of 1% myrrh mouthwash with CHX 0.2% mouthwash in terms of plaque reduction, gingival inflammation control, inflammatory mediator (IL-1β) inhibition, and BOP improvement. Both myrrh and CHX were found to be effective in reducing gingival inflammation and BOP compared to the control solution (0.9% normal saline). In addition, no significant differences between the three experimental groups were found with regards to IL-1β and PI parameters.

The findings of this study confirm the results of earlier studies that the use of myrrh mouthwash can help improve gingival inflammation [15-17, 22, 23]. Our results are, however, slightly contrary to Bassiouny *et al*. [16] and our previous work where superior results were obtained but did not reach statistical significance. In this study, a statistically major decrease in gum swelling was demonstrated in the myrrh group compared to the control. Similar findings were reported in the latest research by Alotaibi *et al*. [17] in which major lower gum swelling was reported in the myrrh group at the final examination. The Alotaibi *et al*. study, however, had a significantly higher reduction in gingival inflammation in the chlorhexidine group than the myrrh group. This contrasts with the results of the current study, as myrrh outperformed CHX in gingival inflammation parameters when compared with control. Such difference could be due to the variation in study design, gingival Index

used, as well as myrrh mouthwash preparation. In addition, Alotaibi *et al*. used a commercially available myrrh mouthwash product in patients with gingivitis or mild periodontitis while we tested a customized preparation in an experimental gingivitis model.

The findings on PI in this study contradict the results of our pilot study. Although both myrrh and CHX had no significant effect on the PI parameter compared to the control, the mean PI value increased in the CHX group after the intervention period while both myrrh and control groups had a decrease. Such increase in PI even after CHX use could be due to the experimental nature of this study, as CHX was used over a plaque-covered surface, and patients refrained from any mechanical plaque control measure for 2 weeks. This has been demonstrated in a similar experimental model by Zanatta *et al*. [24] where 0.12% CHX mouthwash showed a little antiplaque effect on structured biofilm after 21 days of plaque accumulation. Other possible reasons for such discrepancies between groups may be the low number of individuals included in each group or the involuntary use of mechanical plaque control.

Apart from gingival and plaque indices, BOP is another reliable indicator of gingival inflammation and periodontal stability [25]. The significant reduction in BOP reported in this study provides further evidence that myrrh-based mouthwashes can reduce gingival inflammation and may impede the progression of periodontal disease. This is consistent with the findings of an earlier double-blinded study by Saeedi *et al*. [26] They applied myrrh-based toothpaste on bleeding gingiva and reported a significantly lower gingival bleeding compared to controls. In addition, a recent study by Al Eid [27] on wound healing after dental extraction also reported less inflammatory signs and postoperative bleeding in participants treated with myrrh mouthwash than those of the control group.

Myrrh has been suggested to be a potential inhibitor of inflammatory responses [22, 23, 28-31]. It has been shown to have anti-inflammatory effects in carcinoma cells [22, 32] and is thought to exert such effects by inhibiting the production of several inflammatory mediators including IL-1β, IL-6, tumor necrosis factor-α (TNF-α), nitric oxide (NO), and prostaglandin E2 (PGE2). This has been demonstrated in an animal model of cecal ligation and puncture (CLP) by Kim *et al*. [28] where the authors observed that administration of myrrh led to a reduction in CLP-induced mortality and an inhibition of lipopolysaccharide (LPS)-induced peritoneal macrophages. CLP is the most frequently used animal model of sepsis that closely resembles the pathophysiological changes observed in human sepsis. It involves perforation of the cecum to induce peritonitis so that an exacerbated immune response is produced, eventually leading to septic shock [33]. In this study, no significant effect of myrrh mouthwash was observed on IL-1β parameters. This finding slightly contrasts with the findings of Kim *et al*. [28]. However, it is to be noted that Kim *et al*. observed the inhibition of IL-1β, IL-6 in CLP-induced production of inflammatory mediators but not in LPS-induced peritoneal macrophages. Because our experimental model was not a sepsis model, we can say that the findings of this study are consistent with the results of Kim *et al*. Taken together, the anti-inflammatory responses of myrrh need further investigation to better understand its effects on various inflammatory mediators.

Myrrh has also been shown to exhibit antibacterial properties. Over the years, many studies have reported its efficacy in infectious diseases [12, 34-38]. Rahman *et al*. [35] found several strains of *Klebsiella pneumoniae*, *Salmonella enterica*, and *Staphylococcus aureus* sensitive to *Commiphora molmol*. The antimicrobial activity of myrrh also includes oral flora. In addition, a recent study by Sambawa *et al*. [36] suggested that the anti-bacterial efficacy of myrrh was fairly comparable to CHX. The use of myrrh is also found to shorten the time required for wound healing. Al Eid [27] reported an enhancement effect of myrrh mouthwash on wound healing after tooth extraction. In summary, the anti-inflammatory, antibacterial, and wound healing properties of myrrh could explain its superior effects on gingival inflammation found in this study.

