Comparative Evaluation of Antiplaque and Antigingivitis Effects of an Herbal and Chlorine Dioxide Mouthwashes: A Clinicomicrobiological Study

Abstract

Aim: The aim of the present study was to compare the efficacy of herbal mouthwash and chlorine dioxide mouthwash in reduction of plaque and gingivitis. Settings and Design: In a randomized clinical trial, forty patients were randomly selected and divided equally into two groups. Materials and Methods: After professional oral prophylaxis, the clinical parameters plaque index, gingival index, and modified sulcular bleeding index were recorded at baseline, 7th day, 14th day, and 21st day. The plaque samples were collected from gingival sulcus with an absorbent sterile paper point and were stored in a thiglycollate broth, then sent for microbiological examination. The microbial colony-forming units were assessed at baseline, 7th day, 14th day, and 21st day for Streptococcus mutans, Tannerella forsythia, and Fusobacterium nucleatum. Results: There was a statistical significant reduction in both clinical and microbiological parameters were observed with use of both the mouthwashes. However, herbal mouthwash was more effective in reducing the plaque and gingivitis than chlorine dioxide mouthwash. Conclusion: Herbal mouthwash was statistically efficacious in controlling plaque and gingivitis with potent antimicrobial activity.

Keywords: Antiplaque effect, dental plaque, gingivitis, mouthwash, periodontal pathogens

Introduction

Dental plaque is a biofilm with layers of microorganisms contained in a matrix that forms on oral surfaces which is considered as etiologic factor for periodontal diseases. Aerobic and anaerobic bacteria in the dental plaque are incompatible with gingival tissues leading to gingivitis, which may progress to periodontitis, so plaque control becomes essential. Hence, nonsurgical periodontal treatment remains the core component of periodontal therapy. For prevention of periodontal diseases and maintenance of periodontal health, professional control along with self-performed oral hygiene is of critical importance.

The hurdles such as plaque retentive factors, inaccessibility, and tissue-borne bacteria limit the efficacy of self-performed oral hygiene; therefore, chemical plaque control agents have been advocated to prevent dental plaque and gingivitis. Chemical plaque control includes various measures such as sprays, irrigators, varnishes, chewing gums, and mouthwash. Mouthwashes provide a method of depositing an active material for slow release in the mouth. This helps in maintaining an effective concentration of the material in the mouth over a considerable period of time following its use.

Among the various mouthwashes which are available, there is a need for the product with maximum efficacy with minimum side effects. Chlorine dioxide mouthwashes exhibit the required properties for a mouthwash.

Stabilized chlorine dioxide has marked bactericidal effects against oral bacteria associated with gingivitis and periodontitis. The oxidative consumption of critical biomolecules by chlorine dioxide is primarily responsible for its wide range of biocidal activity, and its single electron reduction product (ClO₂-) can also act as a reactive oxidant toward many electron-donating biomolecules (e.g., methionine, pyruvate, urate, and endogenous thiols, such as cysteine).

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However, the relative safe nature of herbal extracts has led to their use in several fields of medicine. Plants and plant isolates such as leaves of *Aloe barbadensis* and *Azadirachta indica*, bark of *Acacia arabica* (reviewer 3) demonstrate effects that are immune enhancing, anti-inflammatory, anticancer, etc.[5] Herbal formulations such as gum massage powder consists of *Cinnamomum zeylanicum*, *Piper nigrum*, *Eugenia caryophyllata*, *Glycyrrhiza glabra*, and *Rubia cordifolia* can provide an option for safe and long-term use. Certain plants used in folk medicine serve as a source of therapeutic agent by having multipotential effects in addition to their antimicrobial activity. Recently, studies have been carried out to find the effectiveness of herbal extracts on established plaque and gingivitis, but the literature regarding this is very limited.[6] Hence, the aim of the present study was to compare the efficacy of herbal mouthwash and chlorine dioxide mouthwash in reduction of plaque and gingivitis.

**Materials and Methods**

**Study design**

In this study, a total of 40 patients with mild-to-moderate gingivitis were recruited from outpatient Department of Periodontology, Rungta College of Dental Sciences and Research, Bhilai, Chhattisgarh, India. Subjects were of age group 20–50 years (24 males, 16 females) (reviewer 5). Sample size was calculated by keeping confidence level to be 95%. The original sample came out to be 19 which were rounded off to be 20 (reviewer 5).

Inclusion criteria: (i) systemically healthy patients; (ii) minimum of 20 teeth present in the dentition with no visible signs of untreated caries; (iii) mild to moderate type of gingivitis; (iv) bleeding on probing present, clinically; (v) no periodontal therapy for the past 6 months.

