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Antimicrobial Properties of Green Tea Extract Against Cariogenic Microflora: An *In Vivo* Study

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ABSTRACT The aim of the present study was to test *in vivo* the effectiveness of an experimental green tea extract in reducing levels of mutans streptococci and lactobacilli in saliva by means of selective culture medium. Sixty-six healthy patients ranging in age from 12 to 18 years were recruited and randomly divided into two groups: Group A ($n = 33$) and group B ($n = 33$). Group A subjects were asked to rinse their mouths with 40 mL of an experimental green tea extract, for 1 minute, three times a day for a week, whereas Group B subjects were asked to rinse with 40 mL of a placebo mouth rinse. Saliva samples were obtained at baseline, 4 days, and 7 days. The counts of mutans streptococci and lactobacilli were investigated by chair-side kits. Data were statistically processed. A regression binary logistic analysis was done. The statistical significance level was established at $P < .05$. The experimental group showed a statistically significant reduction in colony counts of mutans streptococci and lactobacilli relative to the control group. These findings showed the efficacy of a green tea extract against cariogenic oral flora, opening a promising avenue of clinical applications in the preparation of specific and natural anticariogenic remedies.

KEY WORDS: • ***antimicrobial activity*** • ***cariogenic bacteria*** • ***green tea*** • ***polyphenol***

INTRODUCTION

RECENTLY, THERE HAS BEEN increased interest in polyphenolic compounds found in plant foods. This is probably because of the mounting evidence that several of these may have beneficial effects in humans. Polyphenols constitute one of the most common and widespread groups of substances in plants, occurring in all vegetative plant organs and also in flowers and fruits. The main sources of the polyphenols present in the daily intake of the human diet are plants like tea, coffee, cereals, and fruit. Despite their wide distribution, the healthy effects of dietary polyphenols have come to the attention of nutritionists only in the last few years. The main factor responsible for the delayed research on polyphenols is the variety and the complexity of their chemical structures.

The biological properties of polyphenols include antioxidant,[1,2] anticancer,[3–5] and anti-inflammatory[6] effects. Experimental studies strongly support a role of polyphenols also in the prevention of cardiovascular disease, osteoporosis, diabetes mellitus, and neurodegenerative disease.[7]

In the last few years, polyphenols from some edible plants have attracted attention as potential sources of agents capable of controlling the growth of oral bacteria.[8] Subsequent *in vitro* studies on plant extracts suggest an activity against several metabolic activities of mutans streptococci, resulting in a decrease in growth and virulence.[9–12] Smullen *et al.*[13] have shown that extracts from unfermented cocoa, green tea, and red grape seeds have a bacteriostatic effect on *S. mutans* and reduce its adherence to glass.

Studies on the development of antiplaque agents for prevention of dental caries have investigated the effect of some tea preparations and their individual components on the glucan synthesis catalyzed by glucosyltransferase from mutans streptococci. Extracts of green tea and polyphenol mixtures showed appreciable inhibition in the synthesis of insoluble glucan.[14]

Experiments have also demonstrated the inhibition of salivary amylase activity by extracts of a commercial tea. The effect on salivary amylase may contribute significantly to reducing the cariogenicity of starch-containing foods.[15]

A point that should be mentioned is that the *in vitro* property of green tea has also been much investigated previously, but *in vivo* evidence able to establish its real contributions to caries reduction is not consistent.

For these reasons a noninvasive method of *in vivo* investigation has been developed. Therefore, the present study

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was conducted with the aim of evaluating the *in vivo* effectiveness of green tea polyphenols in reducing levels of mutans streptococci and lactobacilli in saliva.

MATERIALS AND METHODS

Subjects and study design

The study population consisted of 66 volunteers, 36 female and 30 male, who were in good physical condition and ranged in age from 12 to 18 years. The participants were recruited from the Department of Pediatric Dentistry of the University of Naples ''Federico II,'' Naples, Italy. They were selected with the following criteria. Inclusion criteria were good general health (ASA I–II) and agreement to comply with study procedures. Exclusion criteria were antibiotic treatment during the 14 days before starting the test, the use of an antibacterial mouth rinse during the 12 hours before the test, the presence of dental fixed orthodontic appliances, and conditions that interfered with the examination procedure (non-cooperating subjects). Participation was voluntary. Patients and their parents received verbal and written explanations of the experimental protocol and the study aims, and they signed written informed consent prior to the start of the study. Permission was received from the appropriate authorities. The study protocol was in accordance with the Helsinki Declaration of Human Rights.