Myrrh mouthwash has the potential to be a suitable alternative to CHX mouthwash for gingival inflammation control due to its low side effect profile, wide availability, and ease of preparation. However, in this study, myrrh was used for a short duration and in low concentration (1%); hence, the possible side effects of its prolonged application need to be determined. Furthermore, this study had several limitations. Firstly, the results of this study are not generalizable due to its small sample size. A large randomized trial with an extended follow-up period would have been more appropriate to make a better and more precise comparison between myrrh and CHX. Secondly, we tested the effectiveness of the myrrh mouthwash in an experimental gingivitis model over a short duration. The development of experimental gingivitis is a difficult process and requires discontinuation of oral hygiene, which is socially unacceptable. Thirdly, this study primarily evaluated the efficacy of myrrh on gingival inflammation; thus, its effects on other periodontal parameters were unknown. Further research is warranted with a large sample and long-term follow-up to establish the findings of this study and determine the efficacy of different concentrations of myrrh (i.e., 2% or 3% myrrh formulation).

Conclusion

Despite several limitations of this pilot study, it can be concluded that myrrh-based mouthwash has an efficacy comparable to the 0.2% CHX mouthwash in reducing gingival inflammation and BOP. Given the side effects associated with the long-term use of CHX, myrrh

mouthwash can be considered a suitable alternative. However, more research is needed to establish its effectiveness on a larger scale.

Acknowledgments: The authors would like to thank Dr. Dua Alsini and Dr Hetaf Toniaz for the help in the study.

Conflict of interest: None

Financial support: None

Ethics statement: The ethical approval of the study was obtained from the Research Ethics Committee at KAUFD (protocol number: 058-15). The protocol for this trial is publicly available at ClinicalTrials.gov (NCT04723732).

References

1. Jiao Y, Tay FR, Niu LN, Chen JH. Advancing antimicrobial strategies for managing oral biofilm infections. Int J Oral Sci. 2019;11(3):1-1.
2. Murakami S, Mealey BL, Mariotti A, Chapple IL. Dental plaque–induced gingival conditions. J Clin Periodontol. 2018;45:S17-27.
3. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. Int Dent J. 2021;71(6):462-76.
4. Figuero E, Nobrega DF, García-Gargallo M, Tenuta LM, Herrera D, Carvalho JC. Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review. J Clin Periodontol. 2017;44:S116-34.
5. Figuero E, Herrera D, Tobías A, Serrano J, Roldan S, Escribano M, et al. Efficacy of adjunctive anti-plaque chemical agents in managing gingivitis: A systematic review and network meta-analyses. J Clin Periodontol. 2019;46(7):723-39.
6. Sakaue Y, Takenaka S, Ohsumi T, Domon H, Terao Y, Noiri Y. The effect of chlorhexidine on dental calculus formation: an in vitro study. BMC Oral Health. 2018;18(1):1-7.
7. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017;3:CD008676.
8. Zhao H, Hu J, Zhao L. Adjunctive subgingival application of Chlorhexidine gel in nonsurgical periodontal treatment for chronic periodontitis: a systematic review and meta-analysis. BMC Oral Health. 2020;20(1):1-2.
9. Seguya A, Mowafy M, Gaballah A, Zaher A. Chlorhexidine versus organoselenium for inhibition of S. mutans biofilm, an in vitro study. BMC Oral Health. 2022;22(1):1-9.
10. Takenaka S, Ohsumi T, Noiri Y. Evidence-based strategy for dental biofilms: Current evidence of mouthwashes on dental biofilm and gingivitis. Jpn Dent Sci Rev. 2019;55(1):33-40.
11. Janakiram C, Venkitachalam R, Fontelo P, Iafolla TJ, Dye BA. Effectiveness of herbal oral care products in reducing dental plaque & gingivitis–a systematic review and meta-analysis. BMC Complement Med Ther. 2020;20(1):1-2.
12. Cao B, Wei XC, Xu XR, Zhang HZ, Luo CH, Feng B, et al. Seeing the unseen of the combination of two natural resins, frankincense and myrrh: Changes in chemical constituents and pharmacological activities. Molecules. 2019;24(17):3076.
13. Mansour G, Ouda S, Shaker A, et al. Clinical efficacy of new aloe vera and myrrh-based oral mucoadhesive gels in the management of minor recurrent aphthous stomatitis: A randomized, double-blind, vehicle-controlled study. J Oral Pathol Med. 2014;43(6):405-9.
14. Al-Mobeeriek A. Effects of myrrh on intra-oral mucosal wounds compared with tetracycline and chlorhexidine-based mouthwashes. Clin Cosmet Investig Dent. 2011;3:53-8.
15. Zahid TM, Alblowi JA. Anti-Inflammatory and anti-plaque effects of commiphora myrrh mouthwash: a preliminary pilot clinical study. Open Dent J. 2019;13(1):1-5.
16. Bassiouny G, Al-Barrak H. The anti-plaque effect of Miswak and myrrh mouthwashes versus chlorhexidine in the treatment of chronic gingivitis; a comparative clinical trial. Med Sci. 2014;9(33):32-7.
17. Alotaibi RA, Aldahlawi S, Alyami FM. The Effects of Commiphora Myrrh Mouthwash Verses Chlorhexidine on Dental Plaque and Gingivitis: A Comparative Study. J Res Med Dent Sci. 2020;8(4):65-70.
18. Dhand NK, Khatkar MS. Statulator: An online statistical calculator. Sample Size Calculator for Comparing Two Independent Means. 2014;6:2021. Available from: http://statulator.com/SampleSize/ss2M.html
19. Trombelli L, Tatakis DN, Scapoli C, Bottega S, Orlandini E, Tosi M. Modulation of clinical expression of plaque-induced gingivitis. II. Identification of "high-responder" and "low-responder" subjects. J Clin Periodontol. 2004;31(4):239-52.
20. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol. 1972;43:38.
21. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J. 1975;25(4):229-35.
22. Mahmoud AM, Germoush MO, Al-Anazi KM, Mahmoud AH, Farah MA, Allam AA. Commiphora molmol protects against methotrexate-induced nephrotoxicity by up-regulating Nrf2/ARE/HO-1 signaling. Biomed Pharmacother. 2018;106:499-509.
23. Younis NS, Mohamed ME. Protective effects of myrrh essential oil on isoproterenol-induced myocardial infarction in rats through antioxidant, anti-inflammatory, Nrf2/HO-1 and apoptotic pathways. J Intercult Ethnopharmacol. 2021;270:113793.
24. Zanatta FB, Antoniazzi RP, Rösing CK. The effect of 0.12% chlorhexidine gluconate rinsing on previously plaque-free and plaque-covered surfaces: A

