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Original Article

Dentistry Section

A Comparison of Antibacterial Efficacy of 0.5% Sodium Fluoride Impregnated Miswak and Plain Miswak Sticks on *Streprococcus mutans* - A Randomized Controlled Trial

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ABSTRACT

Introduction: Miswak (Salvadora persica) is a tooth cleaning stick made from the Salvodora Persica tree. It serves as a natural dentifrice with anti-bacterial, anti-plaque and anti-fungal properties. Fluoride, impregnated in chewing sticks will bestow comparable safety against dental caries as the fluoride containing dentifrice.

Aim: The aim of the present study was to assess and compare the anti-bacterial effect of 0.5% sodium fluoride impregnated miswak and plain miswak sticks on *Streptococcus mutans*.

Materials and Methods: A randomized controlled concurrent parallel triple blind clinical trial was conducted for a period of 8 days. The trial included 30 subjects aged 20–23 years, who were randomly allocated in 1:1 ratio to Group A [0.5% Sodium Fluoride (NaF) impregnated Miswak sticks) and Group B (Plain Miswak sticks) respectively. The participants were instructed to chew miswak sticks for 6 minutes in the morning before breakfast. Unstimulated saliva was collected at baseline and after chewing miswak sticks to estimate *S. mutans* count using Mitis Salivarius Bacitracin agar. Data was statistically analyzed using paired and unpaired t-test.

Results: A statistically significant reduction in *S. mutans* Colony Forming Units (CFU) count in saliva was observed after using fluoridated miswak sticks compared to the baseline count (p=0.001). There was no significant difference in anti-bacterial effect of fluoridated miswak sticks and plain miswak sticks on *S. mutans* count (p=0.58).

Conclusion: The efficacy of miswak impregnated with 0.5% NaF and plain miskaw sticks in reducing *S. mutans* counts in saliva is analogous.

Keywords: Dental caries, Mitis salivarus agar, Natural toothbrush, Oral self care, Salvadora persica

INTRODUCTION

Oral health has a major influence on one's general quality of life and well-being. Several chronic and systemic diseases have been attributed to poor oral health [1]. Poor oral hygiene due to lack of proper knowledge of dental care has led to increased prevalence of dental caries around the world [2]. This increased global burden of oral disease has led to the surge for prudent, effective and cost–effective alternate prevention modalities [1].

In developing countries where dental care facilities are out of reach for most of the population, there is a need for health education and to promote traditional means of oral hygiene maintenance [2]. Oral hygiene methods have been practiced worldwide since olden times. These measures have been adapted in some populations depending on factors, like educational level, cultural background, religious norms and as well as socio-economic status [3]. The widely used methods for maintaining oral health are toothbrushes and dentifrices. Babylonians in 3500 BC are considered as the forerunners of the modern day toothbrushes and toothpicks [4].

In spite of global practice of toothbrush and paste, for developing countries like India these are still a luxury and majority of people have access only to the local home grown oral hygiene aids [4]. Rural population of India which accounts to about 80% of the total population still use datun (chewing stick) to clean their teeth [5]. In India, there are at least six different varieties of datun which are in use viz., *Neem (Azadirachta-indica), Babul (Vachellia-nilotica), Mango (Mangifera-indica), Guava (Psidium-guajava)* and *Dandarasa* [6].

Miswak, belongs to the Salvadora species of Salvadoracea family. Scientifically known as Salvadora persica, it is anti-bacterial, anti-caries, anti-periopathic disinfectant having anti-plaque and anti-fungal properties. In indian subcontinent it is also called by the names like Arak tree, Meswak, Peelu, Kharjal or Jhank [7].

According to World Health Organisation (WHO) Consensus Report on Oral Hygiene, chewing sticks could play a pivotal role in the oral health promotion as they are traditionally acceptable, economical and easily accessible [8,9].