The subjects were randomly distributed into two groups of 33 (Groups A and B). All the subjects received a clinical examination carried out by two professionals, in the same room and using the same dental unit (so that all patients were examined under the same lighting conditions). The presence of tooth decay was assessed by systematic evaluation of each subjects' caries experience using the DMFT index (number of decayed, missing, and filled teeth): in Group A the mean DMFT value was 3.13 ± 1.76; in Group B the mean DMFT value was 3.09 ± 1.89.

Experiment design: Group A. Thirty-three subjects were enrolled in this group. Prior to the start of the experiment, the subjects' salivary concentration of mutans streptococci and lactobacilli was calculated from a sample of saliva in order to establish the baseline levels (T0).

Selective culture medium (CRT Bacteria, Ivoclar Vivadent AG, Schaan, Liechtenstein) was used for detection of the mutans streptococci and lactobacilli counts in saliva.

The test was conducted with the following way:

- Each subject chewed a enclosed paraffin pellet in order to stimulate salivation.
- The saliva was collected in a sterile plastic container and then placed, using a pipette, on blue mitis-salivarius agar with bacitracin for determination of mutans streptococci and on the light culture medium (Rogosa agar) for determination of lactobacilli.
- An $NaHCO_3$ tablet was added to the container. This tablet was able to release CO_2 when it came into contact with moisture, creating favorable conditions for bacterial growth.
- The vial with each agar plate was marked with the name of the patient and the date using a waterproof pen.
- All the vials were placed upright in a Cultura incubator (Ivoclar Vivadent AG) at 37°C (99°F) for 48 hours.

After the collection of the first sample, all participants of Group A were instructed to rinse with 40 mL of an experimental mouth rinse for 1 minute. This procedure was to be repeated three times a day (after breakfast, after lunch, and before sleeping), after normal oral hygiene procedures, for 7 days.

After days 4 and 7 of treatment with the mouth-rinse formulation, the salivary sample was collected again and immediately incubated, according to the step-by-step procedure described above, in order to calculate the colony-forming units (CFU) density (CFU/mL) of mutans streptococci and lactobacilli for each subject during (T1) and immediately after (T2) the treatment.

Therefore, in total, three saliva samples (T0, T1, and T2) were taken for each individual.

During the 7-day experimental period no alterations were made to the subjects' diet and oral hygiene procedures.

Experiment design: Group B. Thirty-three subjects were enrolled in this group. Prior to the start of the experiment, the subjects' salivary concentration of mutans streptococci and lactobacilli was calculated in a sample of saliva, in order to establish the baseline levels (T0) with the same procedure given above for Group A.

After the first collection of sample, Group B participants rinsed with 40 mL of a placebo mouth rinse that did not contain green tea infusion, for 1 minute, three times a day (after breakfast, after lunch, and before sleeping) for 7 days.

Subsequent saliva samples were obtained on days 4 (T1) and 7 (T2) after the beginning of the study.

During the 7-day experimental period no alterations were made to the subjects' diet and oral hygiene procedures.

Mouth-rinse formulation

Two different mouth-rinse formulations were prepared. For Group A (experimental), the mouth rinse was prepared with pulverized *Camellia sinensis* leaves. For each rinsing, 1.6 g of pulverized leaves was suspended in 40 mL of distilled water at 100°C for 3 minutes. After this procedure the mouth rinse was kept at room temperature. For Group B (placebo), 40 mL of distilled water was colored with food dye. Both mouth rinses were put into hermetically sealed plastic bottles.

At the end of the treatments the data were processed with Statistical Package for Social Sciences software version 10.0 (SPSS Inc., Chicago, IL, USA). A regression binary logistic analysis was done. The statistical significance level was established at $P < .05$.

RESULTS

The mean stimulated saliva secretion rate was 1.41 ± 0.53 mL/minute.

The CRT Bacteria test results are expressed as a low ($< 10^5$ CFU) or a high ($> 10^5$ CFU) bacterial count.

Statistical analysis within Group A

Variations in mutans streptococci and lactobacilli CFU density (CFU/mL) at T0, T1, and T2 for the test group are summarized in Figure 1A and 1C, respectively.

The differences in mutans streptococci CFU density (CFU/mL) between T0 and T1 were statistically significant ($P < .001$), between T0 and T2 they were statistically significant ($P < .001$), and between T1 and T2 they were not statistically significant.

The differences in lactobacilli CFU density (CFU/mL) between T0 and t1 were statistically significant ($P < .001$), between T1 and T2 they were not statistically significant ($P < .001$), and between T0 and T2 they were statistically significant ($P < .001$).

