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ORIGINAL RESEARCH

Comparing the effectiveness of Salvia officinalis herbal mouthwash and chlorhexidine in reducing plaque and inflammation: A clinical trial

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Abstract

Background: This study aimed to compare the antiplaque and anti-inflammatory effects of Salvia officinalis (S. officinalis) herbal mouthwash and chlorhexidine (CHX).

Materials and Methods: This double-blind clinical trial was conducted on 63 patients without advanced periodontitis and with at least 20 caries-free teeth without extensive restorations. Patients were assigned randomly to 3 groups (n=21) of 0.2% CHX, S. officinalis mouthwash, and saline. The baseline plaque index (PI) was set to zero by scaling and prophylaxis. After recording the Gingival index (GI), patients learned to use the mouthwash (10 cc) for 60 seconds twice daily for 15 days without using toothbrushing or other plaque control methods. PI and GI were then measured again. Data was analyzed using ANOVA, independent t-test, and paired t-test statistical methods at a significance level of P<0.05.

Results: Groups showed no significant difference in GI (P>0.05) at baseline. At day 15, the PI of the CHX group was significantly lower than the S. officinalis group (P<0.001). The PI of the CHX and S. officinalis groups was also lower than that of the saline group (P<0.05). GI was not significantly different between CHX and S. officinalis groups (P>0.05). The GI of the CHX group was lower than that of the control group (P<0.05) Further significant differences were not noted (P>0.05).

Conclusion: Although inferior to CHX, S. officinalis mouthwash effectively decreased the PI and GI of patients.

Keywords: Salvia officinalis; Chlorhexidine; Gingivitis; Dental Plaque Index

Introduction

Periodontal disease is among the most common infectious diseases caused by complex interactions of subgingival biofilm and immune-inflammatory reactions of the host gingival tissue and periodontium to resist microbial invasion. Gingival inflammation can occur as gingivitis or periodontitis (1,2). Periodontitis is a condition that arises when the periodontal tissues experience damage because of inflammatory-immune responses. Untreated gingivitis can lead to periodontitis, which affects the periodontal ligament and alveolar bone in addition to the gingiva. The presence of bacterial plaque has a significant impact on the formation of gingivitis and periodontitis.

Mouthwashes can effectively supplement toothbrushing and flossing, helping to remove supragingival plaque and prevent gingivitis. A suitable mouthwash should be able to eliminate a wide range of cariogenic and periopathogenic microorganisms without inducing drug resistance. It should also have minimal to no impact on normal oral microflora. (3). Currently, chlorhexidine (CHX) is the gold-standard antimicrobial mouthwash for microbial plaque control (4), which is a biguanide-based chlorophenyl with extensive antimicrobial activity and low toxicity. It is the most effective chemical antibacterial mouthwash approved by the American Food and Drug Administration and American Dental Association recommends dental practices for preventing caries, reducing plaque, disinfecting dentures, and controlling inflammation. (5-7). However, chemical mouthwashes, particularly CHX, have side effects such as tooth staining, unfavorable taste, allergy, altering the sense of taste, oral mucosal burning sensation and dryness, discoloration of tooth-colored restorations, and adverse systemic effects in case of accidental deglutition (6, 8, 9).

Throughout history, medicinal herbs have been used as

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the primary treatment for various ailments. Nowadays, they are often combined with modern medicine and have regained popularity. (10). For those seeking a mouthwash alternative, herbal options are often preferred over synthetic options due to their lower risk of toxicity and side effects when used appropriately. Additionally, herbal mouthwashes are a suitable alternative for patients who are unable or prefer not to use synthetic mouthwashes(8,11-14). Researchers are continuously searching for new and better medicinal plants to improve mouthwash formulations, and to enhance their plaque-fighting abilities.

Sage or Salvia officinalis (S. officinalis) is the most valuable species of the Lamiaceae family with significant therapeutic properties. It is a perennial, evergreen subshrub 30 to 60 cm in height. It naturally grows in dry stony grounds and is native to Asia and North Africa (2). The mincludesperties of sage include its anti-spasm, stringent, relaxant, anti-inflammatory, antiperspirant, and blood glucose-lowering effects (15). Considering the increasing popularity of herbal mouthwashes due to their insignificant side effects in the case of proper use (16), and the complications of synthetic mouthwashes, this study aimed to compare the antiplaque and anti-inflammatory effects of S. officinalis herbal mouthwash and CHX.

Materials and Methods

The research was performed at the Periodontics Department of the School of Dentistry, Islamic Azad University, Isfahan (Khorasgan) Branch with the university›s ethics committee code (IR.IAU.KHUISF.REC.1400.295) and registered in the Iranian Registry of Clinical Trials (IRCT20130812014333N183).

