

## The Effect of Green Tea Mouthrinse in a 4 Day Plaque Regrowth Model in Vivo and Antibacterial Efficacy in Vitro: A Randomized Controlled Trial

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**ABSTRACT:** *Aim:* To evaluate the antibacterial efficacy of green tea extract against two primary colonizers in vitro and to evaluate and compare the antiplaque efficacy in vivo with 0.2% chlorhexidine.

*Materials and methods:* The minimum inhibitory concentration (MIC) of 0.2% chlorhexidine and green tea were determined in vitro using agar dilution method. A double-blinded, parallel, randomized, 4 day plaque regrowth clinical trial was designed and conducted to determine and compare the antiplaque efficacy of 0.2% chlorhexidine (group1) and green tea (group 2). Thirty subjects (15 males, mean age 31.9 years), participated in the clinical trial, and the plaque index (Silness and Loe 1964) were compared at the 5<sup>th</sup> day. Statistical analyses for evaluation of plaque growth and comparison between groups were performed by independent *t* test.

*Results:* Green tea mouthrinse shows effective antibacterial action against two selected primary colonizers. When compared between groups chlorhexidine shows least MIC. Both groups showed an effective reduction in plaque re-growth. When compared between groups, chlorhexidine showed more plaque control efficacy than green tea, but the results were not statistically significant ( $p=0.778$ ).

*Conclusion:* The findings of this study suggest that green tea shows effective antibacterial against two primary colonizers with an antiplaque action which is comparable with 0.2% chlorhexidine.

**KEYWORDS:** antiplaque, antibacterial, green tea, chlorhexidine, mouthrinse.

## **1 INTRODUCTION**

Epidemiological (Ash et al.) and clinical research (Loe et al.) suggest a direct association between dental plaque and chronic gingivitis. [1,2] The initial microbial challenge and the host susceptibility are the main contributing factors to the development of inflammatory periodontal disease. Hence, inhibition of bacterial colonization has a pivotal role in the prevention of periodontal disease. Supragingival plaque (which is essential for subgingival plaque development) control is the mainstay of primary and secondary prevention of periodontal disease. [3]

Mechanical plaque control by tooth brushing is the most ideal way of preventing gingivitis and periodontitis. Unfortunately, most of the subjects fail in following a quality self performed mechanical plaque control.[4] These limitations can be overcome by the adjunctive use of antimicrobial agents which are also effective in preventing growth of biofilm in soft tissue surfaces.[5] Evidence from a meta-analysis strongly support the adjunctive use of antimicrobial agents compared to mechanical plaque control.[6]

Antimicrobial resistance, adverse effects of chlorhexidine (like staining, taste alterations, etc.) redirect the attention of researchers to more traditional ways of treating disease. Recently complementary and alternative medicine (CAM) has become more popular due to the natural way of curing various diseases. [7]

**Green tea (*Camellia sinensis*)** is one of the most popular natural products in the world. Recently, intake of green tea catechins has been shown to have great potential in prevention of various diseases (cardiovascular and cancer development). [8, 9] The benefits of green tea are attributable to the presence of various polyphenols such as (-) -epigallocatechin-3-gallate (EGCG), (-) - epigallocatechin (EGC), (-) - epicatechin -3-gallate (ECG) and (-) - epicatechin. Green tea catechins also showed bactericidal property against *Porphyromonas gingivalis* (Pg) *Prevotella* spp[10] and *Tannerella forsythus* (Tf) and beneficial in preventing periodontal disease.[11] The aim of the present study was to evaluate the antibacterial efficacy of green tea extract against two primary colonizers in vitro and to evaluate and compare the antiplaque efficacy in vivo.

## **2 MATERIALS AND METHODS**

The study was designed in two parts: First part evaluated the minimum inhibitory concentration (MIC) of test (green tea) and control (chlorhexidine) agents by in vitro agar dilution method. Second part was a clinical trial to test the antiplaque efficacy of mouthrinse in a 4 day plaque regrowth model in vivo.

### **2.1 IN VITRO TESTING**

The blood agar plates were prepared using TSA (Trypticase Soy broth-30g, Agar 15g) and 5% defibrinated sheep blood (Fig 1). Bacterial strains were inoculated on blood agar plate and incubated at 37°C at 24-48 hours. The growth of bacteria was then confirmed by observing under a light microscope (Fig 2).