Statistical analysis within Group B

Variations in mutans streptococci and lactobacilli CFU density (CFU/mL) at T0, T1, and T2 for the control group are represented in Figure 1B and 1D, respectively.

The differences in mutans streptococci CFU density (CFU/mL) between T0 and T1, T0 and T2, and T1 and T2, respectively, were not statistically significant.

The differences in lactobacilli CFU density (CFU/mL) between T0 and T1, T0 and T2, and T1 and T2, respectively, were not statistically significant.

Statistical analysis between Groups A and B

At T0 the differences in mutans streptococci CFU density (CFU/mL) between Groups A and B were not statistically significant, whereas at T1 and T2 the differences were statistically significant: T1, odds ratio = 3.12 (95% confidence interval = 1.13–8.60); T2, odds ratio = 4.2 (95% confidence interval = 1.44–11.23) (Table 1).

At T0 the differences in lactobacilli CFU density (CFU/mL) between Groups A and B were not statistically significant, whereas at T1 and T2 the differences were statistically significant: T1, odds ratio = 4.02 (95% confidence interval = 1.44–11.23); T2, odds ratio = 4.24 (95% confidence interval = 1.47–12.16) (Table 2).

DISCUSSION

The primary etiological dental caries agents are known to be several restricted strains of oral bacteria; thus, the majority of current commercial antiplaque products are