Exclusion criteria: (i) subjects on antibiotics within last 6 months; (ii) pregnant women and lactating mothers; (iii) medically compromised patients; (iv) subjects with tobacco consumption in any form; (v) subjects wearing partial dentures or having clinically unacceptable restorations or bridges; (vi) subjects wearing orthodontic appliances; (vii) history of allergy to chemical or any herbal products.

The ethical clearance for the study was provided by an institutionally approved ethical committee and all the subjects were informed about the nature of the study and the probable side effects of the drugs being administered. A written informed consent was obtained from all the subjects after informing about the nature of the study. The study was double blinded with examiner and patients were blinded (reviewer 3).

Total subjects were randomly divided into two groups. Randomization was done using chit system in which were asked to pick a chit which allotted them to that particular group (reviewer 2). Group A patients were distributed an herbal mouthwash HiOra® [Figure 1] (Mouthwash Regular – Manufactured by the Himalaya Drug Company Makali, Bangalore – 562123, India). Each gram of HiOra® mouthwash containing Pilu (*Salvadora persica*) – 5.0 mg, bibhitaki (*Terminalia bellirica*) – 10 mg, nagavalli (*Piper betel*) – 10 mg, gandhapura taila – 1.2 mg, ela – 0.2 mg, peppermint satva – 1.6 mg, yavanisatva – 0.4 mg. Group B patients were provided with chlorine dioxide (Freshclor®) [Figure 2] (mouthwash containing Sodium chlorite [as stabilized chlorine dioxide], sodium phosphate tribasic, purified water, citric acid, sucralose).

The patients were asked to use 15 ml of mouthwash twice daily after intake of food that is breakfast and dinner. They were instructed to swish for 30 s and expectorate and were refrained from use of any other plaque control measures. No dropouts observed.

**Clinical parameters**

**Clinical parameters included**

- Silness and Loe plaque index (PI) (1964)[7]
- Loe and silness gingival index (GI) (1963)[7]
- Mombelli and Ouston modified sulcular bleeding index (1987).[8]

All the parameters were recorded at baseline, 7th day, 14th day, and 21st day. Clinical examination was done by the same periodontist. After recording the clinical parameters at baseline, thorough scaling and root planing was carried out using ultrasonic scalers and hand instruments in patients of both groups. No SRP was done in subsequent visits (reviewer 2).

**Plaque sample collection and microbiological analysis**

Subgingival biofilm samples were obtained from all the subjects [Figure 3]. Subgingival samples were obtained with the help of an absorbent sterile paper point. Proper isolation was done before collection of the sample.
The paper point was placed in the sulcus and was left for 30 s and then was stored in a thioglycollate broth. The samples were then incubated at 37°C for 24 h then samples were centrifuged at 3000 rpm for 5 min and inoculated. A quantitative analysis was done for aerobic and anaerobic bacteria after 24 h of incubation at 37°C. The microbial colony-forming units (CFUs) were assessed at baseline, 7th day, 14th day, and 21st day. The microbial CFUs were assessed for *Streptococcus mutans*, *Fusobacterium nucleatum*, and *Tannerella forsythia*. Media used were Mitis salivarius agar (*S. mutans*) [Figure 4], Brewer’s media (*F. nucleatum*) [Figure 5], bile esculin media (*T. forsythia*) [Figure 6]. The colony counter unit was used for calculation of CFU in microbiological analysis.

**Statistical analysis**

Clinical changes were expressed using the mean difference from initial recording of parameter at baseline to the final outcome after the therapy at 21st day. This was done for the clinical parameters and microbial CFUs which were assessed. One-way analysis of variance was used for the analysis of the change within each group. Comparison of the result among the two groups was done using unpaired t-test.

In both analysis, *P* < 0.05 was considered to be statistically significant. Statistical analysis and data management were performed using SPSS version 18 (IBM, Chicago, IL, USA).

**Results**

**Clinical parameters**

**Plaque Index**

PI scores of both group showed reduction from baseline to 21st day [Tables 1-3 and Graphs 1-3]. Mean reduction observed in Group A was 1.08 which was statistically significant (*P* < 0.001), in Group B was 0.80 (statistically significant, *P* < 0.001). On comparison between two groups, statistically significant difference was observed, mean difference observed among the groups was 0.28 with Group A showing better results.

