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Article Information



Identifiers and Pagination:

Year: 2019 Volume: 13 First Page: 1 Last Page: 5

Publisher Id: TODENTJ-13-1

DOI: 10.2174/1874210601913010001

Article History:

Received Date: 1/10/2018

Revision Received Date: 8/12/2018 Acceptance Date: 27/12/2018

Electronic publication date: 31/1/2019

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Collection year: 2019

Article Metrics

CrossRef Citations: 0

Total Statistics:

Full-Text HTML Views: 3793 Abstract HTML Views: 1727 PDF Downloads: 448

ePub Downloads: 363

Total Views/Downloads: 6331

Unique Statistics:

Full-Text HTML Views: 2141 Abstract HTML Views: 904 PDF Downloads: 307 ePub Downloads: 217

Total Views/Downloads: 3569



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Abstract

Objectives:

To assess the effectiveness of a myrrh containing mouthwash in dental plaque and gingivitis control in comparison to a commercially available chlorhexidine

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mouthwash.

Methods:

This is a randomized double blinded controlled clinical trial involving 12 systemically healthy subjects, age group 18-30 years in which experimental gingivitis was allowed to develop and subjects were assigned randomly into 3 groups (Normal saline), (1% Commiphora myrrh mouthwash), (0.2% chlorhexidine gluconate mouthwash,). Patients were instructed to use only the mouthwashes for 14 days. Plaque Index (PI), and Gingival Index (GI) were assessed before and after the treatment with mouthwashes.

Results:

Results showed no significant reduction in gingival inflammation scores in all groups but Myrrh group showed the highest reduction in inflammation mean score (0.32). A significant reduction was noticed in Plaque reduction in patients using Myrrh mouthwash comparing to the one before intervention visit. However, no significant differences noticed in other groups.

Conclusion:

This preliminary study showed that Myrrh mouthwash resulted in clinical improvement in plaque reduction and gingival inflammation parameters. Myrrh may be considered as a potential therapeutic agent in treating gingivitis. However, further clinical and laboratory studies are needed.

Keywords: Myrrh, Chlorhexidine, Gingivitis, Gingival index, Plaque index, Bleeding.

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1. INTRODUCTION

Dental plaque is a mass of bacteria that starts accumulating on the surface of a tooth as a sticky biofilm. If adequate control measures are not undertaken, the gradual build-up of plaque over time leads to tooth decay and gingival diseases [1, 2]. The most common form of plaque-induced gingival disease is gingivitis, which causes inflammation and bleeding of the gingiva [3]. Gingivitis does not affect the tissues and bone supporting the teeth and is easily reversible with proper dental hygiene practices. However, if it is left untreated, theinflamed and/or bleeding gums ultimately lead to periodontitis, in which tissue damage and bone resorption occur [3-5]. Hence, there is a need for appropriate plaque control measures to eliminate the bacterial biofilm from the tooth surface and prevent it from recurrence. Dental plaque control can be achieved both mechanically and chemically, either alone or in a combination of these two measures [6]. The use of these procedures has been shown to reduce the initiation or progression of the plaque building process [7, 8]. The most frequently prescribed antiplaque and anti-gingivitis chemical agent is Chlorhexidine Gluconate (CHX) containing mouthwashes [9]. CHX has been reported to be a gold standard antiplaque and anti-gingivitis agent and its effects in combination with or

without mechanical plaque control measures have been a wide area of discussion in the literature [6, 7, 9-12]. However, the long-term use of CHX mouthwashes has been found to be associated with several side effects including teeth discoloration, staining, and burning sensation, mucous membrane irritation, and taste disturbance [9-12].

In recent years, the use of herbs as an antiplaque agent is becoming increasingly popular, either as home remedies or as complementary or alternative medicine. Some studies have been conducted to evaluate the effectiveness of herbal mouthwashes in plaque control to avoid the side effects of CHX [13-16]. These studies suggested that herbal formulations might be similarly effective in plaque control compared to CHX mouth washes. Some studies even described herbal preparations as a superior alternative to CHX considering their less side effect profile [15, 17].

