Original Article

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Dentistry Section

Effects of Green Tea on *Streptococcus mutans* Counts- A Randomised Control Trail

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ABSTRACT

Context: Mouth rinses have been in use from time immemorial as a supplement for routine oral hygiene. There are many number of mouth rinses currently available in the market in which many of them possess certain drawback, which has necessitated the search for alternate mouth rinses.

Aim: The aim of the present study was to assess the effect of rinsing with green tea in comparison with chlorhexidine and plain water on *Streptococcus mutans* count.

Setting and Design: A short term, single blinded, cross over randomised control clinical trial.

Materials and Methods: Study includes a total of 30 subjects aged 20 to 25 years divided into three groups that is green tea group, chlorhexidine group, and plain water group. A baseline plaque samples were collected and under supervision of examiner all the subjects rinsed with 10 ml of respective solutions for one minute. Plaque samples were collected at five minutes after rinsing. All the 30 subjects were exposed to all the three rinses with a wash out period of seven days between the interventions. All the samples were sent to microbial analysis.

Results: Wilcoxon matched pair test and Mann-Whitney U test showed that both chlorhexidine and green tea significantly reduced *Streptococcus mutans* colony counts compared to plain water.

Conclusion: The results of present study indicate that green tea mouth rinse proved to be equally effective compared to chlorhexidine which is considered as gold standard. This may also be a valuable public health intervention as it is economical and has multiple health benefits.

Keywords: Green tea, Herbal mouth rinse, *Streptococcus mutans*, Chlorhexidine

INTRODUCTION

Dental diseases remain a significant problem with vast majority of population suffering with consequences of disease at some stage in their lives. Oral cavity harbours wide variety of microorganisms, these are considered crucial for the initiation and progression of dental diseases [1]. Out of which *Streptococcus mutans* is one of the main causes of dental disease. Many efforts have been under taken to reduce bacterial colony counts like good oral hygiene practices including proper cleaning of teeth and mouth rinsing with various mouth rinses. Mouth rinses are solutions or liquids used to rinse the mouth for a number of purposes: (a) to remove or destroy bacteria, (b) to act as an astringent, (c) to deodorise and (d) to have a therapeutic effect by relieving infection or preventing dental caries [2]. The daily use of antimicrobial mouth rinse shown to have significant antiplaque activity would be a meaningful, cost effective addition to mechanical oral hygiene methods and can be a valuable component of oral hygiene regimens [3].

Several ingredients and products have been found to be effective against bacterial activity out of which chlorhexidine is considered as gold standard [4,5]. The benefits of chlorhexidine, a cationic biguanide are based on its bactericidal and bacteriostatic activities. Chlorhexidine has often been used as a positive control during assessment of anticariogenic potential of other agents. However, the side effects of chlorhexidine, primarily staining and taste alteration, limit its potential to be used for long-term use [6]. Due to these drawbacks with chemicals in mouth rinses an increasing number of people all around the world are turning to nature by using natural herbal products. There has been a change in thinking globally with a growing tendency to 'go natural'. The natural phytochemicals present in plants could offer an effective alternative to synthetic preparations. Research has been focused in recent years on herbal medicines owing to their wide range of biological and medicinal activities, higher safety margins and lower costs [7].

Green tea is regarded as a health-promoting beverage possessing a wide spectrum of medicinal benefits. The beneficial effects of green tea are generally attributed to its polyphenol content, particularly catechins which has got diverse pharmacological properties that include anti-bacterial effects, anti-cariogenic effects, anti-oxidative effects etc. [8]. Studies suggested that extracts from green tea might be especially helpful in preventing tooth decay by preventing the development of bacterial plaque. Green tea polyphenols work as anti-plaque agents by suppressing glucosyl transferase, which oral bacteria use to feed on sugar [9]. Hence the aim of the present study was to evaluate the effects of rinsing with green tea on *streptococcus mutans* count in plaque and to compare the efficacy of green tea with that of chlorhexidine mouth wash and plain water on the count of *streptococcus mutans* in plaque.

MATERIALS AND METHODS

Study subjects: Based on pilot study results and using the following formula, 10 subjects in each group would provide 80% power of the study at 5% level of significance.

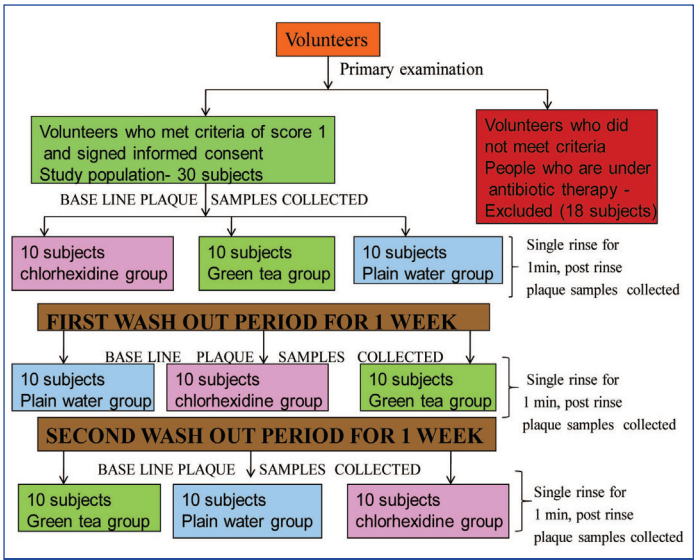
$$N = \frac{(Z\alpha + Z\beta)^2 (S)^2}{2(d)^2}$$

N=sample size; Zα=z value for alpha error; Zβ=z value for beta error; S=standard deviation; d=difference from mean value to be detected.

