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Comparative Antimicrobial Effects of Green Tea and Chlorhexidine Mouthwashes on *Streptococcus mutans* in Young Adults with Dental Caries: An In-Vivo Study

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Abstract

Introduction: Dental caries is a biofilm-mediated disease strongly associated with acidogenic bacteria, particularly Streptococcus mutans. Chlorhexidine is widely used for chemical plaque control, however, side effects limit its long-term use. Catechins like epigallocatechin gallate (EGCG) are abundant in green tea and has attention as a natural antimicrobial alternative.

Methods: This in vivo comparative experimental study included 20 participants aged 22 years with dental caries recruited from the College of Dentistry, University of Babylon, Iraq. Participants were randomly allocated into two groups (n = 10 each): green tea mouthwash and 0.2% chlorhexidine mouthwash. Both groups used the assigned mouthwash twice daily for two weeks. Salivary samples were collected at baseline, after one week, and after two weeks. S. mutans was isolated on Mitis Salivarius Bacitracin agar and quantified as colony-forming units per millilitre. Data were analyzed using Shapiro-Wilk, Friedman, Durbin-Conover, and Mann-Whitney U tests.

Results: Both mouthwashes produced highly significant within-group reductions in salivary S. mutans counts over time ($P < 0.001$). In the chlorhexidine group, mean colony counts decreased from 2546.0 at baseline to 99.2 after one week and 37.0 after two weeks. In the green tea group, counts declined from 2606.3 to 104.5 and 40.9, respectively. No significant between-group differences were observed at baseline, one week, or two weeks.

Conclusion: Green tea mouthwash showed antimicrobial efficacy comparable to chlorhexidine in reducing salivary S. mutans. It may serve as a safe, natural, and cost-effective adjunct for caries prevention.

Keywords: *Streptococcus mutans*, Dental caries, Green tea, Chlorhexidine, Antimicrobial activity, Biofilm inhibition

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Introduction

Dental caries is a complex condition whereby the structure of teeth becomes demineralized as a result of the build-up of acids that are generated by microorganisms in plaque. It is mostly linked with the changes in the oral biofilm, in which acidogenic and aciduric microbes are crucial in the progression of the disease. Of these microorganisms, *Streptococcus mutans* is one that is well known as an important etiological agent because it is able to metabolize dietary carbohydrates, generate organic acids and facilitate enamel demineralization (1,2).

Other acid-tolerant bacteria, like Lactobacilli, in addition to *S. mutans*, can contribute to dental caries development. These microorganisms not only grow in low PH conditions but also increase acid production, which promotes the cariogenic process (3). Thus, dental caries prevention and management cannot be achieved without proper management of these bacteria.

The use of chlorhexidine (CHX) in the dentistry field is regarded as the gold standard antimicrobial agent because it has a wide spectrum of activity and is effective in reducing oral biofilm (4). Its concentration of application is usually between 0.1 and 0.2 percent and it has dose-dependent antibacterial action. Nevertheless, its prolonged use is linked to a number of negative effects such as tooth discolouration, loss of taste, mucosal irritation and the formation of calculus (5). This has led to the quest for other therapeutic agents that have fewer side effects.

The use of green tea (*Camellia sinensis*) as an antimicrobial agent has attracted interest as a natural extract because it is abundant in polyphenolic compounds, especially catechins. They have antibacterial, anti-inflammatory, antioxidant, and anti-cariogenic properties, which are beneficial to oral health. Past researchers have established that catechins have the ability to prevent the growth of bacteria, formation of biofilms and disrupt the cariogenic processes (6, 7).

Although there is an increasing trend in the use of herbal substitutes, very few investigations have directly relate the antimicrobial effect of green tea and chlorhexidine in salivary reduction of salivary *S. mutans*. Therefore, the goal of the current study was to evaluate how well mouthwashes containing green tea and chlorhexidine reduced the levels of *S. mutans* in dental caries patients.

Methods

Study Design and Sample

It was an in vivo comparative experimental study aimed

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at assessing the outcome of green tea and chlorhexidine mouthwashes on salivary *Streptococcus mutans* antimicrobial effect. Twenty participants aged 22 years were enlisted at the College of Dentistry, University of Babylon, Iraq. Participants were divided into two groups (n = 10) at random: the group using green tea mouthwash and the group using chlorhexidine mouthwash. The study did not have any untreated or placebo control group. All participants were taken to the laboratory to provide a salivary sample at the baseline (before treatment), one and two weeks later, to ascertain how many *S. mutans* colonies would change over time.

