Comparative assessment of Cranberry and Chlorhexidine mouthwash on streptococcal colonization among dental students: A randomized parallel clinical trial

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Abstract

Background: Chlorhexidine gluconate mouthwash has earned an eponym of the gold standard against oral infections, but with certain limitations. There is no effective alternative to Chlorhexidine. Cranberry is known to inhibit bacterial adhesion in various systemic infections and acts as a strong antioxidant. However, it is less explored for its dental use. Hence, there is a need to evaluate its effect against oral infections. **Aim:** The aim was to compare the efficacy of 0.2% Chlorhexidine mouthwash with 0.6% Cranberry mouthwash on *Streptococcus mutans*. **Materials and Methods:** This was a double-blind, randomized parallel group clinical trial. Total sample of 50 subjects, aged 18–20 years, were randomly divided into two groups, Group A (25) and Group B (25) were given 10 mL of Chlorhexidine mouthwash and Cranberry mouthwash twice daily, respectively, for 14 days each. The plaque samples, which were taken from the subjects on 1st day and 14th day, were inoculated on blood agar plates and incubated at 37°C for 24–48 h. Number of streptococcal colony forming units were calculated using digital colony counter. The data were subjected to paired *t*-test and unpaired *t*-test at a 5% significance level. **Results:** (1) Chlorhexidine mouthwash showed 69% reduction whereas Cranberry mouthwash showed 68% reduction in *S. mutans* count. (2) No significant difference was seen between Chlorhexidine and Cranberry mouthwash on streptococci. **Conclusion:** Cranberry mouthwash is equally effective as Chlorhexidine mouthwash with beneficial local and systemic effect. Hence, it can be used effectively as an alternative to Chlorhexidine mouthwash.

Keywords: Chlorhexidine, Cranberry extract, *Streptococcus mutans*

Introduction

Oral diseases induced by dental plaque continue to afflict the majority of the world's population. Among them, dental caries is the single most prevalent and preventable oral infectious disease.[1] Dental caries, a destructive condition of the dental hard tissues, if unchecked, can progress and induce death of the vital pulp tissue, with eventual spread of infection to the periapical area of the tooth and beyond, leading to harmful consequences.

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This ubiquitous disease results from the interaction of specific bacteria and constituents of the diet within plaque (a natural biofilm) formed on tooth surfaces. *Streptococcus mutans* is a key contributor to the formation of cariogenic plaque because this bacterium (i) effectively utilizes dietary sucrose to synthesize large amounts of extracellular polysaccharides, (ii) adheres tenaciously to glucan-coated surfaces, and (iii) is also highly acidogenic and acid tolerant.[2-4]

It is believed that reducing the mass of mutans streptococci in dental biofilm could lower the incidence of dental caries. The use of antiadhesion agents that disengage mutans streptococci from the dental biofilm or interfere with their adhesion, without affecting their viability, may prove clinically advantageous, as selective pressure and overgrowth of resistant bacteria would be avoided.

Current methods of combating caries-associated bacteria are mostly broad-spectrum antimicrobials.[5] Chlorhexidine is still the gold standard for its antimicrobial action and high substantiveness, but side effects, such as pigmentation, taste alteration, and the formation of supragingival calculus limit its continued use.[6,7]

There has been a rising interest in naturally derived biologically active compounds that may have potential therapeutic uses in medicine and dentistry.[8,9] Plants have been used in folk medicine for thousands of years, and even

with the advent of modern medicine, products derived from medicinal plants have been the basis for the development of many new lead chemicals for pharmaceuticals.[9-11] Many currently used antibiotics were discovered by screening natural products and compound libraries against whole organisms, which identified bacteriostatic and/or bactericidal properties. Dental medicine has become especially amenable to plant-derived products, driven by evidence that shows that population which regularly incorporate foods or beverages containing certain phytochemicals into their diet have better oral health.[11]

One such herb, Cranberry (*Vaccinium macrocarpon*), is a shrub that grows in the peat bogs of cold regions of Northeastern North America. It is one of North America's three original fruits, the other two being Concord grape (*Vitus labrusca*; also known as fox grape) and blueberry (*Vaccinium* spp.). Cranberries are sold mainly in the form of fresh produce, dried fruit, juice and encapsulated powders. Cranberry extracts are particularly rich in polyphenols,[12] including flavonoids, which have biological properties that can be beneficial to human health.[13]

