Original Article

Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial

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Abstract

Background: Toothbrushes in regular use can become heavily contaminated with microorganisms, which can cause infection or reinfection. There is a need for toothbrush disinfection methods, which are rapidly effective, cost-effective, nontoxic, and that can be easily implemented. **Aim:** To compare the efficacy of 3% neem, garlic of concentration 4.15 mg/mL and green tea of concentration 40 mg/mL with 0.2% chlorhexidine mouthwash as toothbrush disinfectants. **Materials and Methods:** The study was a parallel *in vitro* comparative experimental trial conducted among 75 randomly selected boys aged between 18 years and 21 years. The subjects were divided into five groups, namely, Group I, Group II, Group III, Group IV, and Group V. They were provided with a new set of precoded toothbrushes and nonfluoridated tooth pastes. After 14 days of tooth brushing, the toothbrushes were immersed in antimicrobial solution for 12 h [Group I—distilled water (control), Group II—3% neem, Group III—garlic of concentration 4.15 mg/mL, Group IV—green tea of concentration 40 mg/mL, and Group V—0.2% chlorhexidine] and then subjected to microbial analysis to check the presence of *Streptococcus mutans*. The *t*-test and analysis of variance (ANOVA) were done using Statistical Package for the Social Sciences (SPSS) software version 16. **Results:** All test solutions showed a statistically significant reduction of *Streptococcus mutans* count ($P < 0.001$). There was no statistical difference between the efficacies of neem, garlic, and green tea when compared with chlorhexidine mouthwash ($P > 0.05$). **Conclusion:** Neem, garlic, and green tea are equally efficacious as chlorhexidine and these herbal products can be used as potent alternatives to chlorhexidine as disinfectant for toothbrushes.

Key words: *Antimicrobial, antimicrobial solution, chlorhexidine, disinfection, garlic, green tea, neem, toothbrush*

INTRODUCTION

Toothbrushes are manufactured free of microorganisms. But after a single use, they may become contaminated by a wide array of microorganisms present both in the oral cavity and in the external environment.[1] There

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is a need for disinfection methods, which are rapidly effective, cost-effective, nontoxic, and that can be easily implemented.

With the increasing incidence of drug resistance in the prevalent pathogens and an associated risk with chemotherapeutic agents, it is essential to find an alternative to existing drugs. The herbals, which have known pharmaceuticals properties could be the best source of these alternative drugs.[2] In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world.[3]

The present investigation was designed to study the antimicrobial efficacy of 3% neem, garlic of concentration 4.15 mg/mL, and green tea of concentration 40 mg/mL on *Streptococcus mutans* and to compare with 0.2% chlorhexidine mouthwash, which is considered as the "gold standard" for oral hygiene.

MATERIALS AND METHODS

The study was a parallel *in vitro* comparative experimental trial. The toothbrushes were obtained from subjects aged 18–21 years staying in the boys' hostel of a private engineering college in Chennai, Tamil Nadu, India. The study subjects, the microbiologist, and the statistician were blinded. The purpose of the study was explained to the subjects and the hostel warden and the informed consent was obtained.

After obtaining ethical clearance, a pilot study was conducted with 30 subjects to obtain the feasibility and acceptability and for the sample size. With the help of nMASTER software (developed by the Department of Biostatistics, CMC Vellore for sample size calculation) keeping the power of the study at 90% and alpha error at 5%, a sample size of 13 was obtained for each group. The final sample size was decided to be 15 in each group. As there were four antimicrobial solutions and distilled water being used as the control, the total number of subjects needed for the study was 75.

The subjects aged between 18 years and 21 years, having at least three single-rooted and two multirooted functional teeth per quadrant (third molars excluded) and with decayed, missing, and filled teeth (DMFT) score of <3 were included in the study. Subjects using antibiotics or antiseptic mouth washes for at least 3 months prior to the study, undergoing any dental treatment, with orthodontic or with extensive intraoral prosthesis, medically compromised (e.g., in diabetic patients) and subjects using neem, garlic, green tea, chlorhexidine, and fluoride in any form as an oral hygiene aid were excluded from the study.

Of the total 192 subjects of the age group of 18–21 years residing in the hostel, 158 subjects were eligible for the study. Systematic random sampling was used to select 75 subjects. The preparations of test solutions were done with the help of the guidelines given in the literatures.[4-6]

The 75 subjects were divided into five groups, namely, Group I, Group II, Group III, Group IV, and Group V. Each group had 15 subjects.

