Comparing the antiplaque efficacy of 0.5% *Camellia sinensis* extract, 0.05% sodium fluoride, and 0.2% chlorhexidine gluconate mouthwash in children

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Abstract

**Background:** Dental caries is a multifactorial disease which requires a susceptible host, a cariogenic microflora, and a suitable substrate that must be present for a sufficient length of time. Tea is prepared by the infusion of dried leaves of the tea plant, *Camellia sinensis*, which contains bioactive compounds like polyphenols, flavonoids, and catechins that are thought to be responsible for the health benefits that have traditionally been attributed to tea. These compounds have multidimensional effects such as antibacterial action, inhibitory action on the bacterial and salivary amylase, and inhibition of acid production. **Aims:** The aim of this study is to compare the antiplaque efficacy of 0.5% *C. sinensis* extract, 0.05% sodium fluoride, and 0.2% chlorhexidine gluconate mouthwash in children. **Materials and Methods:** A randomized blinded controlled trial with 60 healthy children of age group 9–14 years was carried out. The subjects were randomly assigned to three groups, i.e. group A – 0.2% chlorhexidine gluconate, group B – 0.05% sodium fluoride, and group C – 0.5% *C. sinensis* extract, with 20 subjects per group. Plaque accumulation and gingival condition were recorded using plaque index and gingival index. Oral hygiene was assessed by simplified oral hygiene index (OHI). Salivary pH was assessed using indikrom pH strips. Plaque, gingival, and simplified OHI scores as well as salivary pH were recorded at baseline, immediately after first rinse, after 1 week, and in the 2nd week. **Statistical Analysis Used:** The data were analyzed using a computer software program (SPSS version 17). Analysis of variance (ANOVA) tests were used to identify significant differences between the means of the study groups. Finally, paired t-tests were used to assess the significance of changes within each group between time periods. Critical *P* values of significance were set at 0.05 and the confidence level set at 95%. **Results:** Mean plaque and gingival scores were reduced over the 2-week trial period in the experimental groups. Antiplaque effectiveness was observed in all groups, the highest being in group C (*P* < 0.05). Chlorhexidine gluconate and tea showed comparative effectiveness on gingiva better than sodium fluoride (*P* < 0.05). The salivary pH increase was sustained and significant in groups B and C compared to group A. Oral hygiene improvement was better appreciated in groups A and C. **Conclusions:** The effectiveness of 0.5% *C. sinensis* extract was more compared to 0.05% sodium fluoride and 0.2% chlorhexidine gluconate mouth rinses. It should be explored as a cost-effective and safe long-term adjunct to oral self-care of patients as it has prophylactic benefits with minimum side effects.

**Key words:** 0.05% sodium fluoride, 0.2% chlorhexidine gluconate, 0.5% *Camellia sinensis*, antiplaque, dental caries

INTRODUCTION

Dental caries is defined as an infectious, microbial disease that is characterized by demineralization of the inorganic portion and the destruction of the organic substances of the teeth. Periodontal diseases are chronic inflammatory conditions characterized by loss of connective tissue, alveolar bone resorption, and formation of periodontal...
pockets as a result of the complex interaction between pathogenic bacteria and the host’s immune response. Periodontitis starts with inflammatory lesions of the gingiva, which, if left untreated, may progress and eventually involve and compromise the entire periodontal apparatus of the affected teeth. Dental plaque is the primary etiologic factor in periodontal disease.[2]

Mechanical plaque control is the most dependable oral hygiene measure, but mechanical oral hygiene methods of plaque removal require time, motivation, and manual dexterity.[9] Hence, the use of the antimicrobial agent is warranted to limit the growth of cariogenic microorganisms and prevent dental caries.[4] The antiplaque agents can be delivered in the form of mouthwashes, dentifrices, chewing gums, gels, and chips. Mouthwashes, a safe and effective delivery system for antimicrobials, can play an important role in plaque reduction.