Criteria for Inclusion and Exclusion

People participated in the study with clinically diagnosed dentary caries and those who have no history of systemic illness. The subjects that had taken antibiotics, antimicrobial mouthwashes or medications that impacted the oral microbiota in the last two weeks were excluded. People who have orthodontic appliances, periodontal disease, or other known allergies to mouthwash ingredients were also avoided to minimize the confounding variables and maintain consistency of the study population.

Intervention Protocol

The participants of each group were requested to apply their given mouthwash twice a day over a period of two weeks. All subjects were instructed to rinse with about 10 millilitres of mouthwash and expectorate after 30 seconds. It was recommended that the participants should not eat or drink within 30 minutes after rinsing to allow maximum effectiveness of the mouthwash.

Salivary Sample Collection

Sterile cotton swabs were used to collect unstimulated saliva samples and carious debris under sterile conditions in participants with clinical signs of dental caries. Swabs were moistened with different points of the mouth and stayed on the point of contact for 1-2 min, and then placed in the sterile tubes with 1 mL of normal saline to be transported. Each sample was inoculated with a standardized volume (10 μL) and allowed to grow on selective culture media, and was incubated under anaerobic conditions (85% N_2, 5% CO_2, and 10% H_2) at 37°C for 48 hours. The *S. mutans* colonies were then counted and declared as the colony-forming units per millilitre (CFU/mL) after incubation (8).

Isolation and Identification of Bacteria

Mitsuis Salivarius Bacitracin (MSB) agar (HiMedia Laboratories, India), supplemented with bacitracin (0.2 units/mL) was used to selectively isolate Streptococcus mutans (8). The colonies were distinguished by their typical morphology and selective growth on the MSB agar.

Preparation of Green Tea Mouthwash

The 13% (w/v) solution of green tea was obtained by dissolving 13 g of commercially available 100 milliliters of sterile saline with green tea heated to about 90°C. The solution was cooled to a room temperature and placed in sterile glass containers before use (9).

Preparation of Chlorhexidine Mouthwash (0.2%)

Chlorhexidine was diluted using the standard volumetric procedures to create a 0.2% solution of chlorhexidine in sterile distilled water. This solution was stirreded to achieve uniformity and filtered through a 5 μm membrane filter to maintain sterility and then used (10).

Ethical Approval

The University of Babylon's College of Dentistry Ethics Committee provided an ethical approval to this study (Ref. No. 77, dated June 22, 2025). All the subjects were informed and signed written informed consent before the collection of samples.

Statistical Analysis

The data was analyzed using the Jamovi software (version 2.6) (11). The Shapiro-Wilk test was used to determine whether the data were normal, but it was refused. Since the data were not normally distributed, non-parametric statistics were used. The Friedman test was employed to compare baseline, one, and two weeks within-group, and Durbin Conover post hoc analysis was utilized for pairwise comparisons. The Mann-Whitney U test was examined using between-group comparisons at each time point. When the *P*-value was less than 0.05, it was considered significant.

Results

Baseline Growth of Streptococcus mutans Colonies

Figure 1 shows the baseline development of the *Streptococcus mutans* colonies on Mitis Salivarius agar and indicates that the bacterial colonies in all the salivary samples are dense and well developed before intervention. This validated a high initial bacterial load of cariogenic bacteria in the participants and gave a standard starting point in determining the antimicrobial action of both chlorhexidine and green tea mouthwash.

Normality Assessment and Selection of Statistical Tests

The result of the descriptive statistics and the Shapiro-Wilk test of the normality of the colony number of *S. mutans* are presented in Table 1. It was found that at least one of the subgroups, namely, the chlorhexidine group in week

Figure 1. *S. mutans colonies* before treatment

Table 1. Descriptive statistics and the Shapiro–Wilk test for *S. mutans* colony counts

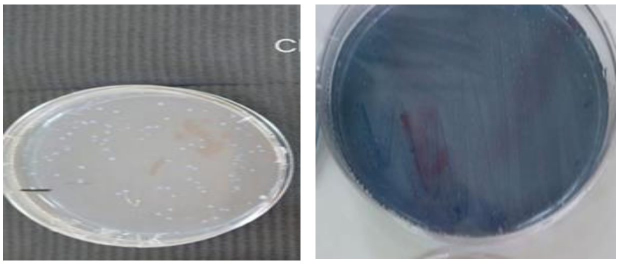
Group and Time Point	N	Mean	Median	SD	Minimum	Maximum	W	*P*-value
CHX before treatment	10	2546.0	2522.5	233.59	2251	2961	0.959	0.777
CHX after 1 week	10	99.2	99.0	7.51	81	108	0.841	0.045
CHX after 2 weeks	10	37.0	37.5	15.29	11	62	0.986	0.988
Green tea before treatment	10	2606.3	2586.0	244.16	2111	2921	0.943	0.582
Green tea after 1 week	10	104.5	100.0	13.27	88	127	0.925	0.404
Green tea after 2 weeks	10	40.9	28.0	31.17	10	90	0.859	0.074

