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EFFECT OF GREEN TEA EXTRACT MOUTHWASH ON STREPTOCOCCUS MUTANS COUNT IN HIGH CARIES RISK DENTAL STUDENTS: A RANDOMIZED CONTROL TRIAL

Randa A. El Naggar * *and* Doaa Gamal Ashour **

ABSTRACT

Context: Dental caries has become one of the most prevalent chronic diseases, with streptococcus mutans count as one of the main causatives. Mouthwashes has been one of the most important methods for bacterial count control. There are many mouthwashes that has been available over the counter but unfortunately, they have many side effects and drawbacks, so this necessitates the search for natural mouthwashes that overcome these side effects with similar antibacterial properties.

Aim: The study's objective was to compare how using mouthwashes of green tea extract versus using chlorhexidine affected the number of Streptococcus mutans bacteria.

Study design: A brief, double blinded, randomized control clinical trial.

Materials and Methods: The study was conducted on a sum of 36 dental students of age range 17 to 23 years that has been split up into two groups; green tea group and chlorhexidine group.

Baseline unstimulated saliva samples were collected, and students received instructions to use 5ml of the designated mouthwash to rinse for 7 days two times daily. And finally come back for a salivary sample after the 7 days period and all saliva samples were sent for microbiological analysis.

Results: independent and paired t-tests demonstrated that green tea and chlorhexidine both decreased Streptococcus mutans count significantly, with no significant difference between both mouthwashes after a week.

Conclusion: Results of this study showed that green tea extract mouthwash has similar anti-bacterial activity as chlorhexidine mouthwash which is considered the gold standard, so green tea mouthwash may be used as a substitute for the chemical mouthwashes.

KEYWORDS: Natural mouthwash, Chlorhexidine, Streptococcus mutans count.

* Lecturer in Conservative Department at Ahram Canadian University.
** Lecturer in Conservative Department at New Giza University

INTRODUCTION

One of the most prevalent chronic diseases worldwide is dental caries. It is a multifactorial disease, that results from many factors including anatomy of the tooth, oral bacterial flora and diet [1]. About 3.5 billion individuals worldwide- three out of four of whom reside in middle-income countries- suffer from dental caries, according to the Global Oral Health Status Report from 2022.

The main bacterial agents in caries development according to several studies are Streptococcus mutans and Lactobacillus. In addition, Actinomyces may promote the caries process [2].

According to different studies about these different microorganisms, streptococcus mutans is the most common bacterial species accompanied with dental caries [3], Therefore, improving oral health will result from lowering the concentrations of salivary Streptococcus mutans in addition to mutans in dental plaque [4].

The main method to keep good oral hygiene and decreasing bacterial count is brushing and interdental cleaning, but most people brush in a wrong way or in a time less than that required for complete removal of dental plaque. One of the most secure and efficient ways to get antibacterial and antiplaque effect is by using mouthwash [3]. Mouthwashes could prevent bacterial colonization, adhesion, and metabolic activity, all of which have an impact on bacterial growth [3].

Due to its broad-spectrum antibacterial activity, chlorhexidine mouthwash is the gold standard antibacterial mouthwash[5]. But Lindhe et al. reported several localized side-effects with CHX use, which restricts its use as a therapeutic agent. These side-effects include tooth discoloration, altered taste perception, irritation of oral mucosa, swelling of the parotid gland, and increases formation of supragingival calculi brought on by the accumulation of organic salts and proteins in saliva [6].

A greater percentage of people worldwide are going back to nature by utilizing natural herbal products because of these problems with the chemicals in mouthwashes[5]. Recently, there has been a significant increase in the usage of natural remedies for treating oral diseases.

Due of its numerous clinical applications, one natural antibacterial agent that has received attention is green tea [1]. According to reports, green tea contains high levels of fluoride and catechin, a bioactive constituent that has anti-bacterial, anti-cariogenic, anti-oxidative, and other effects by preventing the growth of streptococcal bacteria and preventing their adhesion to tooth enamel. Moreover, green tea polyphenols act as antibacterial agent, because it inhibits glucosyl transferase enzyme that the oral bacteria use for feeding.