Chlorhexidine, triclosan, cetyl pyridinium chloride, essential oils, and fluoride-based solution are some of the antimicrobial agents tested against oral microbes.[5-7] Chlorhexidine is the gold standard chemical plaque control agent. Its ability to bind to soft and hard tissues in the oral cavity enables it to act for a long period after application. However, brown discoloration of dentition and restorative material, dorsum of the tongue, taste perturbation, oral mucosal ulceration, unilateral/bilateral parotid swelling, and enhanced supragingival calculus formation have been reported as the side effects of long-term chlorhexidine use.[8,9]

Tea, a product made from the leaf and bud of the plant Camellia sinensis, is the second most consumed beverage in the world, well ahead of coffee, beer, wine, and carbonated soft drinks. Numerous studies have demonstrated that tea possesses antioxidant, antimutagenic, antidiabetic, anti-inflammatory, antibacterial, and antiviral, as well as cancer-preventive properties.[10,11] Green tea is made solely with the leaves of C. sinensis that have undergone minimal oxidation during processing. The most abundant components in green tea are polyphenols, in particular flavonoids such as the catechins, catechin gallates (Cg), and proanthocyanidins.[13] Many of the biological properties of green tea have been ascribed to the catechin fraction, which constitutes up to 30% of the dry leaf weight. These potent antioxidants comprise free catechins, such as (+)-catechin, (+)-gallocatechin, (−)-epicatechin, and (−)-epigallocatechin, and the galloyl catechins, such as (−)-epicatechin gallate (ECg), (−)-epigallocatechin gallate (EGCg), (−)-Cg, and (−)-gallocatechin gallate. Green tea also contains carotenoids, tocopherols, ascorbic acid, minerals such as Cr, Mn, Se, or Zn, and certain phytochemical compounds. The therapeutic and biological activities of catechins reported include lower incidences of various pathological conditions such as cardiovascular disease, stroke, obesity, and cancer. These effects have been attributed, in part, to the antioxidative and free radical scavenging activities of the polyphenolic components of green tea.[13] Studies conducted in the past have shown that the green tea polyphenolic catechins, in particular (−)-(EGCg) and (−)-(ECg), can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency. Evidence is emerging that these molecules may be useful in the control of common oral infections such as dental caries and periodontal disease.[13] Fluoride is an established antimicrobial agent. Because of its antiangiogenic and remineralization properties, it is extensively used in the prevention of dental caries. However, due to risk of ingestion and fluoride toxicity, it is not recommended in small children.[14] Sodium fluoride is regarded as a gold standard of caries prevention. It is extensively used to prevent and treat dental caries due to its antiangiogenic and remineralization properties.[14] This study was conducted to come up with novel and cost-effective mouthwashes that can be used by people for reducing the oral diseases. Tea is commonly used in India. What is required is promotion of the existing resources to gain confidence of local people, as well as efforts to promote oral health. The aim of the present study was to compare the effectiveness of 0.5% tea, 0.05% sodium fluoride, and 0.2% chlorhexidine mouthwashes on oral health in children. The objective of the study was to compare the effects of three mouthwashes on plaque, gingivitis, salivary pH, and oral hygiene status.

**MATERIALS AND METHODS**

A randomized blinded controlled trial was conducted with 60 healthy children of age group 9–14 years was conducted [Figure 1]. Two hundred children of age group 9–14 years were examined for duration of 4 months. Sixty children meeting the eligibility criteria were selected from them and the study was conducted for a period of 2 weeks. Children and their parents were given verbal and written information. Informed consent was obtained from the parents of children prior to the study. The inclusion criteria for the study were: Children having normal occlusion, absence of caries and/or restorations, a healthy periodontium, non-compromised oral health (brushed their teeth twice a day), no history of systemic antibiotic use or topical fluoride treatment within 4 weeks prior to baseline, no regular use of xylitol chewing gum, tea, coffee, or cocoa, no systemic diseases, and absence of orthodontic appliances.
The exclusion criteria were: Children who wore fixed or removable orthodontic appliances or prosthesis, having any type of restorations, had been prescribed antibiotics or other medications in the last 3 months, had undergone periodontal treatment in the previous 6 months, having any systemic illness, and not willing to comply with the study protocol. Subjects who had a mean gingival index (GI) by Loe and Silness in 1963 score of ≥3.0 and mean plaque index (PI) by Silness and Loe in 1964 score of 1.5 were included in the study.

