



Efficacy of Oregano Essential Oil Mouthwash in Reducing Oral Halitosis: A Randomized, Double-Blind Clinical Trial

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ABSTRACT

Background: Halitosis is an unpleasant condition affecting people of all ages that can have negative impact on their social life.

Aim: to evaluate the efficacy of mouthwash containing oregano essential oil in the reduction of oral halitosis compared to Chlorhexidine and distilled water.

Methods: A randomized, double-blind, parallel clinical trial was conducted on 54 dental students with oral halitosis. They were randomly allocated into 3 groups (Oregano, Chlorhexidine and placebo) and instructed to rinse with 15 ml of the assigned mouthwash twice daily for 7 days. Halitosis was measured using two organoleptic methods and BANA (*N*-benzoyl-DL-arginine-naphthylamide) test at baseline and after 7 days.

Results: Both Chlorhexidine and Oregano Groups showed significant reduction of Organoleptic tongue and floss scores and BANA test scores after 7 days while no significant difference was found in placebo group. There was no significant difference in all measurements between Chlorhexidine and Oregano group after 7 days.

Conclusion: It can be concluded that oregano essential oil mouthwash was effective in reducing oral halitosis. It was found to have similar efficacy to CHX in the reduction of oral malodor with no side effects.

Key words: Oregano, Chlorhexidine, Mouthwash, Oral halitosis

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INTRODUCTION

Halitosis refers to any type of unpleasant scent felt from the breath of a person during exhalation and speech [1]. Oral malodor has been known since the Greek and Roman times. The importance of preventing bad breath was described by Islamic and Jewish religions and using of Siwak is considered as a religious practice by Muslims [2]. It is probably the third most common cause for visiting the dentist after dental caries and periodontal diseases due to the negative effects on the patient communication and social life [3].

Halitosis is believed to affect about 25% of the world population [4]. It can be classified

into oral and extraoral in origin. Genuine oral halitosis contributes to more than 80% of the cases and results mainly from tongue coating and periodontitis [5]. Bacterial degradation of the proteins from food, saliva and exfoliated cells are primarily responsible for the production of the volatile sulfur compounds (VSC) in the oral cavity [6]. These bacteria are mainly gram-negative anaerobes like *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythus* and *Fusobacterium nucleatum* [7]. Extraoral halitosis is less frequent and results from systemic diseases, respiratory or gastrointestinal disturbances and metabolic disorders [8].

Oral Halitosis can be assessed by various methods including organoleptic method, gas chromatography, sulfur monitoring and BANA (*N*-benzoyl-DL-arginine-naphthylamide) test [9]. The Organoleptic method is considered

the gold standard in the assessment of halitosis. It's reliable, easy, inexpensive and detect a large variety of odors but less objective compared to gas monitoring devices [10].

The treatment of oral halitosis is mainly directed toward mechanical cleaning and using chemical agents to reduce the microbial load, mask the malodor and decrease the volatility of the malodorous compounds [11]. Mouthwashes are the most used delivery system for the chemical agents because of their simplicity in use and social acceptance [12]. Chlorhexidine (CHX) is considered as the gold standard in the treatment of oral malodor due to its antibacterial activity and substantivity, but it can be used only for short period of time because of adverse effects such as teeth staining and altered taste sensation [13]. Hence there is a need to develop an effective naturally occurring and economical alternative that can be used safely for long period of time with no side effects and such aid can be in form of herbs. *Origanum vulgare* (Oregano) is an important medicinal and culinary herbal that has been used for centuries. Oregano essential oil has been reported to have broad spectrum antimicrobial activity against gram negative and positive bacteria, fungi, and viruses [14]. Carvacrol and thymol are the main components of oregano which have a strong bioactivity including antibacterial, anti-inflammatory and antioxidant [15].

The aim of this study was to evaluate the efficacy of mouthwash containing oregano essential oil in the reduction of oral halitosis compared to positive control (CHX) and negative control (distilled water) using two organoleptic methods and BANA test. We hypothesized that oregano mouthwash would be as effective as CHX in the reduction of oral malodor.

