RESEARCH ARTICLE

Effectiveness of Menthol and Chlorhexidine Mouth Rinses on Reduction of Plaque, Gingivitis, and *Streptococcus mutans* Count in Saliva

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ABSTRACT

Introduction: Menthol is a new product made from mint that is the most effective approach to avoid infections. There has been little human research on using menthol as a mouth rinse ingredient, and there is little information on the subject.

Aim: This study aimed to know the effectiveness of menthol and chlorhexidine mouth rinses in reducing plaque, gingivitis, and *Streptococcus mutans* in saliva among 21 to 45 years old subjects.

Material and method: The study is a placebo-control, parallel-group blind randomized clinical trial consisting of a 2-week period. Forty subjects were selected and randomized into 2 groups consisting of 20 subjects: group A (menthol) and group B (chlorohexidine). The subjects were clinically examined for plaque accumulation using the plaque index (Turesky *et al*.) and gingival inflammation using the gingival index (loe and silness). The saliva samples were collected for estimation of *S. mutans* count on the first day of baseline and after the 14th day using an assigned mouth rinse.

Results: Menthol mouth rinse showed lower scores in plaque index, gingival index, and *S. mutans* count compared to the chlorhexidine mouth rinse which was statistically significant ($p = 0.05$). Mouth rinse with menthol is better compared to mouth rinse with chlorohexidine.

Conclusion: Menthol mouthwash has therapeutic potential and can be prescribed by a dentist as part of routine oral hygiene practice.

Keywords: Chlorohexidine, Gingival index, Menthol, Plaque index, *Streptococcus mutans*.

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INTRODUCTION

The tooth and its supporting structures are affected by a variety of diseases, but plaque-induced inflammatory lesions account for the great common disease and are generally grouped into two categories: gingivitis and periodontitis. The development of gingivitis and periodontitis requires the presence of dental plaque. Individuals must adopt strong oral hygiene routines as the cornerstone of successful periodontal treatment. Subgingival plaque, which is derived from supragingival plaque, is also targeted during periodontal treatment.[1]

The mechanical plaque control approach regulates the most reliable oral hygiene measure is toothbrushing. Lack of skill and individual motivation are two factors that restrict the efficiency of tooth brushing. Oral health has also been improved by using a variety of chemical anti-plaque treatments in various formulations. Mouthwashes have been studied for

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anti-plaque characteristics and are a safe, effective delivery route for eliminating microbes and maintaining oral hygiene.[2]

Bis biguanides are an important group of antibacterial agents among the numerous chemical agents. Because of its significant intra-oral bactericidal and bacteriostatic activity, chlorhexidine digluconate is considered as a broad-spectrum antibacterial action. Chlorhexidine (CHX) digluconate has been used in dentistry for 30 years. It is the most promising anti-plaque and anti-gingivitis chemical compound available today. Gjermo *et al*. found that rinsing twice a day with 10 mL of 0.2% CHX reduced the formation of dental plaque.[3] Unfortunately, these advantages come with drawbacks, the most concerning of which is extrinsic tooth discoloration. Gingival desquamation and burning mucosa have been noted in a few instances. There are new chlorhexidine solutions in various strengths and flavors, as well as alternative anti-plaque agents, has been introduced by several side effects connected with its use.[4]

Dental caries is still one of the most common endogenous dental infections, with considerable socioeconomic consequences. Caries is caused by a complicated interaction between carbohydrates in food and cariogenic microbes in oral biofilms, which is influenced by the quality and quantity of saliva and clinically evidenced by demineralization and destruction of tooth hard tissues. All of the microorganisms linked to caries belong to the normal oral microbiota, which was proven by molecular biologic analysis.[5]

The particular plaque theory, the non-specific plaque hypothesis, and the ecological plaque hypothesis are three prominent explanations for the etiology of caries that have been established. According to the ecological plaque hypothesis, caries is the outcome of a shift in the equilibrium of endogenous microbiota caused by changes in local environmental conditions. It is generally believed that all three parameters (microorganisms, the host, and the environment) are the basic requirement for carious lesions to develop and progress. A wide group of microorganisms was identified from dental lesions, of which *Streptococcus mutans*, *Lactobacillus acidophilus*, and *Actinomyces viscosus* may be considered the main pathogenic species involved in the initiation and development of dental caries and periodontitis.[5,6] With the number of oral diseases due to microbial resistance towards antibiotics, menthol is an alternative mouth rinse which is an alternative treatment for periodontitis and carious lesions by reducing the number of causative organisms.

