ORIGINAL RESEARCH

Comparison of the effectiveness of 0.5% tea, 2% neem and 0.2% chlorhexidine mouthwashes on oral health: A randomized control trial

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ABSTRACT

Background: The aim of this study was to evaluate and compare the effectiveness of 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health.

Materials and Methods: A randomized blinded controlled trial with 30 healthy human volunteers of age group 18-25 years was carried out. The subjects were randomly assigned to 3 groups i.e., group A - 0.2% chlorhexidine gluconate (bench mark control), Group B - 2% neem, and group C - 0.5% tea of 10 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 (OHIS). Salivary pH was assessed by indikrom pH strips. Plaque, gingival, and simplified OHI scores as well as salivary pH were recorded at baseline, immediately after 1st rinse, after 1 week, 2nd week, and 3rd week. The 3rd week was skipped for group A.

Results: Mean plaque and gingival scores were reduced over the 3 week trial period for experimental and control groups. Anti-plaque effectiveness was observed in all groups and the highest being in group C ($P < 0.05$). Neem and tea showed comparative effectiveness on gingiva better than chlorhexidine ($P < 0.05$). The salivary pH rise was sustained and significant in Group B and C compared to Group A. Oral hygiene improvement was better appreciated in Group B and Group C.

Conclusion: The effectiveness of 0.5% tea was more compared to 2% neem and 0.2% chlorhexidine mouth rinse.

Key words: Chlorhexidine gluconate, gingivitis, neem, oral hygiene, plaque, salivary pH, tea

Oral diseases are a costly burden to health-care services, accounting for 5-10% of total health-care expenditures and exceeding the cost of treating other chronic diseases in industrialized countries.[1] In low-income countries, the cost of traditional restorative treatment of dental diseases would probably exceed the available resources for health-care. Therefore, oral health promotion and preventive strategies are clearly more affordable and sustainable. Oral diseases prevalence is very high in India, the culprit being dental biofilm. Therefore, the need exists

for continued research to find safe and effective oral hygiene aids useful as adjuncts to patient oral self-care.[1,2]

Tea, a product made up from 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 possess anti-oxidant, anti-mutagenic, anti-diabetic, anti-inflammatory, antibacterial and antiviral, as well as cancer-preventive properties.[3-5] Azadirachta indica (Neem) has been used to treat infections, skin conditions, and reduce swellings. The antimicrobial properties of neem leaves and seed oil have long been recognized for their benefits to the skin and hair. Neem is commonly used as oral hygiene tool in different parts of the world, which has shown anti-plaque, anti-carious, and antibacterial effects.[6-9]

Mouthwashes are used in dentistry for prevention and curative purpose. Presently available mouthwashes are all medicated and effective. However, the affordability when it comes to a country like India and their side-effects has raised questions. Essential oils and botanical extracts have the potential to

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Access this article online	
Quick Response Code:	**Website:** www.ijdr.in
	DOI: 10.4103/0970-9290.114933

benefit oral health. Unfortunately, many natural products are poorly regulated and many of the available botanical rinses have undergone scant product testing, particularly for efficacy which is also seen with tea and neem.[10-15]

Hence, this study was taken up to come up with novel and cost-effective mouthwashes so that people can use them and the oral diseases are reduced. The use of tea and neem is very common in India. The Neem stick has been used to clean the teeth in India since ancient times and the practice being followed still in many remote areas and villages. Promotion of the existing resources is what is required to gain confidence of local people as well as maintenance of efforts put in to promotion of oral health. The aim of the present study was to compare the effectiveness of the 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health. The objectives being to compare the three mouthwashes on plaque, gingivitis, salivary pH, and oral hygiene status as well as to determine the time specificity after which there is more change in pH during use of mouthwashes. The study was started with null hypothesis.

MATERIALS AND METHODS

This was a triple-blind randomized control parallel design trial [Flow Chart 1]. Ethical clearance was obtained from ethical committee of the institution. Informed consent was obtained from the subjects prior to the study.

Sampling

Based on the secondary data the sample size was estimated to be 30. Below is the sample size calculation using the formula:

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

Δ Confidence interval - 95% (0.95), Power = 80% (0.80), $Z_{0.95} = 1.645$, $Z_{0.80} = 0.84$, μ (mean) = 3.8, μ_0 (mean) = 3.0, $\Delta^2 = 0.64$.