one, demonstrated statistically significant non-normality ($P = 0.045$), meaning that the normality assumption was violated. As a result of this non-normal distribution, parametric tests like ANOVA could not be performed. Thus, non-parametric statistics were used, such as the Friedman test of within-group repeated measurements and Mann Whitney U test of between-group analysis, which guarantees strong and valid analysis of the data.

Effect of Chlorhexidine Mouthwash on S. mutans

Visual Assessment of Bacterial Reduction

Figure 2 shows the reduction in the quantity of *S. mutans* colonies in the presence of chlorhexidine treatment, where the density of colonies is significantly reduced after one week, then further with increase after two weeks. The visual results (Figure 2) show that the bacterial growth has reduced significantly as compared to the baseline (Figure 1), which is a strong indication of the antimicrobial effect of chlorhexidine.



(A) **(B)**

Figure 2. *S. mutans colonies* after CHX treatment (A: one week; B: two weeks)

Within-Group Statistical Analysis

The Friedman test was used to make within-group comparisons of the counts of *S. mutans* colonies at baseline, one week and two weeks, as presented in Table 2. The findings showed a very high statistical significance of the decrease in the number of colonies over time ($\chi^2 = 20.0$, df = 2, $P < 0.001$), and proved that chlorhexidine treatment greatly reduced bacterial counts during the timeframe.

Pairwise Comparisons

Statistically substantial variances were found between all the points of evaluation ($P < 0.001$) as indicated by post hoc pairwise assessments using the Durbin Conover test (Table 3) between the variables of baseline versus one week, baseline versus two weeks and one week versus two weeks. These findings show that the decrease in bacterial count was gradual and statistically significant at all levels of treatment.

Descriptive Changes over Time

As the descriptive statistics in Table 4 show, there is a substantial reduction in the mean numeral of colonies per baseline of 2546.0, to 99.2 and 37.0 after one and two weeks of the application of chlorhexidine, respectively. This drastic drop is indicative of the high antibacterial activity of chlorhexidine in lowering salivary levels of *S. mutans* with time.

Effect of Green Tea Mouthwash on S. mutans

Visual Assessment of Bacterial Reduction

Figure 3 shows the reduction in *S. mutans* colonies with green tea mouthwash treatment, and it can be seen that there is a substantial decrease in the number of colonies after a week and even more after two weeks. Relative to the control condition (Figure 1), these results represent a remarkable uniform antimicrobial effect of green tea.

Within-Group Statistical Analysis

Results of the Friedman test of the green tea group, as indicated in Table 5, revealed that the number of colonies significantly decreased across the three time intervals ($\chi^2 = 20.0$, df = 2, $P < 0.001$), indicating that the mouthwash made of green tea worked well in reducing the level of bacteria over time.

Pairwise Comparisons

Statistically significant changes in all-time points ($P < 0.001$) as indicated by post hoc pairwise comparisons using Durbin Conover test (Table 6) indicated that the decrease in bacteria counts was consistent and persistent during the course of the study.

Descriptive Changes over Time

Table 7 also shows that the average count of *S. mutans* colonies reduced in the baseline (2606.3) to 104.5 in one week and 40.9 in two weeks, with the use of green tea mouthwash. This observation of reduction indicates that green tea was an effective antimicrobial agent of the natural type in decreasing the cariogenic bacterial load.