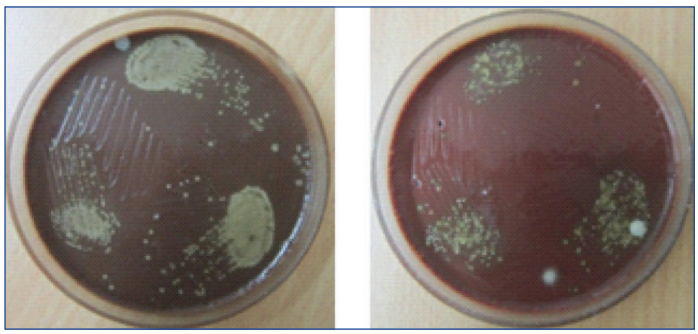
Hence sample size of 30 subjects, belonging to both sexes, ranging in age from 20 to 25 years was selected among students of a dental institute. Commercially available 0.12% chlorhexidine was used as positive control and plain water is used as negative control.

Preparation of Green Tea

Fresh green tea (packing date less than one month) was procured from local market which is available in the form of green tea dip bags. Two percent green tea was prepared with 2 grams of green tea dip bag dipped in 100 ml warm water for five minutes. All the three mouth rinses were dispensed in disposable cups for the participants (10 ml for each participant).



[Table/Fig-1]: Flowchart of methodology



Before rinse After rinse with green tea

[Table/Fig-2 a&b]: Counts of *S.mutans* were expressed as no. of colony forming units/ml, ** Statistically significant*

Groups	Rinse	Mean	Standard deviation	Mean Difference	p-value
Green tea	Before rinse	32500.0	1779.51	5300.00	0.0057*
	After rinse	27200.0	1475.73		
Chlorhexidine	Before rinse	31900.0	1595.13	5500.00	0.0051*
	After rinse	26400.0	1776.39		
Plain water	Before rinse	31200.0	1316.56	200.00	1.0000
	After rinse	31000.0	1414.21		

[Table/Fig-3]: Comparison of before rinse and after rinse *Streptococcus mutans* colony counts of phase 1 in three groups (Green tea, Chlorhexidine, Plain water)
** Statistically significant*

Study Design

The present study is single-blinded (microbiologist-blinded) cross over randomised control trail conducted at the post graduate clinic, department of public health dentistry, in a dental institute of south India. Ethical clearance was obtained from institutes' ethical committee. Prior to start of study a primary examination was conducted using Silness and Loe plaque index. Based on simple random sampling subjects who scored 1 (a film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on tooth surface) were included into the study by obtaining written informed consent. Subjects who did not meet criteria and who are under antibiotic therapy were excluded.

Using random allocation rule participants were divided into chlorhexidine group, green tea group, and plain water group. Cards with group names were kept in sealed envelopes, and all the participants were asked to pick up the sealed envelopes. Envelopes are opened by investigator only after the participant's name was written on the appropriate envelop.

Groups	Treatment	Mean	Standard deviation	Mean Difference	p-value
Green tea	Before rinse	32200.0	1316.6	5600.00	0.0051*
	After rinse	26600.0	1429.8		
Chlorhexidine	Before rinse	31900.0	1370.3	5700.00	0.0051*
	After rinse	26200.0	1316.6		
Plain water	Before rinse	31500.0	1269.3	-200	0.7213
	After rinse	31700.0	1159.5		

[Table/Fig-4]: Comparison of before rinse and after rinse *Streptococcus mutans* colony counts of phase 2 in three groups (Green tea, Chlorhexidine, Plain water)
** Statistically significant*

Groups	Treatment	Mean	Standard deviation	Mean Difference	p-value
Green tea	Before rinse	32000.0	1333.33	5300.00	0.0051*
	After rinse	26700.0	1197.22		
Chlorhexidine	Before rinse	31900.0	1595.13	5700.00	0.0051*
	After rinse	26200.0	1475.73		
Plain water	Before rinse	31200.0	1316.56	100.00	0.2807
	After rinse	31100.0	1549.19		

[Table/Fig-5]: Comparison of before rinse and after rinse *Streptococcus mutans* colony counts of phase 3 in three groups (Green tea, Chlorhexidine, Plain water)
** Statistically significant*

PW-GT	0.2123	0.0002*
PW-Chloro	0.2730	0.0002*
GT-Chloro	1.0000	0.1988

[Table/Fig-6]: : Pair wise comparison of three groups (GT-Green tea, Chloro-Chlorhexidine, PW-Plain water) by Mann-Whitney U test
** Statistically significant*

Before starting intervention a base line plaque samples were collected, 10 ml of each solution were given to the respective groups to rinse for one minute. Plaque samples were collected at five minutes after rinsing. A wash out period of seven days were given and all the subjects were recalled for repetition of above procedure. Hence all the 30 subjects were exposed to all the three rinses with a wash out period of seven days between interventions [Table/Fig-1]. Plaque samples were collected using sterile buds from lingual side of lower molars and this sample were transferred to sterile test tube containing thioglycolate transport media. All the samples were sent for microbiological analysis within half an hour of sample collection.

