EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 70, 1411:1420, APRIL, 2024
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 06-03-2024 • Accept Date : 15-03-2024 • Available online: 05-04-2024 • DOI : 10.21608/EDJ.2024.273718.2966

EFFICACY OF ALOE VERA VERSUS CHLORHEXIDINE 0.12% MOUTHWASHES FOR MANAGEMENT OF BIOFILM INDUCED GINGIVITIS IN FIXED ORTHODONTIC PATIENTS: A RANDOMIZED CLINICAL TRIAL WITH MICROBIOLOGICAL ANALYSIS.

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ABSTRACT

Objective: The objective of this study was to compare the efficacy of Aloe vera (99%) mouthwash with Chlorhexidine (0.12%) mouthwash as adjunctive to self-performed oral hygiene measures in managing biofilm-induced gingivitis in patients under fixed orthodontic treatment.

Methods: Forty patients under fixed orthodontic treatment with biofilm-induced gingivitis were randomly allocated into one of two groups. **Group I (test group, n = 20)** used Aloe Vera mouthwash**,** and **Group II (control group, n = 20)** used Chlorohexidine mouthwash at 0.12%. All patients received the intervention twice daily for a 14-day treatment period followed by a 14-day treatment-free period. The clinical parameters (plaque index PI, gingival index GI, and gingival bleeding index GBI) were measured at baseline, 14 days, and 28 days. Microbiological sampling was also performed on the elastic rings using thioglycolate media.

Results: There was a statistically significant improvement in all clinical parameters in both groups at 14 and 28 days when compared to baseline. The total percentage of change in PI in Group I was (-45,7%) while Group II was (-37.5%), while the difference in PI, GI, and GBI was not significant between groups at different time intervals with p-value ($p>0.05$).

Conclusion Aloe vera mouthwash was as effective as Chlorohexidine in reducing plaque and gingivitis. So, it can be considered a preventive home care therapy in orthodontic patients due to affordability, availability, and sustainability.

KEYWORDS: Aloe Vera, biofilm-induced gingivitis, Chlorohexidine, Orthodontic treatment.

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INTRODUCTION

Orthodontic treatments may induce oral ecologic changes as orthodontic brackets, wires, as well as other appliance components have a significant role in microbial plaque accumulation. and obstructing plaque removal, thus enhancing gingivitis. Therefore, good plaque control is mandatory during fixed appliance therapy ***(Trombelli et al .2018).***

To enhance mechanical plaque control, a chemical agent, such as an antimicrobial mouthwash is incorporated into the oral hygiene measures as evidenced by a plethora of systematic reviews ***(Prasad et al. 2016)***. **Chlorhexidine mouthwash** (CHX) is a cationic chemotherapeutic agent which has the most effective antiplaque agent; it acts by disrupting the extracellular components and the cytoplasmic cell membrane, inducing intracellular components leakage, and interacting with cytoplasmic components (**Kőhidai et al. 2022).** However, with long-term use, several side effects have been reported including staining of teeth and tongue, temporary alteration in taste perception, burning sensation, an increase in calculus deposits, and genotoxicity of buccal epithelial cells ***(Al-Maweri et al. 2020)***.

Research by **Sayar et al. in 2021** has shown that the use of herbal and natural plant products has great effectiveness in the treatment of many oral diseases. The advantage of using herbal medicine is showing fewer side effects than any other type of medication.

Aloe vera (ALO) or Aloe barbadense is a succulent cactus-like plant that belongs to the Liliaceae family. ALO has many pharmacological benefits that are attributed to its ability to enhance wound healing and host immunoregulatory activity, in addition to its anti□inflammatory, antimicrobial, and antioxidant properties ***(Penmetsa et al. 2019).*** Aloe vera is introduced as a natural antiplaque agent that can be used in the treatment of gingivitis with no associated adverse effects. (***Al Marawi et al. 2020)***.

Only a few studies have investigated the efficacy of ALO in orthodontic patients and recommended that more studies delineate the advantages and disadvantages associated with this herbal mouthwash ***(Mokat et al. 2023).*** Hence, the present study evaluated the anti-plaque and anti-inflammatory effect of ALO mouthwash compared to CHX in the management of biofilm-induced gingivitis in subjects with fixed orthodontic treatment.

