



## Comparative Effects of Kemphor and Chlorhexidine Mouthwashes on Tooth Staining and Gingivitis: A Randomized Controlled Crossover Clinical Trial

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### ABSTRACT

**Objectives:** Considering the side effects of chlorhexidine (CHX), which is currently the gold-standard antimicrobial mouthwash, this study aimed to compare the effects of Green Kemphor and CHX mouthwashes on tooth staining and gingivitis.

**Materials and Methods:** This randomized controlled crossover clinical trial evaluated 38 patients requiring CHX mouthwash following oral surgery and periodontal therapy. The patients were randomly assigned to CHX and Kemphor groups (n=19). In CHX group, patients used CHX mouthwash in the first 2 weeks, and after a 4-day washout period, they used Kemphor mouthwash for 2 weeks. This order was reverse in the Kemphor group. Gingivitis was evaluated using the Silness and Loe gingival index (GI), and tooth staining was evaluated by the Lobene index at 0 (baseline), 2 and 4 weeks. Data were analyzed by paired t-test.

**Results:** CHX mouthwash significantly decreased the GI and increased tooth staining (gingival stains, body stains, and stain extent) at 2 weeks (P<0.05). Kemphor mouthwash significantly decreased the GI and increased tooth staining after 2 weeks (P<0.05). The GI in Kemphor group was significantly lower than that in CHX group at 4 weeks (P<0.05). Also, the tooth staining parameters in the Kemphor group were significantly lower than the corresponding values in the CHX group at 2 and 4 weeks (P<0.05).

**Conclusion:** Kemphor had higher efficacy for reduction of GI and caused less tooth staining than CHX; thus, it may be recommended for use as an alternative to CHX.

**Keywords:** Chlorhexidine; Mouthwash; Tooth Discoloration; Gingivitis

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### INTRODUCTION

Smile esthetics plays an important role in facial attractiveness [1]. Tooth discoloration adversely affects dental appearance, and

compromises smile esthetics [2]. Tooth color is determined by the color of dentin as well as intrinsic and extrinsic tooth discolorations [3]. The intrinsic color is determined by the optical

properties of enamel and dentin while the extrinsic color depends on external stains absorbed by the external enamel surface [4]. The most common causes of extrinsic tooth discolorations include poor oral hygiene, dental plaque, dental calculus, consumption of coffee, tea or other coloring agents, and tobacco use [5]. Also, some certain types of bacteria cause green, brown, black, or orange discoloration of tooth surfaces. Moreover, extensive amalgam restorations and iron-containing medications cause black discoloration. Compounds containing nitrate, silver sulfide, and manganese cause gray, yellow, brown or black discolorations while copper and nickel cause green discoloration. Calcium causes yellow, gold, and brown discolorations as well [5]. On the other hand, chlorhexidine (CHX) mouthwash causes the deposition of brown-yellow stains in interproximal areas and next to the gingival margin [6].

Despite the fact that CHX is the gold-standard antimicrobial and anti-plaque mouthwash, many attempts have been made to find an equally effective alternative to CHX due to its side effects such as tooth discoloration. Increasing attention has been recently paid to herbal mouthwashes since evidence shows that some herbal mouthwashes cause significantly lower color change compared with CHX; however, their anti-plaque and antimicrobial properties are still questionable, and may not be comparable to CHX [7,8].

Green Kemphor mouthwash (Pinseque, Spain) contains water, glycerol, sorbitol, xylitol, sodium fluoride, sodium benzoate, benzoic acid, 0.12% CHX digluconate, polyethylene glycol, eugenol, limonene, cinnamal, sodium saccharine, zinc chloride (as stain remover), and vegetable oil. The manufacturer claims that aside from the optimal anti-plaque and anti-bacterial properties, this mouthwash causes less tooth staining than 0.2% CHX, and can be used as an effective mouthwash before and after oral surgical procedures and periodontal therapy. However, information regarding the efficacy of this mouthwash is limited. Considering the patient complaints regarding tooth discoloration caused by CHX,

this study aimed to compare the effects of Green Kemphor and CHX mouthwashes on tooth staining and gingivitis.