In traditional Arabic medicine, myrrh (a mixture of volatile oil, gum, and resin) is a popular herbal compound that has been commonly used to treat a variety of inflammatory conditions for centuries [18]. It is orally biocompatible and is known to have antimicrobial properties [19]. In dental literature, myrrh has been found to be effective in treating several oral conditions such as gum swelling, aphthous sore mouth, and intramucosal wounds [20, 21]. The antimicrobial activity of myrrh against pathogens of the oral cavity has also been demonstrated [22]. Furthermore, a recent study that included myrrh tincture among other herbal extracts in a mouthwash formulation has reported a significant reduction of plaque and gingivitis than baseline [23]. In comparison to 0.2% CHX mouthwash, myrrh has also been found to be slightly superior in reducing plaque build-up and gingival inflammation [24].

However, existing evidence in support of mouthwash formulation containing myrrh as a single active ingredient against plaque and gingivitis is still limited, and studies that so far investigated the effectiveness of myrrh had many methodological limitations. Therefore, this pilot study aimed to assess and compare the plaque and gingivitis control effects of myrrh with a commercially available CHX mouthwash. We hypothesize that myrrh containing mouthwash formulation may be as effective as commercial CHX mouthwash for plaque and gingivitis control.

2. MATERIALS AND METHODS

2.1. Study Design

This was a randomized double-blinded controlled clinical trial carried out at the periodontics department of the King Abdulaziz University Dental Hospital, Jeddah, Saudi Arabia. The study protocol was reviewed and approved by the ethics committee of King Abdulaziz University (protocol number: 058-15). Aims and objectives of this study were clearly explained to all patients. Written informed consent was obtained from participants prior to inclusion.

2.2. Patient Selection

The participants were asked to answer questionnaires which include medical and dental history and 20 healthy patients who are fulfilling inclusion criteria were considered into this experimental gingivitis study. The inclusion criteria were: 18-30 years old, non-smokers, had dentition of ≥20 teeth and a minimum of 5 teeth per quadrant, no gingival swelling, redness or bleeding (signs of gingival inflammation), no probing depth ≥3 mm, and no clinical attachment loss. Exclusion criteria include systemic diseases, lactation or pregnancy, severe mal-aligned teeth, patients with orthodontic appliances, mouth breathers, patients receiving antibiotic therapy (within past six months) or medication such as aspirin or non-steroidal anti-inflammatory (within past one months), patients use oral prophylaxis since past six months. Patients unable to comply with study appointment schedule were also excluded.

Participants were examined for the first time to confirm eligibility and to do dental screening and examination. The first visit includes supra-gingival scaling if needed, prophylaxis and oral hygiene instructions including brushing and flossing 14 days before the study to ensure gingival health, before the start of the experimental period.

At the second visit periodontal examination was done to ensure gingival and periodontal health and patients were instructed to stop brushing teeth for two weeks.

At the third visit (start of the experimental period, day 0), plaque status and gingival status was assessed by using Silness and Loe plaque index [25] and Loe and Silness gingival index [26] respectively. Participants were assigned randomly by simple randomization method using random number generator into one of the three interventional groups: (Normal saline), (1% Commiphora myrrh mouthwash), (0.2% chlorhexidine gluconate mouthwash,). Patients instructed to continue to refrain from brushing and to use 15 ml from the assigned mouthwash which was given in opaque similar unlabeled bottle twice daily for 1 minute. The bottles were distributed to the patients by a dentist other than the examiner. Examiner and assistant have no access to the patient's code. Patients were instructed not to use any other mouthwash and not to eat or drink 30 minutes after using the mouthwash. They were given measuring cup and instructed to shake the bottle before using. Patients, compliance were evaluated with the help of a follow-up sheet given to them.

After 14 days (day 14) patients were reexamined by the same examiner and PI and GI were remeasured. Throughout the study, patients were asked to record any complaints or side effect. Professional scaling, prophylaxis and fluoride application was done at the end of the study.

2.3. Preparation of Mouthwashes

Myrrh mouthwash (1% g) was prepared at advanced technology dental research laboratory, King Abdulaziz University, Jeddah, Saudi Arabia. Myrrh gum resin crystals as identified by light brown crystals were obtained from a local traditional market in Jeddah, Saudi Arabia. The crystals with less impurity were selected, and 50 g were washed with cold water. Crystals then were dried, then diluted in 5 liters of warm distilled water and kept for 24 hours. The solution then was shaken by ultrasonic shaking for 4 hours. The solution then filtered using filter papers and stored in 1 Liter clean dark opaque bottles [24]. Chlorhexidine gluconate 0.2% (Avalon Pharma, Riyadh) and Normal saline 0.9% Sodium Chloride solution, 500 ml (Pharmaceutical solutions industry, Jeddah) commercially available were obtained and used as positive and negative control respectively.