Sampling

Based on the secondary data, the sample size was estimated to be 60. The sample size is calculated using the formula:

\[ n = \frac{2(Z_{0.95} + Z_{0.80})^2}{\Delta^2} + \frac{2(1.645 + 0.84)}{(0.64)^2} = 30 \]

\( \Delta \) Confidence interval = 95% (0.95), power = 80% (0.80), \( Z_{0.95} = 1.645 \), \( Z_{0.80} = 0.84 \), \( \mu \) (mean) = 3.8, \( \mu_0 \) (mean) = 3.0, and \( \Delta^2 = 0.64 \).

Preparation of mouthwash

Commercially available 0.2% chlorhexidine gluconate mouthwash (Periogard, Colgate-Palmolive) was used. Commercially available 0.05% sodium fluoride mouthwash (S-FLO, Dr. Reddy’s Laboratories Limited) was used.

Tea was extracted by combining 31/2 oz. (about seven tablespoons) of green tea with four cups of still (not sparkling) mineral water. This was steeped at room temperature for 1 h and then poured into a lidded container, straining the tea with sieve as it is poured, followed by refrigeration. The loose tea is discarded. The 500 ml concentrated tea is mixed with 1000 ml of distilled water to get 0.5% solution of tea mouthwash.\(^{[17]}\)

The mouthwashes were bottled and coded in similar containers (250 ml), so that the children and investigator were blinded about the kind of mouth rinse used throughout the study. The names of children were written on small pieces of paper by a person not involved in this study and they were shuffled and randomly allocated into three groups (\( n = 20 \) subjects per group) by lottery method. The subjects were randomly assigned to three groups, i.e. group A – 0.2% chlorhexidine gluconate, group B – 0.05% sodium fluoride, and group C – 0.5% \( C. \) sinensis extract, with 20 subjects in each group.

The children were instructed to rinse their mouth after brushing at morning and night, with 20 ml of the mouth rinse containing 0.2% chlorhexidine gluconate mouthwash, 0.05% sodium fluoride mouthwash, or 0.5% \( C. \) sinensis extract mouthwash, for 60 s, twice a day, for 2 weeks. After each application, they were requested not to eat or drink for 1 h. Children’s proper application of mouth rinse was supervised by their parents. After 2 weeks of regular application, the participants were instructed to stop using mouth rinses. It must be noted that the participants were given the same tooth brush and fluoride tooth paste to brush their teeth twice a day during the study. The subjects were requested not to use xylitol-containing products, tea, coffee, cocoa, systemic antibiotics, and topical fluoride treatment for 4 weeks before and during the study. They were also asked to report any change in health status or medicine being used. Any participant violating the rules was excluded.

Before starting the first phase, professional oral hygiene, which included scaling and root planing with polishing, was done and the plaque score was brought to zero. Single trained and calibrated investigator assessed
the baseline plaque by PI, gingival status by GI, and the oral hygiene status was assessed by simplified oral hygiene index (OHIS; John C Greene and Jack R Vermillion, 1964) before the mouthwashes were distributed. \[15,16\] The pH of the saliva was checked by using commercially available pH strips, i.e. indikrom papers, with pH ranging from 2–4.5 to 5.0–7.5. \[17\] The color changes on the pH strips were noted after keeping the strip in the unstimulated saliva for 1 min and matching with the color of standardized color chart given by the manufacturer to represent the pH of saliva. The results of this research indicated that before any intervention, there were no significant differences in the baseline values between three groups. So, it was possible to make a comparison between the effectiveness of three different mouth rinses on the plaque, gingival status, oral hygiene status, and salivary pH.