### MATERIALS AND METHODS

This randomized controlled crossover clinical trial was conducted in Hamadan University of Medical Sciences between 2019-2020. The study protocol was approved by the ethics committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1398.674), and registered in the Iranian Registry of Clinical Trials (IRCT20120215009014N327).

#### ***Trial design:***

This randomized controlled crossover clinical trial evaluated all patients who required the use of CHX mouthwash following oral surgical procedures and periodontal therapy. The results were reported according to the criteria and guidelines of the Consolidated Standards of Reporting Trials and the Cochrane risk of bias tool for assessing the methodological quality of randomized trials.

#### ***Participants, eligibility criteria, and settings:***

The inclusion criteria were as follows: (I) patients requiring the use of CHX mouthwash following oral surgical procedures and periodontal therapy, and (II) plaque index (PI)  $\leq$  30%.

The exclusion criteria were (I) pregnancy, (II) tobacco use, (III) consumption of tea, coffee or cola at least 3 times a day, (IV) patients with systemic diseases who could not undergo surgery or periodontal therapy, (V) use of toothpastes containing CHX, anti-plaque agents, or bleaching agents, (VI) use of any other mouthwash during the study period, (VII) patients with a history of maxillary salivary gland surgery, and (VIII) dental treatment of maxillary anterior teeth.

A total of 38 patients who met the eligibility criteria including 19 males and 19 females between 19 to 58 years were selected by convenience sampling.

#### ***Interventions:***

All patients were briefed about the study and willingly signed informed consent forms prior to their participation. Correct toothbrushing and oral hygiene measures were instructed to

all patients, and they were requested not to use toothpastes containing CHX during the study period. The surgical sites in patients included different parts of the oral cavity except for the anterior segment of the maxilla (six maxillary anterior teeth). Also, the patients had a PI  $\leq$  30%. Considering the need for using the mouthwash after surgery, patients were enrolled the day after their surgical procedure.

Staining of the teeth was determined by the Lobene stain index [9] while gingivitis was evaluated by measuring the gingival index (GI) according to the Silness and L e [10]. The patients were randomly assigned to two groups by block permuted randomization (n=19). The patients in Kemphor group received green Kemphor mouthwash (Pinseque, Spain) in the first 2 weeks. They were requested to rinse 15 cc of the mouthwash twice a day (once in the morning and once in the evening) for 1 minute (according to the manufacturer's instructions) for 2 weeks [11]. They were asked not to rinse their mouth for 1 hour after using the mouthwash. The patients were recalled after 2 weeks. The examiner had no information regarding the type of mouthwash used by the patients. Tooth staining was scored according to the Lobene stain index [9], and GI was determined according to the Silness and L e index [10]. The patients then received dental prophylaxis of the maxillary anterior teeth to eliminate superficial stains. After a 4-day washout period [12], type of mouthwash was changed and the patients were requested to use 0.2% CHX mouthwash (Vi-One, Iran) for 2 weeks. Vi-One contains 0.2% chlorhexidine digluconate, 2% xylitol, and 0.04% thymol. This order was reverse in CHX group, and the patients used CHX mouthwash in the first 2 weeks, and Kemphor mouthwash in the second 2 weeks after a 4-day washout period. Tooth staining was measured again at the end of interventions (Figs. 1 and 2).

Tooth staining was determined at baseline (week 0), 2 weeks and 4 weeks. For this purpose, the tooth surface was divided into two regions: gingival region and body region.

The intensity and extent of staining of the buccal surface of the six maxillary anterior teeth were determined. The two regions were separately scored as follows:

0: No staining; 1: mild staining, 2: moderate staining, and 3: severe staining. The extent of staining was also scored as follows: 0: no staining, 1: staining of up to one-third of the respective region, 2: staining of up to two-thirds of the respective region, and 3: staining of over two-thirds of the respective region [9]. Next, the mean scores of the cervical stains, body stains, and stain extent were calculated, and the mean values of the two groups were compared.

