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CLINICAL TRIAL STUDY

Gingival Inflammatory Indices and Dental Stain Index after Using Aloe Vera-Green Tea Mouthwash, Matrica Mouthwash, or 0.2% Chlorhexidine Mouthwash Compared with Placebo in Patients with Gingival Inflammation

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Abstract:

Introduction:

The purpose of this study was to evaluate and compare the effects of composition of aloe vera-green tea, matrica, and chlorhexidine on gingival inflammatory indices and dental stain index.

Materials & Methods:

In this double-blinded placebo-controlled clinical trial, anti-inflammatory, anti-plaque formation, and dental staining effects of two herbal mouthwashes, including aloe vera-green tea and matrica in comparison with chlorhexidine in 60 patients with plaque induced gingivitis referring to Isfahan University of Medical Sciences, School of Dentistry were evaluated. The indices evaluated in this study were plaque index (Silness and Loe), gingival index (Loe and Silness), bleeding on probing index (Ainamo and Bay) and dental stain index (Lobene stain index). They were evaluated on the first day of using mouthwashes and two weeks later. The obtained data were analyzed using SPSS software version 22. One-way ANOVA, Tukey post hoc, and paired *t* and *Chi*-square tests (α =0.05) were used as appropriate.

Results:

There was no significant difference between the four groups in terms of the mean values of plaque index, gingival index, BOP index, and stain index before the application of mouthwash. However, after mouthwash application, the mean values of indices were significantly different between the four groups. Aloe vera-green tea and chlorhexidine mouthwashes reduced plaque index, gingival index, and bleeding on probing index significantly and there was no significant difference between these two mouthwashes (P>0.05). The effect of matrica mouthwash on plaque index and gingival index was significantly lower than aloe vera-green tea and chlorhexidine (P<0.05). The mean reduction in BOP index was not significantly different between the aloe vera-green tea, chlorhexidine, and matrica groups. Regarding dental stain index, both herbal mouthwashes caused significantly lower dental stain in comparison with chlorhexidine (P<0.05).

Conclusion:

The results of the present study show that aloe vera-green tea mouthwash may be an effective mouthwash owing to its antiplaque and antiinflammatory properties and may be an ideal substitute for chlorhexidine.

Clinical Trial:

The clinical trial code: IR.MUI.REC.1395.3.573

Keywords: Mouthwash, Chlorhexidine, Aloe vera, Green tea, Matricaria chamomile, Gingival inflammation, Tooth discoloration.

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1. INTRODUCTION

Plaque-induced gingivitis is a common disease, which affects more than 90% of all population [1]. The main goal of periodontal disease prevention is to control plaque formation

and prevent gingivitis [2]. However, complete removal of bacterial plaque is quite challenging; therefore, periodontal disease can be prevented by reducing the amount of plaque or changing its composition [3].

Effective prevention of periodontal diseases can be achieved by the mechanical removal of dental plaque using a toothbrush and dental floss. However, a large number of

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people, especially the elderly and disabled populations, may not be able to remove the plaques effectively. Accordingly, different chemical methods have been introduced to facilitate effective plaque control [4]. The gold standard material in chemical plaque control is chlorhexidine. Nevertheless, this substance cannot be used for long periods due to its side effects, such as unusual or unpleasant taste in mouth, tooth/tongue staining, and increased calculus formation [5]. Considering the side effects of chemical mouthwash, there has been a growing interest in using herbal agents with antimicrobial and anti-inflammatory properties [6].

Asphodelaceae is a family of plants that have been used in Greek medicine since ancient times [7]. The medicinal properties of Aloe Vera, which have been examined in animal and *in vitro* studies, include anti-inflammatory, antibacterial, hypoglycemic, anti-arthritis, and anti-ulcer properties. In addition, tissue scar reduction and improved wound healing have been attributed to this plant. Considering these positive characteristics, in addition to simple access, low cost, and no side effects, this plant has become an ideal option for controlling plaque formation and reducing gingivitis [3].

Chandrahas and colleagues showed that aloe vera mouthwash could effectively reduce plaque accumulation, although its anti-plaque and anti-inflammatory effects are less significant than chlorhexidine [3]. Fani and Kohanteb also found that the optimal dose of aloe vera in toothpaste and mouthwash had preventive effects on tooth decay and periodontal disease [8]. Moreover, Kumar and co-workers showed no significant difference in the anti-plaque effects of aloe vera mouthwash and chlorhexidine 0.2% [5].

