The Effect of Miswak and Fluoride Toothpastes on Dental Plaque, A Comparative Clinical and Microbiological Study

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Abstract: Aim: The aim of this randomized, clinical trial was to compare the effect of miswak and fluoride toothpastes on the count of *S. mutans* and lactobacilli in dental plaque and also the effect of both toothpastes on plaque and saliva pH. **Methodology:** Children were randomly allocated into either the fluoride group (n = 20) or the miswak group (n = 20). The antimicrobial effect of both miswak and fluoridated toothpaste used in this study was determined using bacterial count. Two types of media were used for this purpose, Mitis Salivarius Agar Base for *S. mutans*, and tomato agar (ROGOSA AGAR) for Lactobacillus. Plaque and saliva pH were measured using a portable pH meter. Children were asked to brush their teeth twice daily for 2 weeks. **Results:** There was no statistically significant increase in the mean Plaque pH after using fluoridated toothpaste. While miswak group, showed a statistically significant increase in mean plaque pH after 2 weeks. Although the mean saliva pH values of both groups increased slightly yet it was statistically not significant. As regards fluoride group, there was a statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment. While miswak group, showed no statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment. Both fluoride and miswak group, showed a statistically significant reduction in mean $\log_{10}$ values of *Lactobacillus* CFU count after treatment. **Conclusions:** Both toothpastes have a good antimicrobial effect on caries producing bacteria. Miswak toothpaste raised plaque pH; while both toothpastes have no effect on saliva pH.
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Key words: fluoride; miswak; toothpaste; plaque pH; saliva pH; *S. mutans*; lactobacilli

1. Introduction

Dental caries in children is a significant public dental health problem, affecting 60% to 90% of school children in industrialized countries (**WHO, 2003)**.It is not a self-limiting disease and without proper oral hygiene, it will progress until the tooth is destroyed **(*Fejerskov et al., 2003).*** It is a multifactorial disease in which, *streptococci mutans* and *lactobacilli in* dentalplaque, play an important role (***Featherstone, 2000; Selwitz et al., 2007***).

Tooth brushing and flossing removes dental plaque and antiseptic mouthwashes kills some of the bacteria that help in formation of plaque. Good oral hygiene is necessary for the healthy teeth, gum and fresh breath. Fluoride and plant extracts incorporated in toothpastes help to protect teeth by binding with enamel to make it stronger. It is of importance to look at the role plants play in oral hygiene as a number of them have medicinal properties (***Muhammad & Lawal, 2010).***

A study has shown that the concentrations of fluoride in biofilms are significantly increased after brushing with fluoridated toothpaste. The uptake of fluoride into the dental biofilm that was not removed by brushing is regarded as the main cariostatic effect of fluoride containing toothpastes (***Tenuta et al., 2009***).

Miswak *Salvadorapersica*is a small upright evergreen tree or shrub with white branches and aromatic roots, seldom more than 30 cm in diameter and three meters in height, has been used for centuries as oral hygiene tools (***Kassas & Zahran, 1965***).

Chemical analysis of *S. persica* demonstrated the presence of many components exhibiting antimicrobial effect of miswak aqueous extract (***Al-Bagieh et al., 1994)***. Some in *vitro* studies have shown that miswak extracts inhibited growth of various oral aerobic and anaerobeic bacteria, and *C. albicans* (***Al-Bagieh et al. 1994; Al.lafi & Ababneh, 1995***). Inhibition of in *vitro* plaque formation, growth and acid production of various cariogenic bacteria by miswak extracts have also been demonstrated (***Al Sadhan & Almas, 1999***). Miswak extract stimulate parotid saliva flow rate significantly (***Sofrata et al., 2007).***

In vitro studies showed that Miswak extract inhibited the growth of some dental plaque bacteria. And antibacterial effect of the herbal toothpaste was significantly greater than that of the placebo (*P* =0.002). Therefore due to antimicrobial effects of Miswak extract, its use in mouth rinses and toothpastes is highly recommended. ***(Hamid et al., 2007).***