randomized, controlled clinical trial. J Clin Periodontol. 2007;78(11):2127-34.
25. Wada M, Mameno T, Otsuki M, Kani M, Tsujioka Y, Ikebe K. Prevalence and risk indicators for peri-implant diseases: A literature review. Jpn Dent Sci Rev. 2021;57:78-84.
26. Saeedi M, Azadbakht M, Semnani K, Khandan M. Formulation of herbal toothpaste from chamomile and myrrh, a preliminary clinical evaluation on bleeding gum. J Mazandaran Univ Med Sci. 2003;13(40):61-9.
27. Al Eid RA. Efficacy of Commiphora myrrh mouthwash on early wound healing after tooth extraction: A randomized controlled trial. Saudi Dent J. 2021;33(1):44-54.
28. Kim MS, Bae GS, Park KC, Koo BS, Kim BJ, Lee HJ, et al. Myrrh inhibits LPS-induced inflammatory response and protects from cecal ligation and puncture-induced sepsis. Evid Based Complement Alternat Med. 2012;2012:278718.
29. Lebda MA, Mostafa RE, Taha NM, El-Maksoud A, Eman M, Tohamy HG, et al. Commiphora myrrh supplementation protects and cures ethanol-induced oxidative alterations of gastric ulceration in rats. Antioxidants. 2021;10(11):1836.
30. Su S, Duan J, Chen T, Huang X, Shang E, Yu L, et al. Frankincense and myrrh suppress inflammation via regulation of the metabolic profiling and the MAPK signaling pathway. Sci Rep. 2015;5(1):13668.
31. Weber L, Kuck K, Jürgenliemk G, Heilmann J, Lipowicz B, Vissiennon C. Anti-inflammatory and barrier-stabilising effects of myrrh, coffee charcoal and chamomile flower extract in a co-culture cell model of the intestinal mucosa. Biomolecules. 2020;10(7):1033.
32. Qureshi S, Al-Harbi MM, Ahmed MM, Raza M, Giangreco AB, Shah AH. Evaluation of the genotoxic, cytotoxic, and antitumor properties of Commiphora molmol using normal and Ehrlich ascites carcinoma cell-bearing Swiss albino mice. Cancer Chemother Pharmacol. 1993;33(2):130-8.
33. Alverdy JC, Keskey R, Thewissen R. Can the cecal ligation and puncture model be repurposed to better inform therapy in human sepsis?. Infect Immun. 2020;88(9):e00942-19.
34. Kommerein N, Weigel AJ, Stiesch M, Doll K. Plant-based oral care product exhibits antibacterial effects on different stages of oral multispecies biofilm development in vitro. BMC Oral Health. 2021;21(1):1-2.
35. Rahman MM, Garvey M, Piddock LJ, Gibbons S. Antibacterial terpenes from the oleo-resin of Commiphora molmol (Engl.). Phytother Res. 2008;22(10):1356-60.
36. Sambawa ZM, Alkahtani FH, Aleanizy FS, Alqahtani FY. Comparison of Antibacterial Efficacy Chlorohexidine Gluconate and Saudi Myrrh Mouthwashes in the Oral Cavity. Orient J Chem. 2016;32(5):2605-10.
37. Khalil N, Fikry S, Salama O. Bactericidal activity of Myrrh extracts and two dosage forms against standard bacterial strains and multidrug-resistant clinical isolates with GC/MS profiling. AMB Express. 2020;10(1):21.
38. Bhattacharjee MK, Alenezi T. Antibiotic in myrrh from Commiphora molmol preferentially kills nongrowing bacteria. Future Sci OA. 2020;6(4):FSO458.