A Comparative Study between Indigenously Prepared Green Tea and Green Tea in Combination with Triclosan as Mouth Rinse in Reducing *Streptococcus mutans* Count in the Children

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ABSTRACT

Introduction: Maintaining good oral hygiene is of utmost importance for the overall well being of an individual. So, the aim of the present study is to compare the anti-microbial efficacy of indigenously prepared 2% green tea and 2% green tea in combination with 0.03% triclosan mouth rinse on *Streptococcus mutans* colony count in children aged between 7-12 years.

Materials and methods: Unstimulated saliva was collected from 26 healthy children aged between 7-12 years. These were randomly divided into Group 1 receiving green tea mouth rinse and Group 2 receiving green tea and triclosan combination mouth rinse. The *Streptococcus mutans* load was assessed using mitis salivarius bacitracin agar by collecting saliva before and after 30 minutes of the intake of mouth rinses in respective groups. Data were analyzed using student t-test ($p<0.05$ is considered as statistically significant).

Results: There was significant difference between salivary *Streptococcus mutans* count before and after ($p<0.001$) intake of both the rinses. However, the combination of 2 % green tea and 0.03% triclosan showed a significantly greater reduction of *Streptococcus mutans* count than the 2% green tea alone.

Conclusion: There was a significant greater reduction in *S. mutans* count in the combination mouth rinse than the green tea alone. The use of green tea alone, or in combination with triclosan, as mouth rinse appears to be effective for regular use as a part of a daily preventive regime in children.

Keywords: Triclosan, Green Tea, *Streptococcus mutans*, Mitis Salivarius Bacitracin Agar

INTRODUCTION

Prevention of dental caries is one of the hallmarks of contemporary pediatric dentistry. The dental plaque is an important structure as it is a significant contributing factor to the initiation of the carious lesion. *Streptococcus mutans* is the most commonly found microorganism in the oral cavity. It is the main culprit in initiating dental caries. Thus, reducing the levels of *Streptococcus mutans* in the dental plaque will lead to good oral health. Several ingredients and products have been found to be effective against *S. mutans*. Recently, the use of natural products in the treatment of oral conditions has been increased drastically. Many natural ingredients, like green tea (table-1), possess antibacterial properties similar to that of chlorhexidine and are capable of preventing bacterial adhesion, metabolism and colonization and thus inhibits the bacterial growth efficiently.[1,2] It also possess antioxidant properties.[3] Green tea contains nearly 4000 bioactive compounds like flavanoids, tannin, vitamins, fluoride and other mineral salts. Tannins are biosynthetic materials which have a potent antibacterial effect.[4] Triclosan, on the other hand, is a non-ionic phenolic anti-plaque agent. It is a broad-spectrum antimicrobial agent (2,4,4'-trichloro 2'-hydroxydiphenyl ether) which is used to increase the ability of mouthwashes to bind to the oral mucosa, increasing their substantivity and thus their availablility for longer periods of time.[5]

The aim of the present study is to compare the anti-microbial efficacy of indigenously prepared 2% green tea and 2% green tea in combination with 0.03% triclosan mouth rinse on *Streptococcus mutans* colony count in children aged between 7-12 years.

MATERIAL AND METHODS

The present study is single-blinded (microbiologist-blinded) cross over randomized control trail conducted in the Department of Pedodontics and Preventive Dentistry, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, with a sample size of 26 children. Using random allocation rule, participants were divided into green tea group and combination mouth rinse group. Cards with group names were kept in sealed envelopes, and all the participants were asked to pick up the sealed envelopes. Envelopes were opened by investigator only after the participant's name was written on the appropriate envelop. These children had the same dietary pattern and followed similar oral hygiene practices. The protocol of the study was reviewed and approved by the ethical committee of the institution. Informed consent in written was taken from parents of the children who were included in the study.

Exclusion criteria

1. Children who are medically compromised.
2. Children with the history of taking antibiotics three months prior to or during the study period.
3. Children undergoing orthodontic treatment or with an intraoral prosthesis.
4. Children who could not rinse on their own.
5. Presence of any intra oral soft tissue pathology.

Preparation of green tea mouth rinse

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 dip bag of

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2 grams of green tea, dipped in 100 ml of warm water for five minutes.

Preparation of combination mouth rinse (2% green tea and 0.03% triclosan)

30 mg of triclosan was dissolved in 100 ml of water to make its concentration as 0.03%. This solution is then mixed with prepared 2% green tea mouth rinse in 1:1 proportion.

Both the freshly prepared mouth rinses were dispensed in disposable cups for the participants (5 ml for each participant).