100 subjects of 18-25 year age group were screened (by 1st investigator*) to select the subjects based on inclusion and exclusion criteria in July 2010. The subjects (students) staying in the same area (Teerthanker Mahaveer University Campus, Moradabad) with same food habits and same environmental conditions, yet from different hostels (Nursing hostel, pharmacy student's hostel and MBA student's hostel) were selected, so that they don't intermingle with each other. Subjects who gave informed consent, using fluoridated toothpaste, had a mean gingival index (GI)[16,17] (by Loe and Sillness in 1963) score of ≥3.0 and mean plaque index (PI) (by Sillness and Loe 1964)[16,17] score of 1.5 were included in the study. Subjects who were using antibiotics or other medicaments within the last half-year, poor oral hygiene (assessed by oral hygiene index [OHIS] – simplified, 1964 with mean OHIS scores of
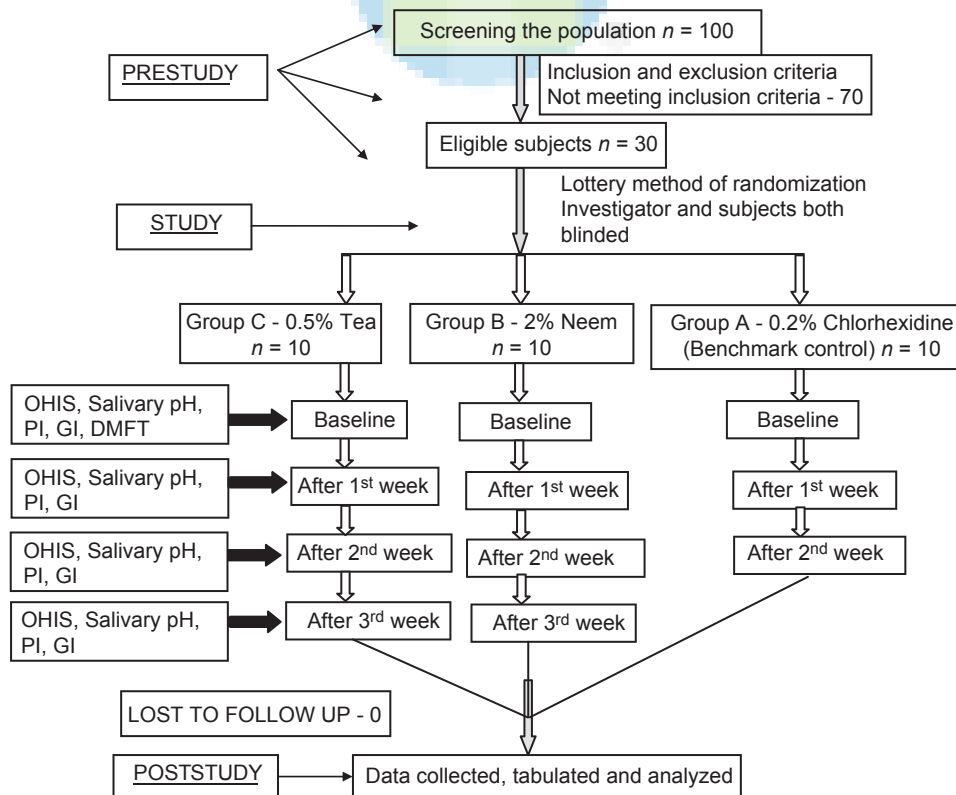
Flow Chart 1: Schematic representation of methodology

3.1-6.0),[17] less than 20 teeth available for evaluation, fixed or removable orthodontic appliances or partial dentures, need for prophylactic antibiotics or antibiotic use within 1 month of randomization, history of immunosuppressive disease, current tobacco use, diabetes, previous use of tea and neem based oral products, periodontal pocket depths > 4 mm and Decayed Missed Filled Teeth more than 3 (assessed by DMFT index, 1938 and WHO modification in 1986 and 1997) were excluded from the study.[17]

Preparation of mouthwash

Commercially available 0.2% chlorhexidine gluconate mouthwash (Periogard, Colgate, NSW, and Australia) was used. 500 ml of mouthwash was given to 10 subjects of Group A.

For 2% neem extracts, 100 g neem sticks was cut in to small pieces and ground to coarse powder in a blender, and stored in containers at room temperature. Later on, the well-soaked (2-4 h in water) neem powder was transferred to a distillation apparatus along with ten parts of water, and the mixture was continuously heated until 60% of the distillate was collected. After cooling, the collected distillate was filtered and dissolved in 1000 mL of distilled water to get 2% neem solution.[14]

Tea was extracted by combining 3 1/2 oz. (about 7 tablespoon) of green tea with 4 cups of still (not sparkling) mineral water. The tea was steeped at room temperature for 1 h and then poured in to the 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.[15]

700 ml (3 weeks supply) of neem and tea mouthwash was provided to each subject in Group B and Group C in the bottle. 500 ml (2 weeks supply) of chlorhexidine gluconate mouthwash was provided to each subject in Group A as chlorhexidine mouthwash use for more than 15 days is not recommended because of its side-effects.[10,13,18]

Oral hygiene instructions

The subjects were instructed to brush twice daily in horizontal scrub method (Norman *et al*., 2002) and to rinse the toothbrushes under running tap water twice after brushing under the supervision of a trained hostel wardens (2 wardens each in 3 hostels). After brushing, the subjects were instructed to use the provided mouthwash. 15 ml of mouthwash was rinsed for 30 s after each brushing. Group A subjects were told to use the mouthwash for 14 days (2 weeks) were as Group B and Group C subjects were told to use the given mouthwashes for 21 days (3 weeks). Unannounced surprise inspections were carried out by the investigator during the use of mouthwashes in the hostel. The subjects were instructed to keep their toothbrushes

separately. Oral hygiene instructions were reinforced after every phase. They were further instructed to avoid rinsing or eating for a period of 60 min after rinsing. All mouth rinses were packaged in to opaque bottles and brown paper bags and no labeling was carried out, thus, blinding the examiner and subject with respect to treatment arm.

