Original Article

Effectiveness of two different herbal toothpaste formulations in the reduction of plaque and gingival inflammation in patients with established gingivitis — A randomized controlled trial*

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A B S T R A C T

Background: Plant based toothpastes have received great attention in reducing gingival inflammation. Studies show contrasting results regarding the effectiveness of these toothpastes. In the present study, the effectiveness of two herbal toothpaste formulations in the reduction of plaque and gingival inflammation was assessed. Nicotine content in the toothpastes was assessed using GCMS.

Material and methods: 50 patients with established gingivitis were included in the study. The subjects were randomly assigned to either the test (Parodontax®) or the control (Colgate® herbal) group. There were 5 drop outs in the study in the control group after baseline examination. No prophylaxis was undertaken prior to commencement of the study, and no attempt was made to modify the participant’s oral hygiene habits. A brief case history was recorded at baseline. The Turesky (1970) modification of the Quigley, Hein (1962) Plaque index (PI), the Loe and Silness (1963) Gingival Index (GI). Unstimulated salivary samples were collected at baseline and 30th day and the pH was measured using a salivary pH meter (CL-51B; Systronics New Delhi, India). Comparisons (intergroup and intragroup) were analysed by the t-test. Groups were also compared regarding age by means of t test, and association between group and sex was verified by means of the chi-square test. All statistical tests employed a level of significance of α = 0.05. There were reports of presence of nicotine and its derivatives in herbal toothpaste after the study was nearing completion. Hence we assessed for the presence of nicotine in both the toothpaste using the methods described by Aggarwal et al.24

Results: When the two groups (test and control groups) were evaluated, after 30 days, the test group presented an average 21.08% reduction in plaque and the control group showed 31.85% reduction in plaque scores. The mean reduction in gingival index (GI) scores was 25.92% and 19.14% in the test and control groups respectively. There was no significant difference between the groups in GI, PI and salivary pH levels. There was no evidence of nicotine or related compounds in both the toothpaste.

Conclusion: Both herbal based dentifrices reduce plaque levels and gingival inflammation. But, it did not alter the pH of the saliva. However, there were no additional benefits of the Parodontax® toothpaste over Colgate® Herbal toothpaste. There was no evidence of nicotine or related compounds in both herbal toothpaste.

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1. Background

Plaque induced gingivitis is the second most common oral disease after dental caries. It is thought to affect at least 75% of the population worldwide.1 Gingivitis begins in the childhood and its prevalence increases with age.2 It has been reported that plaque induced gingivitis is seen in dentate individuals of all age group.3

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Self-performed mechanical plaque removal is a proven method of controlling plaque and gingival disease. However, tooth brushing and flossing are difficult tasks and depend on individual dexterity. Thus, many patients might not be able to completely remove plaque on all teeth surfaces. Mechanical plaque control is a time-consuming procedure, and some individuals may lack motivation for maintaining good oral hygiene. In an effort to enhance the efficacy of mechanical tooth-cleaning procedures, antimicrobial agents have been added to dentifrices.

In recent times, considerable research is being done on mouth rinses and toothpastes based on plant extracts. Parodontax® (GlaxoSmithKline, Middlesex, United Kingdom) has received great attention in reducing gingival inflammation. It is composed of sodium bicarbonate, sodium fluoride (1,400 ppm) and herbal ingredients: Chamomile, (supposed to have anti-inflammatory properties and to decrease gingival inflammation); Echinacea, (is reputed to stimulate the immune response); Sage and Rhatany (anti-hemorrhagic properties); Myrrh, (claimed to be a natural anti-septic); and Peppermint oil (claimed to have analgesic, anti-septic and anti-inflammatory properties). Colgate® herbal toothpaste comprises of calcium carbonate, chamomile, sage, myrrh, eucalyptus and sodium monofluorophosphate.

Some studies reported that Parodontax® is able to reduce plaque and gingivitis, while others showed no significant advantage when compared to a control. In most of the trials conducted thus far, the study subjects received dental prophylaxis and oral hygiene instructions before the commencement of the trial. This may not be true in the majority of the population where plaque removal is unsupervised, hence, increased likelihood of greater plaque accumulation and gingival disease.