The therapeutic applications of cranberries date back to the 17th century, when they were mainly used to relieve scurvy and problems with the stomach and liver.[14] Today, Cranberry juice is commonly recognized as having a preventive effect on urinary infections in women,[15] through the ability of its high-molecular-weight polyphenols (tannins) to inhibit adhesion of the pathogen *Escherichia coli* to the mucosa of the urinary tract.[16-18] These same compounds can also prevent adhesion of *Helicobacter pylori* to the gastric mucosa, thus interrupting a critical stage in the development of gastric ulcers in humans.[19] Some Cranberry extracts also exert an inhibitory effect on the adhesion and infectious capacity of the virus responsible for seasonal influenza.[20] In addition to their impact on certain infectious agents, polyphenolic fractions prepared from cranberries have been shown to inhibit the proliferation of cancerous cells in the mouth, bladder, and prostate and might therefore help to prevent certain forms of cancer.[21,22]

The high molecular weight nondialyzable material (NDM), an active ingredient of Cranberry juice, has shown to reverse the coaggregation of the majority of bacterial pairs; it exhibits tannin-like properties and is highly soluble in water. Precoating of the bacteria with NDM has shown to reduce their ability to form biofilm.[23] Proanthocyanidins and flavonols are the active constituents of Cranberry against *S. mutans*.[12,13,24] A review of the available research suggests that no Indian study has been carried out to check the effect of Cranberry mouthwash *in vivo*.

Taking into consideration, the side-effects of Chlorhexidine and the liking or faith of people for herbal/natural products like Cranberry, the present study was designed to evaluate if Cranberry can be a better choice. The research question was "what is the difference between efficacy of Cranberry and Chlorhexidine mouthwashes on streptococcal colony forming units (CFUs)?" The null hypothesis of the study was that there is no difference in the efficacy of Cranberry and Chlorhexidine mouthwash on streptococcal CFUs. Hence, the present study was conducted with an aim to compare the effect of 0.2% Chlorhexidine mouthwash and 0.6% Cranberry mouthwash on undergraduate dental students.

Materials and Methods

The present study was double-blind, parallel group clinical trial carried out in Department of Public Health Dentistry, ACPM Dental College, Dhule, Maharashtra, India. It included a total of 50 subjects who were enrolled for BDS course (age range of 18–20 years. The ethical clearance for the study was obtained from the ethical committee of the institution, and informed consent was taken from all the participants prior to the study.

Materials used were 0.2% Chlorhexidine mouthwash, 0.6% Cranberry mouthwash, disposable sterile cotton swabs, and sterile test tubes containing saline.

Inclusion criteria

Subjects with good general health, agreement to delay any elective dental treatment including oral prophylaxis, and agreement to comply with the study visits were included in the study.

Exclusion criteria

Subjects with severe mal-alignment of teeth, orthodontic appliances, fully crowned teeth, removable partial dentures; subjects already using mouthwash or dental floss; tobacco consumers, and subjects with medical or pharmacological history that could compromise the conduct of the study were excluded.

Preparation of Cranberry mouthwash

Cranberry extract was procured from Mehta Pharmacy, Ahmedabad. The test mouthwash, that is, Cranberry mouthwash was prepared at the Department of Pharmacology, Annasaheb Ramesh Ajmera Institute of Pharmacy, Dhule. Cranberry mouthwash was prepared by the investigator at 600 mg concentration. This particular concentration was chosen as it produced the maximum zone of inhibition against *S. mutans* among the five different concentrations that were investigated in the previous study (Sethi *et al*., 2011). To prepare a 100 ml of Cranberry mouthwash, 600 mg of Cranberry extract was dissolved in 90 ml of distilled water and 10 ml of alcohol along with 0.1 g $ZnCl_2$, 0.1 g sodium saccharine, 0.05 g menthol, 0.1 g sodium benzoate, and 3 ml of glycerine.