MATERIALS AND METHODS

Sample size calculation & Study design

The sample size calculation was based on 1-month clinical trial results compared ALO to CHX mouthwashes ***(*Yeturu et al. 2016)***. The decrease in the mean PI was considered the primary outcome. To detect a reduction from baseline to 28 days in the mean PI of 0.42 (SD 0.40) with a two-sided 5% significance level and a power of 80%, a sample size of 15 patients per group was necessary, considering an anticipated dropout a total of 40 patients were included. This analysis was performed using G*Power (Version 3.1).

This randomized, comparative, triple-blinded, 2 parallel arm, single-centered study was conducted on 40 male and female patients diagnosed by biofilm induced gingivitis on intact periodontium with bleeding upon gentle probing (BOP ≥10) (**Chapple, et al.2018**) under fixed orthodontic treatment (MBT conventional stainless-steel brackets) in upper and lower arches. Systemically healthy subjects classified by the **American Society of Anesthesiologists (ASA)** as class I ***(Mak et al. 2002)*** guided by (**Cornell Medical Index-Health Questionnaire**)***,*** aged 17-35 years old were recruited from the outpatient clinic of the Oral Medicine, Periodontology, and Oral Diagnosis Department, Faculty of Dentistry, Ain Shams University. Ethical approval for the study was obtained from the Research Ethics

Committee, Faculty of Dentistry, Ain Shams University (FDASU – RECIM / R022121) on the 17th of February 2021. Smokers, Uncooperative patients (not attempting to perform self-performed routine oral hygiene measures), patients taking medications that might cause gingival enlargement as a side effect, those who performed professional mechanical plaque control in the last 6 weeks, pregnant or contraceptive usage, as well as subjects with known allergy to mouthwashes' ingredients as mentioned in the health questionnaire were excluded. The procedure was fully explained to the subjects who signed a written informed consent before starting the treatment.

Randomization & grouping:

The forty eligible participants having biofilm-induced gingivitis and fixed orthodontic appliance were randomly allocated into the following two groups, using a computer-generated randomization list created by (www.Randomizer.org). **Group I (ALO) [test group (n=20)]** followed by oral hygiene measures and instructions at baseline including the use of ALO mouthwash (**Kamath et al.2020**). **Group II (CHX) [control group (n=20)]** followed by oral hygiene measures and instructions at baseline including the use of CHX mouthwash 0.12%. All patients received the intervention twice daily for a 14-day treatment period, then followed by a 14-day treatment-free period

Treatment Protocol:

All eligible patients were given oral hygiene instructions and provided with an oral hygiene kit containing a toothbrush, an interdental brush, dental floss, and a tube of non-fluoridated toothpaste. MBT prescription conventional stainless-steel brackets. All orthodontic procedures were performed by the same operator and devices for all patients by an orthodontic specialist. Patients were instructed to use ALO mouthwash (test group) and CHX mouthwash (control group) for 14 days. Patients were instructed not to rinse with water after the mouthwash to keep the effect for a longer period. Then Participants were asked to stop using the mouthwash for 14 days and not to use any type of mouthwash during the treatment-free period. Patients were instructed to follow the tooth brushing technique modified by Bass two for 2 minutes, in the morning and at night ***(Joybell et al. 2015).***

Assessment

Clinical Assessment

The antiplaque PI **(Silness and Löe 1964)** and anti-inflammatory GI **(Löe, 1967)**, GBI **(Carter and Barnes 1974)** effects were measured for all teeth at 6 sites per tooth using a manual Michigan O probe with William's markings,* The clinical parameters were measured at baseline before any interventions**,** after using mouthwash for 14 days, and on day 28 (after 14 days treatment-free period). Mean values of each parameter were calculated per patient and full charting was plotted for each patient in each follow-up interval.

Extension of the dental plaque biofilm was assessed using intraoral standardized photographs using a Nikon D5300 with the f/3.5-5.6GVR II lens with a frontal profile photograph of the patient. In addition, black cheek retractors were used. The photos were recorded at the end of each period after using the disclosing agent as shown in Figure (1). The Plaque Test of Ivoclar Vivadent, Germany company comprises the fluorescent coloring agent fluorescein. Plaque retained on teeth is exposed as a yellow color, while on gingiva appears as a green color. A blue light source is used under which the teeth appear blue, and the surrounding gingival tissues are slightly dark blue. Hence, plaque is easily distinguished from the surrounding gingival tissues **(Mensi et al 2020).**