Methodology: 26 children aged 7-12 years were selected. A baseline test was performed calculating the number of *S. mutans* colony forming units using Mitis Salivarius Agar after collecting the non-stimulated whole salivary sample (2ml) in sterilized container, in the Department of Microbiology, KCDS, Bengaluru. The media plates were incubated for 48 hours at 37°C. Following incubation, counting of colonies was done using digital colonimeter. The *Streptococcus mutans* count was expressed as a number of colony forming units per millilitre (CFU/ml) of saliva. Children were then divided randomly into 2 equal groups. Group 1 received 2% green tea (Green tea leaf, Tetley, Tata Global Beverages) as mouth rinse and Group 2 received 2% green tea containing 0.03% triclosan mouth rinse, which were indigenously prepared in the Department of Pharmacology. The patients were asked to swish with the prescribed mouth rinse (5ml) for 1 minute. Post rinsing non stimulated whole salivary sample (2ml) was then collected after 30 minutes and again tested for the number of *S. mutans* colony forming units (Figure-1).

STATISTICAL ANALYSIS

Statistical analysis was done to compare both the groups at baseline and after 30 minutes of using mouth rinse using student t- test and data was analyzed using SPSS software (version 20.0). A 'p' value of <0.05 was considered as significant.

RESULTS

The mean, standard deviation, and p-value of the bacterial load levels seen in patients were calculated and compared both pre rinse and post rinse using student paired t- test. The salivary *Streptococcus mutans* count before and after intake of green tea was 37.0 ± 6.325×10³ CFU/ml and 33.77 ± 6.126×10³ CFU/ml respectively and the count before and after the use of combination mouth rinse containing green tea and triclosan was 34.77 ± 7.737×10³ CFU/ml and 27.31± 7.227×10³ CFU/ml respectively (table 2). Microbial analysis of salivary samples indicated that there was reduction in *Streptococcus* colony counts after rinsing with green tea mouth rinse (p=0.429) and green tea and triclosan mouth rinse (p=0.022) compared to baseline, which is statistically significant in both the groups. When comparison was done between two groups with unpaired t-test, there was statistical significant difference between green tea and combination mouth rinse (p < 0.001) where the reduction of *Streptococcus mutans* count following green tea and triclosan mouth rinse was significantly higher than that of the reduction in the green tea mouth rinse alone (table 3).

DISCUSSION

Mouth rinses are widely used to maintain hygiene of the oral cavity. They are recommended for only those who have the ability to swish and expectorate without swallowing. It is important for these products to be safe and effective for regular use in children. Most of the mouth rinses are available 'over the counter' and contain alcohol as one of their ingredients. The major side effects of alcohol containing mouth rinses include the burning sensation, difficulty of use in patients with oral sensitivity and the risk of accidental alcohol ingestion in

Figure-1: Evaluation of bacterial count before and after the intake of green tea mouth rinse (a) and combination of green tea and triclosan mouth rinse (b)

Proteins (15–20% dry weight)	whose enzymes constitute an important fraction.
Aminoacids (1–4% dry weight)	such as teanine or 5-Nethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine.
Carbohydrates (5–7% dry weight)	such as cellulose, pectin, glucose, fructose, sucrose.
Lipids (5-7% dry weight)	as linoleic and linolenic acids.
Sterols	as stigma sterol.
Vitamins	(B, C, E).
Xanthic bases	such as caffeine, theophylline and pigments as chlorophyll and carotenoids.
Volatile compounds	as aldehydes, alcohols, esters, lactones, hydrocarbons, etc.
Minerals and trace elements (5% dry weight)	Such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P,Co, Sr, Ni, K, F and Al.

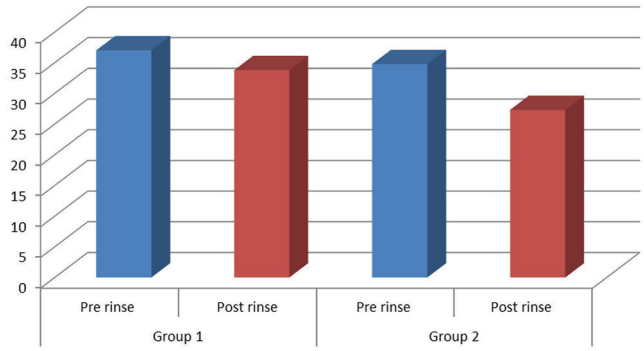
Table-1: Composition of Green Tea
Green tea is reported to have nearly 4000 bioactive compounds,

	Group	Mean	Std. Deviation	t-value	Sig.*
Pre rinse (x 1000 CFU/mL)	1	37.00	6.325	0.805	0.429
	2	34.77	7.737		
Post rinse (x 1000 CFU/mL)	1	33.77	6.126	2.459	0.022
	2	27.31	7.227		

Table-2: Comparison of *Streptococcus mutans* count between pre rinse and post rinse

		Mean	Std. Deviation	t-value	Sig.*
Group 1	Pre rinse (x 1000 CFU/mL)	37.00	6.325	16.067	0.000
	Post rinse (x 1000 CFU/mL)	33.77	6.126		
Group 2	Pre rinse (x 1000 CFU/mL)	34.77	7.737	18.553	0.000
	Post rinse (x 1000 CFU/mL)	27.31	7.227		

Table-3: Comparison of *Streptococcus mutans* count between Group 1 and Group 2.