Comparative Analysis between Chlorhexidine and Green Tea Groups

The results are displayed in Table 8. The Mann-Whitney U

Table 2. Friedman test for chlorhexidine group

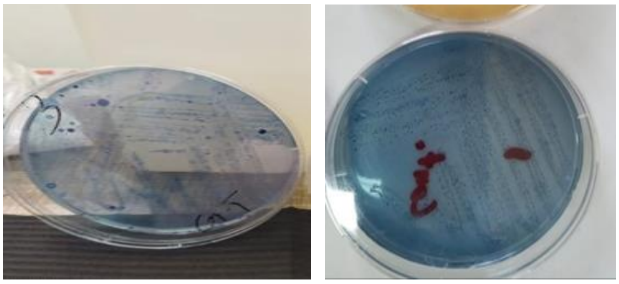
Test	χ^2	df	*P*-value
Friedman test	20.0	2	<0.001

Table 3. Pairwise comparisons for chlorhexidine group

Comparison	*P*-value
Before treatment vs CHX 1 week	<0.001
Before treatment vs CHX 2 weeks	<0.001
CHX 1 week vs CHX 2 weeks	<0.001

Table 4. Descriptive statistics for chlorhexidine group

Time point	Mean	Median
Before treatment	2546.0	2522.5
After 1 week	99.2	99.0
After 2 weeks	37.0	37.5



(A) **(B)**

Figure 3. *S. mutans* after green tea treatment (A: one week; B: two weeks)

test was used to compare the green tea and chlorhexidine groups at the conclusion of each time point. At baseline ($P=0.545$), there were no differences of statistical significance between the two groups, one ($P=0.495$) and two ($P=0.910$) weeks and showed similar antimicrobial efficacy of both treatments.

In general, the chlorhexidine and green tea mouthwashes both showed the reduction in the number of *Streptococcus mutans* colonies with substantial differences among groups (within-group differences highly significant: $P<0.001$) and with regular increases in descriptive values. Nonetheless, there were no statistically substantial alterations in between-group comparisons of the effectiveness, which implies that both treatments had a similar antimicrobial activity in contradiction of *S. mutans*.

Discussion

The current research compared and contrasted the antimicrobial activity of green tea and chlorhexidine mouthwashes in contrast to *Streptococcus mutans*, which is a leading etiological agent of dental caries. The findings indicated that the effects of the two interventions had statistically significant effects on the counts of salivary *S. mutans* colonies at the end of the two-week duration, and the within-group differences were highly significant ($P<0.001$). Nevertheless, there were no statistically significant variances between the two groups at any time, which suggests that green tea had the same antimicrobial

Table 5. Friedman test for green tea group

Test	χ^2	df	*P*-value
Friedman test	20.0	2	<0.001

Table 6. Pairwise comparisons for green tea group

Comparison	*P*-value
Before treatment vs GT 1 week	<0.001
Before treatment vs GT 2 weeks	<0.001
GT 1 week vs GT 2 weeks	<0.001

Table 7. Descriptive statistics for green tea group

Time point	Mean	Median
Before treatment	2606.3	2586.0
After 1 week	104.5	100.0
After 2 weeks	40.9	28.0

Table 8. Between-group comparison using the Mann-Whitney U test

Time point	*P*-value	Interpretation
Before treatment	0.545	No significant difference
After 1 week	0.495	No significant difference
After 2 weeks	0.910	No significant difference

effects as chlorhexidine.

The application of conventional mouthwashes, especially those with chlorhexidine gluconate and alcohol, is widespread, attributed to their broad-spectrum antimicrobial impact, but is commonly linked to such adverse effects as tooth discolouration, distortion of taste perception, and mucosal irritation (12). These restrictions have seen a rise in interest towards natural and herbal alternatives. The safety of the herbal mouthwash is the biocompatibility, low side effects, and bioactive compounds that promote oral health, making herbal mouthwashes safer than other types of mouthwash (13).

The findings of the present research are in agreement with the previous research studies which have indicated that green tea is a viable agent in the inhibition of cariogenic bacteria. Ahmadi et al. (14) observed that green tea-based interventions had a significant reduction in the levels of S. mutans, but the difference in the statistical significance at various periods might be attributed to the difference in the study design and period. Similarly, Salama and Alsughier (15) reported that there was a significant decrease in salivary S. mutans counts based on the use of green tea mouthwash, which supports the application of green tea as a prevention tool in oral health management.

The antimicrobial effect of green tea is mainly credited to the polyphenolic compounds it contains, especially the catechins, including epigallocatechin gallate (EGCG), the most copious and biologically active polyphenolic compound. EGCG was also observed to inhibit glucosyltransferase (GTF), which is a key enzyme in the synthesis of extracellular polysaccharides and biofilm in S. mutans (16). Moreover, EGCG disrupts bacterial metabolic processes and expression of genes, thus decreasing bacterial growth and virulence (17). Moreover, catechins can have bactericidal effects, that is, they cause upheaval of bacterial cell membranes and prevent adhesion to tooth surfaces, which consequently impairs the formation of the plaque (18).