The Silness and L e GI of the six maxillary anterior teeth was scored as follows [10]:

0: Healthy normal gingiva with no observable inflammation, 1: mild inflammation in the form of slight change in gingival color or consistency, mild edema, no bleeding on probing, 2: moderate gingival inflammation and tendency of the gingival margin for bleeding on probing, edema, redness, and gingival hypertrophy; 3: severe gingival inflammation with tendency for spontaneous bleeding, edema, redness, and severe gingival hypertrophy. Eventually, one GI was recorded for each patient, and the mean GI was calculated for the two groups and compared [10].

#### **Outcomes (primary and secondary):**

The main objective of this study was to evaluate tooth staining following the use of the two mouthwashes according to the Lobene stain index [9]. GI, which was measured according to the Silness and L e index [10] was the secondary outcome of the study.

#### **Sample size calculation:**

The sample size was calculated to be 19 in each group according to a study by Sol s et al, [13] assuming alpha=0.05, beta=0.2 and study power of 80%.

#### **Interim analyses and stopping guidelines:**

No interim analyses were performed, and no stopping guidelines were established.

#### **Randomization:**

The patients were randomly assigned to two groups by block permuted randomization.



Fig. 1. Tooth color before and after using CHX first and then green Kempfor (group 1)



Fig. 2. Tooth color before and after using green Kempfor first and then CHX (group 2)

Four block sizes were used in this study. Considering the sample size, 10 blocks were created (actual list length=40). The entire process was performed using the online randomizer calculator.

**Blinding:**

One trained examiner performed all the measurements and recorded the data, who had no information regarding the type of mouthwash used by the patients. Blinding of patients was not possible due to different bottles of the mouthwashes. Thus, the study was conducted as open-labeled. However, this caused no limitation since patients were not in contact with each other.

**Statistical analysis:**

Considering the normal distribution of data confirmed by the Kolmogorov-Smirnov test, paired t-test was applied to compare tooth staining and GI between the two groups.

All statistical analyses were performed using STATA 11 software at 0.05 level of significance.

**RESULTS**

**Participant flow:**

The sample consisted of 38 patients including 19 males and 19 females with a mean age of 36.76±9.96 years (range 19 to 58 years). Figure 3 shows the CONSORT flow diagram of patient selection and allocation to study groups.

**Harms:**

No patients were harmed during the study.

**Subgroup analyses:**

Table 1 presents the baseline GI, gingival stains, body stains, stain extent, and PI of patients in the two groups. The mean GI, gingival stains, body stains, stain extent and PI were not significantly different between the two groups at baseline (P>0.05).

Table 1. Baseline gingival index, gingival stains, body stains, stain extent, and plaque index of patients in the two groups

Parameter	Chlorhexidine	Kempfor	P
Gingival index	0.93±0.54	1.12±0.43	0.246
Gingival stain	0.84±0.39	0.87±0.51	0.826
Body stain	0.40±0.37	0.45±0.34	0.653
Stain extent	1.67±0.87	1.57±0.77	0.719
Plaque index	20.07±6.58	20.84±5.19	0.694

