International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Determining the Antibacterial Substantivity of Green Tea Mouthwash and Comparing it with 0.2% Chlorhexidine Gluconate after a Single Oral Rinse: A Crossover Clinical Trial

Dr. Priyanka Awasare[1], Dr. Rajesh Gaikwad[2], Dr. Veena Viswanadh[3]

[1]Post graduate student, Dept of Periodontology, Government Dental College and Hospital Mumbai, Maharashtra State, India
Corresponding Author Email:

[2]Professor and Head of Department, Department of Periodontology, Government Dental College and Hospital Mumbai

[3]Post Graduate Student, Department of Periodontology, Government Dental College and Hospital Mumbai, Maharashtra state, India

Abstract: ***Introduction: Green tea (Camellia sinensis) originated in China, is one of the most popularly consumed beverages worldwide. it is particularly rich in flavonoids which include catechin. Present study is to identify the substantivity of green tea mouth rinse and duration of its antibacterial substantivity after a single oral rinse and compare it with the substantivity of 0.2% Chlorhexdine gluconate. Methodology: Unstimulated saliva sample (baseline, pre-sample) was collected in sterile penicillin bulbs in the morning 2 hours after routine oral hygiene procedures. A washout period of 1 week was kept between two mouthwashes. The sampling was repeated in a similar manner after every 2 hours for 12 hours. (post 1, post 2, post 3, post 4, post 5, and post 6 samples) and was checked for microbial count. Result and Conclusion: Green tea mouth rinse has an antibacterial effect for 4 to 5 hours after a single rinse where as Chlorhexdine mouthwash remains the gold standard providing maximum antibacterial substantivity for 7 to 8 hours.***

Keywords: Green tea mouthrinse, chlorhexidine mouthrinse, antibacterial substantivity

1. Introduction

Accumulation of dental plaque on the soft tissue approximating the tooth brings on gingivitis, it is the temperate form of periodontal disease [1] .Microbial plaque that accumulate on soft tissue is the substructure of bacterial colonization on the tooth surface and which bring about gingivitis and periodontitis [2] chlorhexine was developed in 1950, which is the most used antiplaque agent. Even so the long term usage of chloehexidine is limited by its altered taste perception and staining of tooth with prolonged usage, as reported by Fardal and turnbell. [3] Though CHX has been the gold standard mouthwash in controlling plaque formation .[4] its inadmissible side effects intensified ability of calculus formation ,bitter taste ,and interference with taste have inspired a search for alternatives. [5] The onset of periodontal disease can be superintended by regular plaque control practices. Mechanical plaque control is the most dependable oral hygiene measure, but mechanical oral hygiene methods of plaque removal require time, motivation and manual dexterity.

Number of the chemical antiplaque agents in various formulations have been tried as adjunct to mechanical measures for improving oral health.[6,7] These antiplaque agents can be dispensed in the form of mouthwashes, dentifrices, chewing gums, gels and chips. Mouthwashes, a safe and effective delivery system for antimicrobials, can play an comprehensive function in plaque minimization. Out of all the antiplaque agents, chlorhexidine is conceded the gold standard agent for its clinical efficacy in chemical plaque control. [8] It has broad antibacterial activity, with very modest toxicity and strong affinity for epithelial tissue and mucous membranes.

Green tea is regarded as a health-promoting beverage possessing a wide spectrum of medicinal benefits. Beneficial effect of green are generally attributed to its polyphenol content, catechins which has got several pharmacological properties that include antibacterial effect, antioxidant effect. In addition, when used as a mouthwash, green tea preparations can obliterate bad breath by suppressing anaerobic bacteria and eradicating the production of volatile sulphur compounds. The present study is designed to determine the sunstantivity of green tea mouth rinse and the duration of its antibacterial substantivity after a single oral rinse and to compare it with the substantivity of 0.2% chlorhexidine gluconate (CHX).

2. Material Method

- In this single-blind, Randomized crossover clinical trial was conducted among 14 participants (students) students staying in the hostel of government dental college and hospital Mumbai.
- The study population was selected based on the inclusion criteria like healthy controls in the age group of 19–25 years with <4 teeth missing and the control having good oral hygiene.
- The individuals with high caries index, any systemic illness, using antibiotics, or any other mouthwashes for 3 months, using orthodontic/prosthetic appliances and with xerostomia were excluded from the study.
- An ethical approval was incurred from the institutional review board, and an informed written consent was

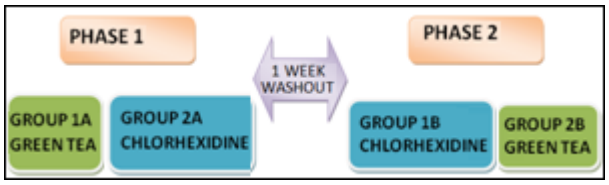
obtained from the students after explaining the methodology of the clinical trial.
- Patient was instructed to do there routiene oral hygiene procedure before carrying out the study.
- All participants were trained and instructed regarding the usage of mouth rinses

Students were instructed to rinse
- 10 ml of 0.2% CHX for 1 min
- 1.3 % green tea was prepared with 1.3 grams of green tea dip bag dipped in 100 ml warm water for five minutes.