* **University of Michigan 'o' probe**

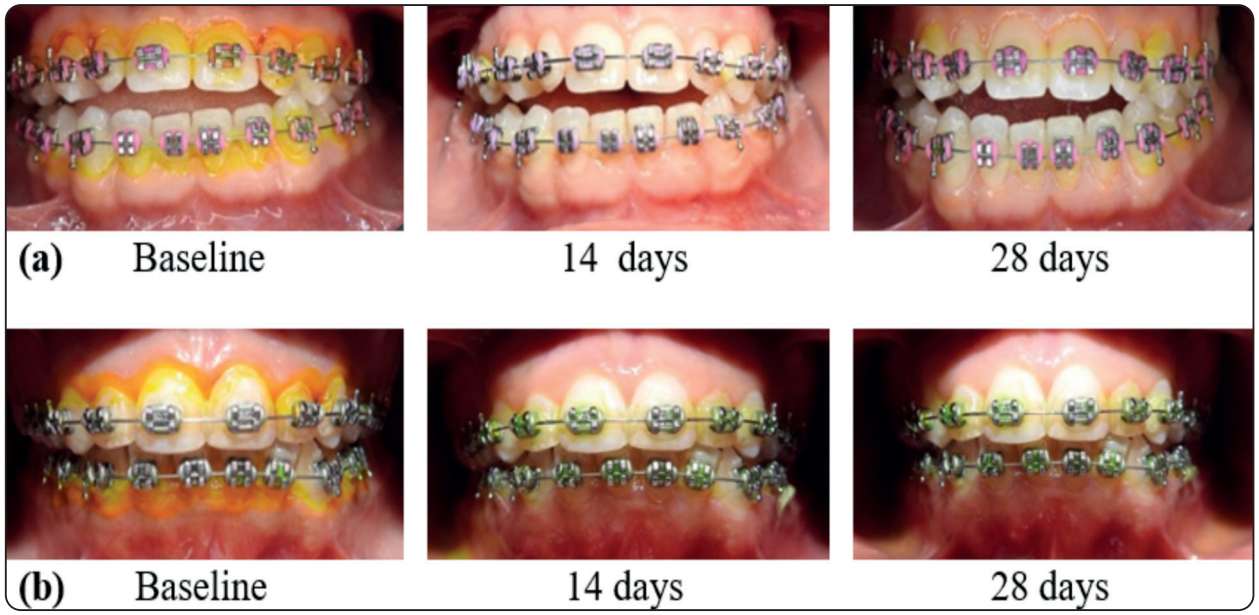

Fig. (1) visual assessment for the extension of dental plaque after rinsing with disclosing agent in patients from (a) study group I (ALO) and (b) control group II (CHX) at different study intervals.

Antimicrobial assessment:

Microbiological sampling was also performed on the elastic rings at the same time intervals used for clinical assessment using thioglycolate media at a temperature of 37ºC, for 24 hours. Then, the optical density was measured by using a 20-spectronic system at 620 nm wavelength ***(Salehi et al. 2006)*.**

Assessment of patient acceptance of the treatment about possible side effects or symptoms.

Patient acceptance was evaluated through a questionnaire. Subjective criteria included (Taste disturbance, burning sensation of tongue, Dryness/soreness, Pruritis/itchiness), and Objective criteria included (Ulcerative lesion, Staining of both teeth and tongue and Allergic reaction). The answer was marked as (0) when the criteria were absent and as (1) when the criteria were present (***Kolhe et al. 2019)*.** Operator perception of side effects was also assessed using the Stain index (SI) ***(Lobene 1968)*,** and the mean SI score and percentage of dental plaque biofilm were then calculated for each case.

Statistical analysis

Analysis of recorded data was done using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as both mean standard deviation and ranges. Also, qualitative variables were presented in numbers and percentages. **The independent-sample t-test** of significance was used when comparing the two means. **A paired sample t-test** of significance was used when comparing related samples. The Comparison between groups with qualitative data was done using both the **Chi-square test** and **Fisher's exact test** instead of the Chi-square test only when the expected count in any cell is less than 5. A p-value of <0.05 was considered statistically significant.

RESULTS

Forty subjects were randomly and equally allocated to one of the two studied groups: Group I (ALO); n = 20 Group II (CHX); n = 20. There were no statistically significant differences between groups regarding either mean age or sex distribution, with a p-value ($p>0.05$). All patients in the study and control group committed to the intervention protocol and the total 3 follow-up visits of the study, there were no dropouts in sampling.