Values are means±standard deviations

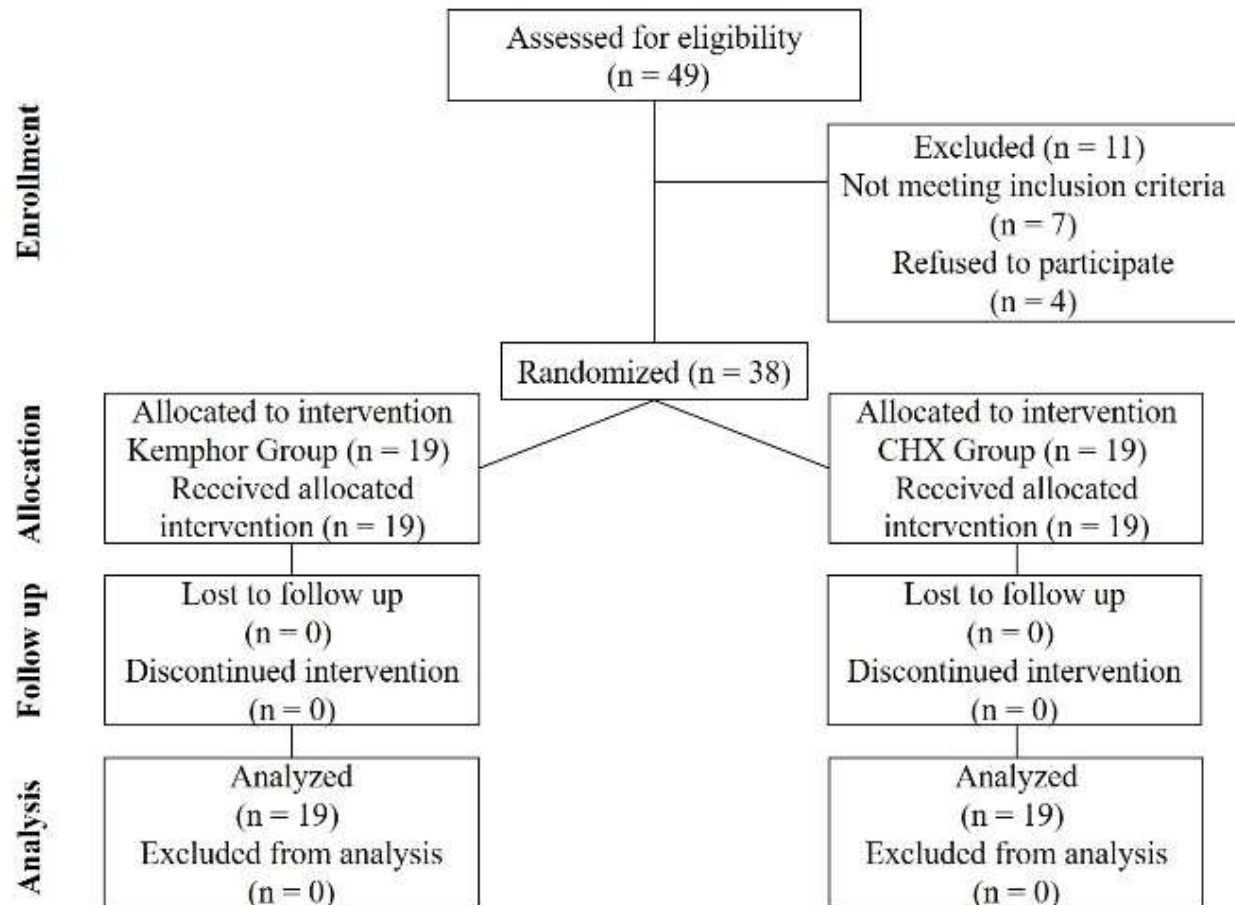


Fig 3. CONSORT flow diagram of patient selection and allocation to study groups

#### **Results in CHX group:**

Regarding GI, the results showed a significant reduction in GI at both 2 ( $P < 0.001$ ) and 4 ( $P = 0.002$ ) weeks, compared with baseline. However, the difference in GI between 2 and 4 weeks was not significant ( $P = 0.649$ ). Gingival stains significantly increased at both 2 ( $P < 0.001$ ) and 4 ( $P < 0.001$ ) weeks, compared with baseline. However, the difference in gingival staining between 2 and 4 weeks was not significant ( $P = 0.073$ ). Body stains significantly increased at both 2 ( $P < 0.001$ ) and 4 ( $P < 0.001$ ) weeks, compared with baseline. However, the difference in body stains between 2 and 4 weeks was not significant ( $P = 0.245$ ). Stain extent significantly increased at both 2 ( $P < 0.001$ ) and 4 ( $P < 0.001$ ) weeks, compared with baseline. The difference in stain extent between 2 and 4 weeks was also significant ( $P = 0.010$ ).