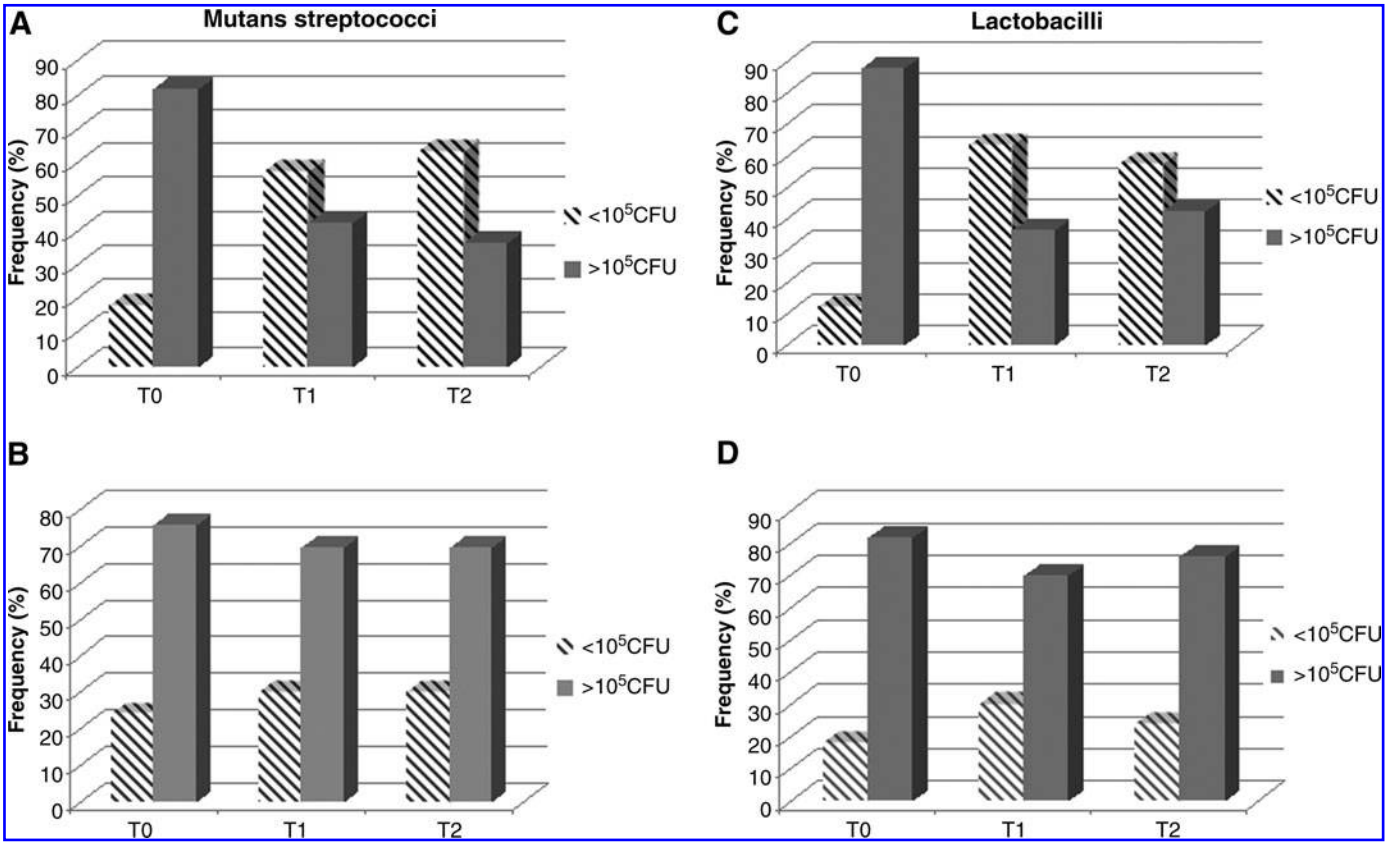

FIG. 1. Variation in mutans streptococci and lactobacilli colony-forming units (CFU) density (CFU/mL) at baseline (T0), 4 days (T1), and 7 days (T2): (**A**) mutans streptococci variation for Group A (experimental); (**B**) mutans streptococci variation for Group B (placebo); (**C**) lactobacilli variation for Group A (experimental); (**D**) lactobacilli variation for Group B (placebo).

Table 1. Statistical Analysis Between Test and Control Groups for Mutans Streptococci Colony-Forming Units Density

			95% confidence interval	
	Significance	*Odds ratio*	*Lower*	*Upper*
T0	.548	0.694	0.211	2.283
T1	.028	3.121	1.133	8.603
T2	.008	4.025	1.442	11.238

antimicrobial compounds, but many antibiotic and chemical bactericides currently used may disturb the bacterial flora of the oral cavity, resulting in induction and overgrowth of antibiotic-resistant bacteria and other opportunistic pathogens such as *Candida albicans*.[16] So, many researchers in the world have been searching for alternatives to prevent the occurrence of this process, for example, using natural substances, derived from food.

The analysis of the literature suggests that diet may influence dental decay experience in two ways: it may inhibit or it may promote the disease. Far more is known about dietary factors that promote dental decay than those that inhibit it.

The studies carried out in these last decades have supported the antibacterial role of polyphenols, but at present their potential use in the control of bacteria responsible for cariogenesis is still under scrutiny.[17]

A relatively larger body of evidence has been accumulated on the effects of tea (particularly green tea) on plaque formation, whereas the data on sources of other polyphenols are at a preliminary stage, although in the case of cranberry numerous findings have been accumulating.[18]

The present *in vivo* study has shown that 60% of subjects using a green tea mouth rinse presented a significant lowering of levels of mutans streptococci and 42.4% of subjects using this green tea mouth rinse presented a significant lowering of levels of lactobacilli compared with the subjects using placebo mouth rinse. This is probably due to the antibacterial properties of polyphenols associated with the inhibition of adherence of bacterial cells to tooth surfaces.[10]

In fact, the results from the present study on the activity of *in vivo* tea extracts against oral microorganisms support the hypothesis that tea exerts an anticaries effect via an antimicrobial mode of action.[19]

Table 2. Statistical Analysis Between Test and Control Groups for Lactobacilli Colony-Forming Units Density

			95% confidence interval	
	Significance	*Odds ratio*	*Lower*	*Upper*
T0	.495	0.621	0.158	2.441
T1	.008	4.025	1.442	11.238
T2	.007	4.241	1.479	12.165

Concerning mutans streptococci, the trend of reduction in the bacterial counts for the test group is directly proportional to the exposure time to green tea mouth rinse. In addition, the slight increase in the salivary lactobacilli CFU density (CFU/mL) observed at t2 relative to t1 in the experimental group, although not statistically significant, should be due to the establishment of an intrinsic mechanism of bacterial resistance.

Our findings reflected what has been found in the literature, where the association between the use of specific foods and reduction of oral cariogenic bacteria has emerged.[20]

Therefore, our study, demonstrating the *in vivo* effect of tea extracts on cariogenic bacteria, could open a promising avenue of application because such extracts are relatively safe, have acceptable taste and odor, and could be used at a reasonable cost in the preparation of specific anticariogenic remedies.

Furthermore, because in the literature there are only a few studies on the *in vivo* antibacterial effect of green tea in humans,[10] an innovative noninvasive *in vivo* investigation has been performed, focused on the applicability of a common beverage with therapeutic effects on human health. Finally, it should be emphasized that the present experimental procedure has demonstrated its validity as far as the original aim of the study is concerned: it is sufficiently sensitive in quantifying variations in mutans streptococci and lactobacilli CFU density (CFU/mL), and it is easy to perform.