This Cross over study was conducted in two phases by keeping 1 week washout period between the Green tea and chlorhexidine mouthrinse.

Phase 1: Group 1A and Group 2A - Green tea mouthwash and Chlorhexidine mouthwash.

1 Week Washout Period



Phase 2: Group1B and Group 2B – Chlorhexidine and Green tea mouthwash given.

- Patients were instructed to do their routine oral hygiene procedures.
- Patients were instructed to not to use any mouthrinse during their routiene oral hygiene procedure.
- Unstimulated saliva sample (baseline, pre-sample) was collected in sterile penicillin bulbs in the morning 2 h after their routine oral hygiene procedures.
- Oral prophylaxis was performed before carrying out the study.
- After that, the individuals were given a randomly selected mouth rinse using a chit pull system .
- Five minutes after rinsing with an allotted mouth rinse, a second sample (post sample) was collected, and both the samples were sent to the Microbiology unit for culture and microbial count. Individuals were asked to continue with their routines without any limitations over their eating and drinking habits.
- The sampling were repeated in a similar manner after every 2 h for 12 h (post 1, post 2, post 3, post 4, post 5, and post 6 samples) and was checked for microbial count.
- This is a crossover study with each participant using all mouthwashes.
- It was repeated for the analysis of microbial count after 0.2% CHX and green tea mouth rinse.

Preparation of Green Tea Mouthwash
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 1.3 grams of green tea dip bag dipped in 100 ml warm water for five minutes. All the two mouth rinses were dispensed in disposable cups for the participants (10 ml for each participant).

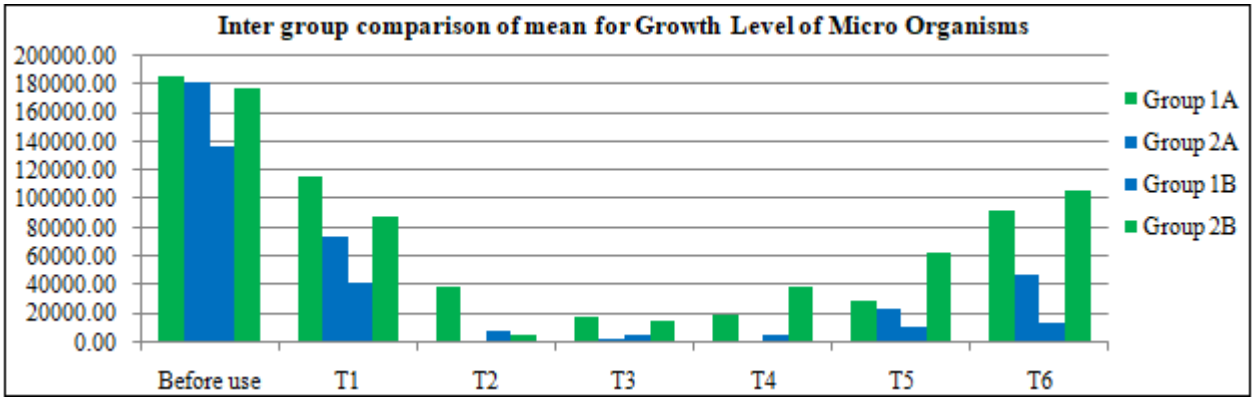
Microbiological analysis
Bacterial count (colony forming units [CFUs]) in each sample will determined by culture and microscopy. Evaluation of microbial load count Bacterial count (colony forming units [CFUs]) in each sample will be determined by culture and microscopy at the pathology lab in Mumbai .

Counting the Colony Forming Units:
For the counting, semi-quantitative method was used. The microbiologist was kept blind to avoid the bias.

Statistical analysis
- Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States).
- Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted. Normality of numerical data was checked using Shapiro-Wilk test & was found that the data did not follow a normal curve; hence **non-parametric tests** have been used for comparisons. Intra group comparison was done using Friedman's (for >2 observations) followed by followed by pair wise comparison using Wilcoxon Signed rank test. For all Data obtained was compiled on a MS Office Excel Sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States).
- Data was subjected to statistical analysis using Statistical package for social sciences (SPSS v 26.0, IBM).
- Descriptive statistics like Mean & SD, Median for numerical data has been depicted.