An in *vivo* study aimed to document changes in plaque pH when an acidic challenge was followed by rinsing with miswak extract showed that classical plaque pH drop after 5% sucrose rinse. The maximum pH drop was recorded after 8 min with a mean minimum pH of 4.5. Rinsing with miswak extract or water at min 9 raised the plaque pH immediately. However, miswak rinse maintained the elevated plaque pH level, while water rinsing did not. The difference in plaque pH between water and miswak sessions was statistically significant. (***Sofrata et al., 2007).***

The aim of this study was to:

1. Compare the effect of miswak and fluoride toothpastes on the count of *S. mutans* and *lactobacilli* in dental plaque.
2. Compare the effect of both types of toothpastes on plaque and saliva pH.

2. Subjects and Methods

Patient selection:

Forty children of both sexes attending the Department of Pediatric Dentistry, Faculty of Oral and Dental Medicine, Cairo University were included in this study. Informed consent from the parents was obtained before inclusion of children in the study. Selected children fulfilled the following criteria:

1. Ranging in age from 7 - 9 years.
2. Permanent first molars and upper central incisors erupted.
3. Apparently healthy with no history of systemic diseases.
4. None of them were under antimicrobial agents since one month of sampling.
5. Number of carious primary teeth ranging between 4 and 8 and no caries in the permanent dentition. (DMF=0 def between 4 - 8)
6. Oral rehabilitation was done.

2. Materials:

- (Dabur MISWAK) herbal toothpaste formulated with pure extract of Miswak.
- (Signal)fluoridated toothpaste containing sodiummonofluorophosphosphate (1450ppm fluoride)..MitisSalivarius Agar Base9(HIMEDIA laboratories Pvt. Ltd. 23, Vadhani Ind. Est., LBS Marge, Mumbai-400 086, India.) Final pH (at 25ºC) 7.0 ± 0.2.
- ROGOSA AGAR (OXOID Ltd., Basingstoke, Hampshire, England) (500g makes 6litres) pH5.4 ± 0.2 at 25^0c.
- (HI 8424 Portable pH/mV/Temperature Meter).

Methods:

All patients were instructed to brush their teeth twice daily, refrain from any other oral hygiene procedures, and continue their routine dietary habits, throughout the duration of the study. They were also instructed to use the assigned toothpaste for 2weeks and refrain from brushing the night before their dental visit in order to allow for plaque accumulation as mentioned in previous studies **(*Edger & Geddes, 1986*).** The patients were examined at the first visit as baseline record and after 2 weeks for comparison.

Pooled plaque samples were collected from buccal surface of clinically sound upper incisors using sterile toothpicks for bacterial count. Plaque samples were placed in the screw capped vial containing 1ml of brain heart infusion broth (BHI).Plaque samples were inoculated on both the agar media. Another plaque sample was collected from buccal surface of clinically sound upper first molarsin a container containing 10 ml normal saline using sterile tooth picks for plaque pH measurement.

Saliva collection:

Children were seated comfortably on the dental chair. Unstimulated (resting) saliva was collected by expectorating in a sterile disposable container over 10 minutes (at base line before the use of toothpaste and after 2weeks).

For standardization of the saliva collection technique the children did not eat or drink (except water) 1 to 2hours before collection and did not perform any physical exercise before collection **(*Sudha et al., 2007).***

The antimicrobial effect of both miswak and fluoride toothpaste used in this study was determined using bacterial count. Two types of media were used for this purpose, Mitis Salivarius Agar Base for *S. mutans*, and tomato agar (ROGOSA AGAR) for *Lactobacillus*.

Counting Bacteria by Dilution and Plating:

Colonies of *S. mutans* appear with morphologic characteristics 0.5 mm raised convex undulated colonies of light blue color with rough margins, granular frosted glass appearance (***Jabbarifaret al., 2005).***

Colonies of *Lactobacillus*characterized bySmall greyish-white, flat or raised, smooth, rough or intermediate (***Janetet al.,* 2003).**

Plates were examined and the numbers of bacterial colonies were counted.