Trial design:

A double-blind randomized clinical trial was conducted in which group 1 used S. officinalis mouthwash, group 2 received CHX mouthwash, and group 3 used saline as a mouthwash. The findings were presented following the CONSORT guidelines for reporting trials.

Participants, eligibility criteria, and settings:

In order to take part in the research, individuals needed to fulfill specific requirements. These requirements involved being within the age range of 20 to 65, possessing a minimum of 20 teeth in good condition without any cavities or major dental procedures, and having moderate gingival inflammation. Participants were excluded of they had a history of systemic diseases, were pregnant or nursing, had taken antibiotics in the last six months, were allergic to antibiotics, had undergone periodontal surgery or scaling in the previous six months, had advanced periodontitis, or smoked. The study included 63 patients who were selected through convenience sampling from those who visited the Periodontics Department of the School of Dentistry at Islamic Azad University, Khorasgan Branch.

Interventions:

Prior to enrollment, every patient received written informed consent. A periodontist diagnosed gingivitis by conducting a dental history review, clinical examination, and measuring clinical attachment loss with a periodontal probe. The plaque index (PI) and gingival index (GI) of each patient were calculated based on Silness and Loe's (17) guidelines. Before the study began, all patients received scaling and prophylaxis to reduce their Pi to zero. Next, the patients were randomly divided into three groups (n=21). Patients in the first group were instructed to use 10cc of 0.2% CHX mouthwash (Iran Naju) twice daily, for 60 seconds each time, for 15 consecutive days, as recommended by the manufacturer. During the 15-day research period, participants were instructed not to use any mechanical plaque control products or toothbrushes. The second group was given S. officinalis mouthwash, and the third group received saline. After the study, the Pi and GI of all participants were reassessed.4 kg of sage was turned into powder, mixed with 6 L of water, and left for 48 hours to extract the essence for S. officinalis mouthwash. We conducted percolation extraction by using 14 litres of solvent. First, we placed a piece of cotton at the bottom of the percolator and added filter paper and glass slabs to ensure homogeneity. The extractions rate was adjusted to 5 mm/minute for every 1 kg of powder until the extract turned colorless. To prevent interference from light, we carefully wrapped every container with aluminum foil to protect the extract. To measure the phenolic compounds, present in the extract, we carefully transferred a volume of 400 µl into a test tube. Next, we added 3 ml of diluted Folin's reagent and placed the solution bain-marie bath set at a temperature of 22°C. After that, we added 3 mm of 6% sodium bicarbonate to the solution and returned it to the bain-marie bath for a total of 90 minutes. To create a concentration calibration curve based on absorbance, we utilized a blank of 400 µl saline and measured the absorbance at a wavelength of 725 nm for each of the standard solutions of gallic acid with concentrations of 50, 100, 150, 200, and 250 µg/ml. Finally, we used the standard curve and the absorbance of the S. officinalis extract to calculate the concentration of phenolic compounds. Synthesis of mouthwash with the aqueous extract of S. officinalis: Experts at the School of Pharmacy, Istahan University of Medical Sciences synthesized a mouthwash using S. officinalis extract with 0.5% phenolic compounds. They used double-distilled saline to reach a volume of 1.8 g of S. officinalis extract with 0.5% phenolic compounds to 2.5L. To improve clarity, they added 20 g of sodium lauryl sulfate, and for stabilization, they added 36 g of methylparaben and 4 g of propylparaben. They also added 0.4 g of mint food coloring, 20 ml of essence, and 80 g of aspartame sweetener to the extract white stirring.

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Synthesis of mouthwash with the aqueous extract of S. officinalis: To synthesize S. officinalis extract with 0.5% phenolic compounds, double-distilled saline was used to reach the volume of 1.8 g of S. officinalis extract with 6% phenolic compounds to 2.5 L. Next, 20 g of sodium lauryl sulfate was added for mouthwash clarity, and 36 g of methylparaben and 4 g of propyl paraben were added for stabilization. Mint food coloring (0.4 g), 20 mL of essence, and 80 g of aspartame sweetener were also added to the extract while stirring. The mouthwash was synthesized by experts at the School of Pharmacy, Isfahan University of Medical Sciences.

Outcomes (primary and secondary):

PI and GI of patients were the primary outcomes of this study. There was no secondary outcome.

Sample size calculation:

The study by Eghbal et al. (16) determined a sample size of 21 in each group with α=0.05, B=0.2, 80% study power, and accounting for 10% dropouts. Patients were chosen through convenience sampling.

Interim analyses and stopping guidelines:

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

Randomization:

Patients were randomly assigned to three groups using a table of random numbers.

Blinding:

The patients were not aware of the type of allocated mouthwash since all mouthwashes were delivered to patients in similar bottles with no name label. The examiner who measured the PI and GI of patients and the statistician who analyzed the data were not aware of the group allocation of patients either.