**Gingival Index**

GI scores from baseline to 21st day, statistically significant reduction was observed within the groups with Group A showing a difference of 0.98 and Group B showed a difference of 0.78. Mean difference among the two groups obtained was 0.20. All results obtained were statistically significant.
Table 1: Changes seen in the plaque index, gingival index, and modified sulcular bleeding index at different time interval in Group A

| Parameter | Mean value at baseline | Mean value at 7th day | Mean value at 14th day | Mean value at 21st day | SD       | P       |
|-----------|------------------------|-----------------------|------------------------|------------------------|----------|---------|
| GI        | 2.19                   | 1.91                  | 1.49                   | 1.21                   | 0.15198  | <0.001  |
| PI        | 2.25                   | 1.84                  | 1.48                   | 1.17                   | 0.16179  | <0.001  |
| mSBI      | 2.5                    | 2.1                   | 1.74                   | 1.37                   | 0.13719  | <0.001  |

SD=Standard deviation, GI=Gingival index, PI=Plaque index, mSBI=Modified sulcular bleeding index

Table 2: Changes seen in the plaque index, gingival index, and modified sulcular bleeding index at different time interval in Group B

| Parameter | Mean value at baseline | Mean value at 7th day | Mean value at 14th day | Mean value at 21st day | SD       | P       |
|-----------|------------------------|-----------------------|------------------------|------------------------|----------|---------|
| GI        | 2.16                   | 1.88                  | 1.61                   | 1.38                   | 0.16432  | <0.001  |
| PI        | 2.26                   | 1.99                  | 1.71                   | 1.46                   | 0.14972  | <0.001  |
| mSBI      | 2.51                   | 2.18                  | 1.95                   | 1.63                   | 0.13828  | <0.001  |

SD=Standard deviation, GI=Gingival index, PI=Plaque index, mSBI=Modified sulcular bleeding index

Table 3: Intergroup comparison of changes seen in the plaque index, gingival index and modified sulcular bleeding index

| Group    | GI      | PI      | mSBI    | P       |
|----------|---------|---------|---------|---------|
| Group A  | 0.98    | 0.80    | 0.20    | <0.001  |
| Group B  | 0.78    | 0.88    | 0.28    | <0.001  |
| Mean difference | 0.20 | 0.28 | 0.25 | <0.001 |

GI=Gingival index, PI=Plaque index, mSBI=Modified sulcular bleeding index

Graph 1: Changes seen in the plaque index, gingival index and modified sulcular bleeding index at different time interval in Group A

Graph 2: Changes seen in the plaque index, gingival index and modified sulcular bleeding index at different time interval in Group B

Modified sulcular bleeding index

Modified sulcular bleeding index scores from baseline to 21st day, statistically significant reduction was observed with Group A showing a difference of 1.13 and Group B showed a difference of 0.88. Mean difference among the two groups obtained was 0.25. All results obtained were statistically significant with $P < 0.001$ [Tables 1-3 and Graphs 1-3].

Changes seen in the PI, GI, and modified sulcular bleeding index at different time interval from baseline to 21st day for

Microbiological parameters

Streptococcus mutans

There was a statistically significant reduction in CFUs of the microorganism with greater reduction in Group A ($8.055 \times 10^{-2}$) than in Group B ($6.057 \times 10^{-2}$) with mean difference of $1.998 \times 10^{-2}$ among the two groups with $P < 0.001$.

Fusobacterium nucleatum

There was a statistically significant reduction in both groups with greater reduction in Group A ($8.891 \times 10^{-2}$) than in Group B ($5.550 \times 10^{-2}$) with mean difference of $3.341 \times 10^{-2}$ among the two groups with $P < 0.001$.

Tannerella forsythia

There was statistically significant reduction in Group A ($8.115 \times 10^{-2}$) and in Group B ($6.123 \times 10^{-2}$). Mean difference of $1.992 \times 10^{-2}$ was observed among the
two groups which was not statistically significant with $P > 0.001$ when comparison of the two groups was done.

Microbial count change of $S.\ mutans$, $T.\ forsythia$, and $F.\ nucleatum$ from baseline to 21st day for Group A are given in Table 4 and Graph 4 and for Group B in Table 5 and Graph 5. Table 6 and Graph 6 show comparison of changes observed among both the groups. Statistically significant difference was observed for all parameters and microbiological assessment except $T.\ forsythia$ which shows a $P > 0.001$ when compared among the two groups.

**Discussion**

In the present study, attempt has been made to establish the role of a natural product in the prevention of gingival disease and to evaluate the efficacy of the product. The

| Table 4: Microbial count change of Streptococcus mutans, Tannerella forsythia, and Fusobacterium nucleatum in Group A |
|-----------------------------------------------|
| **Mean value at baseline** | **Mean value at 7th day** | **Mean value at 14th day** | **Mean value at 21st day** | **SD** | **$P$** |
| $Streptococcus\ mutans$ | $17.689 \times 10^{-2}$ | $13.541 \times 10^{-2}$ | $10.191 \times 10^{-2}$ | $9.634 \times 10^{-2}$ | $1.38506$ | $<0.001$ |
| $Tannerella\ forsythia$ | $17.307 \times 10^{-2}$ | $12.765 \times 10^{-2}$ | $10.085 \times 10^{-2}$ | $9.192 \times 10^{-2}$ | $1.30265$ | $<0.001$ |
| $Fusobacterium\ nucleatum$ | $18.682 \times 10^{-2}$ | $14.592 \times 10^{-2}$ | $11.606 \times 10^{-2}$ | $9.791 \times 10^{-2}$ | $2.39832$ | $<0.001$ |