Mentha species and peppermint oil are the most common natural sources of menthol. Menthol is a covalent organic molecule that can be manufactured synthetically or extracted from peppermint or other mint oils. It is often used in oral hygiene products such as toothpaste, tongue spray, and more broadly as a flavoring food agent (as in chewing gum and candy).[7]

It has been shown to have antibacterial activity against a variety of gram-positive and gram-negative bacteria strains.[8] However, most of the knowledge in the literature is based on in vitro studies and on a limited number of clinical trials. It is high time to develop a simple preparation method of a mouth rinse to prove their efficacy towards clinical and microbiological parameters. Therefore, there is evidence that only a few studies have been conducted on humans where menthol has been an ingredient in a mouth rinse, and thus there is less information on its effectiveness. This study was done to compare the effectiveness of menthol mouth rinse and chlorhexidine mouth rinse in reducing plaque, gingivitis, and *S. mutans* count saliva among the patients.

MATERIALS AND METHODS

A total of 40 subjects aged between 21 to 45 year-old were selected and randomized into 2 groups consisting of 20 subjects: group A (menthol) and group B (chlorohexidine). This study was conducted over 6 months and selected the patient for the study based on inclusion criteria. The inclusion criteria for the selection of subjects are the presence of mild to moderate gingivitis, non-tobacco chewers or smokers, non-alcoholic, and should not be under any medication in the last six months. Teeth that were carious/restored, orthodontically banded, and abutments were not included in the study.

The Institutional Scientific Ethical Board granted ethical approval for this study, and all subjects gave their consent. The Institutional Scientific Ethical Board granted ethical approval to this study, and all subjects gave their consent. All the subjects were clinically examined for plaque accumulation using the plaque index (Turesky *et al*.). Gingival inflammation using gingival index (Loe and silness), and saliva samples were collected for estimation of *S. mutans* count on the first day of baseline and after the 14th day using an assigned mouth rinse.

Preparation of menthol mouth rinse

Menthol crystals (molecular formula $C_{10}H_{20}O$) were purchased from Alpha Chemika company. Alcoholic menthol extract mouth rinse (18 mg%) was prepared by dissolving 18 mg menthol crystals in 0.1 mL of 100% ethanol and, making up the volume to 100 mL of sterile deionized water. This menthol mouth rinse was considered as the case group and chlorhexidine digluconate mouth rinse was used as a positive control group. Both types of mouthwash were coded and looked similar to avoid bias.[9]

All of the subjects were advised to keep brushing their teeth as usual, and the assigned mouth rinses were given to the subjects in their respective groups. Depending upon group, subjects were asked to rinse their mouth with 10 mL of menthol mouth rinse twice a day, around 2 to 5 minutes after brushing for 14 days.

Table 1: Distribution of study subjects based on age and gender

Age	*Male*	*Female*
21–21	0	1
26–30	1	3
31–35	3	1
35–40	9	9
41–45	8	5
Total	21 (53%)	19 (47%)

Table 2: Mean gingival index score and plaque index score for baseline and follow-up after 14 days in case and control group

		Mean ± SD	*Standard error mean*	*p-value*	*Significance*
Gingival index score of case group	Baseline	0.38 ± 0.12	0.03	0.000	Highly significant
	Follow-up	0.18 ± 0.10	0.02		Highly significant
Gingival index score of control group	Baseline	0.40 ± 0.10	0.02	0.000	Highly significant
	Follow-up	0.30 ± 0.09	0.02		Highly significant
plaque index score of case group	Baseline	2.5 ± 0.63	0.16	0.000	Highly significant
	Follow-up	1.51 ± 0.40	0.10		Highly significant
plaque index score of control group	Baseline	2.65 ± 0.42	0.11	0.000	Highly significant
	Follow-up	1.95 ± 0.39	0.10		Highly significant