Tea contains approximately 440 bioactive compounds including polyphenols, which comprise one-third of its components [9]. Naturally, tea exhibits antioxidant, anti-inflammatory, anti-cancer, antibacterial, anti-decay, and anti-gingivitis properties [10]. In addition, green tea has inhibitory effects on the growth of bacteria such as *P. nigrescens*, *P. intermedia*, and *P. gingivalis* [11]. Green tea also reduces the adhesion of *P. gingivalis* to the surface of epithelial cells [12].

Matrica is another herbal mouthwash, which contains chamomile extracts. German chamomile is a plant with antiallergic, anti-inflammatory, bactericidal, and bacteriostatic effects, which can improve wound healing and malodor [13]. Paknejad and others compared the effects of chlorhexidine 0.2% and matrica in patients with chronic periodontitis. They found that chlorhexidine was more effective in reducing pocket depth than matrica, while matrica was more effective in reducing gingival bleeding. Also, both mouthwashes led to tooth staining [14]. Moreover, Kohanteb and colleagues in a study on patients with fixed orthodontic appliance showed that Persica and matrica could effectively reduce the microorganisms around brackets, without inducing any side effects of chlorhexidine, such as tooth staining [15].

With this background in mind, in this clinical trial, the antiinflammatory, anti-plaque, and tooth-staining effects of two aloe vera-green tea and matrica mouthwashes were compared with chlorhexidine mouthwash in patients referred to the School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran.

2. MATERIALS AND METHODS

This clinical trial was conducted on a total of 60 patients (age range, 20-50 years) with gingivitis caused by plaque accumulation, who were referred to the periodontal ward of the School of Dentistry, Isfahan University of Medical Sciences. The inclusion criteria in this study were as follows: 1) systemic health; 2) presence of at least 20 natural teeth in the mouth; 3) presence of all eight anterior teeth (central, lateral maxillary, and mandibular teeth); and 4) O'Leary index greater than or equal to 30% on the first day of mouthwash application (one week after prophylaxis).

On the other hand, the exclusion criteria were as follows: 1) probing depth ≥ 3 mm; 2) severe dental fluorosis; 3) use of anti-inflammatory drugs; 4) cigarette smoking; 5) use of local or systemic antibiotic treatment or antibiotic prophylaxis in the past month; 6) pregnancy; 7) history of allergy to chicory, aloe vera, or green tea; 8) use of anticoagulant drugs (due to the presence of coumarin in chamomile); and 9) restrictions in the use of the modified bass technique for brushing.

After explaining the study procedures to the participants, informed consents were obtained. The participants were allowed to leave the study at any time. Convenience sampling was applied for selecting and assigning the patients to different groups. The patients were then randomly (by random digits table) allocated into four groups of aloe vera-green tea, chlorhexidine, matrica, and placebo (15 patients per group), according to the type of used mouthwash. All of the mouthwashes were bottled in apparently similar containers with opaque covers and none of the patients were informed about the type of the mouthwash they were going to use.

One week before the study, all the patients with gingivitis were subjected to professional cleaning (polishing and scaling if necessary). On the first day of study, plaque index (Silness & Loe) [16], gingival index (Loe & Silness) [17], bleeding on probing (BOP) by sulcus bleeding index (Aainamo & Bay) [18], and stain index (Lobene index) [19] were recorded for all teeth. The participants were trained on how to apply the Modified Bass technique, and its accuracy was examined on the first day of study (one week after training). The use of mouthwash was initiated on the 8th day in the morning after plaque removal or polishing and continued every day for two weeks according to the instructions.

The chlorhexidine, aloe vera-green tea, matrica, and placebo mouthwashes were added to similar bottles and distributed among the participants. The participants were unaware of the content of the bottles. Every day, 50 drops of the aloe vera-green tea mouthwash (Barij Essence, Kashan, Iran) were added to half a glass of water twice a day, once in the morning and once at night, according to the manufacturer's instructions. The mouthwash was rinsed for one minute. On the other hand, in the group using the matrica mouthwash (Barij Essence, Kashan, Iran; IRC1228022708), 50 drops of the mouthwash were added to half a glass of water three times a day in the morning, afternoon, and at night. The mouthwash was rinsed for 30 seconds each time.

In the chlorhexidine group, 10 mL of 0.2% chlorhexidine mouthwash (Iran Nazhu Co., Iran) was used without dilution

twice a day in the morning and at night for one minute. Moreover, distilled water (Barij Essence Co., Iran) was used as the placebo in this study. The participants were asked to avoid foods and drinks for 15 minutes after using the mouthwash. The participants in all four groups were asked to visit two weeks after the start of the study to re-measure gingival inflammatory and tooth staining indices.

Data were analyzed using SPSS software version 22 using one-way ANOVA, Tukey's post hoc test, paired *t* test, and Chi-square test (α =0.05).