#### **Results in Kemphor group:**

Regarding GI, the results showed a significant reduction in GI at both 2 ( $P < 0.001$ ) and 4 ( $P = 0.002$ ) weeks, compared with baseline. The difference in GI between 2 and 4 weeks was also significant ( $P = 0.002$ ). The mean GI, gingival stains, body stains, stain extent and PI were not significantly different between the two groups at baseline ( $P > 0.05$ ). Gingival stains significantly increased at both 2 ( $P < 0.001$ ) and 4 ( $P < 0.001$ ) weeks, compared with baseline. However, the difference in gingival stains between 2 and 4 weeks was not significant ( $P = 0.365$ ). Body stains significantly increased at both 2 ( $P = 0.002$ ) and 4 ( $P = 0.012$ ) weeks, compared with baseline. However, the difference in body stains between 2 and 4 weeks was not significant ( $P = 0.242$ ).

## Tooth Staining: Kemphor vs Chlorhexidine

Stain extent significantly increased at both 2 ( $P<0.001$ ) and 4 ( $P=0.008$ ) weeks, compared with baseline. However, the difference in stain extent between 2 and 4 weeks was not significant ( $P=0.276$ ).

Table 2 shows the mean GI and tooth staining parameters in CHX and Kemphor groups at different time points. Paired t-test showed that at 2 weeks, the mean GI was slightly, but not significantly higher in CHX group ( $P=0.225$ ). At 4 weeks, GI in Kemphor group was significantly lower than that in CHX group ( $P=0.032$ ). The mean gingival staining was significantly higher in CHX group at both 2 ( $P=0.007$ ) and 4 ( $P<0.001$ ) weeks.

The mean body staining was also significantly higher in CHX group at both 2 ( $P=0.022$ ) and 4 ( $P=0.010$ ) weeks. The same results were obtained for stain extent ( $P=0.022$  and  $P<0.001$  at 2 and 4 weeks, respectively).

Independent t-test was applied to assess the

overall effect of treatments irrespective of time point (Table 3). The results showed that the mean GI at the end of the study period was not significantly different between the CHX and Kemphor groups ( $P=0.318$ ). However, the difference in gingival stains ( $P<0.001$ ), body stains ( $P=0.001$ ) and stain extent ( $P=0.002$ ) was significant between the two groups. Considering the crossover design of the trial, the period effect on the intervention was also analyzed (Table 4).

The results showed that the period effect was not significant on GI ( $P=0.150$ ). However, the period effect was significant on gingival stains ( $P=0.015$ ), body stains ( $P=0.05$ ), and stain extent ( $P=0.05$ ).

Table 5 presents the carryover effect on GI and tooth staining parameters in the CHX and Kemphor groups. The results showed that the carryover effect was not significant on any parameter ( $P>0.05$ ).

**Table 2.** Mean difference in gingival index and tooth staining parameters between the chlorhexidine and Kemphor groups at different time points

Parameter	2 weeks				4 weeks			
	CHX	Kemphor	MD	P	CHX	Kemphor	MD	P
Gingival index	0.29±0.32	0.38±0.40	-0.09±0.11	0.225	0.24±0.34	0.08±0.14	0.16±0.08	0.032
Gingival stain	1.77±0.57	1.32±0.49	0.44±0.17	0.007	2.03±0.48	1.38±0.50	0.65±0.16	<0.001
Body stain	1.01±0.61	0.66±0.36	0.34±0.16	0.022	1.15±0.60	0.75±0.35	0.39±0.16	0.010
Stain extent	2.43±0.53	2.00±0.70	0.42±0.20	0.022	2.79±0.35	2.14±0.68	0.65±0.18	<0.001

Values are means±standard deviations.

MD: Mean difference; CHX: Chlorhexidine

**Table 3.** Overall mean difference in gingival index and tooth staining parameters between the chlorhexidine and Kemphor groups

Parameter	Chlorhexidine	Kemphor	Mean difference	P
Gingival index	0.27±0.33	0.23±0.33	0.03±0.07	0.318
Gingival stain	1.90±0.54	1.35±0.54	0.54±0.06	<0.001
Body stain	1.07±0.60	0.71±0.35	0.36±0.11	0.001
Stain extent	2.60±0.48	2.07±0.69	0.52±0.07	0.002

Values are means±standard deviations.