MATERIALS AND METHODS

Study design

This study was a double-blind, parallel 3-arm randomized clinical trial conducted at the Department of Periodontology, College of Dentistry, University of Baghdad, Baghdad, Iraq between January 2020, and December 2020. The study was approved by ethical committee/College of Dentistry/ University of Baghdad in accordance with the Tokyo and Helsinki

declaration of human research (Reference no. 131619 in 2\12\2019). This trial was registered at clinicaltrials.gov (NCT04779502).

Study subjects

The subjects were undergraduate dental students at the College of Dentistry, University of Baghdad. Those who met the inclusion criteria were invited to participate in this study.

The inclusion criteria include age between 19-23 years, organoleptic score ≥ 2 based on Rosenberg scale [16], no systemic diseases and had no use of antibiotic for the last 3 months. The exclusion criteria included smokers, alcoholics, probing pocket depth >6 mm, xerostomia, consuming garlic, or onion two days before, patient with orthodontic appliances and dentures, pregnancy and open carious lesions.

All the participants were informed about the purpose and the methods used in the study and informed consent was obtained.

Sample size was calculated based on data from a similar study [2]. At significant level of 5% and probability power of 80% and to compensate for dropouts it was found that sample size of 54 (15 per group) would be enough to reject the null hypothesis.

Study interventions

The test mouthwash was Oregawash (ZANE HELLAS, Greece) containing oregano essential oil and Corsodyl mouthwash (GlaxoSmithKline Trading Limited, England) containing 0.2% CHX was used as positive control and Distilled water was used as placebo. Oregano mouthwash was prepared by diluting each bottle of Oregawash by 1500 ml of water to obtain 0.2% carvacrol concentration. The mouthwashes were dispensed into identical opaque bottles that were assigned a random sequential letter code (A, B, C) by a third party not involved in the study to ensure blindness of both examiner and participants.

Simple randomization was performed by another person using Microsoft excel (Microsoft Corporation, USA) to generating random numbers that were used to resort the order of the mouthwash groups (A, B, C) and the participants ($n=54$) so that each group will receive a similar number of participants ($n=18$) with 1:1:1 allocation. All the participants were

instructed to rinse twice daily with 15 ml of the allocated mouthwash for 30 seconds, after 30 min of toothbrushing with no change to their routine mechanical dental home care.

Oral malodor assessments

One week before the first assessment, the subjects received instructions to be taken for optimal breath analysis. They were not allowed to eat onion, garlic, spicy food, drink alcohol, or smoke 48 h prior to examination, and not to use chewing gum, perfumed cosmetics that could disguise halitosis.

At the day of the assessment the subjects were instructed not to eat or drink for 2 hours before the examination which was done between 11 am and 1 pm. They were told to bring the mouthwash bottle to determine the remaining volume and to evaluate the compliance.

Oral halitosis was assessed using organoleptic method and BANA test at baseline and after 7 days. Two organoleptic methods were used: 1.Tongue odor test used to determine the halitosis originating from the dorsum of the tongue in which cotton roll was used to scrape the posterior dorsum of the tongue and after 5 seconds the odor is assessed by holding it 5 cm away from the nose [17]. 2.Dental floss odor test used to determine the halitosis originating from the interdental areas. Dental floss pick was passed into the interdental regions of all the teeth and the floss odor is assessed by holding it about 3 cm away from the nose [18].

All the measurements were performed by a single examiner using Rosenberg scale (six-point scale).

BANA test is a rapid test used for the detection of the red complex bacteria (*Porphyromonas gingivalis*,*Treponema denticola* and *Tannerella forsythus*) which are responsible for halitosis due to their proteolytic activity.

Samples from the posterior tongue dorsum was taken on a cotton swab then place on lower part of the BANA strip. The upper part of the strip was

dampened with distilled water with a sterile swab. The strip was then folded and placed in BANA processor for 5 minutes after that it was examined for color change and scored as follows: no color change: 0, light blue: 1, dark blue: 2.