SD=Standard deviation

| Table 5: Microbial count change of Streptococcus mutans, Tannerella forsythia, and Fusobacterium nucleatum in Group B |
|-----------------------------------------------|
| **Mean value at baseline** | **Mean value at 7th day** | **Mean value at 14th day** | **Mean value at 21st day** | **SD** | **$P$** |
| $Streptococcus\ mutans$ | $16.778 \times 10^{-2}$ | $13.898 \times 10^{-2}$ | $12.156 \times 10^{-2}$ | $10.721 \times 10^{-2}$ | $1.36506$ | $<0.001$ |
| $Tannerella\ forsythia$ | $16.175 \times 10^{-2}$ | $12.94 \times 10^{-2}$ | $10.970 \times 10^{-2}$ | $10.052 \times 10^{-2}$ | $1.03081$ | $<0.001$ |
| $Fusobacterium\ nucleatum$ | $15.582 \times 10^{-2}$ | $13.052 \times 10^{-2}$ | $11.606 \times 10^{-2}$ | $10.032 \times 10^{-2}$ | $1.19402$ | $<0.001$ |

SD=Standard deviation
suggested that herbal mouthwash is an effective antiplaque agent (reviewer 2). The main finding of the study showed a significant difference in all the clinical and microbiological parameters assessed from baseline to 21st-day sample, thereby establishing the antiplaque and antigingivitis effects of the mouthwashes.

Evidence shows that the herbs such as Bibhitaki (T. bellerica) one of the ingredients of highly praised ayurvedic compound triphala, used both, internally as well as externally has many of the properties such as astrigent, laxative, used in treatment of skin diseases, and respiratory diseases. Nagavalli (P. betel) shows the antioxidant, anti-inflammatory, antiplatelet, antimicrobial, etc. Pilu (S. persica) shows antioxidant activity. Peppermint that contains menthol activates cold-sensitive TRPM8 (family of Transient receptor potential ion channels, M stands for melastatin) receptors in the skin and mucosal tissues and is the primary source of the cooling sensation that follows the topical application of peppermint oil also used as a flavoring agent in tooth pastes, ice creams, confectionaries, chewing gums, etc., Ela is an effective gargle for bad odour in the oral cavity and dental ailments. The paste is also beneficial for skin diseases, chronic ulcers, and pruritus. Ela oil is used with great benefit in toothache due to infections. Gandhapura taila has analgesic, anti-inflammatory effect used in treatment of joint pains. The herbal mouthwash showed no adverse effects and no staining of teeth with the usage of mouthwash (reviewer 3).

The result of the study is in accordance to the previous study which showed reduction in PI, GI, and gingival bleeding index score by the herbal mouthwash. Chandrahas et al. compared a herbal mouthwash against a positive control and a placebo, herbal mouthwash showed a greater reduction of plaque and GI over placebo group but lesser than positive control; however, the difference was not significant. The present study showed a reduction in all clinical and microbiological parameters by the herbal mouthwash which is in accordance in Chandrahas et al. Sucheta and Bharwani used a herbal formulation as an adjunct to phase I therapy and showed a significant improvement in the scores of clinical and microbiological parameters as compared to scaling and root planing or the use of formulation alone thus establishing the role of herbal products as an adjunctive therapy.

The ability of ClO₂ to reduce malodor is well documented. When sodium chlorite is exposed to the acidic environment, the chlorous acid is released, which exert a high level of microbicidal action and oxidatively consume volatile sulfur compounds, together with their amino acid precursors in the oral cavity, thereby reducing the mouth odor intensity. The persistence of chlorine dioxide is limited and is comparable to chlorhexidine as substantiated by Yates et al. (reviewer 3). In the present study, significant reduction has been observed for different indices, hence establishing its role as potent antimicrobial agent which is in accordance to the previous evidence.

Ghani et al. evaluated the efficacy of chlorine dioxide mouthwash with scaling and root planing, the result showed maximum reduction in plaque accumulation, gingivitis, and halitosis when used with scaling and root planing as compared with either being the only treatment modality, the present study highlights this. In another study comparing chlorine dioxide mouthwash and chlorhexidine mouthwash, results showed significantly greater reduction in plaque growth with chlorhexidine, but chlorine dioxide mouthwash was more preferred by the patients and produced less taste alterations. Limitations of the present study are sample size is small, larger sample size should be considered; duration for the study was short, longer duration investigation should be carried out (reviewer 3).

Conclusion

The results showed that both the mouthwashes are effective as antiplaque and antigingivitis agents. However, herbal mouthwash showed more improved results in all the clinical and microbiological parameters assessed when compared with chlorine dioxide mouthwash.

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Conflicts of interest

There are no conflicts of interest.

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