Table 1. Comparison of mean plaque index and gingival index score at baseline (0 day) and after 14 days.

GI Treatment Groups	GI Baseline (Mean ±SD)	GI After 14 Days (Mean ±SD)	t Statistic	P Value	PI Baseline (Mean ±SD)	PI After 14 Days (Mean ±SD)	t Statistic	P Value
Saline	0.25 ± 0.12	0.18 ± 0.08	1.05	0.36	0.59 ± 0.33	0.70 ± 0.38	-0.87	0.44
Myrrh	0.39 ± 0.37	0.07 ± 0.06	2.05	0.13	0.88 ± 0.15	0.43 ± 0.27	4.90	0.01*
CHX	0.52 ± 0.34	0.36 ± 0.27	2.27	0.10	0.94 ± 0.06	0.72 ± 0.30	1.31	0.27

Table 2. Comparison of Gingival Index (GI) and Plaque Index (PI) at baseline (0 day) and after 14 days for different mouth rinses.

-	Variable	(Saline)	(Myrrh)	(CHX)	_	_
Baseline (0 day)		Mean ±SD	Mean ±SD	Mean ±SD	F	p Value
1.	GI	0.25 ± 0.12	0.39 ± 0.37	0.52 ± 0.34	0.77	0.48
2.	PI	0.59 ± 0.33	0.88 ± 0.15	0.94 ± 0.06	3.13	0.09
After 14 days		_	-	-	_	_
3.	GI	0.18 ± 0.08	0.07 ± 0.06	0.36 ± 0.27	2.84	0.11
4.	PI	0.70 ± 0.38	0.43 ± 0.27	0.72 ± 0.30	1.00	0.40

2.4. Intraexaminer Reliability

The assessment of sulcular depth intraexaminer reliability conducted on a few patients. The examination was done on two visits one week apart. Gingival and plaque indices were done using clinical scenarios and pictures on two different time points. The intraexaminer reliability using the intraclass coefficients were 0.87, 0.91 and 0.84 for gingival, plaque and sulcular depth respectively.

2.5. Statistical Analysis

Statistical analyses were represented as descriptive statistics and performed using the Statistical Package for the Social Sciences (*SPSS*) version 22 (IBM Corp., Armonk, NY, USA) variables were represented as means and standard deviations. Differences between day 0 and 14 were based on Paired t-test with confidence interval of 95%. ANOVA for multiple groups comparison.

3. RESULTS

This study started with a total of 20 patients (13 females and seven males). Three of those did not show up for the second visit, and the five were irregular in compliance and were excluded from the study. The study was conducted with 12 patients (2 Males, and ten females). N = 4 per group. The mean age in years for Myrrh group, chlorhexidine group, and the saline group were 29 ± 12.67 , 23.25 ± 1.5 , 22.75 ± 2.75 years respectively. The study was conducted in 2016.

When comparing results at baseline and after the intervention, there was no significant change in GI in the three interventional groups (p value > 0.05) (Table 1). However, Commiphora Myrrh mouthwash showed the highest reduction in Gingival index mean score (0.32) in comparison to CHX (0.16) and saline (0.07) (P = 0.13) (Table 1). Moreover, the test group (Myrrh group) showed a significant difference (p < 0.05) with respect to PI between baseline and two weeks after intervention (p value < 0.05) (Table 1).

No significant change was detected in the positive control (CHX) or negative control (saline) groups with respect to PI between baseline and two weeks after intervention (Table 2).

For the group comparisons, before intervention (baseline) there was no significant difference between the interventional groups with respect to PI and GI. (p value > 0.05) (Table 2).

4. DISCUSSION

In this study, we assessed the plaque and gingivitis reduction effects of myrrh as a solo agent in an experimental gingivitis model. We compared 0.2% chlorhexidine gluconate, 1% Commiphora myrrh, and Saline mouthwashes clinical parameters. Myrrh and Chlorhexidine were effective compared to placebo in reducing plaque and gingival inflammation clinical parameters.