Therefore, the purpose of this study was to assess the effects of using green tea as a mouthwash vs chlorhexidine on Streptococcus mutans count in saliva.

MATERIALS AND METHODS

Subjects:

36 students were recruited from the Ahram Canadian University, which are systemically healthy, not taking any medications affecting the salivary secretion, not smokers, with no allergy to any of the mouthwash components and have a high risk of caries as determined by the ADA caries risk assessment. Students signed the informed consent and received both written and verbal information about the trial.

Study design:

This clinical trial was a randomized and double blinded design with a one-week period. The students were randomly assigned into two groups using www.randmization.com. In the first phase of the study, group 1 students (N = 18) received instruc-

tions that 30 seconds should be spent rinsing with 5 ml of the chlorhexidine mouthwash two times per day (in the morning and before sleeping) for a week (6), and those in group 2 were instructed (N = 18) to rinse with the green tea mouthwash following the same instructions as the other group. In the second test phase, Samples of the unstimulated saliva were collected into a sterile falcon plastic tube over a 3-5-minute period and were instantly sent to the microbiology laboratory at Ahram Canadian University. Students were instructed to report any side effects or compliance problems by using the mouthwash for 7 days in both groups.

Preparation of green tea extract:

Green tea leaves have been air dried and ground to the required size, and finally extracted by ultrasonic extraction method.

In this method, 100 grams of green tea were transferred to a beaker and 200 mL ethanol was added into it. The beakerwas then placed in a clean ultrasonic bath (PCI India) for facilitating extraction process. Extraction (sonication) was performed at room temperature for 40 minutes (7).

Preparation of green tea mouthwash:

The agar dilution technique was employed to ascertain the MIC and MBC; the MIC for green tea was found to be 62.5 mg/mL (8).

In a beaker, the extract was mixed with propylene glycol & Polysorbate, then 70% sorbitol and glycerin were added. After that, Methyl paraben sodium & propylparaben sodium were dissolved in minimum amount of purified water and added to the bulk. Finally, potassium sorbate was blended in with the mixture after being introduced. Then the pH was adjusted to 7. And the final amount was completed with purified water and mixed well for 10 minutes. Both mouthwashes were filtered by a membrane and put into dark glass bottles tightly closed with a screwcap coded either A or B according to the mouthwash used. (Figure 1)

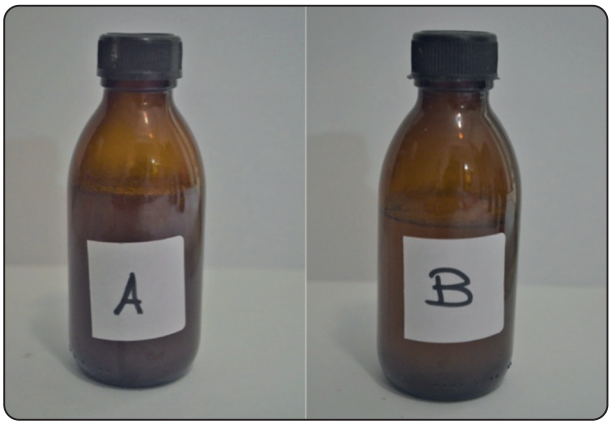

Fig. (1): Mouthwashes in dark glass bottles with codes either A or B according to the mouthwash used.

Saliva samples:

Before using the mouthwash, 2 mL of unstimulated saliva samples were taken. Students were instructed to drool into a sterile falcon tube (Figure 2) while seated upright in the clinic for three to five minutes to gather saliva samples. To avoid any salivary stimulation that would have interfered with the circadian rythm, saliva samples were taken between 10 and 11 in the morning. Additionally, it was instructed to students that they shouldn't eat or drink for an hour before saliva sample collection to reduce salivary stimulation (1).