Exactly 250 ml of 0.2% chlorhexidine gluconate mouthwash, 0.05% sodium fluoride mouthwash, and 0.5% C. sinensis extract mouthwash were provided to each subject in group A, group B, and group C, respectively, in a bottle. Data were collected at baseline, immediately after first rinse, and every week until the second week of study, and assessed for gingival status, plaque, oral hygiene, and salivary pH. Any side effects and acceptability of mouthwashes was recorded with a questionnaire. The questionnaire consisted of four questions (three close-ended and one open-ended) on acceptability or non-acceptability, reason for non-acceptability, any recommendations to change the mouthwashes, and how do they rate the present mouthwash.

Statistical analyses

The data were analyzed using a computer software program (SPSS version 17; SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) tests were used to identify significant differences between the means of the study groups. Finally, paired t-tests were used to assess the significance of changes within each group between time periods. Critical P values of significance were set at 0.05 and the confidence level set at 95%.

RESULTS

Sixty children were recruited in this study. The study was completed without any dropouts. The subjects were within the age group of 9–14 years, with a mean age of 10.94 ± 0.26 years. Age and gender did not show any statistically significant difference between groups and within group, as shown in Table 1. The intra-examiner error was within acceptable limits (kappa coefficient = 0.7) and the power of the study was found to be 0.985 using power and sample size program software. There were no reports of adverse reactions to any of the mouth rinses used.

Table 2 shows the distribution and comparison of baseline characteristics of the four study groups. No statistical difference was observed within as well as between groups in plaque scores, salivary pH, OHIS, and gingival scores. The mean plaque score for group A and group C after the first rinse was 1.45 ± 0.03. The salivary pH was low in group A when compared to others, as shown in Table 3. The least OHIS score was seen with group B, which when compared to others was highly significant (P = 0.002). When comparison was carried out between group A and group C for OHIS scores, the difference was found to be non-significant (P = 8.55 Error-06, NS) at baseline.

The mean plaque score (0.81 ± 0.05) and OHIS (1.11 ± 0.45) were low in group C. The gingival score was high (2.1 ± 0.45) and the pH was low (5.90 ± 0.60) after 1st week in group B when compared to others, as shown in Table 4. The difference in oral hygiene between group B and group C was found to be significant (P = 0.031, S), but no significance was found with groups A and C (P = 0.711). After 2nd week, the lowest plaque was recorded in group C (0.56 ± 0.40) followed by group A (0.64 ± 0.46). The highest salivary pH (6.50 ± 0.8) along with good oral hygiene (0.51 ± 0.54) was found in group C followed by group A (0.88 ± 0.54). Gingival health improved in all the three groups after 2nd week, with least scores recorded in group C (1.10 ± 0.5) as shown in Table 5.

Table 1: Distribution of study subjects by age and gender

| Group | M | F | n | Mean age | SD | Min. age | Max. age | Mean age of M | SD | Mean age of F | SD |
|-------|---|---|---|---------|----|---------|---------|-------------|----|-------------|----|
| A     | 10| 10| 20| 10.7    | 1.87| 9       | 14      | 10.6        | 1.14| 10.8        | 0.84|
| B     | 10| 10| 20| 10.9    | 0.78| 10      | 13      | 10.6        | 1.14| 10.8        | 0.84|
| C     | 10| 10| 20| 11.2    | 1.86| 9       | 14      | 11          | 1.58| 10.6        | 2.15|

ANOVA

P Value 0.759 0.929 0.509

Significant NS NS NS

M=Male, F=Female, n=Number, SD=Standard deviation. *Non-significant (NS) = P>0.05
The salivary pH was high in group C (6.50 ± 0.8) followed by group A (6.45 ± 0.45) [Graphs 1-4] [Tables 6-9].