Statistical analysis:

The Kolmogorov-Smirnov test was applied to analyze the normal distribution of data. The Levene's test was used to assess the homogeneity of variances. Since the normal distribution of data was confirmed (P>0.05), and the assumption of homogeneity of variances was met (P>0.05), comparisons were made by ANOVA (for the comparison of PI at 15 days and GI at baseline), independent t-test (for the comparison of two independent groups), and paired t-test (for comparison with baseline values). All statistical analyses were conducted using SPSS version 24 at a 0.05 level of significance.

Results

Participant flow:

A total of 63 patients participated in this study in 3 groups. The patients were randomly assigned to three groups using a table of random numbers. The mean age of participants was 20-65 years.

Harms:

The study did not result in any harm to patients.

Subgroup analyses:

(Table 1) presents the mean PI and GI of patients in the three groups at baseline and after the intervention. At 15 days, the PI and GI scores were the highest in the saline group followed by the S. officinalis group, and then the CHX group in the studied samples.

Table 1. Mean PI and GI of patients in the three groups at baseline and after the intervention

Variable	Time point	Saline	CHX	S. officinalis	P value
		Mean± SD	Mean± SD	Mean± SD	
PI	Day 15	1.11 ± 0.059	0.79 ± 0.095	0.90 ± 0.045	<0.001
GI	Day 1	0.86 ± 0.113	0.84 ± 0.135	0.86 ± 0.116	0.817
	Day 15	0.89 ± 0.118	0.79 ± 0.123	0.84 ± 0.079	0.027

PI: ANOVA test showed that at 15 days, a significant difference existed in PI of the three groups (P<0.01). Thus, pairwise comparisons were conducted by the LSD post-hoc test (Table 2), which showed that the PI in the CHX group was significantly lower than that in the saline group (P<0.001). Also, the PI in the S. officinalis group was significantly lower than that in the saline group (P<0.001). The PI in the CHX group was significantly lower than that in the S. officinalis group (P<0.001).

GI: ANOVA showed that the GI of the three groups was not significantly different at baseline (P=0.817). However, a significant difference was found in the GI of the three groups at 15 days (P=0.027). Thus, pairwise comparisons were conducted by the LSD post-hoc test (Table 3), which showed that the GI in the CHX group was significantly lower than that in the saline group (P=0.008). No other significant differences were found (P>0.05).

Table 2. comparisons of the groups regarding PI at day 15

Group (I)	Group (J)	Mean difference	P value
Saline	**CHX**	0.320	0.001
Saline	**S. officinalis**	0.207	0.001
CHX	**S. officinalis**	0.113	0.001

Table 3. Pairwise comparisons of the groups regarding GI at day 15

Group (I)	Group (J)	Mean difference	P value
Saline	**CHX**	0.097	0.008
Saline	**S. officinalis**	0.042	0.234
CHX	**S. officinalis**	-0.055	0.124

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The impact of mouthwash type on GI: In the saline group, GI significantly increased at 15 days compared with baseline as shown by paired t-test (P=0.008). However, the results of the t-test showed in the CHX group, GI significantly decreased at 15 days compared with baseline (P=0.031). The results of the t-test showed GI in S. officinalis group decreased at 15 days compared with baseline, but this reduction was not statistically significant (P=0.148).

Effect of type of mouthwash on GI change: The impact of mouthwash type on GI change was observed and analyzed. The results indicated a significant difference among three groups (P=0.003). Further comparison revealed that the CHX group had a greater change in Gl than the saline group (P<0.001). Similarly, the S. officinalis group also had a higher GI change compared to the saline group (P=0.033). However, the difference between the CHX and S. officinalis groups was not significant (P=0.186).

Discussion

There has been a recent increase in interest regarding the use of herbal mouthwashes. One commonly used mouthwash, CHX, has a few drawbacks such as leaving a bitter taste and odor, causing discoloration of teeth and mucosa, and impairing the sense of taste. However, CHX is readily available in Iran. This study aimed to compare the effectiveness of S. officinalis herbal mouthwash and CHX in reducing plaque and inflammation. The study recorded changes in the Plaque Index (PI) after 15 days, as patients had a PI of zero at the study's start. It was not possible to set the Gingival Index (GI) to zero, so the GI values at baseline and after 15 days were compared and reported. The results showed that the mean PI was 0.79±0.09 at 15 days in the CHX group, which was close to the value reported by Mojtahedzade et al, (19) (0.72±0.96). CHX is the standard mouthwash prescribed for periodontal patients and its superior efficacy has been well documented. The mean GI was 0.84±0.13 at baseline in the CHX group, which significantly decreased to 0.79±0.12 at 15 days in the present study. These values were lower than those reported by Ehsani et al, (20) who showed that CHX was significantly superior to a herbal mouthwash evaluated in their study. Variations in the reported results can be due to different study populations and study periods. The mean PI at 15 days was 0.90±0.04 in the S. officinalis group in the present study. This value was 1.23±0.04 in a study by Smullen et al, (21) who used the standard agar diffusion test. They showed that S. officinalis extract inhibited the activity of glycosyltransferase, glucan production, and plaque formation in vitro. They suggested this extract as an anti-plaque agent. Their results were in line with the present findings.