The minimum inhibitory concentration (MIC) was determined against reference strains of two predominant early colonizers on supragingival plaque: *Streptococcus sanguinis* (ATCC 10556) and *Streptococcus oralis* (ATCC 9811). The MIC of green tea and 0.2% chlorhexidine against each test bacterial strain was measured by an agar dilution method. One hundred microlitres of strain suspension were added to the agar plate containing two fold serial dilutions of each agent. This offered the final concentrations of test and control. The plates were then incubated for 18 hours at 35°C.[12]

### **2.2 IN VIVO TESTING**

Green tea mouth rinse was extracted from the leaves using soxhlet extractor with heating of solvent (water). The apparatus are fitted properly and extraction is started with the appropriate heating of solvent. The solvent boiled and converted to vapour and condensed. The hot liquid falls in the drug in extractor and extractor filled with the solvent due to which the level of siphon tube also rises gradually then falls into the solvent flask. The same process is repeated for 2-3 times to obtain the complete extract. Finally extracted green tea was diluted with distilled water to a concentration of 1:1 (5mg/mL) and transferred to a container.

This double-blinded, parallel, randomized, 4 day plaque regrowth clinical trial was conducted in the Department of periodontics, Sri Hasanamba dental college & hospital, Hassan, Karnataka, India. A total of 32 patients (16 males, 16 females, Mean age 31.9 Years) were screened from the out-patient department of periodontics in November 2013. A total of 32 patients were enrolled based on the following inclusion criteria: 1) a minimum of 22 natural teeth, 2) no fixed or removable

appliances, 3) no more than full coverage restorations, and 4) participants who are systemically healthy without any medical or pharmacological history. The protocol for the study was approved by the institutional ethics committee and written informed consent was obtained from the participants before the commencement of the study. The selected subjects were divided randomly into two groups (group 1 and 2) using the lottery method and the participants were masked to the mouthrinse received. Mouthrinses were filled in coded, sealed, matching bottles with printed instructions for usage (Fig 3) and dispensed by a masked dispenser. Clinical assessments were performed by a single examiner who blinded to the study groups.

Group 1: 0.2% chlorhexidine mouthrinse (Hexidine<sup>®</sup>).

Group 2: Green tea mouthrinse.

On day1, all participants received a careful oral examination and then proceeded with scaling and polishing to make the tooth surface free of visible plaque, calculus and extrinsic stain. All patients were instructed to abstain from all forms of oral hygiene measures except the allocated mouth rinse for a period of 4 days. Subjects were asked to rinse 10 ml of prescribed mouth rinse for 60 seconds twice daily (morning and evening after food). On day 5, all patients were evaluated for plaque regrowth by means of Plaque index (PI) (Silness and Loe 1964). Consort flow diagram and study design are shown in Fig 4 and 5 respectively.

### 3 STATISTICAL ANALYSIS

Statistical analyses for evaluation of plaque growth and comparison between groups were performed by independent t test.

### 4 RESULTS

#### 4.1 IN VITRO

The MIC of the two agents is given in Table 1. The lowest concentration of the green tea that inhibited the visible growth of the microorganisms was considered as MIC. The prevention of growth of microorganisms was confirmed using a light microscope. Green tea has shown effective inhibition of microbial growth in pure culture. The MICs of green tea for *S. sanguinis* and *S. oralis* were 512 and 128 $\mu$ g/ml respectively.

#### 4.2 IN VIVO

A total of 32 patients (16 male and 16 female) with an age range of 31.9 years, were enrolled in this study. They were randomly assigned into test and control groups. Thirty patients followed the study protocol and completed the trial period. One patient from each group failed to report on 5<sup>th</sup> day due to personal reasons. No adverse effects were reported in any of the subjects.

Table 2 depicts descriptive statistics of Plaque Index after 4 days. Comparison of plaque index after 4 days is shown in Table 3. Both chlorhexidine and green tea showed an effective reduction in plaque re-growth. When compared between groups, chlorhexidine showed more plaque control efficacy than green tea, but the results were not statistically significant  $p=0.778$  (Fig 6). The mean plaque scores were  $0.55\pm 0.37$ ,  $0.59\pm 0.36$  for group 1 and 2 respectively (Table 2).