All the subjects were provided with dentifrices and toothbrushes during the entire study procedure. All of them were instructed to brush twice daily using a horizontal scrub method for 3 min and to rinse the toothbrushes under running tap water for 20 s after brushing. They were also instructed to keep their toothbrushes in the separately provided disposable glass containers in such a manner that the head of the brush with the bristles should face outside and be left open for drying.

The study was conducted in two stages. In the first stage, for baseline data, all 75 subjects were provided with a new set of precoded toothbrushes and nonfluoridated toothpastes. The toothbrushes were collected back after 14 days. The collected toothbrushes were transferred to the laboratory in separate sterilized pouches. These toothbrushes were aseptically introduced into test tubes containing thioglycolate medium for 4 h such that only the head of the brushes was immersed in the medium and subjected to microbial analysis. This was done to obtain the baseline analysis.

In the second stage, the 75 subjects were again provided with a new set of toothbrushes and nonfluoridated toothpastes. The toothbrushes were collected back after 14 days. The collected toothbrushes were transferred to the laboratory in separate sterilized pouches. The toothbrushes collected from Group I were aseptically introduced directly into test tubes containing 5 mL of distilled water (control) in which only the head of the toothbrush was immersed in the medium. Similarly, the toothbrushes collected from the subjects in Group II, Group III, Group IV, and Group V were immersed in the test tubes containing 3% neem, garlic of concentration 4.15 mg/mL, green tea of concentration 40 mg/mL solutions, and 0.2% chlorhexidine mouthwash. After 12 h of immersion in the solution, the toothbrushes were subjected to microbial analysis.

Microbial analysis

The toothbrushes were introduced into test tubes containing thioglycolate medium and kept in these for 4 h. The samples were then vortexed for 15 s to dislodge the bacteria from the bristles to the media using a cyclomixer (Remi Elektrotechnik Limited). The solution was inoculated using a platinum inoculation loop. 10 µL of the vortexed sample was being streaked on mitis salivarius agar and incubated in an anaerobic jar with gas pack system (Remi Elektrotechnik Limited) for 48 h at 37°C in an incubator. Plates were opened after 48 h and the colonies were counted using colony counter (Remi Elektrotechnik Limited) and were expressed as number of CFU/mL of the media [Figure 1].

Statistical analysis

Data were entered in Microsoft Excel and the analysis was performed using SPSS software version 16 (IBM Corporation). The *t*-test was used for comparing the baseline and test values and analysis of variance (ANOVA) was used for multiple group comparison. In all the above tests, $P < 0.05$ was accepted as indicating significance.

RESULTS

All the 75 subjects were present from the beginning to the end of the study. The power of the study was kept at 90%. The colony-forming units (CFUs) in the study ranged 0–8.8 × 10^3. The mean level of contamination of toothbrush by *Streptococcus mutans* in each group at the baseline and after decontamination with different solutions were given in Table 1. The percentage of reduction done by difference solutions is given in Figure 2.

When comparing the mean percentage reduction in *Streptococcus mutans*, CFUs between the control group and each experimental group (II, III, IV, and V), the differences noted were all statistically very highly significant ($P < 0.001$). When the mean percentage reduction produced by experimental Groups II, III, and IV were compared with experimental Group V, the differences noted were not statistically significant ($P > 0.05$) [Table 2].

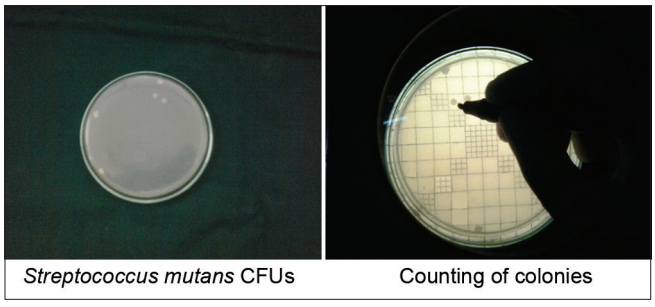

Figure 1: Picture of colony counting

DISCUSSION

In the study, subjects were taken from the hostel because it is easy to supervise the method of brushing, duration of time, methodology of storing, and it was easy to collect all the toothbrushes as they all stayed under one roof. Toothbrush and toothpaste were used in the study as an oral hygiene aid, which is the most commonly used aid and is most effective, safest device to remove dental plaque.[4]

Transmission of *Streptococcus mutans* occurs even through dentifrices.[4] To avoid any such factors confounding the result of this study, each subject was given an individual toothbrush and toothpaste. For sterility control, four new toothbrushes that were freshly removed from the packets were subjected to microbial analysis. None of the unused toothbrushes had the colonies of *Streptococcus mutans*, suggesting that the origin of these microorganisms is from the oral cavity.

