The Comparison of Antimicrobial Effect of New Herbal with Standard Toothpaste with Their Influence on Gingival Health Indexes: A Randomized Clinical Trial

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Abstract

Background: Dental caries, gingivitis, and periodontitis are considered the most common oral and dental health problems in different parts of the world.

Objectives: The present study aimed to evaluate the antibacterial activity, plaque index, and gingival index determination of herbal toothpaste compared with standard toothpaste.

Methods: The present study is a randomized clinical trial of 60 participants of 18 - 28 years and a minimum of 24 healthy teeth at Birjand Dental School that were randomly divided into two groups, herbal and standard toothpaste (n = 30 each group); only participants were blind. All subjects brushed their teeth for 3 minutes 2 times a day for seven months. The plaque index (PI) and gingival index (GI) for each participant also, saliva samples were taken from the subjects for different parts of the world.

Results: This study was conducted on 60 patients in two study groups. No significant statistical difference was observed between age and gender in the two studied groups. No significant statistical difference was observed between age and gender in the two studied groups. Our study showed that the mean number of S. mutans in the two groups was not significantly different (P > 0.05), but the mean number of Lactobacillus spp. in the group of herbal toothpaste at three months (P = 0.02) and seven months (P = 0.002) was significantly less than standard toothpaste. Also, the mean PI and the GI indices in the herbal toothpaste group after use for five months (P = 0.02) (P = 0.03) and seven months (P < 0.001) (P = 0.03), respectively, were significantly lower than standard toothpaste.

Conclusions: Throughout the 7-month trial, herbal toothpaste showed higher antimicrobial activity against Lactobacillus spp. than standard toothpaste, effectively reducing dental plaque and gingivitis. Furthermore, no undesirable reactions to toothpaste were reported during the trial. Therefore, it was concluded that possibly novel herbal toothpaste could be an alternative for controlling dental plaque and gingivitis. Further studies are needed.

Keywords: Periodontal index, Herbal, Toothpaste, Lactobacillus spp.

1. Background

Dental caries is still high in many developing countries, and almost half of the world's population, or 3.5 billion people, suffers from tooth decay (4). Current evidence shows that risk factors for developing dental caries, including plaque formation, fermentable carbohydrates, cariogenic bacteria, the quantity and quality of saliva, host susceptibility, poor oral hygiene, and duration, play a role in the onset and progression of dental caries (5).
Growing evidence suggests that oral pathogens are identified as a key element in the formation of dental caries (6). *Streptococcus mutans* is one of the most effective cariogenic bacteria, which has a key role in dental caries by adhesion to oral and dental tissues; herein, carbohydrate fermentation results in lactic acid production and demineralization of enamel (7). Since Mechanical plaque removal is one of the most accepted methods of controlling plaque, there are many mechanical aids to remove or control dental plaque, including toothbrushes, floss, mouth rinses, and dentifrices (8). Brushing with toothpaste containing various chemical formulations by reducing the number and activity of dental plaque is considered the most common form of dental disease prevention worldwide (9).

Toothpaste is classified as a drug and, according to American Dental Association (ADA), pastes gels or powders used with a toothbrush for cleaning dental plaque and improving oral health (10). Commonly, a wide range of chemical agents, such as fluoride in toothpaste, is used for inhibitory effects by destroying the cell wall and disrupting enzymatic activity in plaque formation (11). However, it can be associated with adverse outcomes, including chemical resistance to the antibacterial agents also lead to local toxic effects such as irritation and desquamation of the oral mucosa (stomatitis, glossitis, gingivitis) or systemic side effects such as allergic toxic reactions (acute or chronic) and even dental staining (12).

Considering that the phytochemicals isolated from the plants have a large number of active biological components, they may be a good alternative to Barberry and *Zizyphus jujuba*, which are found in Birjand (a sunny city in eastern Iran, the capital of South Khorasan province). Berberine is the main compound of berberis, an isoquinoline alkaloid produced by *Berberis vulgaris* L. cultivated in Asia and Europe with different pharmacological effects such as anti-inflammatory, antimicrobial, antifungal, and antiviral effects in both in vitro and in vivo studies (13). Also, *Z. jujuba* Mill, which is cultivated mainly in Europe and most of Asia many biologically active components, especially alkaloids and Saponin, which have antioxidant, anti-inflammatory, and antiviral properties and also improve immune function (14).

2. Objectives

Since chemical agents such as fluoride in toothpaste are associated with adverse outcomes, including chemical resistance to the antibacterial agents, the phytochemicals isolated from the plants as herbal toothpaste seem to be a good alternative. Thus, the present study aimed to evaluate the antibacterial activity, plaque, and gingival index determination of herbal in comparison with standard toothpaste against *S. mutans* and *Lactobacillus* spp.