Methodology

The 30 subjects were randomly (lottery method) allocated to the 3 groups by a 2nd investigator# (Group A [benchmark control] - 0.2% chlorhexidine gluconate mouthwash, Group B - 2% neem mouthwash, Group C - 0.5% tea mouthwash). Single trained and calibrated investigator$ assessed the baseline plaque by PI (Silness P and Loe H, 1964), gingival status by GI (Loe H and Silness J, 1963) and the Oral Hygiene Status was assessed by OHIS (John C Greene, Jack R Vermilion, 1964) before the mouthwashes were distributed. The pH of the saliva was checked by using commercially available pH strips i.e. indikrom papers ranging from 2-4.5 to 5.0-7.5.[19] The color changes on the pH strips were noted after keeping the strip in the un stimulated saliva for 1 min and matching with the color of standardized color chart given by the manufacturer to represent the pH of saliva.

Baseline data followed by immediately after 1st rinse and every week until the 3rd week of study was assessed for gingival status, plaque, oral hygiene, and salivary pH. Any side-effects and acceptability of mouthwashes was recorded by the questionnaire. The questionnaire consisted of 4 questions (3 close-ended and 1 open-ended) asking for acceptability or non-acceptability, reason for non-acceptability, any recommendations to change the mouthwashes and how do they rate the present mouthwash.

Repeat examinations was done on 5 subjects for measuring Gingival Index, Plaque Index, Salivary pH and OHIS (one day between examinations) to establish intra-examiner error and inter examiner calibration, which was carried out by 1st investigator*. In the process of study (July 2010-September 2010), if any untoward things happened for the study subject, medical care was arranged in Teerthanker Mahaveer Hospital, which was situated in the same campus as of study subjects and the study subject would be dropped out of study. The study subjects who required the dental treatment were provided free of cost after the study was completed.

Statistical analyses

The data were carried out using a computer software program (SPSS version 17, Chicago, USA). 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 a confidence of 95%.

RESULTS

For the study purpose, 30 subjects were recruited and completed the study without any drop outs. That accounted to 10 subjects (5 males, 5 females) in each of 3 groups. The subjects were with the mean age of 20.94 ± 0.26. Age and gender did not show any statistical significant difference between groups and within group as shown in Table 1. The intra-examiner error was within acceptable limits (kappa co-efficient = 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 3 study groups. The overall mean DMFT of the subjects in the study were found to be 1.18 ± 0.05. No statistical difference was observed within as well as between groups in DMFT, plaque scores, salivary pH, OHIS, and gingival scores. The mean plaque scores for all the 3 groups after 1st rinse was 1.45 ± 0.03. The salivary pH was low in Group C when compared to others as shown in Table 3. The least OHIS score was seen with Group A, which when compared to others was highly significant (P = 0.002). When comparison was carried out between Group A and Group B for OHIS scores the difference was found to be non-significant (P = 8.55E-06, NS) at baseline. A significant (P = 0.0002, S) relation was also obtained between Group A and C, but no difference was observed between Group B and Group C immediately after 1st rinse.

The mean plaque score (0.81 ± 0.46) and OHIS (1 ± 0.71) were low in Group C; gingival score was low in Group B (1.78 ± 0.44) whereas, Group A had a low pH (5.22 ± 0.36) when compared to others after 1st week [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 seen with A and B (P = 0.711) or A and C (P = 0.151) [Table 4]. After 2nd week the lowest plaque was recorded in Group C (0.22 ± 0.44) followed by Group B (0.56 ± 0.53). A significant difference was obtained between Group A and Group B (P = 0.005, S) as well as Group B and Group C (P = 0.01, HS). There was no difference between A and C (P = 0.063, NS). The highest salivary pH was recorded in Group C (6.06 ± 0.46) and good oral hygiene was seen in Group C (0.56 ± 0.53) followed by Group B (0.89 ± 0.78). Gingival health

Table 1: Distribution of study subjects by age and gender

Group	Male	Female	n	Mean age	SD	Min age	Max age	Mean age of males	SD	Mean age of females	SD
A	5	5	10	20.7	1.87	18	24	20.6	1.14	20.8	0.84
B	5	5	10	20.9	0.78	20	22	20.6	1.14	20.8	0.84
C	5	5	10	21.2	1.86	19	24	21	1.58	20.6	2.15
ANOVA											
P value*				0.759				0.929		0.509	
Significant				NS				NS		NS	