**DISCUSSION**

Mouth rinses are widely used as an adjunct to mechanical oral hygiene procedures for their analgesic, anti-inflammatory, antimicrobial and anticariogenic activity. The most commonly used mouth rinses in children for therapeutic purposes are 0.05% sodium fluoride and 0.02% chlorhexidine gluconate. Fluoride is an established antimicrobial agent; because of its anticariogenic and remineralization properties, it is extensively used in prevention of dental caries. However, due to risk of ingestion and fluoride toxicity, it is not recommended in small children.[14] Chlorhexidine is known to be the “gold standard” mouth rinse against cariogenic flora. Many clinical trials have shown that the taste of chlorhexidine mouth rinse is not well accepted by children.[8,9] It also produces brown staining of teeth and affects the mucus membrane and tongue.[8,9] The most commonly prescribed concentration is 0.2%, which was, therefore, used in the study.

There is always a quest for new and improved products, with emphasis being placed on natural/nature identical products. Scaling and root planing are effective in altering the flora; green tea catechin has also been shown to be effective in altering the flora and acting as an adjunct to scaling and root planing.[19]
Oxidative stress plays a vital role in the pathogenesis of periodontal disease as well as many other disorders, and it is believed that antioxidants can defend against inflammatory diseases.\(^{[20]}\) Numerous health benefits of green tea and its constituents have been reported. It is a powerful antioxidant and has anti-inflammatory properties. Catechin was found to have antiplaque and antibacterial properties and contributed in caries
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### Table 9: Comparison of gingival index within groups A, B, and C

| Baseline characteristics | Group A (mean±SD) | Group B (mean±SD) | Group C (mean±SD) | F (ANOVA) | P | Inference |
|--------------------------|-------------------|-------------------|-------------------|-----------|---|-----------|
| Gingival index (baseline)| 2.68              | 2.54              | 2.34              | 0.210526  | 0.811475 | NS†      |
| Gingival index (1st rinse)| 0.68              | 2.04              | 2.14              | 1.3       | 0.28905 | NS†      |
| Gingival index (1st week)| 1.78              | 2.1               | 1.6               | 1.7815    | 0.16145 | NS†      |
| Gingival index (2nd week)| 1.17              | 1.5               | 1.10              | 0.67469   | 0.4625  | NS†      |

†Non-significant (NS) = P>0.05, *significant (S) = P<0.05. SD=Standard deviation

prevention and gingival enhancement. Rasheed and Haider described the antibacterial effect of green tea catechins against Streptococcus mutans bacteria and stated that catechins are of great value in the reduction of S. mutans and caries prevalence.[21]

The purpose of the study was to assess and compare the effectiveness of 0.5% C. sinensis extract, 0.05% sodium fluoride, and 0.2% chlorhexidine mouthwashes on the oral health of children. This was a triple-blind study wherein the investigator, study subjects, as well as the statistician were not aware to which group the subjects belonged and coding was done for each group and individual. The results of this research indicated that before any intervention, there were no significant differences in the baseline values between three groups. So, it was possible to make a comparison between the effectiveness of the three different mouth rinses on the plaque, gingival status, oral hygiene status, and salivary pH. No side effects or mishappenings were observed during the study procedure.

0.5% tea was used, so that the concentration does not change the taste but should cause maximum inhibition of variables. In the present study, 0.5% C. sinensis extract had the maximum desired effect when compared to 0.05% sodium fluoride and 0.2% chlorhexidine gluconate. The plaque level was brought to 0.56 at the end of 2nd week from baseline (1.52), when compared to chlorhexidine. The salivary pH increase was more in tea group. The oral hygiene status improved from poor to good. Tea group had an upper hand when it came to gingival status, as the response was very good and quick when compared to sodium fluoride or chlorhexidine, which also showed significant effects.

Various mechanisms have been explained for the effect of tea on gingival health. Green tea catechin has been shown to be bactericidal against Porphyromonas gingivalis and Prevotella spp. in vitro. Tea catechins containing galloyl radicals possess the ability to inhibit both eukaryotic and prokaryotic cell-derived collagenase, an enzyme that plays an important role in the disruption of the collagen component in the gingival tissues of patients with periodontal disease.[22,23] Catechin derivatives have been reported to inhibit certain proteases of P. gingivalis and may reduce periodontal breakdown.[24] Green tea catechins have also been shown to inhibit protein tyrosine phosphatase in Prevotella intermedia.[25] EGCg has been reported to inhibit the production of toxic metabolites of P. gingivalis. A study has shown that purified tea polyphenols inhibited in vitro growth and H₂S production of P. gingivalis and Fusobacterium nucleatum associated with human halitosis.[26]