In this study, the number of *Streptococcus mutans* ranged 5 × 10^3–8.8 × 10^3 CFUs in the baseline. This shows

Table 1: Mean CFUs of *S. mutans* in different groups

Group	Baseline* Mean±SD†	After decontamination* Mean±SD	Mean difference* Mean±SD
Distilled water	7.3±1.14	7.14±0.96	0.16±0.88**
Garlic	7.04±0.62	0.93±1.2	6.25±1.41***
Neem	6.87±0.88	0.28±0.64	6.59±0.92***
Green tea	6.77±0.78	1.13±1.67	5.63±1.77***
Chlorhexidine	6.9±0.5	0.57±1.06	6.33±1.08***

**Level of contamination of toothbrush by S. mutans (×10^3 CFU), **Not significant (P>0.05), ***Significant (P<0.001). †SD=Standard deviation*

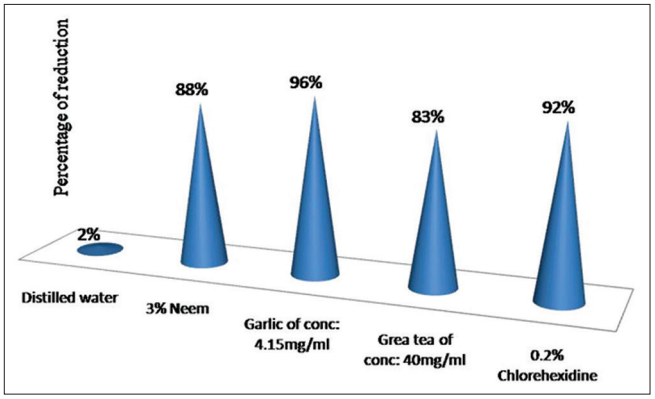

Figure 2: Mean percentage reduction of *S. mutans* CFUs in different groups

Table 2: Comparison of the mean percentage reductions in *S. mutans* count between different groups after decontamination

Group*	Mean reduction (%)	1	2	3	4	5
Distilled water	2	-	P=0.000^{+}	P=0.000^{+}	P=0.000^{+}	P=0.000^{+}
Garlic	96	-	-	P=0.34^{++}	P=0.96^{++}	P=0.27^{++}
Neem	88	-	-	-	P=0.62^{++}	P=0.07^{++}
Green tea	83	-	-	-	-	P=0.69^{++}
Chlorhexidine	92	-	-	-	-	-

**1-distilled water, 2-garlic, 3-neem, 4-green tea, 5-chlorhexidine. $^{+}P<0.001$ (Significant). $^{++}P>0.05$ (Not significant)*

that the toothbrush that disturbs or reduces the existing oral microflora may at the same time introduce other microorganisms such as *Streptococcus mutans* in the oral cavity. This disturbance of the existing flora may result in the removal of competitive organisms and thus, increases the possibility for *Streptococcus mutans* to colonize.[7,8]

In this study, three herbal disinfectant media were compared with chlorhexidine (gold standard antimicrobial agent[4-6]) to know the efficacy in reducing contamination of the toothbrush.

Various media are used to culture the microorganisms. Thioglycolate medium, peptone water, tryptone water, nitrate broth, etc., are some of the enriched culture media. In this study, thioglycolate medium was used for the growth of *Streptococcus mutans* due to its availability and it is also a good transport medium.

The toothbrushes from the subjects were collected after 14 days. However, different study intervals such as 5 h, 6 h, 24 h, and 48 h and 3 days, 5 days, 14 days, 15 days, 24 days, and 28 days, and 3 months have been followed in other studies.[4] In this study, 14 days was selected because in a study conducted by Sogi *et al*., maximum microbial contamination was found in toothbrushes after 14 days and 28 days.[9]

In the study, the toothbrushes were kept in antimicrobial solutions for 12 h. Various studies showed 5 min, 12 h, 20 h, 24 h, etc., as the soaking time.[4,9] 12 h was selected for this study by considering the fact that in real-life scenario it is advisable to brush twice daily, i.e., once in every 12 h. Hence, the toothbrushes could be dipped in the disinfectant during the interval of tooth brushing.