The results of clinical trials on established gingivitis patients will represent the vast majority of the dentifrice users. It has been shown that the pH of total saliva changed significantly towards an alkaline range when herbal products were used. Nicotine, a compound commonly seen in Tobacco is well known for its addiction and dependence potential. Nicotine increases blood pressure, heart rate and respiratory rate by facilitating the release of catecholamines. It increases the levels of free fatty acids, blood sugar and interferes with antioxidant activity. It is known to cause addiction and dependence potential. Nicotine increases blood pressure and respiratory rate by facilitating the release of catecholamines. It increases the levels of free fatty acids, blood sugar and interferes with antioxidant activity. It is known to cause addiction and dependence potential. Nicotine increases blood pressure and respiratory rate by facilitating the release of catecholamines. It increases the levels of free fatty acids, blood sugar and interferes with antioxidant activity.

2. Methodology

This was a randomised, double blinded, parallel arm controlled trial. Subjects for the study were recruited from all the patients reporting to the outpatient, Department of Periodontics, Yenepoya Dental College, Yenepoya University. Institutional Ethical Clearance was obtained prior to the study (YUE C 139/25/7 113). The clinical trial was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. This study was registered in Clinical Trial Registry of India [CTRI/2013/10/004120 [Registered on: 31/10/2013]]. The first enrolment for the study was done on 27/07/2013 and the study was completed on 20/03/2015. Informed consent was taken prior to participation in the study. The outline of the study protocol is given in Fig. 1. The entire study protocol can be accessed at [http://ctri.nic.in].

Based on previous studies, a sample size of 40, at 80% power and 95% confidence interval was estimated. 10% drop out rate was estimated and adjusted in the sample size. Hence 50 subjects (25 for each group) who fulfilled the inclusion criteria were recruited for the study. The inclusion criteria for the study were: Age ≥18 years, minimum of 14 teeth, good general health, baseline plaque score mean > 1.5 (Turesky modification of Quigley Hein Index, 1970), established gingivitis (Loe and Silness index mean score > 1). Exclusion criteria were presence of advanced periodontal disease (CPITN code 4 teeth), smokers, use of orthodontic appliances, use of antibiotics in previous 3 months, use of mouth rinses in previous 3 months, allergy to toothpaste or Parodontax herbal dentifrice and Colgate herbal dentifrice.

The subjects were randomly assigned to either the test (Parodontax® toothpaste) or the control (Colgate® Herbal toothpaste) group. The random allocation sequence was done by one of the authors (V.A.B) using coin toss method. The random allocation sequence was not revealed to the main investigator (R.H.) until the dentifrices were assigned to the participants. The main investigator enrolled the subjects and assessed the study variables. Blinding and allocation concealment was controlled by the investigator (S.N.R.). He (S.N.R.) distributed the toothpastes in plain white tubes, as “group A” and “group B” tubes. The two investigators (R.H. & V.A.B) and study subjects were unaware of the contents of each tube. The contents of each tube were revealed only after the completion of the study period. Subjects in the test group received a toothpaste tube containing 90 g of Parodontax® toothpaste (GlaxoSmithKline, Middlesex, United Kingdom). Subjects in the control group received a toothpaste tube containing 90 g of Colgate® Herbal toothpaste (Colgate — Palmolive India Limited, Mumbai, India) containing calcium carbonate, chamomile, sage, myrrh eucalyptus and sodium monofluorophosphate. The control dentifrice was modified by the investigator (S.N.R.).