3. RESULTS

In this study, 36 women and 24 men with the age range of 20-50 years were recruited. The results of one-way ANOVA showed no significant difference in the mean age of the participants between the four groups (P=0.74). The results of *Chi*-square test also showed no significant difference in the sex distribution of participants between the four groups (P=0.91).

According to the results of one-way ANOVA, there was no significant difference between the four groups in terms of the mean values of plaque index (P=0.78), gingival index (P=0.33), BOP index (P=0.57), and stain index (P=0.14) before the application of mouthwash. However, after mouthwash application, the mean values of plaque index (P<0.001), gingival index (P<0.001), BOP index (P=0.007), and stain index (P=0.007), and stain index (P<0.001) were significantly different between the four groups. The mean indices before and after the use of mouthwash in each group are presented in Table 1.

In the placebo group, the mean value of stain index was not significantly different before and after the use of mouthwash. Also, there was no significant difference in other indices, which highlights the accuracy of negative control in the study. The mean changes in the plaque index, gingival index, BOP index, and stain index before and after the use of mouthwash in the four groups are presented in Table **2**.

Comparison of mean changes based on one-way ANOVA showed significant differences between the four groups in the mean changes of indices before and after the use of mouthwash.

Multiple comparisons using Tukey's post hoc test showed that the mean values of plaque index and gingival index in the aloe vera-green tea and chlorhexidine groups were significantly higher than the values reported in the matrica and placebo groups. On the other hand, there was no significant difference between the matrica and placebo groups or aloe vera-green tea and chlorhexidine groups. The mean reduction in BOP index was not significantly different between the aloe vera-green tea, chlorhexidine, and matrica groups. However, the mean reduction in inflammatory indices was significantly higher in these three groups, compared with the placebo group. The mean changes in the stain index of the chlorhexidine group was significantly higher than the other three groups; nevertheless, the mean changes in the stain index were not significantly different between the other three groups (Table **3**).

4. DISCUSSION

The purpose of this study was to determine and compare the mean values of gingival inflammatory indices and tooth staining before and after the use of aloe vera-green tea, matrica, and 0.2% chlorhexidine mouthwashes. The results showed no significant difference in the mean values of gingival indices between the chlorhexidine and aloe vera-green tea mouthwashes. Based on the findings, the herbal compound did not result in tooth staining. On the other hand, the effectiveness of matrica mouthwash was significantly less than chlorhexidine. There was also a slight decrease in the gingival inflammatory indices of the placebo group, which might be attributed to an increase in the patients' attention to oral hygiene during the use of mouthwash or after plaque removal and prophylaxis. This finding is consistent with the results reported by Jenabian and colleagues [20].

Table 1. The mean values of plaque index, gingival index, bleeding on probing index, and stain index in the groups before and after the use of mouthwash.

0	X7 · 11	Before the Use of Mouthwash		After the Use of		
Groups	Variables	Mean	SD	Mean	SD	- P-value
A1 /	Plaque index	0.82	0.44	0.36	0.34	< 0.001
	Gingival index	1.32	0.28	0.62	0.32	< 0.001
Aloe vera-green tea	BOP index	0.56	0.27	0.28	0.26	< 0.001
	Stain index	0	0	0.02	0.01	0.17
	Plaque index	0.81	0.37	0.24	0.12	< 0.001
Chlorhexidine	Gingival index	1.31	0.40	0.50	0.23	< 0.001
Chiornexidine	BOP index	0.54	0.21	0.21	0.13	< 0.001
	Stain index	0.04	0.02	0.37	0.06	< 0.001
	Plaque index	0.74	0.32	0.55	0.24	0.002
	Gingival index	1.12	0.41	0.74	0.22	0.004
Matrica	BOP index	0.46	0.19	0.25	0.18	< 0.001
	Stain index	0.06	0.03	0.15	0.05	0.054

Gingival Inflammatory Indices and Dental Stain Index after using mouthwash

(Table 1) contd.....

Groups	Variables	Before the Use of Mouthwash		After the Use o	<i>P</i> -value	
	variables	Mean	SD	Mean	SD	<i>P</i> -value
Placebo	Plaque index	0.90	0.49	0.76	0.49	0.06
	Gingival index	1.17	0.31	1.01	0.29	0.11
	BOP index	0.53	0.22	0.46	0.18	0.15
	Stain index	0.09	0.04	0.09	0.04	1

BOP: Bleeding on Probing

Table 2. The mean changes in the plaque index, gingival index, bleeding on probing index, and stain index before and after the use of mouthwash in the four groups.