Neem possesses various medicinal properties, and is widely available in most rural and urban areas of developing countries. In this study, 3% neem was found to be an effective antimicrobial solution against *Streptococcus mutans* on toothbrush bristles (87% reduction). This was in line with the studies conducted by Balappanavar *et al*.[4] and Padma K Bhat *et al*.[10] This could be due to the presence of polyphenolic tannins present in the extract, which could effectively bind to the surface-associated bacterial proteins, resulting in bacterial aggregation and loss of glucosyltransferase activity. This bacterial aggregate effectively reduces the count of *Streptococcus mutans*. A study conducted by Aarati *et al*. proved that both aqueous and alcoholic extracts of neem have significant antibacterial activity against *Streptococcus mutans*.[11]

Garlic showed the maximum reduction (96%) in the *Streptococcus mutans* count in the present study. The antibacterial activity of garlic is due to the presence of allicin. Till now, not much studies have been reported as per the literature search, which have used garlic for decontaminating toothbrush. The result of this study is in concurrence with the study conducted by Amin *et al*.,[12] Prabhakar *et al*.,[13] and Yu-Ying Chen *et al*.[5] Nikolic *et al*. reported that allicin possesses strong anti-*Streptococcus mutans* activity.[14] Fani *et al*. reported that mouthwash containing garlic could be used for the prevention of dental caries.[15] Despite the antimicrobial effects of garlic extract, side effects such as unpleasant taste, halitosis, and nausea were reported.[16]

Green tea polyphenols have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties in numerous human, animal, and *in vitro* studies.[15] In the present investigation, green tea showed 84% reduction in the *Streptococcus mutans* count and has been proved to be effective in decontaminating toothbrushes. This anti-*Streptococcus mutans* property of green tea is due to the presence of polyphenolic compounds in green tea. Not many studies have been reported as per the literature search, which have used green tea for decontaminating toothbrushes. Several studies have indicated that green tea inhibits growth, acid production, metabolism, and glucosyltransferase enzyme activity of *Streptococcus mutans*.[10]

Chlorhexidine is a cationic agent that exhibits broad spectrum antimicrobial effect and is a benchmark control in various studies. In the present investigation, chlorhexidine showed 92% reduction in the *Streptococcus mutans* count. Chlorhexidine kills bacteria by disrupting the cell membrane.[17] In studies conducted by Bhat *et al*.[1] and Nanjunda Swamy *et al*.,[18] chlorhexidine produced 100% reduction of the *Streptococcus mutans* count. The study conducted by Suma Sogi *et al*. found that chlorhexidine produces 88% bacterial reduction in toothbrushes after 14 days.[9] But the result of the present study was not in concurrence with the study reported by Balappanavar *et al*.[4] and Padma K Bhat *et al*.[10] where the percentages of reduction in *Streptococcus mutans* count were only 64% and 65%, respectively, which were less than the present study.

As per the present results, neem, garlic, and green tea have been proved to be as effective disinfectants as chlorhexidine for toothbrushes. Kudva *et al*. reported that garlic and chlorhexidine are effective against *Streptococcus mutans*.[19] Fani *et al*. showed that the inhibitory activity of garlic on *Streptococcus mutans* was comparable with chlorhexidine.[15] Groppo *et al*. also reported that the garlic and chlorhexidine had a similar effect on *Streptococcus mutans*.[12] In contrast to the present result, Priya Subramaniam *et al*. reported that aqueous extract of green tea shows more reduction of *Streptococcus mutans* than chlorhexidine.[20]

In the present investigation, garlic showed the maximum reduction in the *Streptococcus mutans* count. It produced 96% reduction followed by chlorhexidine (92%), neem (88%), and green tea (83%). Distilled water showed only a 2.2% reduction. When comparing the mean difference and also the mean percentage reduction obtained in *Streptococcus mutans* colony count between the groups, only the comparison with distilled water showed a statistically very high difference. But when comparing the mean difference or mean percentage reduction in CFUs of *Streptococcus mutans* in garlic, neem, and green tea with chlorhexidine, the differences were not statistically significant from each other. So the present investigation showed that immersion of toothbrush for 12 h in 3% neem, garlic of concentration 4.15 mg/mL, and green tea of concentration 40 mg/mL were as efficacious as 0.2% chlorhexidine for disinfection. So neem, garlic, and green tea can be used at par with chlorhexidine as a disinfectant for toothbrush.

CONCLUSION

Based on the results of the study, it can be concluded that neem, garlic, and green tea are equally efficacious as chlorhexidine and these herbal products can be used as potent alternatives to chlorhexidine as disinfectants for toothbrushes.

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Conflicts of interest

There are no conflicts of interest.

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