No prophylaxis was undertaken prior to commencement of the study, and no attempt was made to modify the participant’s oral hygiene habits. All subjects were instructed to apply 15 mm length (in order to standardise the quantity used) of the toothpaste on the brushing surface of the toothbrush and brush twice daily (Morning and night) for a period of 5–10 min for 30 days. A brief case history was recorded at baseline. Unstimulated salivary samples were collected at baseline and 30th day, with the patient sitting with the head down and the mouth open to allow the saliva to drip from the lower lip into a beaker for 2 min to collect about 2 ml of saliva over a given time and the pH was measured using a salivary pH meter (CL-51B; Systronics, New Delhi, India). At baseline and on the 30th day, the amount of plaque and gingival inflammation was measured on all teeth, at the buccal, mesial, distal and lingual aspects, with the exception of the third molars. The subjects were stained for plaque using an erythrosine disclosing solution and cotton swabs. The amount of plaque was scored using the Turesky14 (1970) modification of the Quigley, Hein15 (1962) index, following which the gingival inflammation was recorded using the Loe and Silness (1963) Gingival Index (GI). All measurements were recorded by the main investigator (R.H.), who was previously calibrated. For calibration, two measurements were performed with 1-h interval. Intra-examiner calibration was performed in 5 patients until an 80% agreement was obtained. There were 5 drop outs in the study, 3 of them had moved abroad and 2 of them refused to come after baseline examination.

2.1. Extraction of nicotine from Parodontax® dentifrice and Colgate® herbal dentifrice

Nicotine was extracted from the control and Test tooth paste was done as described by Agarwal and Rajagopal cited by Agarwal and Ray. Nicotine was extracted and isolated from the control and test tooth paste by taking 25 g of toothpaste in a conical flask. 15 mL of NH₃ (strong) was added to the flask, followed by 250 mL.
chloroform and then the flask was sealed with a cap. The ammonia was added to humidify the sample. Then the flask was kept in the mechanical shaker for about 1 h. It was followed by handshaking for 2 min. Then the chloroform. The extract was then filtered out and dried. The samples were stored in Eppendorf tube. The samples were sent to SAIF, IIT, Mumbai, India ltd for further analysis.

2.2. Method for identification of nicotine

The samples were further analysed using GC-MS (GC — Agilent Mahe, MS — Jeol). Electron ionization mode was used (EI was 70 eV, Solvent used was Chloroform (CHCL3)). Ion source temperature was 200 °C, the injector temperature was 250 °C, and transfer line temperature was 260 °C. The sample injection was done using split
ion mode. The carrier gas used was helium with a flow of 1 ml/min. HP5 Column was used with a length of 30 m, 0.25 mm internal diameter, film thickness was 0.25 micron. Full scan mass spectra was used with a mass range of 40–400 m/z for analyte identification.

3. Results

Statistical analysis was performed using IBM Statistical Program for Social Sciences Version 16.0 (SPSS Inc., Chicago Illinois, USA). The data were found to be normally distributed and comparisons (intergroup and intragroup) were analysed by the t-test. Data obtained from the study are represented in the tables. Groups were also compared regarding age by means of t test, and association between group and sex was verified by means of the chi-square test. All statistical tests employed a level of significance of α = 0.05. The primary outcome was to assess the efficacy of the test and control toothpaste on gingivitis and assess the pH of saliva at baseline and 30th day.

A total of 50 participants were enrolled in the study (n = 25 in each group). Only 45 participants completed the 30 day study period. The test group had 18 females and 7 male participants whereas the control group had 12 females and 8 male participants. The mean age in the test group (n = 25) was 23.84 ± 9.218 and in the control group (n = 20) the mean age was 24.35 ± 7.028. There was no statistically significant difference between the groups in relation to age (p = 0.839) and sex (p = 0.396). Hence the groups were matched at the baseline [refer Table 1].

At baseline, there was no significant difference in plaque scores between the two groups. On intergroup comparison, there was highly significant reduction in PI scores in test and control group after 30 days. The test group showed 21.08% reduction in plaque (p = 0.00), whereas the control group showed 31.85% reduction in plaque scores (p = 0.00) [refer Table 2]. Between group comparison showed significant difference (p = 0.02). The control group showed a significantly higher reduction in plaque scores [refer Table 3].

There was no significant difference in the Gingival Index between the groups at baseline. On intergroup comparison, there was highly significant reduction in PI scores in test and control group after 30 days. The test group showed 25.92% (p = 0.00) and the control group showed 19.14% reduction in GI scores (p = 0.001) [refer Table 2]. Between group comparison showed no significant difference (p = 0.365) [refer Table 3].