Data analysis

Data analysis was performed using Statistical Package of Social Science (SPSS) (version 26, IBM, USA). Shapiro-Wilk test was used to assess the data for normality of distribution. The data obtained from organoleptic tongue and floss scores and BANA test failed to show normality and were described by median and quartiles. Intergroup comparison for the variables at baseline and after treatment was done using Kruskal-Wallis test. Then, Bonferroni post hoc test was used to determine the difference between each pair of the interventions. Intragroup comparison for the variables between baseline and after treatment was done using Wilcoxon signed-rank test. The mean percentage reduction was calculated using the formula $[(\text{pre} - \text{post}) / \text{pre}] \times 100$ for various variables. The Spearman's rho rank correlation coefficient was used to show the correlation between the different variables at baseline. The statistical differences were significant when $P < 0.05$.

RESULTS

A total of 90 participants were assessed for oral halitosis and only 54 patients met the inclusion criteria and completed the study. Demographic data of the study groups at baseline are shown in Table 1.

Organoleptic tongue measurements

There was no statistical difference between the three groups at baseline ($P=0.81$). Intragroup comparison between baseline and after 7 days showed statically significant reduction of organoleptic tongue scores in CHX group ($P=0.0001$) and Oregano Group ($P=0.0001$). While placebo group showed no significant difference ($P=0.05$) (Table 2).

Table 1: Baseline Demographic data of the study groups.

Variable	Groups			Total	
	Oregano	Placebo	CHX		
Gender	Male (N%)	8(44.4)	11(61.2)	6(33.3)	25(46.3)
	Female (N%)	10(55.6)	7(38.8)	12(66.7)	29(53.7)
	Age (mean \pm SD)	21.5 \pm 1.2	21.3 \pm 1.1	22 \pm 0.9	21.6 \pm 0.2

Organoleptic floss measurements

Intergroup comparison showed no significant difference at baseline ($P=0.66$). Intragroup comparison between baseline and after 7 days showed significant reduction of organoleptic floss scores in CHX group ($P=0.0001$) and Oregano Group ($P=0.0002$) while Placebo group showed no significant difference ($P=0.07$) (Table 3).

BANA test measurements

At baseline there was no significant difference among the groups ($P=0.5$). Intra group comparison between baseline and after 7 days

showed significant reduction of BANA test scores in CHX group ($P=0.001$) and Oregano Group ($P=0.002$) while there was no significant difference in placebo group ($P=0.65$) (Table 4).

Correlation

Spearman correlation used to determine correlation between variables at baseline. Strong positive correlation was found between organoleptic tongue scores and BANA test ($r=0.56$) and between organoleptic tongue scores and organoleptic floss scores ($r=0.46$) while weak positive correlation was found between organoleptic floss scores and BANA test ($r=0.25$) (Table 5).

Table 2: Inter and intra group comparison of Organoleptic tongue scores and mean percent reduction.

	Value	Oregano	Placebo	CHX	P value*
Baseline	Median	3	4	4	0.81
	25%	3	3	3	
	75%	4	4	4	
	Mean ± SD	3.50 ± 0.78	3.66 ± 0.84	3.55 ± 0.85	
7 days after	Median	2	3	1	P<0.0001
	25%	1	3	0	Pairwise comparisons
	75%	2	4	1.25	CHX-Oregano 0.08†
	Mean ± SD	1.77 ± 0.54	3.33 ± 0.76	0.94 ± 0.72	CHX-Placebo P<0.0001†
Mean percent reduction		47%	9%	73%	Oregano-Placebo 0.001†
P value **		0.0001	0.05	0.0001	

** Significant at ($p < 0.05$) by Wilcoxon Signed Ranks Test

*Significant at ($p < 0.05$) by Independent-Samples Kruskal-Wallis Test

† Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 3: Inter and intra group comparison of Organoleptic floss scores and mean percent reduction.