Graph-1: Evaluation of *S. mutans* count pre rinse and post rinse in Group 1 and Group 2

children.[6] Adair (2006) recommended the use of alcohol free preparations over those containing alcohol.[7] Herbal mouth rinses have received special attention because of being non synthetic and non-chemical, and they have been long used in traditional medicine. Green tea has been considered a medicine and a healthy beverage since ancient times.[8] Hence in this study, two mouth rinses were used that were alcohol free and contain natural ingredient, green tea.

Streptococcus mutans plays the chief role in causing dental caries. Green tea extract applied topically inhibits *Streptococcus mutans* bacteria. Tea leaves contain fluoride which is known to prevent dental caries.[9] Other than fluoride, several green tea polyphenols have preventive effects on dental caries. Epicatechin gallate (ECG), epicate-chin (EC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) are the catechins present in the green tea. The most abundant catechin in green tea is epigallocatechin-3-gallate (EGCG) and also it is the most active catechin. ECG and EGCG strongly inhibit GTase which is used by bacteria to synthesize water soluble and insoluble glucans, thus inhibiting adherence of the bacteria to tooth surfaces.[10]

Awadalla et al (2011) in his study showed that there was a statistically significant difference among subjects pre- and post-rinsing with 2% green tea for 5 min concerning *S. mutans* count and the pH values of saliva and plaque.[11]

Takashi (2005) stated that rinsing with green tea regularly exhibit reduction in plaque *S. mutans* levels and also inhibit the cellular adhesion to teeth and he concluded that these effects collectively play great part in caries prevention as well.[12]

Triclosan, on the other hand, has broad spectrum antimicrobial activity and is effective against *S. mutans* at low concentration.[13] It is used to increase the ability of mouthwashes to bind to the oral mucosa, and thus be available for longer periods of time, thereby increasing their substantivity.[5] Triclosan has inhibitory effects on plaque which is mediated by its antimicrobial action. Jenkins *et al* (1991) compared the magnitude and duration of salivary bacterial count reductions produced by a single rinse of 0.2% triclosan, 1% sodium lauryl sulfate (SLS) and 0.2% chlorhexidine mouthwashes. They found that there was considerable reductions in bacterial counts which remained significant for 3 hours with triclosan and for 7 hours with SLS and chlorhexidine.[14]

In the present study, a statistically significant reduction in salivary *S.mutans* count was obtained with both the 2% green tea mouth rinse and the combination mouth rinse. However, the combination of 2% green tea and 0.03% triclosan showed a significantly greater reduction of *Streptococcus mutans* count than the 2% green tea alone. The green tea mouthwash is reported to have no evidence of irritation or burn, shows similar antibacterial effects as compared to chlorhexidine and is more safe and economical.[11] Moreover green tea is 5-6 times cost-effective, easy to prepare and can be used as home care product. When it is combined with triclosan, it gave extraordinary results due to the synergistic action of the antimicrobial rinses. In developing countries like India, green tea mouth rinse can be a good preventive home therapy. This study was carried out on a small sample which is the limitation of this study. More extensive studies over varying time periods and with larger samples should be carried out to establish the efficacy of green tea mouth rinse in the prevention of dental caries and better oral hygiene.

CONCLUSION

Proper and regular hygiene is required to prevent dental problems. The treatment for dental problems is expensive and cannot be afforded by poor people. So, these types of natural products which are of low cost and have almost negligible side effects in children are a great help to the society.

In the present study,

- Significant reduction in the salivary levels of *S. mutans* was observed with green tea mouth rinse and the combination of green tea and triclosan mouth rinse.
- There was significantly greater reduction in *S.mutans* count in the combination mouth rinse than green tea alone.
- The use of green tea alone or in combination with triclosanas mouth rinse appears to be effective for regular use as a part of daily hygiene regime in children.

However, further studies are recommended in this field for green tea and triclosan application in children younger than 6 years and evaluation of any potential adverse effects with long term use of these mouth rinses.

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