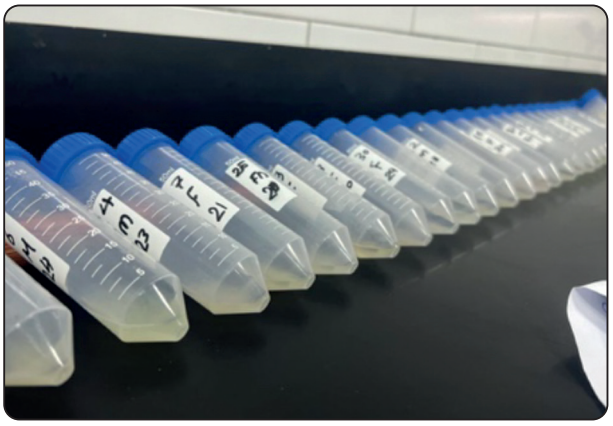

Fig. (2) Saliva samples in Falcon tubes

Microbial evaluation:

The microbiology lab received test tubes with salivary samples in a box with cold packs. CFUs of *Streptococcus mutans* were calculated on nutrient agar (Figure 3).

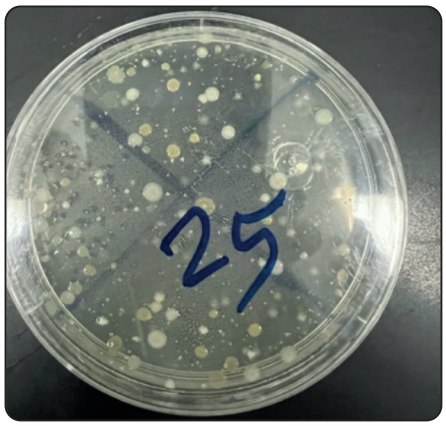

Fig. (3) Streptococcus mutans bacterial colonies on nutrient agar plates

Undiluted samples were inoculated on to nutrient agar plates. At 37°C, these plates were all incubated with atmosphere of 10% CO2. On agar plates, different bacterial colonies had been discovered after 24-48 hours of incubation. The total number of colonies was counted, and the viable count was calculated.

In table (2), all data were reported as minimum, maximum, mean, and standard deviation for both groups. SPSS 16® (Statistical Package for Scientific Studies), GraphPad Prism, and Windows Excel were used to conduct the statistical analysis. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to investigate the given data for normality, and the results showed that the significant level (P-value) was insignificant since P-value > 0.05 showed that the given data came from a normal distribution (parametric data). Consequently, a paired t test was used to compare before and after, whereas an independent t test was used to compare differences between groups.

RESULTS

A randomized control clinical trial with double blinding was performed on 36 dental students. Following the use of chlorhexidine mouthwash, a microbiological analysis of saliva samples revealed a decrease in Streptococcus mutans colony counts from (678.27±467.55) to (205.47±231.75) as P=0.0001* and in green tea there was a statistically significant decrease from (618.27±416.50) to (239.47±275.46) as P=0.0001* compared to baseline. [Table 1].

No statistically significant difference was found between chlorhexidine and green tea when pairwise comparison was performed between both groups using a Paired t test, with difference between them P >0.05. [Table 2].

TABLE (1) Comparison between before and after using Paired t test in both groups:

		Min	Max	M	SD	Paired Differences					P value
						MD	SD	SEM	95% CI		
									L	U	
Group A (CFU/ml)	Before	36.00	1520.00	678.27	467.55	472.80	385.45	99.52	259.35	686.25	0.0001*
	After	0.00	784.00	205.47	231.75						
Group B (CFU/ml)	Before	36.00	1328.00	618.27	416.50	378.80	268.74	69.39	229.98	527.62	0.0001*
	After	0.00	844.00	239.47	275.46						

Min: minimum Max: maximum M:mean SD: standard deviation MD: mean difference
SED: standard error difference CI: confidence interval L:lowere arm U: upper arm *Significant difference as P<0.05

TABLE (2) Comparison between two groups regarding before, after and difference using Independent t test in both groups:

	Group A		Group B		MD	SED	95% CI		P value
	M	SD	M	SD			L	U	
Before (CFU/ml)	678.27	467.55	618.27	416.50	60.00	161.68	-271.18	391.18	0.713
After (CFU/ml)	205.47	231.75	239.47	275.46	-34.00	92.95	-224.39	156.39	0.717
Difference (CFU/ml)	472.80	385.45	378.80	268.74	94.00	121.32	-154.52	342.52	0.445