There was no significant difference in the salivary pH levels at baseline or after 30 days on intergroup and between group comparisons (p > 0.05). The normal salivary pH was maintained. No adverse reactions were observed during the trial. None of the volunteers reported any reaction or discomfort related to the toothpastes. But, they complained of unpleasant taste in the initial period of the study but they found the taste to be acceptable at the latter stage of the study and this did not deter them from continued usage of the toothpaste.

3.1. Nicotine identification

Gas chromatography mass spectroscopy was done to detect the presence of nicotine in both the control and test toothpastes. But full scan or split scan analysis which compares retention time, molecular ion peak and base peak did not show evidence of nicotine in both the toothpastes (refer Figs. 2–3).

4. Discussion

In recent times, there has been a renewed interest in using herbal based products. In the indigenous systems of medicine, different components of different plants have been used in medicinal preparations to clean teeth or to treat oral diseases including periodontal disease.17–19 Herbal-based toothpastes are as effective as the conventionally formulated dentifrice in the control of plaque and gingivitis.13 The efficacy of Colgate® herbal dentifrice has been evaluated in previous study.13 Hence it was chosen as control tooth paste.

### Table 1
Study sample data.

|          | Test group, n % | Control group, n % | p value |
|----------|-----------------|--------------------|---------|
| Sex      |                 |                    |         |
| Male     | 7 (28%)         | 8 (40%)            | 0.396 NS* |
| Female   | 18 (72%)        | 12 (60%)           |         |
| Age (years) | 25 (100%)    | 20 (80%)           | 0.396   |
| Mean age | 23.84 ± 9.21 years | 24.35 ± 7.02        | 0.839 NS** |

NS – not significant, *Chi square test, **t test.

### Table 2
Intragroup comparisons of PI, GI, pH values at baseline and 28 days in the test and control.

| Group   | N   | Mean  | Std. deviation | Median (IQR) | Change (%) | Wilcoxon signed rank test Z value | p value |
|---------|-----|-------|----------------|--------------|------------|----------------------------------|---------|
| Test    |     |       |                |              |            |                                  |         |
| Baseline| 25  | 1.991 | 0.443          |              | 21.08      | 4.37                             | 0.000   |
| 30 days | 25  | 1.571 | 0.356          |              | 25.92      | 4.10                             | 0.000   |
| Control |     |       |                |              |            |                                  |         |
| Baseline| 20  | 2.066 | 0.443          |              | 31.85      | 3.92                             | 0.000   |
| 30 days | 20  | 1.408 | 0.316          |              | 19.14      | 3.46                             | 0.001   |
| Test    |     |       |                |              |            |                                  |         |
| Baseline| 25  | 1.468 | 0.329          |              | 25.92      | 4.10                             | 0.000   |
| 30 days | 25  | 1.088 | 0.289          |              | 25.92      | 4.10                             | 0.000   |
| Control |     |       |                |              |            |                                  |         |
| Baseline| 20  | 1.493 | 0.327          |              | 19.14      | 3.46                             | 0.001   |
| 30 days | 20  | 1.207 | 0.114          |              | 19.14      | 3.46                             | 0.001   |
| Test    |     |       |                |              |            |                                  |         |
| Baseline| 25  | 6.932 | 0.349          |              | 0.55       | 0.39                             | 0.696   |
| 30 days | 25  | 6.970 | 0.333          |              | 0.55       | 0.39                             | 0.696   |
| Control |     |       |                |              |            |                                  |         |
| Baseline| 20  | 7.084 | 0.399          |              | 0.22       | 0.243                            | 0.808   |
| 30 days | 20  | 7.099 | 0.345          |              | 0.22       | 0.243                            | 0.808   |