Significant (S)=*P*<0.05, Non-significant (NS)=*P*>0.05

Table 2: Distribution and comparison of baseline characteristics of subjects

Baseline characteristics	A (mean±SD)	B (mean±SD)	C (mean±SD)	F value (ANOVA)	P value	Inference
Plaque index	1.52±0.06	1.52±0.06	1.55±0.03	0.615751	0.547649	NS
Salivary pH	5.17±0.5	5.00±0.61	4.94±0.58	0.539432	0.589233	NS
OHIS	4.1±1.10	4.3±1.77	4.11±1.45	0.064401	0.937773	NS
Gingival index	2.67±1.00	2.5±0.71	2.44±0.73	0.210526	0.811475	NS
DMFT	1.11±0.78	1.22±1.20	1.33±1.0	0.109091	0.897091	NS

Significant (S)=*P*<0.05, Non-significant (NS)=*P*>0.05, DMFT=Decayed missed filled teeth, OHIS=Simplified oral hygiene index

Table 3: Distribution and comparison of mean values immediately after first rinse

Characteristics	A (mean±SD)	B (mean±SD)	C (mean±SD)	F value (ANOVA)	P value	Inference
Plaque index	1.45±0.12	1.42±0.14	1.47±0.08	0.371661	0.774098	NS
Salivary pH	6.28±0.44	6.06±0.53	5.89±0.6	1.565217	0.227425	NS
OHIS	0.67±0.71	2.67±1.12	2.1±1.2	8.065744	0.001793	S
Gingival index	0.18±0.53	2.11±0.6	2±0.5	1.3	0.28905	NS

Significant (S)=*P*<0.05, Non-significant (NS)=*P*>0.05, OHIS=Simplified oral hygiene index

Table 4: Distribution and comparison of mean values after 1 week

Characteristics	A (mean±SD)	B (mean±SD)	C (mean±SD)	F value (ANOVA)	P value	Inference
Plaque index	1.22±0.12	0.93±0.36	0.81±0.46	0.277356	0.8412	NS
Salivary pH	5.22±0.36	5.33±0.61	5.5±0.35	0.830097	0.44683	NS
OHIS	2.44±0.53	1.33±0.71	1±0.71	16.04717	2.56E-05	S
Gingival index	2.11±0.33	1.78±0.44	1.89±0.33	1.831978	0.166127	NS

Significant (S)=*P*<0.05, Non-significant (NS)=*P*>0.05, OHIS=Simplified oral hygiene index
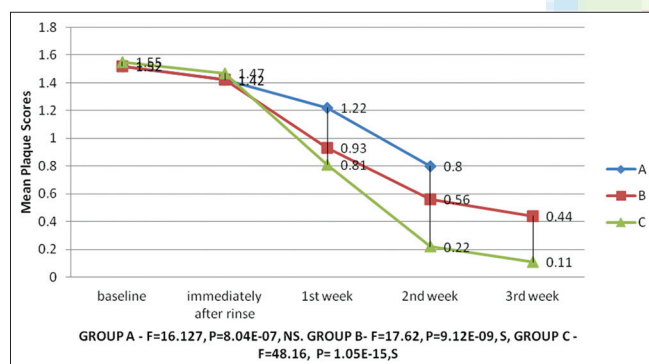
improved in all the 3 groups after 2nd week with least scores in Group C (1.11 ± 0.6) and Group B (1.11 ± 0.6) as shown in Table 5. Both Groups B and C showed good oral hygiene though the least score was seen in Group C after 3rd week [Table 6]. The plaque score exhibited by Group C (0.11 ± 0.33) was very low and was highly significant. The salivary pH was high in Group C (6.44 ± 0.39).

Graph 1 shows the mean changes in plaque at different time intervals between the three groups. The intragroup comparison at different time periods showed a statistically significant difference in all the groups as shown in Graph 1. In Group A, when the differences where compared significant differences were observed between baseline and immediately after 1st rinse ($t = 2.119905$, $P = 4.17E-13$, S), baseline and 1st week ($t = 2.119905$, $P = 4.08E-11$, S) as well as between baseline and 2nd week ($t = 1.745884$, $P = 0.04399$, S). Drop in plaque scores were seen from immediately after 1st rinse to after 1st week ($t = 2.119905$, $P = 2.59E-10$, S) and 2nd week ($t = 2.119905$, $P = 0.040666$, S) as well which was statistically significant. The decrease in plaque score from 1st week to 2nd week was also significant ($t = 2.119905$, $P = 0.002119$, S).

In Group B, the decrease in plaque values at immediately after 1st rinse ($t = 2.119905$, $P = 6.8E-12$, S) and after

1st week ($t = 2.119905$, $P = 0.004067$, S) when compared to baseline was considered significant whereas, the low plaque level after 2nd week ($t = 2.119905$, $P = 0.862655$, NS) and 3rd week ($t = 2.119905$, $P = 0.657293$, NS) were non-significant when compared with baseline. The decline from 1st rinse to 1st week ($t = 2.119905$, $P = 0.001126$, S) was statistically significant whereas not at 2nd ($t = 2.119905$, $P = 0.477347$, NS) and 3rd week ($t = 2.119905$, $P = 0.908957$, NS). However, the drop from 1st week to 2nd week ($t = 2.119905$, $P = 0.00995$, S) and 3rd week ($t = 2.119905$, $P = 0.028851$, S) was significant. Followed suite for from 2nd week to 3rd week ($t = 2.119905$, $P = 0.002515$, S).