M:mean ***SD: standard deviation*** ***MD: mean difference*** ***SED: standard error difference***
CI: confidence interval ***L:lowere arm*** ***U: upper arm***

DISCUSSION

The current study's objective was to ascertain how green tea mouthwash has an impact on the quantity of Streptococcus mutans in saliva of dental students. A total of 36 healthy students between the ages of 17 and 21 participated in the study. They represented university students who were at a high risk of developing dental caries because the majority of them spent more than six to eight hours outside each day and frequently consumed sugary foods, candies, and fizzy beverages. Additionally, practicing brushing and other plaque-control strategies while they are away from home is not practical.

Due to its lower content of bicarbonate ions, unstimulated saliva was chosen for collection in this investigation, in order to overcome the bias of the buffering action of saliva.

Green tea has been chosen as the natural herb for formulation of the mouthwash as the previous reports proved that using green tea extract as a mouthwash had valuable effect on decreasing caries incidence by reducing bacterial attachment to the tooth structure, suppressing cariogenic microorganisms. Furthermore, green tea catechins aid in restoring salivary pH to the neutral level, and this is not a good opportunity for bacteria to grow.

According to the current study findings green tea extract mouthwash was found to have significant decrease in streptococcus mutans count. This result agrees with Thomas et al. who discovered that rinsing with green tea significantly lowers the number of Streptococcus mutans due to its inhibitory effect on cariogenic bacteria because of the catechins content [9]. Also, Ahmadi et al discovered that the number of Streptococcus mutans had significantly decreased as well with green tea mouthwash due to the polyphenolic compounds found in the green tea that prevents bacterial adhesion on tooth structure[10]. In addition to Salama et al. who stated that the main reason for the significant decline in the number of streptococcus mutans is due to applying it locally which prevents adhesion of bacteria on the tooth structure [11].

The most prescribed concentration of chlorhexidine mouthwash, 0.12%, has been shown in numerous clinical studies to be the gold standard, and was employed in the study. In this study, chlorhexidine mouthwash has significant reduction in streptococcus mutans count, this agrees with Hedge et al. because of the adsorption of chlorhexidine to extracellular polysaccharides [3]. Also, in accordance with Shah et al. who found that chlorhexidine has significant reduction in bacterial count because at low concentration it causes leakage of the cell wall in the microorganisms and because of the precipitation of cytoplasm brought on by protein cross-linking at greater doses, it is bactericidal [12].

The current study findings have disclosed that there was no significant difference between both mouthwashes regarding decrease in number of bacteria, this is in accordance with Neturi et al. who indicated that both mouthwashes have the same effect on streptococcus mutans count [5], but this result is in disagreement with Thomas et al. who stated that using green tea as a mouthwash has significant decrease in streptococcus mutans count than chlorhexidine mouthwash [9], and also this result disagrees with Hedge et al. who stated that the chlorhexidine mouthwash group significantly lowered bacterial count more than the green tea mouthwash group [3].

Nevertheless, using green tea mouthwash overcame the drawbacks of chlorhexidine mouthwash such as taste alteration, staining and bacterial resistance and has the advantage of low-cost preparation which is more convenient than purchasing chlorhexidine mouthwash.

CONCLUSION:

Based on the outcomes of our study, we may come to the conclusion that chlorhexidine and green tea mouthwash have great antimicrobial efficacy against *Streptococcus mutans*, so green tea extract mouthwash could be regarded as a good substitute mouthwash to overcome drawbacks of chlorhexidine mouthwash. However, further studies could be beneficial to observe if there are any adverse effects on the long-term usage of this mouthwash with a larger sample size.

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Conflicts of interest :

There aren't any conflicting interests.

Ethics approval:

The ethics committee of research of Faculty of Dentistry, Ahram Canadian University reviewed and approved the research protocol and permitted the procedures asserted in the study.

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