*p < 0.005 – Statistically significant, *NS – Not significant, HS – Highly significant.
It is important that clinical trials verify the efficacy of any new product, instead of simply assuming that the product is efficient based on laboratory studies. Previous studies have compared the efficacy of herbal-based toothpaste against conventional toothpaste. Hence we compared two herbal-based toothpastes. This provides more homogeneity in comparison. To the best of our knowledge, this is the first randomized controlled clinical trial in a subset of Indian population assessing the efficacy of Parodontax® toothpaste and used a proven herbal-based toothpaste as control. The composition of the two toothpastes used is almost similar. This makes comparison novel and results credible. The clinical variables assessed were the Plaque index (PI), Gingival index (GI) and Salivary pH at the baseline and 30 days. There was a highly significant intragroup reduction in both the plaque index scores and the gingival index scores within both the groups (p < 0.000). There was no statistically significant difference in the salivary pH range in the test and control groups (p < 0.645 and p < 0.885). Intergroup comparison showed a statistically significant difference between the test and control groups only in relation to the plaque index scores (p < 0.025). The control group showed a higher percentage change than the test group, this could be because of a slight difference in the composition between the test and control toothpaste formulation. The results of our study were in partial agreement with other studies that reported a significant intragroup reduction in the plaque index and gingival index scores but no significant difference on intergroup comparison. It has been reported that increased periodontal disease is associated with reduced salivary flow rate and increased salivary pH. This increase in pH could be due to supersaturation of saliva with calcium and phosphate salts. It has been noted that the use of herbal products displaced the pH of the total saliva into the alkaline range, whereas in our study, we noted no significant change in the salivary pH levels. In our study, we noted that Parodontax® toothpaste significantly reduced plaque and gingival inflammation in established gingivitis and did not affect salivary pH. However, it did not show additional benefit when compared to Colgate® Herbal toothpaste. The dropouts in the control group might have had some influence on the results. The principal ingredients of Parodontax® and Colgate® Herbal toothpaste have several medicinal properties. However, data pertaining to the substantivity of these products cannot be found in literature. Results of other clinical studies have confirmed the anti-plaque and anti-gingivitis properties of herbal dentifrices. In a study done by Al-Kholani AI, Parodontax® toothpaste was more effective for the reduction of dental plaque than Silica toothpaste; however, differences were non-significant. He attributed this effect to the three pillars of Parodontax® toothpaste which include attack, defend, and fortify. Our study compared two herbal toothpastes while a majority of the studies have compared herbal with a non-herbal toothpaste. Longitudinal randomized clinical trials with split mouth design need to be conducted to validate the results of our study.

Agarwal et al compared eight dentifrices and reported the presence of nicotine in some commonly used toothpastes and tooth powders. Nicotine, a principal component of tobacco has many well-known side effects. Addiction is one of the important side effects. In their study, they reported that nicotine is highly addictive and many toothpaste manufacturing companies utilize this property by incorporating tobacco products during packaging and marketing.

### Table 3

| Group | Mean ± S.D. | Change (%) | Mann–Whitney Z value | p value |
|-------|-------------|------------|----------------------|---------|
| Test  | 0.420 ± 0.341 | 21.08 | 2.182 | 0.029 |
| Control | 0.658 ± 0.341 | 31.85 | Sig* |
| Plaque index scores | | | |
| Test | 0.381 ± 0.351 | 25.92 | 0.880 | 0.379 |
| Control | 0.286 ± 0.338 | 19.14 | NS* |
| Gingival index scores | | | |
| Test | 0.038 ± 0.407 | 0.55 | 0.480 | 0.631 |
| Control | 0.016 ± 0.471 | 0.22 | NS* |
| pH Values | | | |
dentifrices. Hence, we assessed the presence of nicotine in our study. But our study in contrast to Agarwal et al did not show the presence of nicotine in both the toothpastes used in the study. The subjects did complain of unpleasant taste with both the dentifrices in the initial usage period but they did not discontinue the usage. This could be an area of concern when using herbal based dentifrices. But, taste assessment and its influence on the selection of dentifrices were beyond the scope of the study. This apparently trivial but important issue needs to be addressed in the future studies.

4.1. Limitations of the study

The results of this study have to be interpreted with caution as the sample size is small and sampling frame may not represent the general population. The dropouts in the control group, it could have influenced the study results.

5. Conclusion

Based on the results of this study we can conclude that both herbal based toothpastes caused significant reduction in plaque levels and gingival inflammation. The control tooth paste (Colgate® Herbal toothpaste) showed significantly higher reduction in plaque levels. The pH of the saliva was not altered. The Parodontax® toothpaste resulted in reduction of plaque and gingival inflammation on established gingivitis. There was no evidence of presence of nicotine in both the toothpastes.

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