CONCLUSIONS

The present study demonstrated that daily use of a mouthwash of green tea infusion could reduce the salivary levels of mutans streptococci and lactobacilli, which are the most virulent cariogenic pathogens in the oral cavity. This approach could be an alternative strategy for the prevention of dental caries.

More studies, particularly *in vivo* and *in situ*, are necessary to establish conclusive evidence for the effectiveness of polyphenols against dental caries with the aim of improving oral health; it is essential to determine the nature and distribution of these compounds in our diet and to better identify which of the hundreds of existing polyphenols are likely to provide the greatest effects. As the evidence of therapeutic effects of dietary polyphenols continues to accumulate, it is becoming more and more important to understand the nature of *in vivo* absorption and metabolism.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Luczaj W, Skrzydlewska E: Antioxidative properties of black tea. *Prev Med* 2005;40:910–918.
2. Frei B, Higdon JV: Antioxidant of tea polyphenols in vivo: evidence from animal studies. *J Nutr* 2003;133:3275S–3284S.

3. Krishnan R, Maru GB: Inhibitory effect(s) of polymeric black tea polyphenol fractions on the formation of [^{3}H]-B(a)P-derived DNA adducts. *J Agric Food Chem* 2004;52:4261–4269.
4. Yamane T, Nakatani H, Kikuoka N, *et al*.: Inhibitory effects and toxicity of green tea polyphenols for gastrointestinal carcinogenesis. *Cancer* 1996;77:1662–1667.
5. Zhang G, Miura Y, Yagasaki K: Effects of dietary powdered green tea and theanine on tumor growth and endogenous hyperlipidemia in hepatoma-bearing rats. *Biosci Biotechnol Biochem* 2002;66:711–716.
6. Sang S, Lambert JD, Tian S, *et al*.: Enzymatic synthesis of tea theaflavin derivatives and their anti-inflammatory and cytotoxic activities. *Bioorg Med Chem* 2004;12:459–467.
7. Scalbert A, Manach C, Morand C, Rémésy C, Jiménez L: Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* 2005;45:287–306.
8. Taguri T, Tanaka T, Kouno I: Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biol Pharm Bull* 2004;27:1965–1969.
9. Brighenti FL, Luppens SB, Delbem AC, *et al*.: Effect of *Psidium cattleianum* leaf extract on *Streptococcus mutans* viability, protein expression and acid production. *Caries Res* 2008;42:148–154.
10. Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M: Anticaries effects of polyphenolic compounds from Japanese green tea. *Caries Res* 1991;25:438–443.
11. Hirasawa M, Takada K, Otake S: Inhibition of acid production in dental plaque bacteria by green tea catechins. *Caries Res* 2006; 40:265–270.
12. Matsumoto M, Minami T, Sasaki H, Sobue S, Hamada S, Ooshima T: Inhibitory effects of oolong tea extract on caries-inducing properties of mutans streptococci. *Caries Res* 1999;33: 441–445.
13. Smullen J, Koutsou GA, Foster HA, Zumbé A, Storey DM: The antibacterial activity of plant extracts containing polyphenols against *Streptococcus mutans*. *Caries Res* 2007;41:342–349.
14. Hattori M, Kusumoto IT, Namba T, Ishigami T, Hara Y: Effect of tea polyphenols on glucan synthesis by glucosyltransferase from *Streptococcus mutans*. *Chem Pharm Bull* 1990;38:717–720.
15. Kashket S, Paolino VJ: Inhibition of salivary amylase by water-soluble extracts of tea. *Arch Oral Biol* 1988;33:845–846.
16. Gunsolley JC: A meta-analysis of six-month studies of antiplaque and antigingivitis agents. *J Am Dent Assoc* 2006;137: 1649–1657.
17. Ferrazzano GF, Amato I, Ingenito A, De Natale A, Pollio A: Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). *Fitoterapia* 2009;80:255–262.
18. Bodet C, Grenier D, Chandad F, Ofek I, Steinberg D, Weiss EI: Potential oral health benefits of cranberry. *Crit Rev Food Sci Nutr* 2008;48:672–680.
19. Li JY, Zhan L, Barlow J, Lynch RJ, Zhou XD, Liu TJ: [Effect of tea polyphenol on the demineralization and remineralization of enamel in vitro]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2004;35: 364–366.
20. Hamilton-Miller JM: Anti-cariogenic properties of tea (*Camellia sinensis*). *J Med Microbiol* 2001;50:299–302.

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2. Gabriella Gazzani, Maria Daglia, Adele Papetti. 2011. Food components with anticaries activity. *Current Opinion in Biotechnology* . [CrossRef]