### 5 DISCUSSION

The landmark studies by Loe et al. and Theilade et al. demonstrates a direct association between microbial plaque accumulation and initiation of gingivitis. [2, 13,14] Later, the role of microorganism (Socransky et al) and individual host susceptibility to the development of inflammatory periodontal disease (Hart et al.) was established. [15,16] However, the main strategy of periodontal treatment still focuses on elimination of microbial plaque followed by meticulous oral hygiene measures.

Green tea catechins are well known for its anti oxidative, antimicrobial, anticariogenic and anti carcinogenic activity. Epigallocatechin in green tea has been found to be beneficial in deodorizing methyl mercaptan ( $\text{CH}_3\text{SH}$ ), a volatile sulfur compound (VSC) which causes halitosis.[17] Various polyphenols in green tea are also effective against periodontal pathogens, especially *P. gingivalis*<sup>10</sup> and also inhibits bone resorption.[18] The level of catechins in tea varies according to the collection and processing of the leaves. The maximum level of catechins is detected in green tea (non fermented) than

oolong tea (semi fermented) and black tea (fermented).[19] The tea leaves which are fresh and produced by drying and steaming, preserve the catechins by preventing oxidation. The present study was designed to evaluate the efficacy of green tea against two primary colonizers. The MIC of green tea and chlorhexidine was determined for chosen bacteria. Then the antiplaque efficacy of green tea was evaluated and compared with chlorhexidine.

Both microorganisms show susceptibility to chlorhexidine and green tea. Compared to green tea, chlorhexidine demonstrated lesser MIC. The inhibitory effects of green tea on streptococcus species in vitro suggest its action in controlling plaque growth. When considering the clinical efficacy of two agents for antiplaque action, chlorhexidine showed better reduction in plaque regrowth than green tea. But there was no statistical significant difference between the groups ( $p=0.778$ ). As the vitro and in vivo results were supported by each other, it would be reasonable to say that green tea is as efficacious as chlorhexidine in inhibiting plaque regrowth.

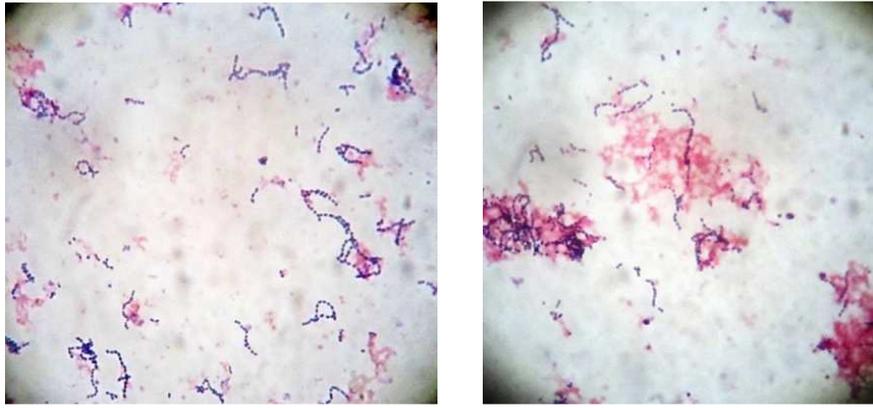
Studies conducted by Sakanaka et al. and Rasheed and Haider clearly showed the inhibitory effects of green tea catechins (MIC in the range of 50-1000 $\mu$ g/ml) on dental caries causing microorganisms like *S. mutans* and *S. sobrinus*. [20,21]The proposed mechanisms of action of catechins were by inhibiting the proliferations of streptococcus species, by inhibiting bacterial glucosyl transferase and preventing adherence to enamel. You SQ investigated the use of 0.2% green tea solution as a rinse and found a significant reduction in plaque index which is similar to the present study. [22]He also showed that there were no developments of resistance in repeated cultures. Thus, green tea can be used as an effective antiplaque agent especially in patients who are susceptible to gingivitis.

The varied benefits of green tea, such as easy availability, reduced cost; less staining, antibacterial property and less resistance make this natural product an effective therapeutic agent against periodontal disease. Further long-term trials with large samples and antibacterial action of green tea against other primary colonizers are needed to provide more promising evidence.