There was a statistically significant reduction in Clinical parameters (PI, GI, and GBI) scores in both

groups at 14 and 28 days when compared to baseline. The PI score showed the highest mean percentage reduction, wherein the ALO group was (45,7%), while the CHX group was (37.5%). A significantly higher reduction of PI and GI was found in the ALO group, while a significant reduction in GBI was found in the CHX group. However, the difference was not statistically significant between groups at baseline, 14 days, and 28 days refer to **(table 1).**

Regarding Microbiology x 10^3 at baseline, 14 days, and 28 days, the difference between groups was not statistically significant. However, in both groups, there was a statistically significant percentage change in the ALO and CHX groups with (98%) and (99.8%) respectively, refer to **Table (2).**

TABLE (1) Descriptive statistics and test of significance for the mean differences and percentage changes of Clinical parameters (PI, GI, GBI) between study intervals.

PI score	**Group I (ALO)**		**Group II (CHX)**		**P-value Between MD**
	MD	**% change**	**MD**	**% change**	
Baseline -14 days Treatment period	-0.66± 0.11	-35.5%	-0.54± 0.11	-30.7%	0.523 ^{NS}
14 days - 28 days Treatment free period	-0.19± 0.03	-15.8%	-0.12± 0.02	-9.8%	0.898^{NS}
Baseline - 28 days Whole Study period	-0.85± 0.16	-45.7%	-0.66± 0.11	-37.5%	0.361^{NS}
Total % change	-45.7%		-37.5%		0.778^{NS}
P-value	0.000^{HS}		0.000 ^{HS}		
GI SCORE	**Group I (ALO)**		**Group II (CHX)**		**P-value**
	MD	**% change**	**MD**	**% change**	
Baseline -14 days Treatment period	-0.62± 0.11	-31.8%	-0.45±0.09	-28.0 %	0.061 ^{NS}
14 days -28 days Treatment free period	0.09± 0.01	6.8%	0.13±0.02	11.21%	0.266^{NS}
Baseline - 28 days Whole Study period	-0.43± 0.08	-22.1%	-0.22±0.04	-13.7%	0.417^{NS}
Total % of change	-22.1%		-13.7%		0.380
P-value	0.000 ^{HS}		0.014^{S}		
GBI SCORE	**Group I (ALO)**		**Group II (CHX)**		**P-value**
	MD	**% change**	**MD**	**% change**	
Baseline - 14 days Treatment period	-0.46±0.08	-24%	-0.43±0.09	-24.2%	0.468 ^{NS}
14 days - 28 days Treatment free period	0.07±0.01	4.79%	0.03±0.01	2.2%	0.491^{NS}
Baseline - 28 days whole Study period	-0.29±0.06	-15.1%	-0.4±0.07	-22.5%	0.106^{NS}
Total % of change	-15.1%		-22.5%		0.285^{NS}
P-value	0.019^{s}		0.000^{HS}		

Using: t-Independent Sample t-test for comparing between two groups

Using: t-Paired Sample t-test for comparing between time intervals

MD: Mean difference

NS: Non-significant; S: Significant; HS: Highly significant

TABLE (2) Descriptive statistics and test of significance for the difference and percentage changes of bacterial load (CFU) between study intervals.

Colony Forming Unit (CFU) Mean ± SD*10^3	**Group I (ALO)**		**Group II (CHX)**		**P-value**
	MD	**% change**	**MD**	**% change**	
Baseline -14 days Treatment period	-27437.5±4664.4	-99.8%	-22567.7±4739.2	-100.0%	0.630^{NS}
14 days - 28 days Treatment free period	7.0±1.0	12.4%	5.0±0.9	9.3 %	0.130^{NS}
Baseline - 28days Whole Study period	-26930.6±5116.8	-98%	-22517.7±3828.0	-99.8%	0.119^{NS}
Total % of change	-98%		-99.8%		0.599 ^{NS}
P-value	0.002^{S}		0.003^{S}		

Using: t-Independent Sample t-test for comparing between two groups

Using: t-Paired Sample t-test for comparing between time intervals MD: Mean difference

NS: Non-significant; S: Significant; HS: Highly significant

None of the patients in the ALO group reported any side effects, so there was a statistically significantly higher frequency of side effects reported by the patient in the CHX group compared to the ALO group. The highest percentage of reported side effects in the CHX group was tongue staining, while itching was the least reported side effect, refer to **Table (3).**

The total percentage change of SI in the ALO group was (0.00%), while in the CHX group was (9.80%) with a high statistical difference between groups at baseline, 14 days, and 28 days as shown in **Table (4).**

TABLE (3) Comparison between two groups regarding side effects.