In Group C, the significant decrease in plaque scores was seen from baseline to 1st rinse ($P = 3.89E-16$, S), 2nd week ($P = 0.039198$, S) and 3rd week ($P = 0.001126$, S) but not significant with after 1st week ($P = 0.116603$, NS). Same way the decrease in plaque from 1st rinse to 1st week ($P = 0.047749$, S) and 3rd week ($P = 0.005726$, S) was significant whereas non-significant for 2nd week ($P = 0.110257$, S). The drop in plaque scores from 1st week to 2nd ($P = 0.072218$, S) and 3rd ($P = 0.135205$, NS) week are non-significant but the drop from 2nd to 3rd week ($P = 0.000187$, VS) is found to be significant.

Graph 2 depicts the inter-and intra-group comparison of salivary pH. There was rise in salivary pH from baseline immediately after rinse in all the 3 groups but came down after 1 week but maintained higher than that of baseline. At the end of 2nd and 3rd week the rise in pH was seen again consistently in all the 3 groups. The pattern observed was statistically significant in all the 3 groups. The difference in salivary pH in Group A from baseline to immediately after rinse ($P = 5.58E-08$, S), 1st week ($P = 0.000102$, VS) and 2nd week ($P = 6.36E-07$, S) was significant. The rise in pH after 1st rinse to 1st week ($P = 0.774242$, S) was not significant but was significant with 2nd week ($P = 0.004127$, S). The change in pH from 2nd week to 3rd week was also significant ($P = 1.54E-07$, S). In Group B, the pH rise from baseline to all the time periods until 3rd week was consistently significant (after rinse $P = 1.02E-06$, S. 1st week

Graph 1: Inter and intragroup comparison of plaque scores. Repeated measures ANOVA, Unpaired *t*-test. Significant (S) = $P < 0.05$, Non-significant (NS) = $P > 0.05$

Table 5: Distribution and comparison of mean values after 2nd week

Characteristics	A (mean±SD)	B (mean±SD)	C (mean±SD)	F value (ANOVA)	P value	Inference
Plaque index	0.8±0.46	0.56±0.53	0.22±0.44	4.846154	0.015909	S
Salivary pH	6.01±0.49	5.67±0.35	6.06±0.46	1.672789	0.206596	NS
OHIS	1.11±0.78	0.89±0.78	0.56±0.53	1.560422	0.2228	NS
Gingival index	1.22±0.83	1.11±0.6	1.11±0.6	0.764706	0.475289	NS

Significant (S)=*P*<0.05, Non-significant (NS)=*P*>0.05, OHIS=Simplified oral hygiene index

**Table 6: Distribution and comparison of mean values after 3rd week**

Characteristics	B (mean±SD)	C (mean±SD)	t value (unpaired *t*-test)	P value	Inference
Plaque index	0.44±0.53	0.11±0.33	2.100922	0.001809	HS
Salivary pH	6.28±0.44	6.44±0.39	2.109816	1.9E-05	S
OHIS	0.22±0.44	0.11±0.33	2.119905	0.000187	HS
Gingival index	1.00±0.5	1.00±0.5	2.109816	0.565165	NS

Significant (S)=*P*<0.05, Non-significant (NS)=*P*>0.05, HS=Highly significant, OHIS=Simplified oral hygiene index
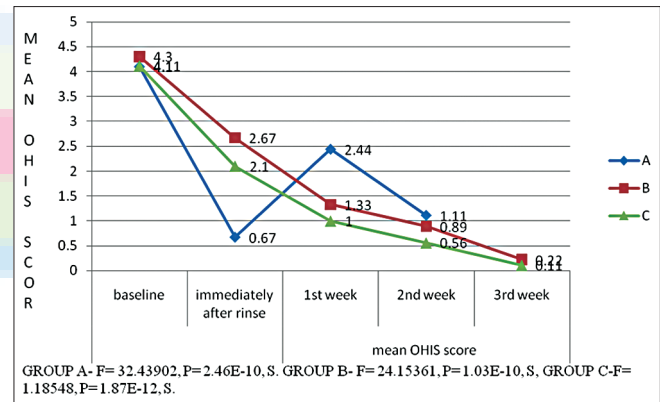
$P = 0.000285$, VS. 2nd week $P = 2.65$E-06, S. 3rd week $P = 1.07$E-07, S). The pH after 1st rinse to 1st week decreased, which was non-significant ($P = 0.317683$, S) whereas 2nd week ($P = 0.010688$, S) and 3rd week ($P = 6.69$E-05, S) had a significant difference with after rinse. The increase in pH from 1st week to 2nd week ($P = 3.57$E-05, S) and 3rd week ($P = 8.63$E-07, S) is statistically significant as well as from 2nd week to 3rd week ($P = 2.31$E-07, S). In Group C, the changes in salivary pH from baseline to after rinse ($P = 3.18$E-06, S), 1st week ($P = 3.96$E-06, S), 2nd week ($P = 2.52$E-07, S), and 3rd week ($P = 1.09$E-08, S) is statistically significant. The pH alteration immediately after 1st rinse to 1st week ($P = 0.018214$, S), 2nd week ($P = 0.00029$, HS), and 3rd week ($P = 7.19$E-06, S) is also found to be statistically significant. The rise in pH from 1st week to 2nd week ($P = 5.55$E-07, HS) and 3rd week ($P = 6.58$E-09, HS) was significant as well as the change in pH from 2nd week to 3rd week was also significant ($P = 3.78$E-06, HS).

