



Effect of Green Tea and Chlorhexidine Mouth Rinses on Cariogenic Bacteria

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Abstract

This study aimed to evaluate the effectiveness of green tea mouthwash in comparison to mouthwashes containing chlorhexidine on the clinical isolated Lactobacilli. Samples from 22-year-old University of Babylon College of Dentistry students were gathered to evaluate caries bacteria. They had been divided up, into three groups: the control group, the green tea mouthwash group, and the chlorhexidine mouthwash group. Each group consists of thirty people. The colony counts of *Lactobacillus* were measured before and after the intervention (one and two weeks), and the data was analyzed using the ANOVA and post hoc tests. After a week of treatment, the Anova and Post Hoc Tests showed that the chlorhexidine and green tea groups of *Lactobacillus* differed significantly, (P -value < 0.05). Nevertheless, no significant changes (P -value > 0.05) were seen between groups for *Lactobacillus* after two weeks of treatment. Green tea mouthwash effectively reduces the salivary levels of Lactobacillus and can be incorporated in dental products as an effective anticariogenic agent.

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Introduction

Dental caries is most common disease in the world and occur from the breakdown of tooth structure due to an excessive acid from bacteria of plaque on teeth. When change occur in biofilm is the cause of dental caries. Streptococcus mutans is acidogenic bacteria breaks the hydroxyapatite crystals in teeth, transformation the sucrose into acids, and lead to decalcification. Lactobacilli bacteria progress the caries process. A lot of numbers of chemical agents can change the oral microbiota and have negative effect [1,2]. Acidogenic and acid-tolerating bacteria, such as lactobacilli, are linked to dental caries. Different species of lactobacilli in oral microbiota digest the carbohydrate and produce acid [3]. Detection of caries in early stages and prevention of caries is the goal of modern practical strategies. It has long time believed that chlorhexidine is the best

mouthwash, but have the side effect such as teeth staining, altered taste perception, formation of supragingival calculus and ulcers in oral mucosal [4,5]. Tea takes from the leaves of the plant Camellia sinensis. Green tea (Camellia sinensis), consist of catechins and polyphenol components, green tea has properties of antibacterial, antiviral, antimutagenic, anti-inflammatory, and antioxidant against infections of periodontal tissue. In vitro studies found the polyphenolic chemicals called catechins and the aflavins, which are extracted from green tea have biological properties in prevention of dental caries [6,7]. Therefore, the goal of this study was to assess how green tea and chlorhexidine mouthwashes inhibited Lactobacillus bacteria to control and prevent of dental caries among 22-23 years-old students from the Dentistry College of Babylon University.

Materials and Methods

In this study design to assess cariogenic bacteria, Samples were taken from 22-year-old fifth-stage dental students at the University of Babylon to evaluate caries bacteria. They were divided into three groups: the control group, the green tea mouthwash group, and the chlorhexidine mouthwash group. Each group consists of thirty people. To assess the bacterial count, samples were obtained from the control group, the two groups using the green tea and chlorhexidine mouthwash, and both before and after using the mouthwash.

Salivary samples collection

Using sterile cotton swabs, saliva samples and caries debris were taken from dental students had symptoms of dental caries. For transportation, the swabs were placed at carious locations, left for one to two minutes, removed, and put in a sterile vial with one milliliter of

saline. Saliva and caries debris were streaked on various isolation media in a loopful (10 μ L). Lactobacilli incubated for 24 hours at 37°C. The inoculation plates were incubated anaerobically (85% N₂, 5% CO₂, and 10% H₂) at the appropriate temperatures. Following bacterial culture, the numbers of Lactobacillus bacteria were measured in colony-forming units (CFUs) then registered for every sample as 1.5×10^5 CFU/mL on plates with transmitted light illumination were manually counted [8] (Figures 1 and 2). Bacterial isolation and identification samples were plated on Man Rogosa Sharpe (MRS) medium and cultured for 48–72 hours at 37 °C. Following incubation, isolated colonies were selected at random for purification. Gram stain was used for microscopic analysis to check the resulting pure colonies for LAB [9]. Methyl red, indole synthesis in peptone water, medium, urease activity, and gelatin liquefaction were utilized to identify each isolate based on its morphological, physiological, cultural, and biochemical traits. Berge's Manual of, Systematic Bacteriology describes [10,11].

Preparation of Green Tea

100 milliliters of saline heated to about 90 degrees Celsius and 13 grams of commercially fractionated green tea were used to make a 13% green tea infusion. Sterile glass containers were used to store the fluid [12].

Ethical Approval

This study according to Ethical Committee, University of Babylon, College of Dentistry (Ref. No. 77).

Statistical Analysis

ANOVA and Post Hoc tests were used in all comparisons with alpha set at 0.05.



Figure 1. MRS agars of *Lactobacillus* colonies before treatment.

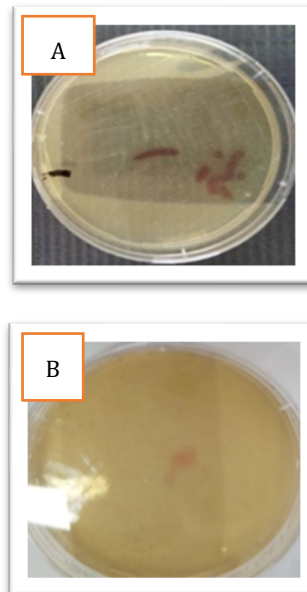


Figure 2. MRS agar of *Lactobacillus* colonies after 0.2% CHX treatment (A: one week; B: two weeks).