Invention of fluoride therapy played a major role in caries preventive strategies over five decades [10]. Topical fluoridation is the most commonly utilized method by which fluoride products are directly applied to the tooth surfaces either in the clinic or at home; this treatment modality has shown reduction in caries prevalence to about 30% [11]. Fluoride dentifrice is the commonest form of topical fluoride used worldwide [12]. The benefit of fluoride in reducing caries experience is throughout life [13]. Although dentifrices are widely used in the world, in developing countries, its use is limited among people, especially rural population because of their cost and compliance issues [14]. This problem can be overcome by fluoridating indigenous oral hygiene aids like miswak. Fluoride if present in chewing sticks will confer similar protection against dental caries as fluoride dentifrice. However,

there is limited scientific information on anti-bacterial property of fluoridated miswak.

Thus, the present study was conducted to test the hypothesis that there is no difference in the anti-bacterial effect of plain and 0.5% Sodium Fluoride (NaF) impregnated miswak against *Streptococcus mutans*.

MATERIALS AND METHODS

A randomized controlled concurrent parallel triple blind clinical trial was conducted in the Department of Public Health Dentistry, Krishnadevaraya College of Dental Sciences and Hospital, Bengaluru, Karnataka, India. The sample was drawn through non-probability sampling technique. The sample size of 30 subjects was selected based upon an earlier study by Yavagal PC et al., [15]. Subjects, aged between 20-23 years, who fulfilled the following eligibility criteria, were selected from the Krishnadevaraya College of Dental Sciences and Hospital.

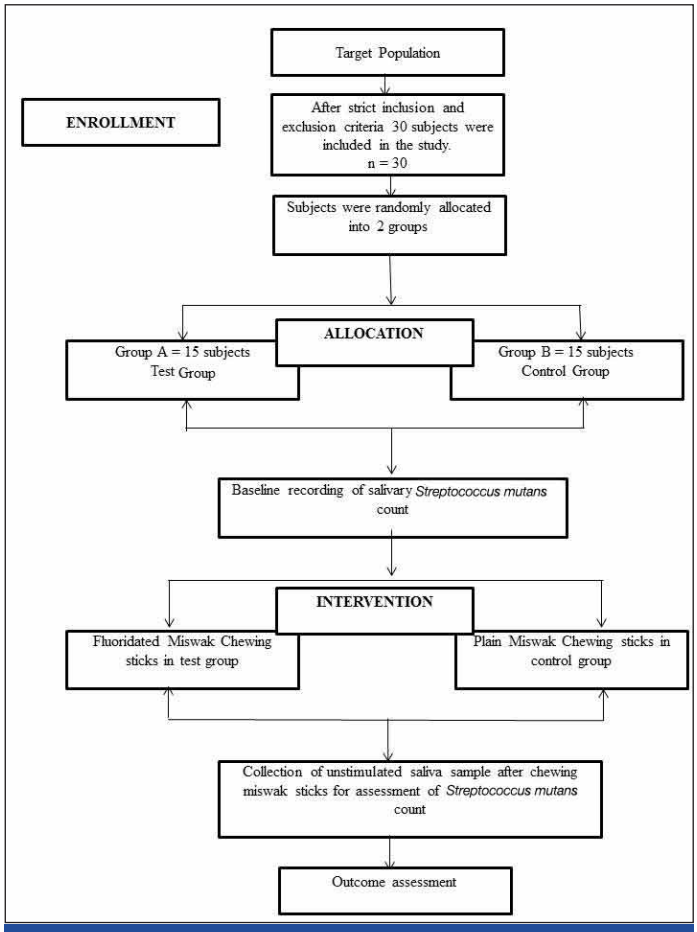
Inclusion Criteria:

1. Subjects in the age group of 20–23 years
2. Healthy non-smokers
3. Subjects with good manual dexterity

Exclusion Criteria:

1. Systemically compromised
2. Subjects undergoing orthodontic treatment
3. Subjects under antibiotic therapy or using anti-microbial mouthwash for past one week.

These students were randomly assigned using lottery method into two groups with 15 in each group (Group A and Group B) [Table/Fig-1]. Study participants were refrained from eating or drinking two hours before using miswak sticks to standardize the saliva collection technique. Informed consent was taken from the participants and ethical clearance was obtained from the institutional ethical committee.