Chlorhexidine Gluconate, a known antiplaque and antigingivitis agent, has been investigated extensively in the literature [27-29]. Different modes of delivery and concentrations of CHX were studied [27, 28, 30]. Despite the delivery mode, the mechanism of action is the same, CHX disrupts the bacterial cell membrane and interfering with the cell osmolarity causing cell death [31]. 0.2% chlorhexidine was shown to have a more antiplaque effect than 0.12%, but it has more esthetic concerns among other side effects which might affect the patient's compliance [27]. In Saudi Arabia only 0.2% CHX mouthwash is available, and thus it was used as a positive control in this study.

Myrrh has been used by patients widely in Saudi Arabia as an alternative medicine for different conditions and purpos [32]. Several herbal types of mouthwash and a

mouthwash combination which includes Myrrh tincture among other herbal extracts shown to be effective in treating gingivitis [13, 33, 34]. In the present study, we evaluated Myrrh mouthwash as a solo treatment agent. All the participants were instructed to stop all oral hygiene measures except the interventional mouthwashes. Mouthwash amount, duration of use, and frequency were standardized, and the participants were asked to follow the instructions. Randomization and exclusion of significant risk factors such as smoking in which might affects the gingival status were done to reduce confounding. Based on the results of this initial study, Myrrh mouthwash was effective in reducing plaque and gingivitis.

The clinical findings of this study showed that using myrrh mouthwash reduces plaque. Although not statistically significant, it also showed superior results in gingival inflammation in Myrrh group compared to CHX and Saline. In a similar study by Bassiouny *et al.* [24], Their study results although was not statistically significant but it showed superior results for myrrh over Chlorhexidine in the reduction of both plaque and inflammation but was in combination with mechanical cleaning [24].

The reduction in inflammation in Myrrh group and its superior clinical results, when compared to the saline group, may be attributed to its anti-inflammatory properties. Kim *et al.* reported that Myrrh exerted an anti-inflammatory effect by increasing bacterial clearance and inhibiting proinflammatory cytokines production such as IL-1 β , IL-6, and TNF- α [35]. In addition to myrrh anti-inflammatory properties, myrrh showed anti-bacterial properties. In an animal study, the myrrh group showed no bacterial colonization in contrast to the control group when used in oral wound healing [21]. Another group, have found that Myrrh mouthwash had a potent antimicrobial effect against oral flora and some species of microorganisms and results were comparable to some extent with Chlorhexidine Gluconate [36] Both anti-inflammatory and antibacterial proprieties could explain the antiplaque and antigingivitis effect of myrrh. It should be noted, myrrh was used for short-term and in low concentration as a mouthwash to investigate the effect on gingivitis in this study, so its prolonged effect that might influence the results differently was not tested [21].

Myrrh been shown to be biocompatible with oral tissues and promotes healing by promoting earlier remodeling [21]. The healing proprieties of Myrrh may be explained by its induction of maturation and activation of WBCs [37]. Myrrh used previously to treat infection and inflammations traditionally [38]. Moreover, Myrrh has been shown to promote dermal fibroblasts proliferation and increase the expression of the mRNA expression of collagen III [39].

The availability of Myrrh, ease of preparation, effectiveness and fewer side effects could make it an alternative mouthwash. The main limitation of this pilot study is the small sample size. Our study started with twenty participants, in which eight of them were excluded due to failure in following oral hygiene measure. Discontinuation of oral hygiene to develop the experimental gingivitis is difficult and socially not accepted. To the authors' knowledge, this pilot study is the first study to evaluate the effectiveness of myrrh mouthwash as a solo treatment agent for short-term use to reduce plaque and gingival inflammation. Further larger scale and long-term clinical and biochemical studies are needed to confirm the efficacy and safety of myrrh mouthwash on gingival and periodontal health as an alternative to other antiplaque and anti-gingivitis agent.

CONCLUSION

Overall, the results of this pilot study, even with its limitation, support our hypothesis and illustrate that Myrrh mouthwash has potential as an antiplaque and antigingivitis therapeutic agent. Further detailed clinical, laboratory, safety studies are needed to confirm its effectiveness on a larger scale.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was reviewed and approved by the ethics committee of King Abdulaziz University (protocol number: 058-15).

HUMAN AND ANIMAL RIGHTS

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No animals were used in this research. All human 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.

CONSENT FOR PUBLICATION

Written informed consent was obtained from participants prior to inclusion.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Sara Basuhail, Dr. Nora Khan and Dr. Rahaf Alghamdi who helped in data collection and entry.

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