Results

There were no significant differences between the colonies of *Lactobacilli* before and after one week of CHX therapy ($P > 0.05$), nonetheless there were significant differences amid the colonies before and after two weeks of CHX treatment ($P < 0.05$). Additionally, Table 1 indicates that there were no significant differences between the first and second weeks of CHX administration ($P > 0.05$).

Following green tea treatment (one and two weeks), MRS agar of *Lactobacillus* colonies are shown in Figure 3.

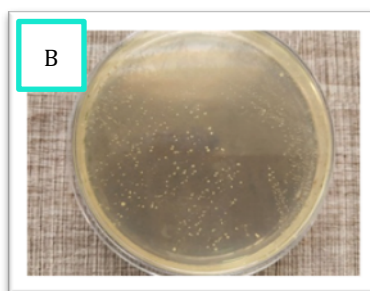


Figure 3. *Lactobacilli* colonies after green tea treatment (A: one week; B: two weeks).

It was found significant differences after treatment (one week and two weeks) with green tea mouthwash for *Lactobacillus* colonies ($P < 0.05$), the same result was reported between one and two weeks of treatment, as illustrated in Table 2.

Results shown in Table 3 significant differences between groups and within groups ($P < 0.05$) for *Lactobacilli* colonies after one week of treatment.

Following two weeks of treatment for *Lactobacilli*, there were no significant differences ($p > 0.05$) between the groups (Table 4).

Discussion

This present study evaluated the effects of mouthwashes with green tea mouthwash in comparison to 0.2% chlorhexidine on the levels of *Lactobacilli* in saliva at one- and two-weeks intervals. In this study, it was found following one week of treatment, significant differences between groups and within groups (P value < 0.05) for *Lactobacilli* colonies. Following two weeks of treatment, Regarding *Lactobacilli*, there were no significant differences ($p > 0.05$) among the groups. However, another investigation discovered According to the study's findings, the number of lactobacilli decreased statistically [13].

Sajadi et al. [14] investigated the effects of mouthwash containing green tea and found that, in comparison to fluoride and chlorhexidine gel, green tea extract gel was more effective in decrease numbers of cariogenic bacteria over an extended period. Tao et al. [15] demonstrated that tooth cavities can be prevented by munching gum enhanced through green tea polyphenols. Behfarnia et al.'s 2016 study [16] demonstrated that chewing gum made from green tea reduce blood interleukin β 1 and plaque indices. Green tea contains polyphenols that block growth of bacteria such as *Lactobacilli*, *S. mutans*, and *S. sobrinus* [17]. By changing the fibrils and fimbriae, the polyphenolic chemicals inhibit the bacterial adhesion on teeth surface [18]. Conversely, catechins help to significantly lower the plaque index by inhibiting glucosyltransferase [19]. According to the results, green tea inhibits *Lactobacillus* bacteria by altering their phenotypic, which prevents the bacteria from adhering to the tooth surface [20,21].

Conclusion

Green tea mouth wash is considered as a viable mouthwash, in which this mouthwash has been shown to have antibacterial properties, and it helps to effectively lower the salivary levels of *Lactobacillus* colonies this attributed to

green tea components lead to this better results.

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Table 1. Mean, SD, standard error, mean difference, and P value of *Lactobacilli* colonies before and after treatments with chlorhexidine (Group1).

CHX	Mean	Standard Error	CHX	Mean Difference	Standard Error	P value
before treatment	81.7000	12.32797	before treatment one week after treatment	27.00000	16.09746	0.232
one week after treatment	54.7000	11.76818	two weeks after treatment	59.40000*	16.09746	0.003
two weeks after treatment	22.3000	9.91077	One week after treatment two weeks after treatment	32.40000	16.09746	0.128
F	6.827					
P-value	0.004					

Table 2. Mean, standard deviation, standard error, mean difference, and P-value of *Lactobacilli* colonies before and after treatments with green tea (Group2).

Green tea	Mean	Standard Deviation	Standard Error	Green tea	Mean Difference	Standard error	P-value
before treatment	274.5000	72.07750	22.79291	Before treatment one week after treatment Two week after treatment	183.10000* 268.40000*	19.49887 19.49887	0.000 0.000
one week after treatment	91.4000	22.30695	7.05408	One week after treatment two weeks after treatment	85.30000*	19.49887	0.000
two weeks after treatment	6.1000	3.21282	1.01598				
F	98.929						
P-value	0.000						

Table 3. Mean, standard deviation, and standard error of group1 (CHX) and Group 2 (green tea) after one week treatment.

	Mean	Standard Deviation	Standard Error	F	P-value
Group 2 <i>Lactobacilli</i> one week after treatment	91.4000	22.30695	7.05408	7.080	0.001
Group 1 <i>Lactobacilli</i> one week after treatment	54.7000	37.21424	11.76818		

Table 4. Mean, standard deviation and, standard error of group I (CHX)and group II (green tea) after two weeks of treatment for *Lactobacilli* colonies.

Groups	Mean	Standard Deviation	Standard Error	F	P-value
Group II <i>Lactobacilli</i> two week after treatment	6.100	3.2128	1.0160	2.700	0.06
Group I <i>Lactobacilli</i> two week after treatment	22.300	31.3406	9.9108		