Evidence of other studies also supports the antibacterial potential of green tea. Previous research has indicated that the numbers of S. mutans decreased to considerable extents when green tea preparations were applied (19). Sajadi et al. (20) found green tea extract to have a better long-term antibacterial effect than fluoride and chlorhexidine gels. Furthermore, Tao et al. (21) showed that chewing gum enriched with green tea polyphenols could significantly decrease the incidence of caries, and Behfarnia et al. (22) found that inflammatory markers and plaque indices improved with the use of green tea-comprising products.

Green tea has an antimicrobial effect that is not just limited to *S. mutans* since polyphenols have been observed to prevent the growth of various cariogenic pathogens, such as *S. sobrinus* and Lactobacilli (23). Bacterial adhesion is also affected by these compounds as they modify the surface structures like fibrils and fimbriae and inhibit colonization of the tooth surface (24). Additionally, the catechins prevent the activity of glucosyltransferase, which helps in the decrease of the plaque formation and the total number of bacteria (25-28).

The gold standard antimicrobial agent is still chlorhexidine because of its bactericidal and bacteriostatic effects. It works by destroying bacterial cells membranes, heightening permeability and precipitation of intracellular proteins, resulting in cell death in bacteria. The high decrease in *S. mutans* noted in the chlorhexidine group of this study is in tandem with the proven effectiveness of this agent, but its application in the long term is restricted due to its adverse effects, which include staining and taste change.

Clinically, the similar efficacies of green tea and chlorhexidine in this study imply that green tea could be an effective alternative natural approach to controlling cariogenic bacteria. It is less expensive, more available and less risky in terms of side effects and can be utilized in long term preventive dentistry.

Although these are encouraging results, some shortcomings have to be noted. The limited time of the study and relatively small sample used can limit the extent to which the findings can be generalized. Also, the lack of a control group does not provide the opportunity to completely differentiate the effect of treatment and natural changes over time. The research should incorporate more sample sizes, extended follow-ups and a control group in the future in order to enhance the validity of the results.

The future studies ought to also consider how to maximise green tea preparations with known active compounds concentrations and the possibility of using green tea in combination with other antimicrobial agents. Moreover, more sophisticated molecular methods may be used to explore the overall effects of these interventions on the oral microbiome.

Conclusion

The current study has shown that both the green tea and chlorhexidine mouthwashes are efficient in lowering salivary concentrations of *Streptococcus mutans* significantly in two weeks. The within-group analysis showed that the bacterial colony counts had significantly reduced between the baseline and the one week and further two weeks in both treatment groups, which showed a long-lasting antimicrobial effect. Notably, the between-group comparison revealed that there was no statistically significant variance between the efficacy of green tea and chlorhexidine at any time interval, which indicates that green tea has a similar antibacterial effect to the conventional gold-standard agent. The antimicrobial effect of green tea is observed to be due to its bioactive polyphenolic compounds, especially epigallocatechin gallate (EGCG) that inhibit bacterial adhesion, disrupt biofilm formation, and disrupt important enzyme pathways that cause cariogenic activity. These processes help decrease *S. mutans* and underscore the possibilities of using green tea as a natural treatment option in oral health care. Since green tea mouthwash is equally effective and has less risk of causing adverse effects, it can be used as a safe, cost-effective and biocompatible substitute to chlorhexidine as a long-term preventive and management tool against dental caries. Nevertheless, it is advised that additional research on a bigger sample group and longer follow-up durations should be conducted to confirm these results and consider their wider clinical implications.

Authors' Contribution

Conceptualization: Luma Jasim Witwit, Hanan Salman Hassan
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Formal analysis: Ammar Abdul Sattar Hamza, Hanan Salman Hassan
Investigation: Ammar Abdul Sattar Hamza
Methodology: Luma Jasim Witwit, Hanan Salman Hassan
Project administration: Luma Jasim Witwit
Resources: Hanan Salman Hassan
Software: Ammar Abdul Sattar Hamza
Supervision: Luma Jasim Witwit
Validation: Hanan Salman Hassan
Visualization: Ammar Abdul Sattar Hamza
Writing–original draft: Ammar Abdul Sattar Hamza
Writing–review & editing: Luma Jasim Witwit, Hanan Salman Hassan

Conflict of Interests

No conflicts of interest are declared by the authors.

Ethical Approval

This study was approved by the Research Ethics Committee of the College of Dentistry, University of Babylon (Approval Ref. No. 77, dated June 22, 2025). Written informed consent was obtained from all participants prior to sample collection.

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