Index Values Before and After the Use of Mouthwash	Aloe Vera-0	Green Tea	a Chlorhexidine		Matrica		Placebo		<i>P</i> -value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	<i>r</i> -value
Plaque index	-0.46	0.08	-0.57 ^a	0.08	-0.20	0.05	-0.14	0.07	< 0.001
Gingival index	-0.70	0.10	-0.81	0.08	-0.38	0.11	-0.15	0.05	< 0.001
Bleeding on probing index	-0.29	0.06	-0.33	0.05	-0.21	0.03	-0.08	0.03	< 0.001
Stain index	0.02	0.01	0.33	0.06	0.09	0.05	0	0	< 0.001

Table 3. Tukey's post hoc test for multiple comparisons.

Dependent Variable	(I) Solution	(J) Solution	Mean Difference (I-J)	Std. Error	Sig.
		Chlorhexidine	0.11200	0.09850	0.668
	Aloe vera-green tea	Matrica	-0.26133*	0.09850	0.049
Dia ang Judan		Placebo	-0.31933*	0.09850	0.010
Plaque Index	Chlorhexidine	Matrica	-0.37333*	0.09850	0.002
	Chiornexidine	Placebo	-0.43133*	0.09850	0.000
	Matrica	Placebo	-0.05800	0.09850	0.935
		Chlorhexidine	0.11133	0.12535	0.811
Gingival Index	Aloe vera-green tea	Matrica	-0.31600*	0.12535	0.037
		Placebo	-0.54267*	0.12535	0.000
	Chlorhexidine	Matrica	042733*	0.12535	0.006
	Chiornexidine	Placebo	-0.65400*	0.12535	0.000
	Matrica	Placebo	-0.22667	0.12535	0.280
		Chlorhexidine	0.04733	0.05968	0.857
	Aloe vera-green tea	Matrica	-0.08133	0.05968	0.528
Bleeding on probing Index		Placebo	-0.21067*	0.05968	0.005
Bleeding on probing index	Chlorhexidine	Matrica	-0.12867	0.05968	0.148
	Chiomexiaine	Placebo	-0.25800*	0.05968	0.000
	Matrica	Placebo	-0.12933*	0.05968	0.045
		Chlorhexidine	-0.31267*	0.05268	0.000
	Aloe vera-green tea	Matrica	-0.07467	0.05268	0.494
Stain Index		Placebo	0.02000	0.05268	0.981
Stain Index	Chlorhexidine	Matrica	0.23800*	0.05268	0.000
	Chiornexiaine	Placebo	0.33267*	0.05268	0.000
	Matrica	Placebo	0.09467	0.05268	0.286

*The mean difference is significant at the 0.05 level.

The anti-inflammatory and immune-modulating effects of aloe vera have been attributed to polysaccharides [21, 22]. Aloe vera also contains compounds with confirmed antibacterial effects, including pyrocatechol, cinnamic acid, and ascorbic acid [23]. The positive effect of this plant on the plaque index can be attributed to these compounds. On the other hand, one of the main components of green tea is epigallocatechin (EGC), which plays a major role in its antiinflammatory and anti-cancer activities through inhibiting the nitrous oxide cycle [24]. It has also been shown that green tea and catechins have bactericidal activities against oral pathogens [25]. Therefore, the anti-inflammatory and antibacterial effects observed in the present study can be attributed to these components of green tea.

One of the major components of chamomile is bisabolol, which can repair mucosal lesions [26]. In addition, apigenin in the extract of chamomile has anti-inflammatory and antioxidant properties, which inhibits the activities of hyaluronidase, cyclooxygenase, and collagenase, involved in gum inflammation and bleeding [27]. The reduced bleeding during probing can be attributed to this flavonoid. Overall, previous studies have reported different results regarding the antimicrobial and anti-plaque effects of chamomile. These discrepancies might be related to the use of different concentrations of this plant.

The results of the present study are in line with the findings reported by Sargolzai and co-workers, who examined the effects of aloe vera-green tea and chlorhexidine mouthwashes on gingival inflammatory indices [28]. Both mouthwashes significantly decreased the plaque index, gingival index, and sulcus bleeding index, and no significant differences were found in their effectiveness. However, in their study, the effect of aloe vera-green tea mouthwash on the gingival index was greater than that of chlorhexidine, which is contrary to the findings of the present study. In their study, the use of mouthwash was initiated on the day of plaque removal. In fact, the reduction in inflammatory indices could be related to plaque removal. However, in our study, the participants were given one week for the improvement of gum health after plaque removal and polishing.