3. Result and Discussion

0.2% CHX shows statistically significant results in terms of CFU count at different times, as compared to baseline. in intra Group 1A, Group 1B, Group2A and group2B comparison statistically highly significant difference seen for the values between the time intervals ($p<0.01$) with higher values at before use & least at T3. There was a statistically **non significant** difference seen for the values between the time intervals ($p>0.05$) when it compared it among the entire time interval. [Table1] [Table 2] [table3] [table4]. 0.2% CHX also exhibited a statistically significant difference ($P < 0.005$), when compared with Green tea mouth rinse [graph 1]. Green tea mouth rinse presented with a statistically significant result ($P < 0.005$) in the initial hours only. However, post this period, the results with Green tea mouth rinse were noted to be statistically non significant.



Graph 1

Plant extracts have been widely used in topical and oral applications for disease treatment. The phenolic compound in green tea may block the growth of bacteria responsible for tooth cavity and plaque formation [9, 10]. Studies have demonstrated that, in situ, 0.2% CHX has a greater immediate antibacterial effect and substantivity than other antiseptics used in the oral cavity [11-12] our study also demonstrated the same. Its antibacterial mode of action is explained by the fact that the positively charged bis-biguanide molecule gets rapidly attracted by the negatively charged bacterial tooth surfaces and oral mucosal cell surfaces, increasing substantivity through controlled release of the agent. [13,14] The persistence of CHX on the oral surfaces and its ability to suppress salivary bacterial counts was demonstrated to last for than 12 h. [15]

Thus, CHX in a mouth rinse (0.12% or 0.2% solution) is administered at 12-h intervals and retains its ability to retard/prevent plaque formation. [16] In this clinical crossover trial, 0.2% CHX substantivity was noticed till the 7th h. At the 8th h, the count of CFU was found to be increased. The persistence of CHX on the oral surfaces and its ability to suppress salivary bacterial counts was demonstrated to last for than 12 h. [17] Thus, CHX in a mouth rinse (0.12% or 0.2% solution) is administered at 12-h intervals and retains its ability to retard/prevent plaque formation. [18] In this clinical crossover trial, 0.2% CHX substantivity was noticed till the 7th h. At the 8th h, the count of CFU was found to be increased. These results were in accordance with König et al., [19] Boulos et al.,[18] , Addy M et al., [20] and Tomás et al.[21] found that the practice of eating, chewing, and drinking significantly decreased the substantivity of 0.2% CHX, with complete recovery of the salivary flora at 3–7 h after the mouth rinse. This study demonstrated that 0.2% CHX was the most effective agent both in terms of magnitude of effects and duration of action.

Supanee Rassameemasmaung (2012) concluded that after using for 4 weeks, green tea mouthwash could significantly reduce VSC level in gingivitis subjects without causing remarkable side effects. [22] Okamoto et al., suggested that green tea catechins may have the potential to reduce periodontal breakdown resulting from potent proteinase activity of porphyromonas gingivalis.[23] Mathur, et al. Conclusions: Green tea based mouthwashes can be considered an alternative to CHX mouthwashes in sustaining oral hygiene, especially because of the added advantages provided by such herbal preparations.[24] Kaur, et al. supports the effectiveness of green tea catechin mouthwash as an antiplaque agent. It should be explored as a cost-effective, long-term antiplaque rinse with prophylactic benefits. [25] A study by Kudva et al. in 2010 on local drug delivery of green tea catechin showed that there was significant reduction in pocket probing depth and reduction in the number of various periodontopathogenic bacteria such as Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Fusobacterium species and Capnocytophaga. [26]

In present study, Green tea shows an antibacterial activity of 4-5 hrs whereas chlorhexidine remains the gold standered. However contrary Chlorhexidine, substantivity of green tea has not evaluated so far.Therfore this study imposed to determine the substantivity of the green tea mouth rinse.