Side effect	**Group I (ALO)**		**Group II (CHX)**		**p-value**
	No.	**%**	**No.**	**%**	
Taste disturbance	0	0.0%	5	25.0%	0.017^{S}
Burning sensation	0	0.0%	5	25.0%	0.017^{S}
Dryness	0	0.0%	3	15.0%	0.072
Itching	0	0.0%	2	10.0%	0.147
Ulcer	0	0.0%	4	20.0%	0.035^{S}
Staining of tongue	0	0.0%	7	35.0%	0.004^{S}
Allergy	0	0.0%	2	10%	0.147

Using: t-Independent Sample t-test for comparing between two groups

Using: t-Paired Sample t-test for comparing between time intervals MD: Mean difference NS: Non-significant; S: Significant; HS: Highly significant

TABLE (4) Descriptive statistics and test of significance for the difference and percentage changes of SI between study intervals.

SI score	**Group I (ALO)**		**Group II (CHX)**		P-value
	MD	**% change**	**MD**	**% change**	
Baseline -14 days Treatment period	0.02±0.00	1.50%	0.17±0.04	9.8%	0.002^{S}
14 days - 28 days Treatment free period	-0.02±0.000	-1.5%	0.00±0.00	0.00%	0.000^{HS}
Baseline - 28 days Whole study period	0.00±0.00	0.00%	0.17±0.03	9.8%	0.000^{HS}
Total % of change	0.00		9.80		0.020^{S}
P-value	0.635 ^{NS}		0.009^{S}		

Using: t-Independent Sample t-test for comparing between two groups

Using: t-Paired Sample t-test for comparing between time intervals MD: Mean difference

NS: Non-significant; S: Significant; HS: Highly significant

DISCUSSION

Orthodontic patients are more prone to biofilm accumulation resulting in clinical signs of gingivitis. The retention effects of orthodontic appliances limit mechanical biofilm control. Therefore, the use of chemical agents as an adjunct to oral hygiene measures will be a valuable option for these patients ***(Sabatoski et al. 2015)***.

A systematic review showed that chemical plaque control resulted in effective plaque control, decreased bacterial count, and gingivitis in patients undergoing fixed orthodontic treatment ***(Zhang et al. 2018).***

The present randomized controlled and comparative clinical trial aimed to assess both the antiplaque and anti-inflammatory effects of the ALO mouthwash in comparison with CHX in orthodontic patients.

ALO is a potential anti-bacterial and anti-inflammatory agent ***(Chhina et al.2016)***. Moreover, ALO oral hygiene products are widely used in many countries. It is available as toothpaste, gels, and also as mouth rinses ***(Al Marawi et al.2020).*** For this reason, ALO mouthwash formulation was used as an intervention in the test group of this study.

CHX gluconate at 0.12% concentration is the "gold" standard antimicrobial mouth rinse as well as the most effective anti-plaque mouth rinse ***(James et al. 2017). So,*** *CHX (0.12%) was the intervention of choice as a positive control in the current study.*

The primary outcome of the present study was the reduction of plaque scores (PI, SI, Visual extension of plaque) and subsequently gingival inflammation scores (GI, GBI) in fixed orthodontics patients because the main aim of periodontal therapy prophylaxis is the effective plaque control for prevention and treatment of periodontal diseases. Most of the traditional mouth rinses have various side effects. So, the evaluation of the adverse effects of both interventions is one of the objectives of this trial.

In the current study, ALO group showed a significant decrease in PI, GI, and GBI at 14 and 28 days from baseline. The significant decrease in clinical parameters is comparable to those reported by ***Kamath et al 2020,*** who stated that GI, PI, and BOP were significantly decreased when compared to baseline at 21 days and 35 days in both groups. Moreover, **Ayesha** ***et al 2022,*** stated that both CHX and ALO groups showed a reduction in PI and GI scores significantly.

In addition, the results of the present study agreed *with* ***Chandrahas*** **et al.** ***2012,*** who stated that the ALO has shown a significant reduction in PI, GI, and GBI at 7, 14, and 28-day intervals. Also, **Gu*pta et al.2014, Chhina et al. 2016, and Vangipuram et al 2016*** reported that ALO and CHX mouth rinses are equally effective in reducing PI. However, the difference was not statistically significant as all the compounds possessed anti-inflammatory and antibacterial properties.