Mean was counted from duplicate for each sample.

Real bacterial number=50ml (20 micron) x dilution factor x count /ml.

Measurement of plaque and saliva pH by sampling method:

A portable pH meter was used for measurements.
(HI 8424 Portable pH/mV/Temperature Meter).

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. A logarithmic transformation (log_{10} transformation) of each CFU count was performed to normalize the data before statistical evaluation because of the high range of bacterial counts. Student's t-test was used to compare between the two groups. Paired t-test was used to study the changes after treatment within each group. Percent change data showed non-normal (non-parametric) distribution, so Mann-Whitney *U* test was used to compare between the two groups. This test is the non-parametric alternative to Student's t-test.

3. Results

The mean age of children in group I (fluoride toothpaste) was (7.8±0.8), and group II (miswak toothpaste) was (7.8±0.9). (*P*-value=0.967).

Plaque pH

Table (1), shows the mean plaque pH of both groups before and after treatment: the mean pH value at baseline (before treatment) was (4.3±0.4) in fluoride group and (4.6±0.6) in miswak group. The mean pH value after treatment was (4.4±0.4) and (4.8±0.5) respectively.

As regards fluoride group, there was no statistically significant increase in the mean pH after treatment. While miswak group, showed a statistically significant increase in mean pH after treatment.

As regards the percent change, no statistically significant difference was noted between both groups. Table (2), figure (1)

Saliva pH

Table (3), shows the mean saliva pH of both groups before and after treatment, the mean pH value at baseline (before treatment) was (7±0.7) in fluoride group and (7.1±0.4) in miswak group. The mean pH value after treatment was (7.2±0.6) and (7.2±0.5) respectively.

Both fluoride and miswak group, showed no statistically significant increase in the mean pH after treatment.

Although the mean saliva pH values of both groups increased slightly yet it was statistically not significant, Table (4).

As regards the percent change, no statistically significant difference was noted between both groups, Figure (2).

Table (1): The mean values of plaque pH before and after treatment in both groups

Group / Period	Fluoridated toothpaste		Miswak toothpaste	
	Mean	SD	Mean	±SD
Before treatment	4.3	0.4	4.6	±0.6
After treatment	4.4	0.4	4.8	±0.5
P-value	0.063		0.033*	

**: Significant at P ≤ 0.05*

Table (2): The mean values and percent change of plaque pH in both groups

Group / Period	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean	±SD	Mean	±SD	
Before treatment	4.3	±0.4	4.6	±0.6	0.041*
After treatment	4.4	±0.4	4.8	±0.5	0.018*
Percent increase	3.7	±5.7	4.6	±8.5	0.375

**: Significant at P ≤ 0.05*

Table (3): The mean values in saliva pH before and after treatment in both groups.

Group / Period	Fluoridated toothpaste		Miswak toothpaste	
	Mean	±SD	Mean	±SD
Before treatment	7	±0.7	7.1	±0.4
After treatment	7.2	±0.6	7.2	±0.5
P-value	0.304		0.056	

**: Significant at P ≤ 0.05*

Table (4): The mean values and percent change of saliva pH in both groups.

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean	±SD	Mean	±SD	
Before treatment	7	±0.7	7.1	±0.4	0.688
After treatment	7.2	±0.6	7.2	±0.5	0.834
Percent increase	2.4	±5	1.3	±3.1	0.942

**: Significant at P ≤ 0.05*

***Streptococcus mutans*count**

Table (5), shows the mean $\log_{10}$ SM count of both groups before and after treatment, the mean $\log_{10}$ SM count at baseline (before treatment) was (6±1.3) in fluoride group and (6.4±1) in miswak group. The mean $\log_{10}$ SM count after treatment was (5.6±1.3) and (6.1±1.2) respectively.

As regards fluoride group, there was a statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment. While miswak group, showed no statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment.

As regards the percent change, no statistically significant difference was noted between both groups. Table (6), and figure (3).