Table 3: Comparison of the difference in the mean reduction of gingival index score and plaque index score for baseline and follow-up after 14 days in case and control group

	Mean ± SD	*Standard error mean*	*p-value*	*Significance*
Gingival index score in case and control	-0.115	0.0473	0.04	Significant
plaque index score case and control	-0.441	0.139	0.008	Significant

Ask the subjects to chew paraffin wax to collect and stimulate the whole saliva and spit into calibrated disposable plastic cups. The sample was dispatched to the microbiology department for estimation of *S. mutans* level. The sample was inoculated on the dry mitis salivarius agar with potassium tillurite medium and bacitracin. The plates were incubated at 37 ℃ in a 5 to 10% carbon dioxide jar for 48 hours. The number of colonies forming units (CFU/mL) of *S. mutans* (CFU/mL) in saliva was determined.

All the data were entered in the MS-excel and the results were analyzed using SPSS version 22.0. A paired sample t-test was applied to compare menthol and chlorhexidine groups, where the significance level was set at $p < 0.05$.

RESULTS

Add total off are a total of 40 subjects who participated in the study between the age of 21 to 45 years. The number of males was 21 comprising 53% and the number of females was 19 comprising 47% (Table 1).

The mean score for the gingival index at baseline was 0.38 for the menthol group and 0.4 for the chlorhexidine group; similarly, after 14 days was 0.18 for the menthol group and 0.30 for the chlorhexidine group (Table 2). The difference in the mean gingival index score between baseline and after 14 days in the menthol group and chlorhexidine group was found statistically significant where *p* is 004 (Table 3).

The mean score for plaque index at baseline was 2.50 for the menthol group and 2.65 for chlorhexidine group; similarly, after 14 days was 1.51 for the menthol group and 1.95 for chlorhexidine group (Table 2). The difference in the mean plaque index score between baseline and after 14 days in menthol group and chlorhexidine group was found statistically significant where p is 008 (Table 3).

The mean *S. mutans* count in saliva at baseline was 1,33824 CFH/mL for the menthol group and 98093 CFH/mL for the chlorhexidine group; similarly, after 14 days 91204 CFH/mL for menthol group and 78946 CFH/mL for chlorhexidine group (Table 4). The difference in the *S. mutans* count in saliva between baseline and after 14 days was statistically significant for the menthol group and chlorhexidine group where *p* is 0.02 (Table 5).

During the research, no negative side effects or mucosal lesions were reported (Table 6).

DISCUSSION

The effectiveness of newer natural or artificial chemical items is proven by several studies on their use in the treatment of oral diseases. Menthol is one of the components which is derived from the mint and is also called peppermint oil. Menthol is a chemical that has awakened the interest of the pharmaceutical and food industries in recent decades. It's a crystalline, transparent, or white-colored terpenoid found in the EO of *Mentha* spp plants like peppermint. It's a terpenoid with a crystalline, transparent, or white appearance found in the EO of *Mentha* species, such as peppermint.[10,11]

Chlorhexidine (CHX) digluconate is the most effective anti-plaque and anti-gingivitis dental medicine. It is reported that rinsing with 0.2% CHX of 10 mL twice a day inhibits the formation of dental plaque and he also documented that it acts as an anti-gingivitis, but it has side effects like extrinsic tooth staining alteration of taste and desquamation of mucosa.