The mean GI in the S. officinalis group was 0.86±0.11 at baseline and 0.84±0.09 at 15 days in the present study. These values were lower than the corresponding values (1.83±0.04 and 1.78±0.02, respectively) in a study by George et al (22). The reduction in GI was not significant in the present study. However, George et al. (22) reported a significant reduction of GI in the group using S. officinalis toothpaste. They reported that S. officinalis toothpaste was as effective as regular kinds of toothpaste in plaque control and reduction of GI. Although their findings were in line with the present results, differences in the efficacy of S. officinalis in the two studies can be due to the use of different forms of extract (mouthwash versus toothpaste) and different concentrations. Moreover, it may be due to scaling treatment conducted at baseline and its effect on the measurement of GI at 15 days. If the study period were longer, this difference could become significant.

Based on the study, it was observed that the group treated with CHX exhibited lower PI levels compared to the saline and S. officinalis groups after 15 days. Furthermore, the S. officinalis group recorded a significant decrease in PI levels in comparison to the saline group. Another study conducted by Eghbal et al. (16) revealed that S. officinalis displayed more efficacy in combating common oral bacteria than CHX, with optimal antibacterial effects. Furthermore, Beheshti-Rouy et al. (23) analyzed the clinical effects of S. officinalis mouthwash on the count of Streptococcus mutans in plaque samples taken from 71 students aged between 11-14 years. The results showed that the use of S. officinalis mouthwash significantly decreased the count of dental plaque Streptococcus mutans. The researchers suggest that S. officinalis mouthwash can be used as an adjunct for plaque control and caries prevention.

In the present study, the change in GI was significantly greater in the CHX and S. officinalis groups than in the saline group. However, the difference in this regard was not significant between the CHX and S. officinalis groups. Alfahdawi et al. (24) assessed the effect of S. officinalis extract on pathogenic bacteria, in comparison with a chemical mouthwash and found that pure S. officinalis essential oil was more effective than its mouthwash and methanolic extract against bacteria; its aqueous extract had no significant antibacterial activity. Their results were different from the present findings regarding no significant effect of aqueous extract of S. officinalis, which may be due to differences in geographical region from which S. officinalis was collected, technique of extraction, or concentration of extract used.

In the present study, the GI in the CHX group was significantly lower than the saline group at 15 days. However, the difference in GI was not significant between the CHX and S. officinalis groups at 15 days. Fawzi et al. (25) evaluated the anti-inflammatory

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effects of S. officinalis mouthwash on gingivitis and minor aphthous ulcers at 1, 3, and 6 days after use. They confirmed its anti-inflammatory effects and reported that this mouthwash can be suggested as an effective anti-inflammatory product for patients with gingival inflammation and minor aphthous ulcers.

In the present study, GI significantly increased at 15 days compared with baseline in the saline group while GI significantly decreased at 15 days in the CHX group compared with baseline. This reduction, however, was not significant in the S. officinalis group. The lack of a significant change in GI in the S. officinalis group can be due to the low concentration of extract. Future studies on higher concentrations of this extract and its other formulations (such as toothpaste or varnish) are required to further elucidate this topic.

Although S. officinalis reduced plaque and gingivitis compared to saline, CHX is still the gold standard anti-plaque agent. Considering the favorable results obtained in the present study regarding the positive effects of S. officinalis mouthwash on PI and GI, and the confirmed antimicrobial activity of this extract in previous investigations (16), further studies are recommended on other properties of this mouthwash and its optimal concentration to pave the way for its widespread clinical application.

The focus of this study was limited to examining the effects of the aqueous extract of S. officinalis. However, future studies need to investigate the potential impact of the hydroalcoholic extract of S. officinalis on oral infections, PI, and GI. Additionally, this study only evaluated one concentration of the extract, so further research is needed to compare the effects of different concentrations. Although this study compared the mouthwash's impact on PI and GI to CHX, future research should evaluate its effects on other clinical parameters and compare it to other commercially available mouthwashes. Lastly, it would be advantageous to compare the effects of S. officinalis to other herbal mouthwashes such as Persica.

Conclusion

Although S. officinalis mouthwash was found to be effective in reducing the PI and GI of patients, its efficacy was still lower than that of CHX.

Conflict of Interests: None

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