**Table 4.** Period effect on gingival index and tooth staining parameters in the chlorhexidine and Kemphor groups

Parameter	2-4w period effect on chlorhexidine group	2-4w period effect on Kemphor group	Mean difference	P
Gingival index	0.22±0.23	0.13±0.25	0.13±0.08	0.150
Gingival stain	0.35±0.62	- 0.68±0.25	- 0.67±0.16	0.015
Body stain	- 0.20±0.52	0.47±0.46	1.04±0.15	0.05
Stain extent	0.27±0.64	- 0.78±0.62	1.06±0.21	0.05

Values are means±standard deviations

**Table 5.** Carryover effect on gingival index and tooth staining parameters in the chlorhexidine and Kemphor groups

Parameter	2-4 week carry over effect on chlorhexidine group	2-4 week carry over effect on Kemphor group	Mean difference	P
Gingival index	0.40±0.45	0.66±0.71	- 0.25±0.20	0.102
Gingival stain	3.12±0.89	3.39±0.94	- 0.26±0.30	0.184
Body stain	1.70±0.81	1.82±0.88	- 0.12±0.28	0.308
Stain extent	4.56±1.05	4.80±0.96	- 0.24±0.34	0.240

Values are means±standard deviations

## DISCUSSION

This study compared the effects of Green Kemphor and CHX mouthwashes on tooth staining and gingivitis. The results showed significant reduction of GI at 2 and 4 weeks compared with baseline in CHX group with no significant difference between 2 and 4 weeks. In line with this finding, Herrera [14] in a clinical trial reported that use of CHX for 4 weeks improved gingival inflammation. Also, Mali et al. [15] reported significant improvement of GI and PI after using 0.2% CHX. Similar results were reported by Najafi et al, [16] Ripari et al, [17] and Sreenivasan and Prasad [18].

Biofilm-producing microorganisms such as streptococci play a fundamental role in development of gingivitis and periodontitis [19]. CHX has a broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria and fungi. Depending on its concentration, CHX can have bacteriostatic (in lower concentrations) or bactericidal (in higher concentrations) effects [20]. CHX is a positively-charged chemical agent that forms electrostatic bonds to the cell wall of negatively charged bacteria. Resultantly, the bacterial cell wall is compromised, making the

bacteria susceptible to osmosis. In higher concentrations, the cell wall is degraded and CHX enters the bacterial cell and attacks its cytoplasmic membrane, causing membrane damage, which increases the permeability of bacterial cell and eventual cell death [20,21]. Moreover, CHX prevents the formation of bacterial biofilm on tooth and gingival surfaces; however, it has lower efficacy in elimination of mature biofilm [22].

The results of the present study also indicated significant reduction of GI in Kemphor group after 2 and 4 weeks, with no significant difference between 2 and 4 weeks. Kemphor mouthwash also contains CHX but at a lower concentration (0.12%) than the pure CHX mouthwash used in our study. It also contains polyethylene glycol, which has confirmed antibacterial activity exerted by lysis of the cell wall of bacteria such as staphylococci [23]. Polyethylene glycol is also used as an antimicrobial agent in food industries [24]. Kemphor mouthwash also contains fluoride. Dang et al. [25] highlighted the role of fluoride as an antimicrobial agent in reduction of microbial oral and dental infections, and pointed to its significant antibacterial activity against *Streptococcus mutans*. Thus, fluoride

is extensively used in the formulation of cariostatic toothpastes. It appears that the antimicrobial effects of sodium fluoride are attributed to acidification of bacterial cytoplasm. Also, sodium fluoride has inhibitory effects on the glycolytic pathway enzymes in bacterial cells, and subsequently impairs energy production by inhibition of metabolic pathways [26]. Kemphor mouthwash has herbal products such as eugenol, limonene, and cinnamal. Eugenol increases the lysosomal activity and damages the cell membrane and cell wall of Gram-positive and Gram-negative bacteria. It also inhibits lipid peroxidase and hydroxyl radicals, and has antimicrobial effects [27,28]. Limonene has antimicrobial activity as well [29,30].