The inter and intra group comparison of OHIS is shown in Graph 3. In Group A, there is fall in oral hygiene scores from baseline immediately after 1st rinse followed by increase in score after 1 week and again a downfall in score after 2nd week, which was found to be statistically significant. However, the Group B and Group C scores were consistently decreased from baseline until the end of 3rd week which was also found to be significant. In Group A, the drop in scores from baseline was significant with immediately after rinse ($P = 6.19$E-05, S) and 2nd week ($P = 0.000583$, HS) and non-significant with 3rd week ($P = 0.137785$, NS). The decrease in oral hygiene from immediately after rinse and 1st week ($P = 6.01$E-08, S) as well as 2nd week ($P = 0.000818$, S) is statistically significant. Whereas, the improvement in oral hygiene from 1st week to 2nd week is non-significant ($P = 0.304594$, NS). In Group B, the improvement in oral hygiene from baseline and immediately after rinse was non-significant ($P = 0.452963$, NS) whereas when compared to baseline the 1st week ($P = 0.011504$, NS), 2nd week ($P = 0.003118$, NS), and 3rd week ($P = 0.000232$, NS) the relation was significant. After 1st rinse the oral hygiene improvement compared after 1st week ($P = 0.460677$, NS) and 2nd week ($P = 0.106514$, NS) were not significant but was significant with 3rd week ($P = 0.002371$, S). The improvement in oral hygiene from 1st week to 2nd week ($P = 0.13341$, S) and 3rd week ($P = 0.694446$, S) is not significant as well as between 2nd and 3rd week ($P = 0.281663$, S). In Group C, when compared with baseline a significant relation was seen with 1st week ($P = 0.001223$, HS), 2nd week ($P = 0.000142$, VS) and 3rd week ($P = 1.73$E-05, S) but not with immediately after 1st rinse ($P = 0.139511$, NS). After rinse and 3rd week was significant ($P = 0.036228$, S) whereas 1st week ($P = 0.821453$, NS), 2nd week ($P = 0.242855$, NS) and after rinse were non-significant. When compared with 1st week and 2nd week ($P = 0.077039$, NS) as well as 1st week and 3rd week ($P = 0.675497$, NS) no significant improvement in oral hygiene was noticed. A significant oral hygiene improvement was seen from 2nd week to 3rd week ($P = 0.016681$, S).
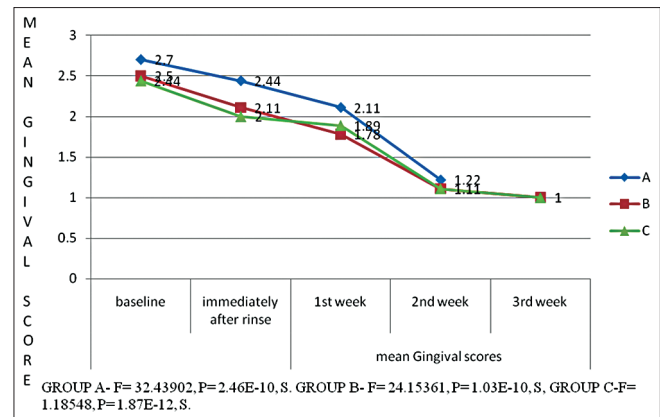
The Graph 4 shows the gingival status changes in different groups at different time periods. The gingival status improved from baseline until the 3rd week, which was found to be statistically significant in all groups with maximum increase in gingival condition in Group C. In Group A, there was improvement in gingival condition

GROUP A- F= 17.28323, P=4.06E-07, S. GROUP B- F=11.44719, P=1.71E-06, S, GROUP C- F=14.25, P=1.37E-07, S.
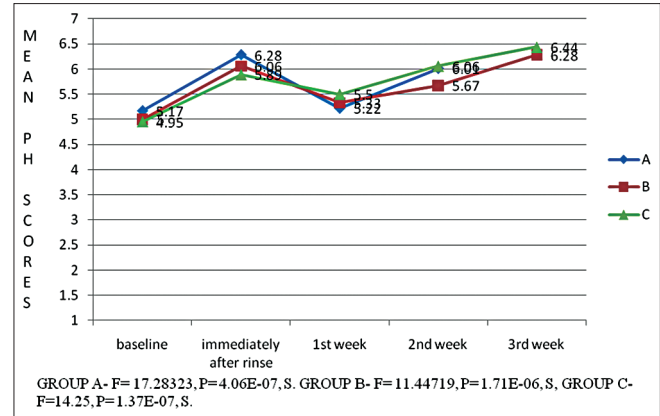
Graph 2: Inter- and intra-group comparison of salivary pH. Repeated measures ANOVA, Unpaired *t*-test. Significant (S) = $P < 0.05$, Non-significant (NS) = $P > 0.05$

GROUP A- F= 32.43902, P=2.46E-10, S. GROUP B- F=24.15361, P=1.03E-10, S, GROUP C-F= 1.18548, P=1.87E-12, S.