***Lactobacilli* count**

Table (7) shows the mean $\log_{10}$ LB count of both groups before and after treatment, the mean $\log_{10}$ LB count at baseline (before treatment) was (2.4±0.4) in fluoride group and (2.5±0.5) in miswak group. The mean $\log_{10}$ LB count after treatment was (2.2±0.5) and (2.3±0.4) respectively.

Both fluoride and miswak group, showed a statistically significant reduction in mean $\log_{10}$ values of *Lactobacilli* CFU count after treatment.

As regards the percent change, no statistically significant difference was noted between both groups. Table (8), Figure(4).

Table (5): The mean $\log_{10}$ values of *S. mutans*CFU count in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste	
	Mean	±SD	Mean	±SD
Before treatment	6	±1.3	6.4	±1
After treatment	5.6	±1.3	6.1	±1.2
P-value	0.026*		0.301	

**: Significant at P ≤ 0.05*

Table (6): The mean log10 values of *S. mutans* CFU count and percent change in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean $\log_{10}$	±SD	Mean $\log_{10}$	±SD	
Before treatment	6	±1.3	6.4	±1	0.259
After treatment	5.6	±1.3	6.1	±1.2	0.198
percent reduction	4.8	±3.2	2.8	±1.8	0.884

**: Significant at P ≤ 0.05*

Table (7): The mean $\log_{10}$ values of *Lactobacilli* CFU count in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste	
	Mean	±SD	Mean	±SD
Before treatment	2.4	±0.4	2.5	±0.5
After treatment	2.2	±0.5	2.3	±0.4
P-value	<0.001*		0.002*	

**: Significant at P ≤ 0.05*

Table (8): The mean $\log_{10}$ values and percent change of *Lactobacilli* CFU count in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean $\log_{10}$	±SD	Mean $\log_{10}$	±SD	
Before treatment	2.4	±0.4	2.5	±0.5	0.513
After treatment	2.2	±0.5	2.3	±0.4	0.466
percent reduction	39.8	±20.9	35.4	±21.7	0.532

**: Significant at P ≤ 0.05*

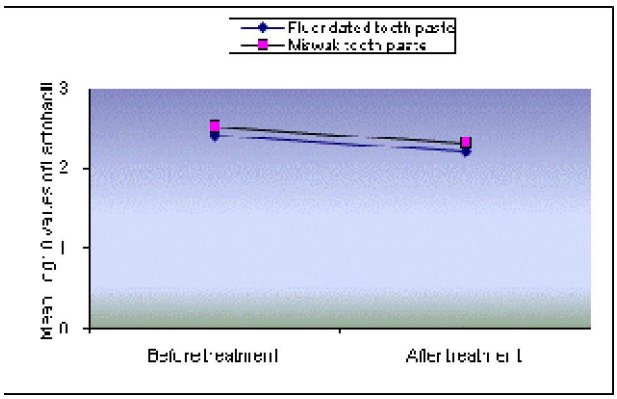

Figure (4): Changes after treatment in $\log_{10}$ values of *Lactobacilli* CFU count in both groups

4. Discussion

Children were selected from the Department of Pediatric Dentistry, Faculty of Oral and Dental Medicine, Cairo University to ensure comparable socioeconomic and feeding habits of all participants. Selected children ranged in age from 7-9 years as they can rinse and spit decreasing the risk of swallowing toothpaste.

Plaque samples were collected as they do not require sophisticated equipment, collected in a simple method and are efficiently used on a large number of subjects (***Sofrata, 2010).***

In miswak group the results were in agreement with **(*Sofrataet al., 2007),*** who demonstrated that rinsing with Miswak extract had a neutralizing effect on plaque pH after a previous sucrose exposure. The data indicated that rinsing with Miswak extract raised the plaque pH for a more prolonged time as compared to water rinsing.