Fluoride is an established antimicrobial agent and has anticariogenic and remineralization properties. So, it is extensively used in prevention of dental caries. Fluoride prevents dental caries through different processes. It inhibits adhesion of S. mutans to the tooth structure and, therefore, inhibits insoluble dextran production by the bacteria. It inhibits tooth demineralization and also remineralizes incipient carious lesions. However, due to risk of ingestion and fluoride toxicity, it is not recommended in small children.[14] Chlorhexidine is known to be the “gold standard” mouth rinse against cariogenic flora. Its ability to bind with soft and hard tissues in the oral cavity enables it to act for a long period after application. However, brown discoloration of dentition and restorative material, dorsum of the tongue, taste perturbation, oral mucosal ulceration, unilateral/bilateral parotid swelling, and enhanced supragingival calculus formation have been reported as the side effects of long-term chlorhexidine use. Many clinical trials have shown that the taste of chlorhexidine mouth rinse is not well accepted by children.[18,3] The most commonly prescribed concentration is 0.2%; hence, this was considered in the study.

The findings of a study on 6–16-year-old children showed that regular daily consumption of green tea rich in catechin (576 mg per can) had no side effect on children’s health. However, it can decrease obesity and cardiovascular risk factors in fat children.[27] There have been other studies on the safety of catechin and no side
effects have been found.\(^{28-30}\) Whereas high amount of fluoride ingestion may lead to acute poisoning and its low repeated ingestion causes fluorosis, especially in children. So, sodium fluoride mouth rinse is not recommended for children younger than 6 years as they may swallow it.\(^{14}\) Hence, this study is conducted on 8–12-year-old children.

It has been shown that the earlier the colonization of \(S.\) \(mutans\) in the mouths of children, the higher the caries prevalence at 4 years of age. In fact, the earlier transmission of \(S.\) \(mutans\) occurs, the higher the caries risk at older ages.\(^{31,32}\) Therefore, prescribing a safe and harmless antibacterial agent in children has an important role in prevention of dental caries at older ages.

Based on the results of this study and owing to fewer side effects along with good acceptability of green tea in comparison to sodium fluoride and chlorhexidine, it seems that green tea can be used with less concern compared to sodium fluoride and chlorhexidine mouth rinses in children. It is recommended that studies with larger sample size on chlorhexidine, sodium fluoride, and tea mouthwashes should be encouraged to assess their efficacy, dosage, toxicity, exact concentrations, formulas for patient recommendation, and long-term effectiveness.

**CONCLUSION**

In developing countries, the cost of traditional restorative treatment of dental diseases usually exceeds the available resources for oral health care. Prevalence of oral diseases is very high in India. Therefore, continued research is going on to find safe and effective oral hygiene aids for oral self-care of patients. Mouthwashes are used in dentistry for prevention and curative purposes, but their affordability and side effects have raised questions. Considering the fact that the mouth rinses available presently in market are chemical based, costly, and have side effects, which restricts their use especially in India, a cost-effective and easily available herb as an adjuvant to oral hygiene maintenance may have a far-reaching effect on the prevention as well as prevalence of oral diseases. The promotion of botanical herbs with fewer side effects and low cost may motivate the patient for oral hygiene maintenance.

The results of the study indicate that mouthwash prepared from green tea has a comparable antiplaque efficacy to chlorhexidine gluconate when used for a period of 14 days. Furthermore, green tea mouthwash has better taste and no known side effects that are found with fluoride and chlorhexidine mouthwashes. So, it can be used on a daily basis as an alternative for chlorhexidine gluconate and sodium fluoride as an antiplaque, anticariogenic, and remineralizing agent. More studies with bigger sample sizes and different variables are required to explore the role of extract of \(C.\) \(sinensis\) as an adjunct to oral health care.

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