[Table/Fig-1]: Flow diagram of study protocol according to CONSORT.

In the trial, all the three i.e., the primary investigator, study subjects and the trial statistician were unaware of the study details.

Intervention Procedure

Collection of miswak sticks: Fresh miswak sticks were purchased from the local market. Fifteen sticks were randomly selected and it was made sure that they resemble in their size, diameter and colour. Each miswak stick was cut into two equal parts using a scalpel. One part was used as plain miswak and the other part was impregnated with 0.5% NaF. The sticks were then coded by an independent investigator who was not aware of the study method and grouping. NaF impregnated miswak sticks were coded as A (A1, A2….A15) while plain miswak sticks were coded as B (B1, B2…B15).

Preparation of fluoridated miswak sticks: The miswak sticks were then placed in a sterile bottle containing 500ml of 0.5% NaF solution for a day. After impregnation, the miswak sticks were removed from the bottle using forceps and placed on a filter paper at room temperature overnight for 12 hours in order to dry.

Collection of Saliva Sample

Baseline sample: All the study participants were instructed to expectorate 2ml of unstimulated saliva in a sterile container.

Intervention (chewing miswak): The study participants were asked to peel off the bark of the Miswak stick to about half an inch from the tip and then chew the tip gently until it becomes like a brush. They were instructed to move the Miswak stick around the dentition for six minutes.

Test sample: After the intervention (chewing miswak sticks) the study participants were instructed not to swallow the saliva but to spit 2ml of unstimulated saliva in the sterile container. The sterile containers were coded and sent for the microbiological analysis.

Microbiological assessment: The saliva sample was vortexed in the vortex mixer (REMI M101) for one minute. A 100µl of the sample was transferred to 9.9ml of sterile Brain Heart Infusion Agar (BHA) broth and vortexed again for one minute. The 100µl of the diluted sample was transferred to a sterile Mitis Salivarius Bacitracin Agar (HI MEDIA, Mumbai) and spread uniformly using a sterile L spreader (TARSONS, Kolkata). Inoculated culture media were incubated at 37°C in a candle extinction jar for 48 hours. The colonies on the agar plate were observed. Small convex deep blue colonies were further studied by gram stain and identification tests. *S. mutans* were identified by gram stain morphology of gram positive cocci occurring in chains. They were confirmed by a positive mannitol and sorbitol fermentation. The colonies were counted using a digital colony counter (LABTRONICS) and the Colony Forming Unit per ml (CFU/ml) of the saliva sample was calculated.

Debriefing: At the end of the study the details regarding group allocation and results were disclosed to the study subjects.

STATISTICAL ANALYSIS

The data was analyzed using SPSS (Statistical Package for Social Sciences) software version 20.1. The significance level was fixed at 5%. Frequency distribution, mean, and Standard Deviation (SD) were generated. Paired t-test (two-tailed, dependent) was used to compare the mean differences at the baseline and after the intervention within each group. Unpaired t-test (two-tailed, independent) was used for comparison of mean difference in *S. mutans* between the groups (NaF impregnated miswak sticks group and plain miswak sticks group).

RESULTS

The Group-A, fluoridated miswak group, consisted of total 6 male (40%) and 9 female subjects (60%), and the Group B, plain miswak stick group, had 5 male (33.3%) and 10 female subjects (66.7%)

Group	Chewing Miswak	Mean *S. mutans* Count (Mean±SD)	Mean Difference (Mean±SD)	95% CI of the Difference	t-value	p-value
Group A (NaF impregnated Miswak) n=15	Before	932.33± 455.64	508.40± 369.39	303.84-712.96	5.330	0.001
	After	423.93± 230.32				
Group B (Plain Miswak) n=15	Before	901.47± 377.02	417.60± 250.45	278.91-556.29	6.458	0.001
	After	483.87± 339.40				

[Table/Fig-2]: Comparison of *S. mutans* counts (CFU/ml x 103) within the group pre- and post- chewing miswak stick.
Statistical test applied: Paired t test

A study by Bhat PK et al., using miswak extract rinse reported a significant reduction in both *S. mutans* and Lactobacilli count [19].