	Value	Oregano	Placebo	CHX	P value*
Baseline	Median	3	3	3	0.66
	25%	3	3	2.75	
	75%	4	4	4	
	Mean ± SD	3.38 ± 0.91	3.27 ± 0.75	3.11 ± 0.75	
7 days after	Median	2	3	1	P<0.0001
	25%	1	2	0	Pairwise comparisons
	75%	2	4	2	CHX-Oregano 0.06†
	Mean ± SD	1.94 ± 0.93	2.88 ± 0.90	1.05 ± 0.87	CHX-Placebo P<0.0001†
Mean percent reduction		42%	11%	66%	Oregano-Placebo 0.03†
P value **		0.0002	0.07	0.0001	

** Significant at ($p < 0.05$) by Wilcoxon Signed Ranks Test

*Significant at ($p < 0.05$) by Independent-Samples Kruskal-Wallis Test

† Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 4: Inter and intra group comparison of BANA test and mean percent reduction.

	Value	Oregano	Placebo	CHX	P value*
Baseline	Median	2	1	1	0.5
	25%	1	1	1	
	75%	2	2	2	
	Mean ± SD	1.55 ± 0.51	1.44 ± 0.51	1.33 ± 0.59	
7 days after	Median	1	1	1	0.005
	25%	1	1	0	Pairwise comparisons
	75%	1	2	1	CHX-Oregano 0.34†
	Mean ± SD	1.0 ± 0.48	1.38 ± 0.60	0.66 ± 0.59	CHX-Placebo 0.002†
Mean percent reduction		35%	4%	50%	Oregano-Placebo 0.18†
P value **		0.002	0.65	0.001	

** Significant at ($p < 0.05$) by Wilcoxon Signed Ranks Test*Significant at ($p < 0.05$) by Independent-Samples Kruskal-Wallis Test

† Significance values have been adjusted by the Bonferroni correction for multiple tests.

Table 5: Spearman correlations.

Variable	Organoleptic tongue scores	Organoleptic Floss scores	BANA test
Organoleptic Tongue scores	1	0.462**	0.565**
Organoleptic Floss scores	0.462**	1	0.258
BANA test	0.565**	0.258	1

**Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

This study was designed to assess the effectiveness of Oregano essential oil mouthwash against oral halitosis compared to CHX and distilled water. The result of this study showed that Both CHX and oregano resulted in the reduction of oral malodor compared to the placebo.

Halitosis was measured using organoleptic method and BANA test of the tongue. Organoleptic assessment of the exhaled air is considered uncomfortable for the examiner and the patient and alternatively organoleptic evaluation of tongue and floss malodor were used [17,19]. The dorsum of the tongue is regarded as the main source of oral halitosis due to the presence of the papillary structures which can retain food and support bacteria [20]. Although tongue cleaning has been reported by many studies to be the most effective in reduction of oral malodor, it only affected the food supply and not the bacterial load [21,22]. Therefore, antimicrobial agents should be used to provide a long-term effect.

CHX group showed the highest reduction of organoleptic tongue and floss scores and BANA test scores after 7 days. This was consistent with the results reported by similar studies on the effect of CHX against oral halitosis [8,17,23]. Oregano group also showed significant reduction of both organoleptic scores and BANA test

results that were comparable to those of CHX mouthwash. The reduction could be attributed to the antibacterial activity of the oregano essential oil. The inhibitory effect of Carvacrol against oral and periodontal pathogens has been demonstrated by many studies [24,25]. In vitro study showed that carvacrol at concentration of 1% inhibited the growth of *Porphyromonas gingivalis* which might explain the reduction in the result of BANA test [26]. Placebo group showed no significant difference between baseline and after 7 days. There was a positive correlation between organoleptic scores and BANA test at baseline and this is consistent with the correlation reported by Pham et al. [27].

Limitations of this study includes the short follow up period of 7 days and relatively small sample size and the target population was dental student with age limit of 18-23 years. Further studies are required to determine the long-term effect of Oregano essential oil mouthwashes on large sample size and different population and age ranges using more objective methods of assessment like gas chromatography.

CONCLUSION

It can be concluded from the results of this study that oregano essential oil mouthwash was effective in reducing oral halitosis. It was found to have similar efficacy to CHX in the reduction

of oral malodor with no side effects. It could be used as safe and effective alternative to CHX in the management of oral halitosis that possibly can be used for long period of time specially when regular follow ups are difficult or in special groups who have difficulties in practicing mechanical oral hygiene.

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