In the present study, one of the most important adverse effects of chlorhexidine, *i.e.*, tooth staining, was compared with herbal mouthwashes. Unlike chlorhexidine, there was no significant increase in the stain index of aloe vera-green tea and matrica mouthwashes. It is worth mentioning that matrica caused a more significant increase in this index, compared with aloe vera-green tea mouthwash. In this regard, Paknejad and colleagues in their study concluded that matrica, similar to chlorhexidine, led to tooth staining [14], whereas Salehi and co-workers showed that matrica did not cause any tooth staining [15]. So far, no study has examined the staining caused by aloe vera or green tea.

Apart from the study by Sargolzai and colleagues, no other study has yet examined the aloe vera-green tea mouthwash. Nevertheless, each of these herbal compounds has been separately studied *in vitro* and *in vivo*. In 2011, Vidya Dodwad and Komal Arora in a clinical-microbiological study evaluated the effects of aloe vera gel and mouthwash on *Streptococcus* species, *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*. The results indicated that the aloe vera gel was more effective in reducing the bacterial count, compared with the aloe vera mouthwash.

In another clinical study on patients with chronic periodontitis, it was concluded that the effectiveness of aloe vera gel was greater than that of the aloe vera mouthwash [29]. Moreover, Chhina and colleagues in 2016 compared the clinical anti-plaque efficacy of aloe vera with chlorhexidine and reported similar anti-plaque effects. Also, the adverse effects of chlorhexidine were not observed in aloe vera. These findings are consistent with the present results [30].

The improvement of plaque index and bleeding indices after the use of green tea mouthwash is consistent with previous reports by Pistorios and co-workers in 2003 and Hamilton and others in 2001 [25, 31]. Moreover, the findings of this study are consistent with the results reported by Jenabian and co-workers in 2012, comparing the effects of green tea and placebo mouthwashes on the inflammatory and plaque indices [20]. In both studies, the plaque and gingival indices were significantly affected.

The catechin compounds in green tea inhibit the activity of peptidases and collagenases [11]. Also, the low level of tannin and vitamin K can lead to a reduction in the gingival index [10]. The results of the present study are consistent with a study by Radafshar and colleagues in 2015, comparing the effects of green tea and chlorhexidine 0.2%. In both studies, green tea was as effective as chlorhexidine in reducing plaque accumulation and gingivitis, without causing any tooth discoloration [32].

The present study showed that matrica mouthwash had significant effects on the BOP index, similar to chlorhexidine; however, it did not significantly affect the plaque or gingival indices. These findings are inconsistent with the results reported by Pourabas and co-workers in 2010 regarding the effects of matrica on bacterial plaque and gum inflammation, as they showed that this mouthwash had significant effects on plaque accumulation and gingival inflammation [33].

Furthermore, Paknejad and others compared the effects of chlorhexidine 0.2% and matrica in patients with chronic periodontitis during one month of mouthwash application. They concluded that chlorhexidine was more effective in reducing pocket depth than matrica, whereas matrica was more effective in reducing the gingival index, which is comparable to the results of the present study [14]. In addition, Azimi and colleagues in 2016 compared the antibacterial effects of matrica and chlorhexidine in hospital in patients with intra-tracheal tubes and found that chlorhexidine caused a more significant reduction in the accumulation of bacterial colonies, which is similar to the antiplatelet effect reported in this study [34].

One of the major limitations of this study was our inability to blind the participants due to differences in the taste of mouthwashes. Since the exact mechanism for antimicrobial effects of herbal mouthwashes is still unknown, microbiological studies are recommended. In addition, further clinical studies are suggested for comparing combined use of aloe vera and green tea and each herbal product alone. Considering the differences in the frequency of matrica application and its method of use with other mouthwashes, consultation with the manufacturer is suggested to reconcile the differences.

CONCLUSION

Although both herbal mouthwashes had favorable effects on gingival indices, it can be concluded that aloe vera-green tea mouthwash had similar effects to chlorhexidine; however, the effectiveness of matrica was lower than the other two mouthwashes. Both herbal mouthwashes had less significant effects on the stain index. These findings showed that aloe vera-green tea mouthwash could be a suitable alternative to chlorhexidine due to its anti-inflammatory and anti-plaque properties and lack of side effects; on the other hand, matrica was not a proper alternative. Overall, more clinical studies are necessary to support the present findings.

ETHICS APPROVAL AND CONSENT TO PARTICI-PATE

The study protocol was approved by Ethics Committee of Isfahan University of Medical Sciences.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013. (http://ethics.iit.edu/ecodes/node/3931)

CONSENT FOR PUBLICATION

Patients were briefed about the study and signed informed consent forms.

AVAILABILITY OF DATA AND MATERIAL

Data sharing is not applicable in this study as no new datasets were generated in the research.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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