This purpose of the study was to compare the effect of the menthol (18%) and chlorohexidine (0.2%) on clinical parameters

Table 4: Mean streptococci mutans count for baseline and follow-up after 14 days in case and control group

		Mean ± SD	*Standard error mean*	*p-value*	*Significance*
Mean streptococci count of case group	Baseline	133824 ± 51012	13171	0.000	Highly significant
	Follow-up	91204 ± 40606	10484		
Mean streptococci count of control group	Baseline	98093 ± 25837	6671	0.000	Highly significant
	Follow-up	78946 ± 17460	4508		

Table 5: Comparison of the difference in the mean reduction of streptococci mutans count for baseline and follow-up after 14 days in case and control group

	Mean ± SD	*Standard error mean*	*p-value*	*Significance*
Streptococci mutans count in Case & Control	34493	12989.99	0.02	Significant

Table 6: Observation of side effects at 5th, 10th, and 14th day in case and control group

Sl No	*Side effects*	*Case group*	*Control group*
1	Erythematous mucosa	0	1
2	Soreness of mucosa	0	1
3	Bleeding	0	0
4	Mouth burning	0	1
5	Oral dehydration	0	1
6	Desquamation of mucosa	0	2
7	Dark spots on tooth	0	16
8	Taste alteration	0	8

like gingival and plaque indices and microbiological evaluation such as the level of *S. mutans* in saliva.

The current study found that using menthol extract as a mouth rinse resulted in reduction of gingival, plaque indices after the mouth rinsing where the significance *p-value* is <0.001. similarly, CHX mouth rinse had a similar anti-gingivitis and anti-plaque efficacy. The results were in agreement with studies conducted among adults on clinical parameters like means of plaque, bleeding and gingival indices scores were recorded on baseline examination and after the period of rinse by Nidhal Ali *et al.* and they observed that a significant reduction in clinical parameters scores after the use of menthol extract mouthrinse as compared to the use of the CHX group.[9] Few more authors like Shetty PR *et al.*, and Pistorius A *et al.* were also identified that there will be a reduction in gingival inflammation after the use of the herbal extract.[12,13]

The difference noted in the mean score reduction of gingival index and plaque index at the follow-up between the menthol group and chlorhexidine group was statistically significant (p= 0.05). This could be due to the fact that menthol has many mechanisms of action as compared to chlorhexidine.

The present study showed that there is a reduction of *S. mutans* count score from the saliva sample of the menthol mouth rinse users compared to chlorhexidine mouth rinse users.

We would like to conclude that menthol can aid in the killing of pathogenic microbes and aid in the prevention of oral infection. The oral administration of menthol may promote oral health by inhibiting the growth of pathogenic bacteria and modifying mucosal immunity in the oral cavity. Christine AC *et al.*, state that essential oil like eucalyptus and menthol-containing mouthwashes have a better and deeper bacterial reduction in laboratory biofilm models when compared with several other types of mouthwash recommended for daily use and therefore provides support for the clinical superiority in controlling the plaque biofilm.[14,15]

One of the limitations of the study is that a number of microorganisms obtained from carious lesions, such as *S. mutans*, *Lactobacillus acidophilus*, and *Actinomyces viscosus*, have been associated in the formation of dental caries in medical literature. But we have considered only *S.* mutants because it contributes a major role in the initiation and progression of dental caries.

CHX is considered to be the excellent mouthwash because of its notable anti-plaque effects with few local side effects like staining, alteration of taste sensation, etc.[4,12] But based on our study, menthol was more effective compared to chlorohexidine. However, we are not aware of the menthol mouth rinse's adverse effects. Based on this, short-term study there was no report of any adverse effects of menthol mouth rinse. Therefore, we need to progress towards a long-term (12–24 months) randomized clinical trial with a larger population to determine menthol's effectiveness in treating and preventing periodontal diseases and dental caries.

This study concludes that the 18% menthol mouth rinse significantly reduces gingivitis and superior effectiveness towards anti-plaque/antigingivitis compared to the chlorhexidine mouth rinse to be utilized as adjuncts to mechanical oral hygiene practises.

CONCLUSION

In conclusion, the effectiveness of menthol mouth rinse in reducing plaque accumulation, gingival inflammation, and *S. mutans* count is better in comparison to chlorhexidine. Furthermore, no reports about the negative effect of menthol on oral health were reported in the medical literature and in our study. Therefore, menthol mouth rinse had potential therapeutic value to prevent oral disease, and dentists can be recommended to use it in their dental practice.

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