Another study by Almas K et al., showed similar results where significant reduction in *S.mutans* counts was observed after using miswak extract mouthrinse [20].

LIMITATION

This study is confined by certain limitations. The smaller sample-size and the influence of oral hygiene practices and dietary habits on the study results cannot be ignored. Albeit it can be recommended that NaF impregnated miswak stick may offer itself as an affordable and effective oral hygiene aid and can be used as an effective vehicle for fluoride delivery in countries where Miswak use is common or a tradition.

Chewing Miswak	Group	Mean *S. mutans* Count (Mean±SD)	M.D.	95% CI of the Difference		Levene's Test for Equality of Variances		t-value	p-value
				Lower	Upper	F	Sig.		
Before	Group A	932.33±455.64	30.86	-281.92	343.66	0.280	0.601	0.202	0.841
	Group B	901.47±377.02							
After	Group A	423.93±230.32	-59.93	-276.87	157.01	0.955	0.337	-0.566	0.576
	Group B	483.87±339.40							

[Table/Fig-3]: Comparison of *S. mutans* counts (CFU/ml x 103) between the two groups.
Statistical Test applied: Unpaired t test

study. The mean age of the study participants in Group A and Group B was 21.47±1.25 and 21.53±1.25 respectively.

[Table/Fig-2] shows the difference in *S. mutans* count of each group before and after chewing miswak sticks. A statistically significant reduction in *S. mutans* count was observed after using fluoridated miswak sticks (Group A) as compared to the baseline count ($p<0.001$). Similarly in Group B (plain miswak sticks), the decrease in mean *S. mutans* count from before chewing to after chewing miswak sticks was found to be statistically significant ($p<0.001$). It was observed that there was no statistically significant difference in the mean of *S. mutans* counts (CFU/ml x 10^3) between the two groups ($p>0.05$) [Table/Fig-3].

DISCUSSION

This study was based on the concept that chewing sticks are frequently utilized in many countries including India, for cleaning purposes and are often used up to 5 times/day. The idea of fluoridating Miswak chewing sticks in order to have a dual effect, combining the mechanical cleaning and the anti-cariogenic effect achieved through fluoride release into the oral cavity allures scientific research.

As this is an in-vivo study, it was important to fix the NaF solution at a safer concentration. In the current research work Miswak sticks were impregnated with 0.5% NaF were utilized. Baeshan H and Birkhed D considered this to be safe and effective in their study, where different concentrations of NaF were used to impregnate Miswak sticks [16].

S. mutans strains were considered in the study as they are most commonly implicated microorganisms in the causation of dental caries [17]. Mitis Salivarius Bacitracin agar was used for inoculation of *S. mutans* as it is considered to be a standard media to be used for respective strains [18].

The results of the present clinical study indicate that 0.5% NaF impregnated miswak was as effective as plain miswak chewing sticks in reducing the *S. mutans* count in saliva. There are very few studies that report on the anti-bacterial efficacy of fluoridated Miswak chewing sticks. A randomized controlled trial conducted by Yavagal PC et al., in 2014 showed a significant decline in salivary *S. mutans* count but not in salivary Lactobacilli count [15].

CONCLUSION

Sodium fluoride impregnated miswak sticks were able to reduce the *S. mutans* counts as effectively as plain Miswak sticks.

The public health aspect of this study is that fluoridated miswak chewing sticks may have a great role in community oral health programmes, for the prevention of caries in rural areas especially in communities which do not have access to community water supply, where water fluoridation and fluoride supplement programmes are not feasible. The use of miswak conforms not only to the subject of primary healthcare approach, but also to certain cultural, long-established, societal, and religious beliefs. Use of fluoridated miswak into the healthcare system will significantly help the masses with financial constraints, as well as limited oral health care facilities. Further research on the role of fluoridated miswaks as a preventive medicament should be conducted in large populations for example school children.

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