It accumulates in the bacterial cell membrane and compromises its integrity [31]. More importantly, limonene inhibits biofilm formation by *Streptococcus mutans* [32]. Cinnamal has antimicrobial properties against oral microorganisms as well [33]. It disintegrates the bacterial cell wall, inhibits biofilm formation, impairs ATP synthesis, and causes eventual bacterial cell death [34]. Cetylpyridinium, which is a quaternary ammonium compound, is also present in the composition of Kemphor. It is an antimicrobial agent with strong effects on a wide range of Gram-positive and Gram-negative bacteria [35]. Since gingival inflammation and dental plaque are directly caused by the activity of microorganisms, such antimicrobial agents can significantly decrease GI and control dental plaque.

In the present study, GI and PI were not significantly different between CHX and Kemphor groups at 2 weeks. However, at 4 weeks, GI was significantly lower in Kemphor group than CHX group, which may be due to the activity of herbal compounds and cetylpyridinium in the composition of Kemphor. In the present study, tooth staining significantly increased at 2 and 4 weeks, compared with baseline in the CHX group. However, tooth staining at 4 weeks was the same as that at 2 weeks (except for stain extent). Tooth staining due to the use of CHX

has been extensively reported in the literature [14,16,36]. Breakdown of CHX molecule releases para chlorophenyl, which deposits on the tooth and soft tissue surfaces, causing a yellow to brown discoloration. Also, CHX triggers the Maillard reaction as the result of which, melanoidin brown pigments are produced from foods and cause tooth discoloration. Formation of pigmented metal sulfides following denaturation of pellicle proteins caused by CHX is also responsible for tooth discoloration. Deposition of chromogens in the diet along with CHX is another mechanism of tooth staining by CHX [6].

In the present study, tooth staining significantly increased at 2 and 4 weeks, compared with baseline in Kemphor group, with no significant difference between 2 and 4 weeks. This finding can be due to the presence of CHX in the composition of Kemphor. However, a significant difference was noted in tooth staining between CHX and Kemphor groups, such that tooth staining at 2 and 4 weeks was significantly lower in Kemphor group. This finding can be attributed to lower concentration of CHX in Kemphor compared with the pure CHX mouthwash used in our study since evidence shows higher tooth discoloration in use of higher concentrations of CHX [16]. Moreover, other constituents of Kemphor may interfere with tooth staining. Kumar et al. [37] showed that sodium fluoride and chloride decreased tooth staining by CHX. Sodium fluoride plays a role in tooth whitening and can decrease or even eliminate tooth stains [38]. Also, evidence shows that mouthwashes containing chloride decrease dental calculus, which has a direct correlation with tooth staining [39]. Moreover, zinc ion prevents plaque formation and decreases tooth staining as such [37]. Zinc chloride is used as stain remover in the composition of Kemphor. However, further studies are still required on the mechanism of action of zinc chloride in elimination of tooth staining. Mauland et al. [40] assessed the side effects of 0.2% CHX mouthwashes with and without an anti-discoloration system after periodontal surgery. They found that the CHX mouthwash without an anti-discoloration system resulted



in significantly lower plaque and gingival index. Considering the crossover design of this clinical trial, the carryover effect was also evaluated to make sure that the washout period completely eliminated the effects of previous treatment.

The results revealed no significant carryover effect on any parameter. Thus, the results reported for the CHX group could be attributed to the pure effect of using CHX. The same was true for Kemphor. The period effect on the parameters was also analyzed to find out whether the passage of time affected the parameters or not. The results showed no significant period effect on GI. However, this effect was significant on tooth staining. But, since the effect of both mouthwashes on tooth staining was significant, the period effect could be neglected. Small sample size was a limitation of this study. Similar future studies with a larger sample size are required on other mouthwashes containing stain removers.

## CONCLUSION

Kemphor appeared to have higher efficacy for reduction of GI and caused less tooth staining than CHX; thus, it may be suitable for use as an alternative to CHX; however, further investigations on larger sample sizes are required to cast a final judgment.

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## CONFLICT OF INTEREST STATEMENT

None declared.

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