Graph 3: Inter- and intra-group comparison of simplified oral hygiene index. Repeated measures ANOVA, Unpaired *t*-test. Significant (S) = $P < 0.05$, Non-significant (NS) = $P > 0.05$

GROUP A- F= 32.43902, P=2.46E-10, S. GROUP B- F=24.15361, P=1.03E-10, S, GROUP C-F= 1.18548, P=1.87E-12, S.

Graph 4: Inter- and intra-group comparison of gingival scores. Repeated measures ANOVA, Unpaired *t*-test. Significant (S) = $P < 0.05$, Non-significant (NS) = $P > 0.05$

from baseline to immediately after rinse ($P = 0.055606$, S), which was significant whereas, the improvements in 1st week ($P = 0.224017$, NS) and 2nd week ($P = 0.320934$, NS) when compared with baseline was not significant. After 1st rinse to the end of 1st week ($P = 0.005495$, S) a significant decline in gingival scores was see, which was not observed when compared with 2nd week ($P = 0.508618$, NS). When the 2nd and 3rd week were compared a non-significant increase in gingival status was observed ($P = 0.715219$, NS). In Group B, a statistical significant decline in gingival scores was seen from baseline to 1st rinse ($P = 0.049865$, S) where this significance was not seen when compared with 1st week ($P = 0.256526$, NS), 2nd week ($P = 0.304594$, NS) and 3rd week ($P = 0.150069$, NS) from baseline. After 1st rinse and at the end of 1st week ($P = 0.016321$, S) the significant increase in gingival condition was seen were non-significant relation is seen with 2nd week ($P = 1$, NS) and 3rd week ($P = 0.675497$, NS) when compared with after 1st rinse. No significant relation obtained when 1st week is compared to 2nd ($P = 0.198445$, NS) as well as 3rd week ($P = 0.332195$, NS). However, the fall in gingival scores from 2nd week to end of 3rd week was statistically significant ($P = 0.003575$, S). In Group C, when baseline was compared with after 1st rinse ($P = 0.077039$, NS), 1st week ($P = 0.114742$, NS) and 2nd week ($P = 0.304594$, NS) there was no statistically significant difference however, with 3rd week it was significant ($P = 0.00013$, HS). When after 1st rinse was compared with 2st week ($P = 0.675497$, NS) no significant relation was obtained but with 1st week ($P = 0.000414$, VS) as well as 3rd week ($P = 0.000414$, VS) significance was obtained. The 2nd and 3rd week were compared the result being non-significant increase in gingival status ($P = 0.346414$, NS).

DISCUSSION

The study was carried out to assess and compare the effectiveness of 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health. This was a triple-blind study where in the investigator, study subjects as well as the statistician was not aware to which group the subjects belonged and coding was done for each group and individuals. No side- effects or miss happenings were seen during study procedure.

Comparison with other studies could not be carried out as the material and concentrations used are different as well as the age group for the study and time intervals varied for every study. Since, the GI has been the most widely used index in studies investigating oral hygiene products,[9,11,13,18] it was included in this study to permit comparison between studies.

0.2% Chlorhexidine
Chlorhexidine digluconate is, to date, the most thoroughly studied and the most effective anti-plaque and anti-gingivitis agent. However, several side-effects associated with its use have stimulated the search for alternative agents. For this reason only it is taken as a benchmark control for various mouthwashes. The most commonly prescribed concentration is 0.2% hence, this was considered in the study.[18,20-22]

As expected the mean plaques scores reduced from baseline to 3rd week. The lowest plaque was recorded after the 1st rinse. The drop was found to be significant. Same goes with gingival scores, were significant reduction of gingivitis was seen from score 2.7 at baseline to 1.4 at the end of 2nd week. Oral hygiene was poor at baseline for subjects, after use of mouthwash oral hygiene improved to good. There was a drastic reduction in oral hygiene scores immediately after the rinse when compared to Group B and C may be due to the effect of very vigorous swishing and rinsing of mouth rinse, which had removed the debris in this group and this was not controlled for the 3 groups. However, after the rinse the OHIS score was more than other groups due non-compliance of subjects in rinsing the mouth wash properly. The salivary pH also was increased to 6.01 from 5 at baseline. This increase was throughout the time period. Our studies are in concurrence with other studies.[18,20-22]