Similarly another study showed that rinsing with Miswak extract resulted in protracted elevation of plaque pH (>6.0). The difference in plaque pH between Miswak extract and water rinse was statistically significant at 30 minutes ($p< 0.01$) (***Sofrata, 2010).***The elevated plaque pH with Miswak rinse could be due to a buffering capacity of the Miswak extract, salivary stimulation due to Miswak taste, and/or antibacterial activity against acid-producing bacteria. Also (**Edgar & O'Mullane, 1996**) explained that Miswak stimulated salivary secretions as it has a relatively strong taste, thus washing out and diluting acids.

Although the mean saliva pH values of Miswak and fluoride groups increased slightly yet it was not statistically significant. As regards the percent change, no statistically significant difference was noted between both groups.

On the contrary, ***(Dąbrowska, 2005)*** found that fluoride contained in oral hygiene preparations cause increase salivary pH. Differences in results between studies can be due to lack of compliance with the use of the assigned dentifrice which can occur in home-use studies.

Pooled plaque samples were used to determine levels of *S. mutans* because it is likely to be the most reliable method in children due to higher odds ratio between caries and *S. mutans* in plaque samples compared to saliva (***Sanchez-Pertez & Costa-Gio, 2001***). Plaque samples were collected from buccal surface of upper incisors as they have high concentration of plaque by sterile toothpick in accordance to ***Wennerholm et al., 1995***.

Decrease in the mean bacterial count has-been observed in both groups, toothpastes have reduced *S. mutans* and *Lactobacilli* count when used over a period of 2weeks. This substantiates the antibacterial properties of both toothpastes.

Similar results were noted in previous studies ***(Paiet al., 2004;Jabbarifar et al., 2005)*** where fluoride products containing500ppm and 1000ppm of fluoride, showed significant reductions of up to 50% after 21 days.

Similarly several studies demonstrated the antibacterial effect of miswak on *S. mutans* and found that *S. persica*has an antibacterial effect on different types of bacteria, including *mutansstreptococci* ***(Al-Lafi & Ababneh, 1995; Almas et al., 1997; Almas, 1999; Almas & Al-Bagieh, 1999).*** Another study demonstrated an immediate antibacterial effect of Miswak with significant reduction of *S. mutans*and non-significant reduction in *Lactobacilli* when comparing Miswak sticks with toothbrush ***(Almas& Al-Zeid, 2004).***

Contradicting our results ***(Petersson et al., 1991)*** foundno difference in levels of *S. mutans* or lactobacilli between subjects using or not using different fluoridated toothpastes. Also ***(Faiez, 1995)*** found that toothpaste containing Miswak extract was significantly more effective compared to fluoridated toothpaste,and attributed the anticaries effect of miswak to its fluoride content.

On the contrary testing the antibacterial effect of fresh Miswak pieces embedded in inoculated agar plates indicated that Miswak had very strong antibacterial activity. The inhibitory effect of miswak was most pronounced on *P. gingivalis, A. actinomycetemcomitans*, and *H. influenzae*, less on *S. mutans* and least on *Lactobacillus acidophilus,* which indicated that Miswak extract was more effective against Gram negative than Gram-positive species ***(Sofrata, 2010).***

Furthermore, the results from these studies cannot be directly compared as the Miswak sources and the concentrations predations are different, also method of extract preparation. However, the chemical compositions of *S. persica*roots and the exact amounts of each component are contradictory (***Ezmirly & Seif-Elnasr, 1981; Abdel- Wahab et al., 1990; Bader et al., 2002).***

Conclusion

From the present study the following conclusions can be drawn:

1. Both toothpastes have a good antimicrobial effect on caries producing bacteria, thus can be used in children as a regular home care preventive aid in combating dental caries.
2. Miswak toothpaste raised plaque pH; suggesting a positive role in the reduction and prevention of dental caries.
3. Both toothpastes have no effect on saliva pH.

Recommendations

1. Long term studies are needed to quantify the efficacy of miswak toothpaste among children.
2. Further researches with larger sample sizes are needed to evaluate the benefits of Miswak toothpaste use for children.
3. More researches are needed to study the combined effect of Miswak and fluoride in different oral hygiene products.
4. Further studies are needed to evaluate the effect of Miswak toothpaste on preschool children of high caries risk.

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