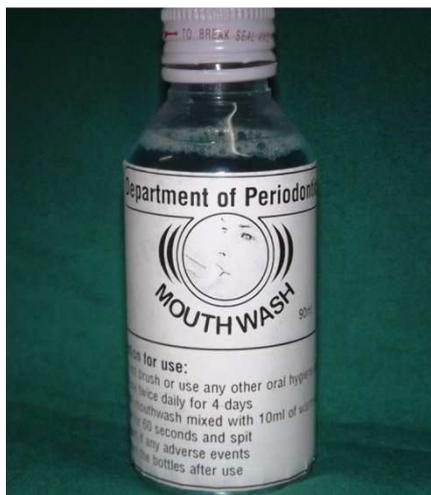
## **6 FIGURES**



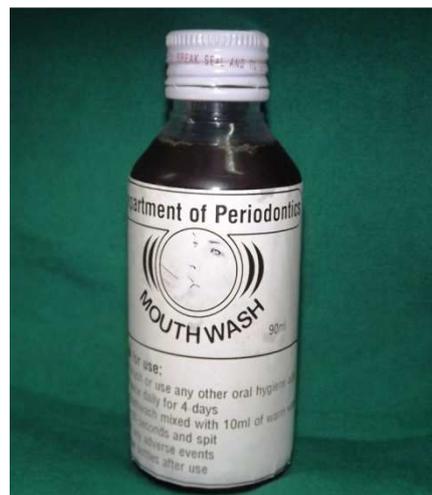
**Fig. 1. *Streptococcus* species grown on agar plate**