Study design

Based on the data obtained from pilot study and fixing α at 5% ($P < 0.05\%$), β at 20% and power at 80%, the sample

size obtained for each group was 25; thus effective sample size was 50. The entire sample size of 50 subjects were randomly divided into Group A (25 subjects) who were given Chlorhexidine mouthwash® (Welldent, Purple Remedies, Ahmedabad, Gujarat, India) (designated as A) and Group B (25 subjects) who were given Cranberry mouthwash (designated as B) employing lottery method. This was a double-blind study as the investigator was unaware about the sampling of the groups and study subjects were unaware about the mouthwash they were using. At the start of the study period, baseline recordings, that is, streptococcal CFU/ml before using mouthwash was determined for each subject by obtaining plaque samples.

Method of collection of plaque

The patient was asked to rinse thoroughly with plain water, and a jet of water spray was used to eliminate any debris present on the tooth surface. The plaque samples were subsequently obtained from buccal surfaces of premolars and molars of subjects using disposable sterile cotton swabs. The samples were transferred to a sterile tube containing 1 mL of 0.15 M saline solution. These specimens were stored in ice bags at 2°C to prevent denaturation and transported to the lab within 15 min where they were processed immediately.

The plaque samples, dissolved in saline, were inoculated on blood agar plates and incubated in an incubator at 37°C for 24–48 h. Numbers of streptococcal colonies were calculated using digital colony counter (Lab Hosp Colony Counter Digital LHC 06).

Then, subjects were instructed to rinse for 14 days, twice daily, morning after breakfast and night before going to bed, with 10 ml (undiluted) of the assigned mouth rinse for 30 s and then expectorate the rinse. A measuring cup was provided to the children to dispense 10 ml of the assigned mouth rinse.

Again after 14 days, the plaque samples were taken and inoculated on blood agar plates to determine the colony count.

Statistical analysis

Intragroup comparison for evaluation of streptococcal CFU count before and after using mouthwash was done using paired *t*-test, whereas intergroup comparison for difference in reduction between the two mouthwashes was done using unpaired *t*-test.

Results

At baseline, mean no. of CFU/ml for Chlorhexidine group were 44.3 and after use of mouthwash for 14 days were 13.8. Baseline mean CFU/ml for Cranberry group were 41.2 and after 14 days were 13.3. There was statistically significant ($P < 0.001$) difference between baseline mean no. of CFU/ml and after 14 days in both Chlorhexidine and Cranberry groups [Table 1].

For Chlorhexidine group, mean reduction in CFU/ml was 30.4 that is, 69% reduction in CFU/ml after use of Chlorhexidine mouthwash; whereas mean reduction in CFU/ml in Cranberry group was 27.9 that is, 68% reduction after use of Cranebrry mouthwash. This Intergroup comparison, however, did not show any significant difference between Chlorhexidine and Cranberry mouthwash on microbial CFU/ml ($P = 0.07$) [Table 2].

Discussion

Numerous drugs and drug delivery systems have been tested for their effect on dental biofilm formation and maturation. The most common of them contain antibacterial agents, which reduce the number of viable microorganisms in the biofilm.[25] Although effective, such antibacterial applications have several undesirable side effects. Manipulation of the oral bacterial ecology by altering bacterial adhesion in biofilm-without affecting their viability-represents a novel targeting approach. The anti-adhesion strategies are based on antibodies, adhesion site analogs, and receptor analogs.[26] Anti-adhesion agents reduce the total mass of causative microorganisms but do not affect the viability of the oral bacteria, thereby decimating the development of resistant strains or secondary infections. Thus, application of anti-adhesion agents appears to be a promising approach in oral hygiene.

Cranberry juice has been used in herbal medicine as an anti-infection agent, especially for urinary tract infections (UTIs). The NDM constituent of the juice exhibits anti-co-aggregation activity against a variety of oral bacteria.[27] This provided the impetus to assess the effectiveness of Cranberry as an anti-adhesion agent against *S. mutans* for the present study.

Nutrient and antioxidant capacity

Cranberries have moderate levels of vitamin C, dietary fiber and the essential dietary mineral, manganese, as well as a balanced profile of other essential micronutrients [Table 3].