3. Methods

3.1. Study Setting

The present study is a randomized control clinical trial conducted at Birjand Dental School, Birjand, Iran, on 60 participants aged 18 - 28 years who were simply randomly divided into two groups of herbal and standard toothpaste (*n* = 30 each group).

3.2. Sample Collection

According to the results obtained from the study of Esfahanizadeh et al. (15) and using the formula for comparing two averages and considering 90% power and 99% confidence *S*1 = 0.2, *S*2 = 0.23, *d* = 0.2 and taking into account the 10% drop in the number of samples about 30 people in each group was calculated.

Participants were admitted to the study if meeting the following criteria: age 18 - 30 years and a minimum of 24 healthy teeth. Exclusion criteria included systemic disease, using drugs, periodontal disease, pregnancy, breastfeeding, smoking, and a history of allergies to toothpaste or herbal medicine.

3.3. Toothpaste Preparation

A non-Iranian toothpaste (crest cavity protection) and herbal toothpaste prepared from Berberine and Saponin (main compounds of Barberry and *Z. jujuba*) respectively in the department of Pharmacology, Mashhad University of medical sciences were used in this study. All toothpaste was prepared in the lab section of the department of Pharmacology and separately packaged in label-free tubes with the same color of herbal toothpaste for two groups as those participants were completely blind. All subjects brushed their teeth for 3 min 2 times a day with the Bass method.

3.4. Gingival Health Assessment

GI and PI index: To determination of the GI at the onset of the study and 3, 5, and 7 months after toothpaste usage, each of the four areas of the facial, mesial, distal, and lingual teeth is examined and received a score from zero to 3 as normal gum and without inflammation: Zero; mild inflammation: Slight color change and very little edema without bleeding during the probe: 1; moderate inflammation: Edema and inflammation with bleeding during the probe: 2; and severe inflammation: Severe and marked edema and inflammation, ulceration and tendency to bleed spontaneously: 3.
PI for each tooth was determined as the average score of 4 tooth levels (buccal/labial and lingual surfaces of teeth) and was scored as without plaque: zero, a very thin plaque clinging to the edge of the free gum and adjacent teeth: 1; medium accumulation of plaque on the edge of the gums visible to the naked eye: 2; and excessive plaque buildup between the gingival pockets or on the teeth and gingival margins: 3 (16). Then, oral cavity was examined for any allergic or adverse reaction on hard and soft tissues.

3.5. Sampling and Microbial Assay

Saliva sampling: Saliva samples were taken from the participants 4 times onset of the study, 3, 5, and 7 months after using toothpaste. The participants were educated not to eat or drink except water and not perform physical exercise for at least two h before the saliva collection. Participants were asked to swallow their saliva and at least 1 ml of their non-induced saliva collection in a sterile container for 5 minutes (17). The saliva sample was then transferred to the laboratory in less than 30 minutes for S. mutans and Lactobacillus spp. culture.

3.5.1. Bacteria Culture

The collected saliva was diluted ten-fold as serial dilution by sterile normal saline. Diluted saliva was cultured in duplicate on Mitis salivarius bacitracin (MSB) agar (Himedia, India) supplemented by potassium tellurite and MRS agar (Merck, Germany) for isolation of S. mutans and Lactobacillus spp., respectively. The plates were incubated in a candle jar with a microaerophilic atmosphere at 37° for 48 h. Colonies of S. mutans identified with morphologic characteristics (0.5 mm convex undulated colonies of light blue color). Colonies of Lactobacillus spp. were characterized by small grayish-white. Colony-forming units (CFUs) per ml of saliva were calculated based on the mean of the counted colonies and saliva dilution rates (18).

3.6. Statistical Analysis

Obtained data were analyzed using SPSS 19 software. The normality of the data was evaluated using Kolmogorov-Smirnov statistical test. Mann-Whitney test was used to compare the two groups with each other, and Friedman and Wilcoxon’s tests were to analyze the changes during the onset of the study and three, five, and seven months after toothpaste usage. The criterion for the significant difference was P < 0.05.

4. Results

This study was conducted on 60 patients in two study groups. The mean age in the herbal toothpaste group was 23.5 ± 2.1 years, and in the standard toothpaste group was 23.3 ± 2.01 years (P = 0.56). In the herbal and standard toothpaste groups, 60% and 50% of subjects were male, respectively (P = 0.43). So no significant statistical difference was observed in terms of age and gender in the two studied groups.