**Fig. 2.** Growth of bacteria shown under microscope (100x)



**Group 1**



**Group 2**

**Fig. 3.** Mouthrinse filled in identical bottles

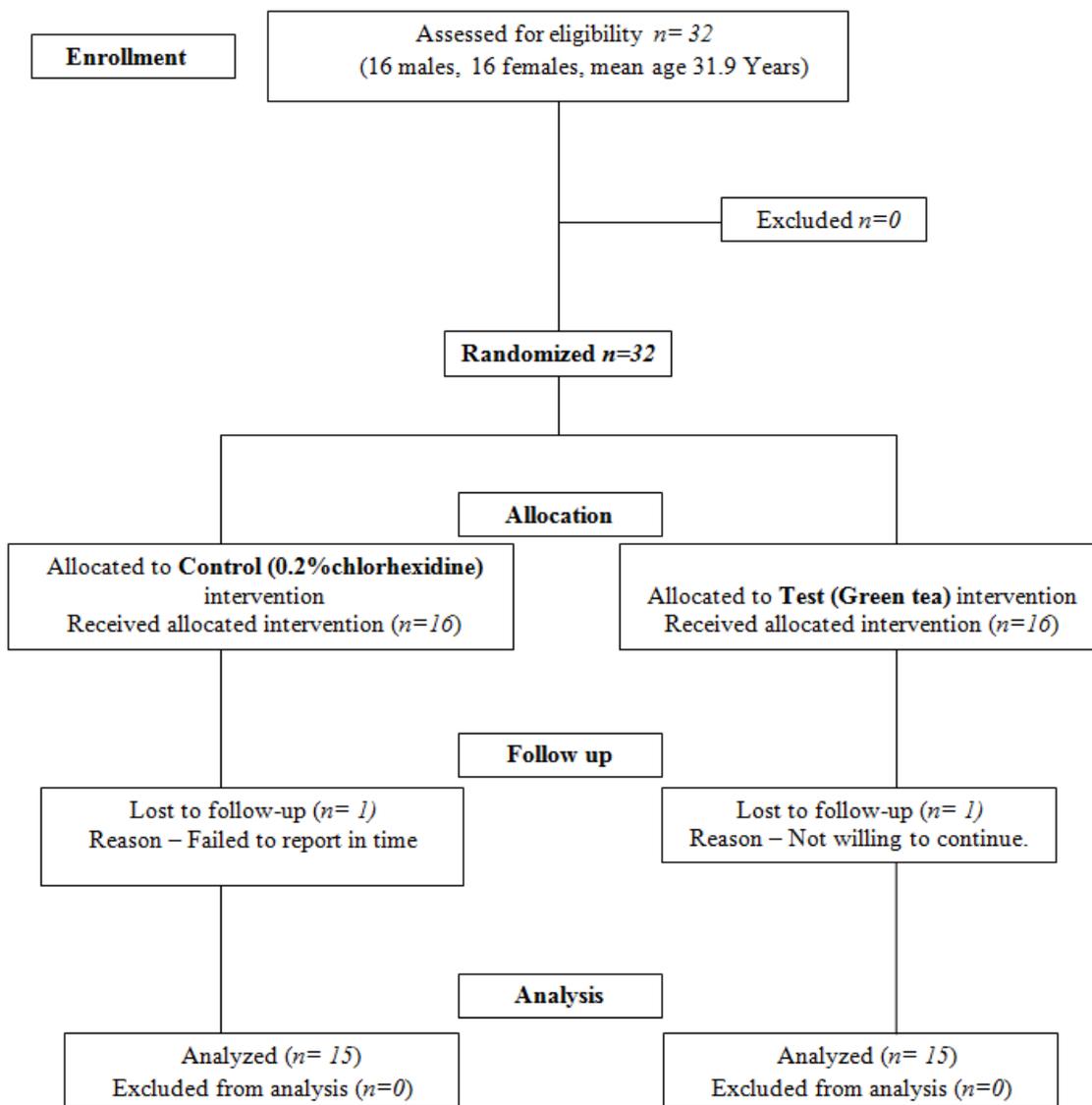


Fig. 4. Study flow diagram

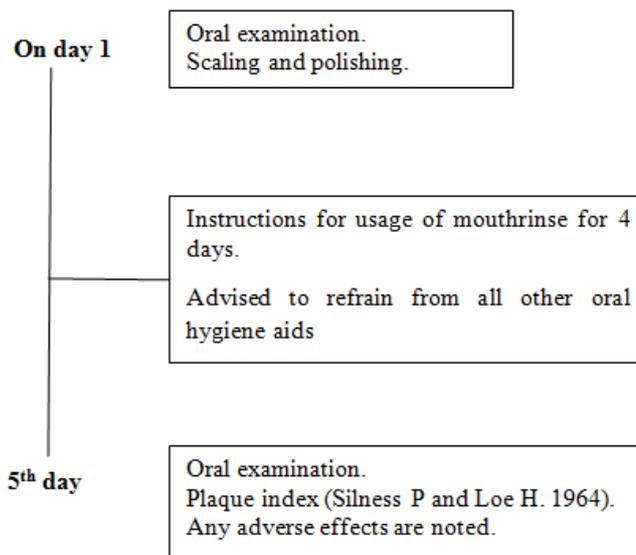


Fig. 5. Study design

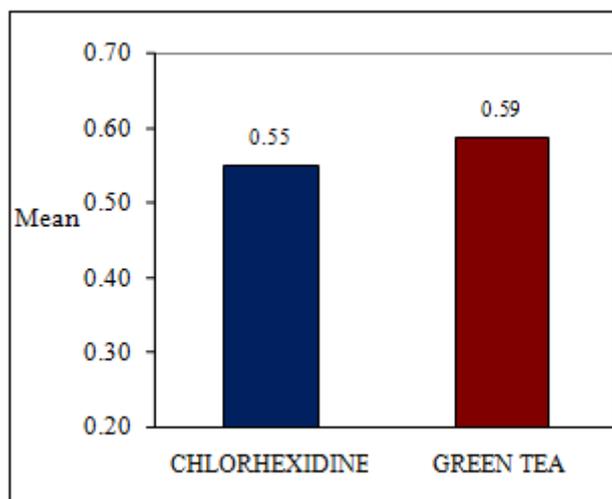


Fig. 6. Comparison of Plaque Index after 4 days between groups

7 TABLES

Table 1. MIC of test agents against oral microorganisms

Microorganisms tested	ATCC Reference No.	0.2% Chlorhexidine	Green tea
<i>Streptococcus sanguinis</i>	10556	62	512
<i>Streptococcus oralis</i>	9811	62	128

Table 2. Descriptive statistics of Plaque Index after 4 days based on group

Group	Mean	SD	Median
Chlorhexidine	0.55	0.37	0.49
Green Tea	0.59	0.36	0.50

**Table 3. Comparison of Plaque Index after 4 days between group**

Group	Mean	SD	N	t	p
Chlorhexidine	0.55	0.37	15	0.29	0.778
Green Tea	0.59	0.36	15		

## 8 CONCLUSION

Within the limitations of the study, it was demonstrated that green tea is safe and effective in inhibiting oral bacteria and also in reducing plaque regrowth.

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