The results of the current were also comparable to a study done by ***Sharma et al. 2018,*** there was no statistical difference in the results observed when Triphala and CHX were compared in 210 patients after 7 and 15 days ***(Sharma et al.2018).***

The anti-plaque efficacy of ALO is related to its ability to inhibit oral microorganisms such as streptococcus strains, actinomyces viscous, and candida ***(Al-Maweri et al.2020).*** In addition, the ALO contains many anti-inflammatory components that have an inhibitory effect on inflammatory precursors including bradykinin and histidine, thus subsiding inflammation and edema ***(Sayer et al. 2021).***

Moreover, ALO contains hyaluronic acid, mannose-6-phosphate, dermatan sulfate, and vitamin C, which enhance the synthesis of collagen, fibroblast activity, and wound healing, further contributing to its anti-inflammatory properties in gingival inflammation. While CHX depends mainly on its anti-plaque effects in reducing gingivitis ***(*****Almarawi** ***et al. 2020).***

The result of the study done by **Yetru et al 2016** was inconsistent with the result of the present study and showed superior results of CHX in decreasing GI, PI, and GBI in comparison with ALO. However, **yetru *et al 2016*** found that CHX is more effective compared to ALO in decreasing plaque score and gingival inflammation.

The improvement in clinical parameters that subjects of the current trial experienced is not specifically related to the therapeutic properties of the ALO, but also may be associated with a behavioral change; this is known as the Hawthorne effect which commonly exists and may lead to overestimated results. Participants enrolled in oral hygiene studies usually improve their self-performed oral hygiene measures irrespective of the product they receive, and this may have a good impact on the results of the test group ***(Abdulraheem et al. 2018).***

The microbiological analysis showed a significant decrease in both groups at 14, and 28 days compared to baseline with the total percentage of change in ALO group being (98%), while in the CHX group was (99.8%) with an insignificant difference between the two groups. This was inconsistent with **Paul et al. 2019**, who counted the number of colony-forming units (CFUs) on blood agar plates, and reported no significant difference was observed between the CHX and ALO groups in any sites (P = 0.456) (**Paul et al. 2019)**

Moreover, the results of the current study agreed with ***Mohamed et al.2020*****,** who concluded that the total bacterial count in both ALO and CHX was decreased with a significant difference from the baseline to 14 days and 28 days ***(Mohamed et al.2020).***

The antibacterial effect of ALO may be associated with many of its components including ascorbic acid, cinnamic acid, p-coumaric acid, and pyrocatechol. Pyrocatechol, which is a hydroxylated phenol harms microorganisms, this may be attributed to the location and number of hydroxyl groups in the phenol group. Thus, the increase in hydroxylation further increases its toxic effect on microorganisms. Moreover, phenolics work by damaging cell membranes and denaturing proteins. The effectiveness of the phenols increases in the presence of organic material. In addition to its great ability to adhere to the surface for a long time; thus, potentiating its antibacterial and tuberculocidal properties ***(Lawrence et al.2009).***

The results of the present study demonstrate that ALO mouthwash is biocompatible and well tolerated with very limited adverse effects reported in comparison with CHX mouthwash which showed a statistically significant higher frequency of side effects in taste disturbance (25%), burning sensation (25%), ulcer (20%), staining of the tongue (35%) and allergy (10%). In contrast, many participants in the CHX group experienced numerous side effects. ***Chhina et al 2016,*** stated that in CHX group 40% of teeth were stained and 25% of subjects reported taste disturbance compared to ALO group in which no stains and only 2% altered taste perception was observed (**Chhina et al.2016**).

Additionally, ***Gupta et al 2014***, concluded that ALO group has no side effects in comparison to 70% of teeth stains and 65% altered taste perception in CHX group. The findings of the current study reinforced previous studies that reported significant side effects of CHX ***(Durbakula et al 2018, James et al. 2017, Zhang et al. 2018).***

The novelty effect has a great impact, in which participants are motivated by using new substances to improve their oral hygiene measures. However, in other studies, a lack of compliance with the correct use of the mouthwash was observed.

The current study was limited by a short follow-up period and investigating the effect of ALO on specific periodontal pathogens.

CONCLUSION

The current study concluded that ALO mouthwash was as beneficial as CHX and showed promising results in decreasing plaque count, treating gingivitis reducing the number of CFU with no side effects. Therefore, it may have been considered a suitable alternative to CHX.

Funding

Self-funding.

Conflict of interest

All authors declare no conflicts of interest in this study.

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