The levels of S. mutans and Lactobacillus spp. in saliva at the onset of the study and 3, 5, and 7 months after toothpaste usage are shown in Tables 1 and 2. Most participants had high levels of salivary S. mutans and Lactobacillus spp. at baseline. The results showed that the mean number of S. mutans and Lactobacillus spp. significantly decreased in both herbal and standard toothpaste groups compared to baseline after seven months of follow-up. There was no statistically significant difference between S. mutans levels in the three follow-up times between the herbal and standard toothpaste groups. In the case of Lactobacillus spp., the results show a significant decrease in the number of Lactobacillus spp. in the group using herbal toothpaste compared to the standard in 3 months (P = 0.02) and seven months (P = 0.002) of follow-up.

The mean of PI and GI at the baseline and 3, 5, and 7 months after herbal and standard toothpaste usage was shown in Tables 3 and 4. The results showed that the PI and GI significantly decreased in both herbal and standard toothpaste groups compared to baseline after seven months of follow-up. The PI significantly decreased in the herbal toothpaste group compared to the standard in the 5-month (P = 0.02) and 7-month (P < 0.001) follow-ups. Also, the GI significantly decreased in the herbal toothpaste group compared to the standard in the 5-month and 7-month follow-ups (P = 0.03).

5. Discussion

Since plaque is considered the main etiological factor for several oral diseases, optimal plaque control, including mechanical control, is important for preventing gingivitis, periodontitis, and dental caries (19). Hence, various chemical substances have been developed to control periodontal diseases that may lead to harmful local and systemic side effects when used for a long time (11, 12). Therefore, research has recently been done on alternative products to synthetic chemicals.

The results of our study showed that the mean of PI and GI was significantly reduced in herbal and standard toothpaste groups after seven months of follow-up examination as compared to baseline. Our study is in line with a randomized clinical trial study by Taghavi et al., in which PI and GI scores had significant differences between baseline and follow-up examinations (20). Also, the comparison between the two groups in our study showed that The
### Table 1. Comparison of Streptococcus mutans Count Between Herbal Toothpaste and Standard Toothpaste

| Group/Time       | Standard Toothpaste       | Herbal Toothpaste       | Mann-Whitney (P Value) |
|------------------|---------------------------|-------------------------|------------------------|
| Baseline         | 60.3 ± 81.1 [5126.35]     | 62.4 ± 75.8 [7215.95]   | 0.56                   |
| Three months after | 43.8 ± 60.7 [4895.24]   | 35.8 ± 51.9 [4980.16]   | 0.46                   |
| Five months after | 25.8 ± 42.1 [4057.20]    | 70.1 ± 48.3 [3659.12]   | 0.56                   |
| Seven months after | 19 ± 26.2 [1940.12]      | 22.9 ± 25.5 [1940.75]   | 0.54                   |
| Friedman test (P value) | < 0.001                 | < 0.001                 |                        |
| Wilcoxon’s test (P value) | < 0.001; All times together are significant | < 0.001; All times together are significant |                        |

* Values are expressed as mean ± SD [median (Q1 - Q3)].

### Table 2. Comparison of Lactobacillus spp. Count Between Herbal Toothpaste and Standard Toothpaste

| Group/Time       | Standard Toothpaste       | Herbal Toothpaste       | Mann-Whitney (P Value) |
|------------------|---------------------------|-------------------------|------------------------|
| Baseline         | 15 ± 52.818 [0.54 - 6]    | 30.1 ± 22.4 [110.5 - 3.9] | 0.77                   |
| Three months after | 4.5 ± 7.7 [1.8 - 1.1]    | 2.96 ± 4.03 [1.6 - 4.2]  | 0.02                   |
| Five months after | 1 ± 3.5 [0.5 - 3.4]      | 0.758 ± 0.944 [0.6 - 1.5] | 0.26                   |
| Seven months after | 1 ± 1.9 [2.27 - 2.6]     | 1.08 ± 0.705 [0.4 - 0.8] | 0.002                  |
| Friedman test (P value) | 0.001                   | 0.001                   |                        |
| Wilcoxon’s test (P value) | 0.001; All times together are significant | 0.001; All times except 5 with seven months are significant |                        |

* Values are expressed as mean ± SD [median (Q1 - Q3)].

### Table 3. Comparison of Plaque Index Between Herbal Toothpaste and Standard Toothpaste

| Group/Time       | Standard Toothpaste       | Herbal Toothpaste       | Mann-Whitney (P Value) |
|------------------|---------------------------|-------------------------|------------------------|
| Baseline         | 0.65 ± 0.81 [1 - 0]       | 0.59 ± 0.7 [1 - 0]      | 0.43                   |
| Three months after | 0.58 ± 0.7 [0 - 1]       | 0.47 ± 0.5 [0 - 1]      | 0.08                   |
| Five months after | 0.51 ± 0.75 [1 - 0]      | 0.43 ± 0.23 [0.25 - 0]  | 0.02                   |
| Seven months after | 0.51 ± 0.47 [0 - 1]      | 0 ± 0 [0 - 0]           | < 0.001                |
| Friedman test (P value) | 0.003                   | 0.003                   |                        |
| Wilcoxon’s test (P value) | 0.005; 0.029; 0.034; 0.021; < 0.001; All times except onset with three months is significant | 0.005; 0.029; 0.034; 0.021; < 0.001; All times except onset with three months is significant |                        |