Intra group comparison for values for Group 1 (A)
There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) with higher values at before use & least at T3. **Pair wise comparison** There was a statistically significant / highly significant difference seen for the values between the time intervals ($p<0.01$, 0.05) between T2, T3, T4, T5, T6 time interval with –

Before use and T6 - T3, T6 - T4, T6 - T5. There was a statistically **non significant** difference seen for the values between the time intervals ($p>0.05$) when it compared it among the entire time interval. [Table 1]

Table 1: Intra group comparison for values for Group 1A

	Mean	Std. Deviation	Minimum	Maximum	Chi square value	p value of Friedman Test
Before use	185714.29	29920.530	120000	200000	25.467	0.000**
T1	115714.29	93738.491	15000	200000		
T2	38571.43	71521.226	2000	200000		
T3	18571.43	1718.249	15000	20000		
T4	19428.57	786.796	18000	20000		
T5	29000.00	29670.412	2000	80000		
T6	92857.14	63170.216	20000	200000		

Intra group comparison for values for Group 2 (A)

Table 2: Intra group comparison for values for Group 2A

	Mean	Std. Deviation	Minimum	Maximum	Chi square value	p value of Friedman Test
Before use	137428.57	88315.452	2000	200000	21.419	0.002**
T1	41485.71	59414.685	200	150000		
T2	8942.86	10362.570	200	20000		
T3	5657.14	8257.291	200	20000		
T4	6171.43	7899.729	200	20000		
T5	10571.43	8695.921	2000	20000		
T6	14142.86	8335.238	2000	20000		

There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) with higher values at before use & least at T2 **Pair wise comparison** There was a **statistically significant / highly significant difference** seen for the values between the time intervals ($p<0.01$, 0.05) between T1 ,T2 ,T3 ,T4 ,T5,T6 - Before use and T2 ,T3 compared with T1, and T5 ,T6 compared with T2 and T5 ,T6 compared with T3 and T5 , T6 compared with T4 and T6 compared with T5. There was a statistically **non significant difference** seen for the values between the time intervals ($p>0.05$) between T4 - T1, T5 - T1, T6 - T1, T3 - T2, T4 - T2, T4 - T3. [Table 2]

Table 3: Intra group comparison for values for Group 1B

	Mean	Std. Deviation	Minimum	Maximum	Chi square value	p value of Friedman Test
Before use	181428.57	22677.868	150000	200000	34.420	0.000**
T1	73171.43	85589.752	200	190000		
T2	714.29	878.310	200	2000		
T3	1657.14	1727.095	200	5000		
T4	1214.29	949.436	200	2000		
T5	23285.71	4151.879	18000	30000		
T6	46428.57	22119.804	20000	80000		

Intra group comparison for values for Group 1(B)

There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) with higher values at before use & least at T3 .**Pair wise comparison** There was a statistically significant / highly significant difference seen for the values between the time intervals ($p<0.01$, 0.05) between T1 ,T2 ,T3 ,T4 ,T5,T6 - Before use. There was a statistically non significant difference seen for the values between the time intervals ($p>0.05$) between T2,T3 ,T4 T5 ,T6 with T1 and T3 ,T4,T5,T6 with T2.and T4 ,T5 ,T6 with T3 and T5 - T4,T6 - T4 and T6 - T5.[table 3]

Intra group comparison for values for Group2 (B)

There was a statistically highly significant difference seen for the values between the time intervals ($p<0.01$) with higher values at before use & least at T2 **Pair wise comparison** There was a statistically significant / highly significant difference seen for the values between the time intervals ($p<0.01$, 0.05) between T2 - Before use,T3 - Before use,T4 - Before use,T5 - Before use and T6 - Before use,T2 - T1,T5 - T2,T6 - T2,T4 - T3,T5 - T3,T6 - T3,T6 - T4 There was a statistically non significant difference seen for the values between the time intervals ($p>0.05$) between T1 - Before use,T3 - T1,T4 - T1,T5 - T1,T6 - T1,T3 - T2,T5 - T4,T6 - T5.[table 4].

Table 4: Intra group comparison for values for Group 2B

	Mean	Std. Deviation	Minimum	Maximum	Chi square value	p value of Friedman Test
Before use	177142.86	38606.686	100000	200000	31.057	0.000**
T1	87714.29	91039.761	10000	200000		
T2	5914.29	9057.488	200	25000		
T3	14885.71	17592.368	200	50000		
T4	39285.71	16938.263	20000	60000		
T5	62857.14	26276.914	20000	100000		
T6	105714.29	42761.799	80000	200000		

4. Conclusion

Green tea mouthwash had an antibacterial effect for 4–5 hrs, After a single rinse with no eating and drinking restrictions over the day, It can be used for at least 3 times daily for its maximum antibacterial effect. Chlorhexidine remains the gold standard providing maximum antibacterial substantivity of 7–8 h. As green tea catechin mouthwash because of its better taste and no known side-effects can be used on a daily basis as anti-plaque agent. It should be explored as a long term antiplaque rinse with prophylactic benefits.

Financial support and sponsorship- Nil

Conflicts of interest- There are no conflicts of interest.

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