Table 1: Baseline and final microbial CFU/ml of the both the groups

Group	Before use	After use (14 days)	Mean difference	Percentage reduction	*t**	*P*
Chlorhexidine (Group A)	44.3±7.5	13.8±2.4	30.5	69	26.97	<0.001, HS**
Cranberry (Group B)	41.2±5.8	13.3±1.8	27.9	68	32.1	<0.001, HS**

*Significant ($P<0.05$); **Highly significant ($P<0.001$). CFU: Colony forming units

Table 2: Mean and percentage reduction in microbial CFU/ml score for both the groups

Group	Mean reduction	Percentage reduction	*t**	*P*
Chlorhexidine (Group A)	30.5±5.6	69	1.80	0.07, NS
Cranberry (Group B)	27.9±4.3	68		

*Significant (P<0.05); NS: Not Significant. CFU: Colony forming units

Table 3: Nutrient and antioxidant capacity of Cranberry per 100 g

Energy	46 kcal
Fiber, total dietary	4.6 g
Sugars total	4.04 g
Calcium	8 mg
Magnesium	6 mg
Phosphorus	13 mg
Potassium	45 mg
Sodium	2 mg
Vitamin C, total ascorbic acid	13.3 mg
Vitamin A, IU	60 IU
Carotene, beta	36 mcg

Source: USDA National Nutrient Data Base

The present study was designed to compare the efficacy of 0.6% Cranberry mouthwash with 0.2% Chlorhexidine mouthwash on colonization of *S. mutans*. *S. mutans* have a central role in the etiology of dental caries,[28] because these can adhere to the enamel salivary pellicle and to other plaque bacteria.[29] Since Cranberry is known to inhibit bacterial adhesion,[30] the present study assessed the effect of Cranberry mouthwash on adhesion of *S. mutans* to the tooth surface.

All the subjects taken for the study were residing in the same hostel, thereby eliminating the bias occurring due to different eating patterns. Each trial period was restricted to 14 days to prevent tooth staining associated with prolonged usage of Chlorhexidine.

The Cranberry mouthwash in addition to Cranberry contained distilled water, ethyl alcohol, zinc chloride, sodium saccharin, menthol, sodium benzoate, and glycerine. Zinc chloride is known to be an anti-halitosis agent. Sodium saccharin acts as a sweetening agent. Menthol is used as a flavoring agent while Sodium benzoate acts as a preservative. Glycerine is used as a humectant. A mixture of ethyl alcohol and water is used as a solvent. Chlorhexidine mouthwash contained Chlorhexidine, sodium fluoride, and zinc chloride as main ingredients dissolved in a pleasantly flavored aqueous base.

Results of the study showed almost similar effect of both the mouthwashes on *S. mutans* CFU/ml. This indicates that Cranberry mouthwash is equally effective as Chlorhexidine mouthwash with beneficial local and systemic effects as discussed earlier.

The results of this study are in conjunction with that of an earlier study[31] which found out that the Cranberry constituent inhibited the adhesion of streptococci to saliva-coated hydroxyapatite. The data suggested that the ability to reduce *S. mutans* count *in vivo* is due to the anti-adhesion activity of the Cranberry constituent. Other supporting studies[32] concluded that NDM fraction of Cranberry juice inhibited 80–95% of biofilm formation among the streptococci studied (*S. sobrinus*, *S. mutans*, *S. criceti*, *S. sanguinis*, *S. oralis* and *S. mitis*). The anti-adhesion effect of Cranberry on *S. mutans* was also supported by an *in-vitro* study[23] which found significant inhibition zones associated with various concentration of Cranberry extract.

Recommendations

Further clinical trials on Cranberry mouthwash need to be conducted on larger sample size to assess its safety and adverse effects. If further clinical trials on Cranberry mouthwash are conducted using swish and swallow method, there is a wide possibility that along with improvement in oral health, it can have additional systemic benefits like prevention of UTI, urinary bladder cancers or gastric ulcers.

Conclusion

This study, therefore, suggests that herbal products like Cranberry can prove to be effective or better alternatives to Chlorhexidine in improving the oral health with added systemic benefits and minimal side effects. Further scope lies in the long-term evaluation of the advantages and side effects of such herbal extracts.

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