2% Neem
Neem contains trimethylamine, chlorides, nimbidin, azadarachitin, lectin, fluorides in large amounts and silica, sulfur, vitamin C, tannins, saponins, flavonoids, and sterols in small quantities. The antibacterial and antiseptic properties of neem have been proved in various studies on health.[14,23] In the present study, 2% neem was used so that the taste should not be a hindrance for its use with maximal inhibition of bacteria and plaque. It was seen that the significant reduction in plaque was seen from baseline (1.52) to 3rd week (0.4). The reduction was better than that of chlorhexidine. The salivary pH was also maintained high when compared to chlorhexidine levels. The gingival condition was reduced from severe gingivitis to no gingivitis by the end of 3rd week. The gingival response was better for neem than compared to chlorhexidine. The oral hygiene status also changed from fair to good. Some studies have given same results.[13,24]

0.5% Tea
Originating from China, tea has gained the world's taste in the past 2000 years. The economic and social interest of tea is clear and its consumption is part of many people daily routine, as an everyday drink and as a therapeutic aid in many illnesses. Ancient Asian cultures have consumed green tea as a beverage for over 4000 years. Drinking tea has become associated with life-style and living habits of more than 80% of the population, though it is brewed differently to suit one's taste and life-style. The first clue to the oral health benefits of tea came from studies in the 1940 s to 50 s showing fluoride to be the active component.[25] Reports suggested not only fluoride but also tannins contributed to the inhibitory effect.[2,26-28]

0.5% of tea was used so that the concentration should not change the taste but should have maximum inhibition of

variables. In the present study 0.5% tea had the maximum desired effect when compared to neem and chlorhexidine. The plaque level was brought to 0.1 at the end of 3rd week from baseline (1.51). Though, not much difference was observed in the salivary pH level at the end of 3rd week with neem (6.3, 6.4, respectively). However, when compared to chlorhexidine at the end of 2nd week only the salivary pH rise was more in tea group. The oral hygiene status improved from poor to good. Tea group had upper hand when it came to gingival status as the response was very good and quick when compared to neem or chlorhexidine, which was significant also. May be the catechins, tannins, and astringent effect present in the tea have carried out wonders to gingival health.

Comparison with the other studies could not be carried out as, tea as a mouth rinse has not been studied separately. In combination with other herbal mouth rinses, the effect is similar to our study (Soukoulis *et al*., 2004).[2] Two studies have been reported with that of tea tree oil with similar results and on the same variables, which we have seen the effect.[11,12] A recent human study investigated the effect of tea polyphenols in the form of chew candies on gingival inflammation over a 4-week period. The approximal plaque index and sulcus bleeding index were determined at the end of day 7 and 28. These authors suggested that tea polyphenols might exert a positive influence on gingival inflammation however, the results were not statistically significant.[29]

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 the 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.[30,31] Catechin derivatives have been reported to inhibit certain proteases of *P. Gingivalis* and may reduce periodontal breakdown.[32] Green tea catechins have also been shown to inhibit protein tyrosine phosphatase in *Prevotella intermedia*.[33] EGCG has been reported to inhibit production of toxic metabolites of *P. Gingivalis* (Sakanaka S *et al*., 2004)[34] have shown that purified tea polyphenols inhibited *in vitro* growth and H_2S production of *P. gingivalis* and *Fusobacterium nucleatum* associated with human halitosis.

Acceptability and substantivity

When the acceptability questionnaire was given to the subjects 80% subjects had no problem in using the chlorhexidine mouthwash followed by tea (78%) and neem (60%). The constraints usually coated for chlorhexidine was taste and smell whereas, for tea, the constraint was bitterness with color was slightly repulsive. For neem, it was taste which caused drawback. However,

if given a choice, the subjects had no problem using tea and neem mouthwash. Additives can be added to neem and tea to reduce the bitterness, which has been usually followed in Ayurveda system in India. Though, no specific test was employed for the substantivity, reduction in plaque and gingival score as well oral hygiene scores were taken as criteria for the longer action of the mouthwashes.

More studies with larger sample size on neem and tea mouthwashes should be encouraged to assess its efficacy, dosage, toxicity, exact concentrations, formulas for patient recommendation, and long-term effectiveness. The current investigators recognize several potential influencing variables in the present study. The participants' involvement in the study may have been a motivating factor for improvement in their habitual oral hygiene practices; and similarly, participants may have thought that the investigators expected to see a reduction in scores and hence strived to achieve that reduction through their oral hygiene efforts.

CONCLUSION

All the 3 mouthwashes used in our study were found to be effective against the plaque, gingivitis, oral hygiene, and salivary pH. When between group comparisons was carried out 0.5% tea showed better effectiveness followed by 2% neem and then 0.2% chlorhexidine mouthwash. Though, all the 3 groups did not exhibit mean changes greater than one unit from one time period to another at baseline it did approach it for several data sets. Considering the fact that the mouth rinses available presently in market are chemically based, costly, and have side-effects, which restricts their use especially in India, a cost-effective and easily available herbs as adjuvant to oral hygiene maintenance may have a far reaching effect on the prevention as well as prevalence of oral diseases.[35] The promotion of botanical herbs with fewer side-effects may motivate the patient for oral hygiene maintenance.

ACKNOWLEDGMENT

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