Microbiological Analysis

Microbial analysis was done in Department of Microbiology, Government Medical College in South India. Plaque samples were diluted with sterile saline in a ratio of 1:1000 and streaked on chocolate agar plates, this plates are incubated for 48 hours at 37°C and number of bacterial colonies counted [Table/Fig-2a,b].

STATISTICAL ANALYSIS

Mann-Whitney U test and Wilcoxon matched pairs test were used and data was analysed using SPSS software (version 16). A 'p' value of <0.05 was considered as significant.

RESULTS

A crossover, single blinded randomised control clinical trial was conducted on 30 subjects of mean age 22.4 ± 1.75. Microbial analysis of plaque samples indicated that there is reduction in *streptococcus* colony counts after rinsing with chlorhexidine (p=0.0051; p= 0.0051; p=0.0051) and green tea (p=0.0057; p=0.0051; p=0.0051) compared to baseline, which is statistically significant in all the three phases [Table/Fig-3-5]. No significant differences were seen in *S.mutans* levels among the plain water group [Table/Fig-3-5]. When pair wise comparison was done among three groups with Mann-Whitney U test, there is no statistical significant difference between green tea and chlorhexidine (p=0.1988) where as green tea and

chlorhexidine are more effective when compared to plain water (p=0.0002) which is statistically significant [Table/Fig-6].

DISCUSSION

Herbal mouth rinses have received special attention because of being non chemical and non synthetic, and they have been long used in traditional medicine. Green tea has been considered a medicine and a healthful beverage since ancient times. The traditional Chinese medicine has recommended this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and in general, to prolong life. Green tea leaves contain three main components which act upon human health: xanthic bases (theophylline), essential oils and polyphenolic compounds [10].

Oral diseases including dental caries, periodontal diseases, and tooth loss may significantly impact a person's overall health. Among these dental caries is a multifactorial infectious disease in which nutrition, microbiological infection and host response play important roles. Studies have shown that green tea possess diverse pharmacological properties which includes anti-bacterial, anti-viral, anti-oxidative, anti-inflammatory, anti-cariogenic, anti-aging [9]. Some animal studies have such as studies conducted by Linke and LeGeros indicated that frequent intake of green tea can significantly decrease caries formation, even in the presence of sugars in diet [11]. Okamoto et al., suggested that green tea catechins may have the potential to reduce periodontal breakdown resulting from potent proteinase activity of *porphyromonas gingivalis* [12]. From some decades, the determination of bacterial counts has been a test accepted by the scientific community to investigate the antibacterial effect of mouth rinses.

Axelsson and lindhe have shown that chlorhexidine mouth wash is effective in reducing plaque and gingivitis [13]. Menendez et al., have shown that chlorhexidine is very effective against *S.mutans* in dental plaque [3]. Salehi et al., have compared the antibacterial effects of persica mouthwash with that of standard chlorhexidine on *S.mutans* [14]. Hence, the gold standard mouthwash was used as control in this study to assess the effect of green tea mouth rinse on *streptococcus mutans* count. In this study there was a definitive reduction in the *S.mutans* count in plaque, after rinsing with green tea which is similar to trail conducted by You SQ [15].

In this study the chlorhexidine group showed a slightly greater statistically significant reduction of *streptococcus mutans* count in plaque than green tea mouth rinse. However, green tea has certain advantages over chlorhexidine: it does not stain; it has no lingering after taste, no bacterial resistance and causes no allergy. Moreover green tea is 5-6 times cost-effective, easy to prepare and can be used as home care product [7]. Green tea mouth rinse can be a good preventive home therapy in developing countries like India. This study was carried out on a small sample which is the limitation of this study. More extensive studies with larger samples and over varying time periods should be carried out to establish the efficacy of green tea mouth rinse in the prevention of dental caries.

The following conclusions were derived from this study:

- Reduction in *S.mutans* count was seen in both the green tea and chlorhexidine groups
- Statistically significant reduction in the mean *S.mutans* counts were slightly more in chlorhexidine group than in the green tea group when compared to plain water group.

CONCLUSION

Healthy teeth are fundamental for the proper functioning of the human body. Proper and regular hygiene is required to prevent dental problems. The treatments for dental problems are expensive and cannot be afforded by poor people. So, these types of natural products, which are of low cost, are a great help to society. It is necessary to carry out research into these products and make available to every part of the country.

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