* Values are expressed as mean ± SD [median (Q1 - Q3)].
| Onset with seven months. |
| Onset with five months. |
| Three months with five months. |
| Three months with seven months. |

### Table 4. Comparison of Gingival Index Between Herbal Toothpaste and Standard Toothpaste

| Group/Time       | Standard Toothpaste       | Herbal Toothpaste       | Mann-Whitney (P Value) |
|------------------|---------------------------|-------------------------|------------------------|
| Baseline         | 1.07 ± 0.64 [1 - 1.25]    | 1.07 ± 0.69 [1 - 1.2]   | 0.99                   |
| Three months after | 1.03 ± 0.49 [1 - 1]      | 0.87 ± 0.51 [1 - 1]     | 0.39                   |
| Five months after | 0.87 ± 0.35 [1 - 1]      | 0.61 ± 0.49 [1 - 1]     | 0.03                   |
| Seven months after | 0.8 ± 0.41 [1 - 1]      | 0.53 ± 0.51 [1 - 1]     | 0.03                   |
| Friedman test (P value) | 0.013                   | < 0.001                 |                        |
| Wilcoxon’s test (P value) | 0.031; 0.008; 0.003; 0.002; 0.008 | 0.003; 0.002; 0.008 |                        |

* Values are expressed as mean ± SD [median (Q1 - Q3)].
| Onset with seven months. |
| Onset with five months. |
| Three months with seven months. |
| Onset with five months. |
Many studies have been conducted on reducing *S. mutans* and *Lactobacillus* spp. significantly decreased in both groups of using herbal and standard toothpaste compared to baseline after seven months of follow-up, but there was no statistically significant difference between *S. mutans* levels in the three follow-up times between the herbal and standard toothpaste groups. Our result is in line with Patil et al.’s study on 100 students of 4-6-year-old children using Himalaya herbal dental cream or cheerio gel containing fluoride (n = 50), despite the 90% reduction of saliva *S. mutans* after a 5-month follow-up period in two groups, no statistically significant difference observed between the groups (26). Our study showed that the herbal toothpaste was more effective than the standard in reducing *Lactobacillus* spp., especially after seven months of use. Many studies have been conducted on reducing *S. mutans* and *Lactobacillus* spp. using commercial and herbal toothpaste. Our result is in line with the Biria et al.’s study (28) that using herbal toothpaste containing bamboo salt and non-herbal conventional toothpaste leads to a significant reduction of *S. mutans* and *Lactobacillus* spp., after a 4-week follow-up; however, unlike our study, it is not statistically different between the two groups in the case of *Lactobacillus* spp. It seems that the duration of using herbal toothpaste is an effective factor in reducing the amount of *Lactobacillus* spp. Mehta et al. study (27) on 55 children aged 8-14 years showed herbal (Freshol) mouthwash and chlorhexidine can significantly reduce the amount of *S. mutans* saliva, PI, and GI score during periods of 10 days. Our study is in line with Ferrazzano et al. (29) on 66 healthy individuals of 12-18 years in two groups of green tea extract, or placebo mouth rinse exhibited a statistically significant reduction in colony counts of *Lactobacilli* saliva sample relative to the control group. First, in this study, only two of the important cariogenic bacteria in dental biofilm formation were examined so that it can study other cariogenic bacteria at the molecular level. Second, the effect of new toothpaste in this study was not examined on special groups, including elderly subjects also pregnant and breastfeeding women.

5.1. Conclusion
Our study showed considering the new herbal toothpaste for seven months in vivo that relatively well-controlled gingivitis and dental plaque also, there were no adverse effects it can be used effectively. Of course, more studies are needed to evaluate its effectiveness.

5.2. Limitation
First, in this study, only two of the important cariogenic bacteria in dental biofilm formation were examined so that it can study other cariogenic bacteria at the molecular level. Second, the effect of new toothpaste in this study was not examined on special groups, including elderly subjects also pregnant and breastfeeding women.

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#### Footnotes

**Authors’ Contribution:** Conception or design of the work, N. A, H. A, E. Sh, Z. T.; Analysis or interpretation of data
for the work, Gh. R. Sh.; Drafting the work or revising it critically for important intellectual content, ZT, E. Sh, H. A.; Final approval of the version to be published, N. A, H. A. E. Sh, O. R, Gh.R. Sh, Z. T.; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved, N. A, H. A. E. Sh, O. R, Gh. R. Sh, Z. T.

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