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Research Article

# ANTIMICROBIAL EFFECT OF MISWAK STICKS AND 0.5% SODIUM FLUORIDE IMPREGNATED MISWAK STICKS ON *STREPTOCOCCUS MUTANS* AND *LACTOBACILLI* - A RANDOMIZED CONTROLLED TRIAL

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### ABSTRACT

**Purpose:** To assess and compare the in vivo antibacterial property of Miswak sticks and 0.5% Sodium Fluoride (NaF) impregnated Miswak sticks against *Streptococcus mutans* (*S.mutans*) and *Lactobacillus*.

**Materials and methods:** A randomized controlled clinical trial (parallel group design) was conducted involving 20 subjects who were randomly allocated to Group A (0.5% NaF impregnated Miswak sticks) and Group B (Plain Miswak sticks). Subjects were instructed not to use any oral hygiene aids containing fluoride for one week before the test and not to eat or drink two hours before they chewed either plain or fluoride impregnated miswak sticks for 6 minutes in the morning before breakfast. Pooled saliva was collected at baseline and after chewing miswak sticks and analysed for antimicrobial activity against *Lactobacilli* and *Streptococcus mutans*.

**Results:** Microbiological results showed significant reduction in the salivary *S.mutans* count after using fluoridated miswak stick compared to baseline saliva sample [  $p=0.006$  ]. A reduction in the lactobacillus count was seen in both the groups compared to the baseline levels but, the difference was not statistically significant.

**Conclusion:** A significant reduction between pre-test and post-test was found only for salivary *S.mutans* count ( $p=0.006$ ) in 0.5%NaF impregnated miswak sticks group but not for salivary *Lactobacilli* count. Sodium fluoride when impregnated to miswak sticks did not significantly add to the antimicrobial efficacy.

**Keywords:** Miswak sticks, 0.5% Sodium Fluoride, *Streptococcus mutans*, *Lactobacilli*

### INTRODUCTION

Oral hygiene maintenance through regular removal of dental plaque and deposits is an essential factor in the prevention of dental caries. Oral hygiene practices vary across different populations, societies and cultures<sup>1</sup>. Despite worldwide use of toothbrush and paste, in developing countries like India even now, majority of the population use indigenous aids for teeth cleaning. Reasons for this include cost, availability, customs and religions<sup>2</sup>.

Chewing sticks (miswak) continue to be one of the important oral hygiene aids used among certain sections of population in India, majority being Muslims. Chewing sticks were used by the Babylonians some 7000 years ago; they were later used throughout the Greek and Roman empires and have been used by Jews, Egyptians, and Muslims. Miswak is harvested from the plant *Salvadora Persica*. Previous study has reported the

antibacterial effect of miswak on cariogenic bacteria and also periodontal pathogens<sup>3,4</sup>. World Health Organisation also recommends and encourages the use of chewing sticks for oral hygiene maintenance in areas where their use is customary as they are inexpensive, culturally acceptable, taste is not unpleasant, and have been reported to have antiplaque and many pharmacological properties<sup>5,6</sup>. Few clinical studies comparing mechanical plaque removal by the miswak sticks and the modern toothbrush report that miswak is as good as or more effective than tooth brushing in reducing plaque and gingivitis<sup>7</sup>.

Dental caries is one of the most common oral diseases and a major public health problem in many countries<sup>8,9</sup>. Fluoridated products like toothpastes, mouthwashes, rinses, gels, varnishes and flosses have contributed immensely towards prevention of dental caries but their use is limited among people because of their cost, side effects and compliance issues<sup>10,11</sup>. Perhaps,

these issues may be circumvented by fluoridating indigenous oral hygiene aids like miswak. In this regard, exploration of literature revealed very few studies which have tried to test the anticariogenic properties of fluoride impregnated indigenous aids. Hence, this study assessed and compared the in vivo antibacterial property of miswak sticks and 0.5% Sodium fluoride (NaF) impregnated miswak sticks against *streptococcus mutans* (*S.mutans*) and *lactobacillus*.

**Research Hypothesis (H1):**

“When used as chewing sticks the antibacterial efficacy of 0.5% NaF impregnated Miswak sticks on salivary *Streptococcus mutans* and salivary *Lactobacillus* is different when compared to plain Miswak sticks”.

**Null Hypothesis (H0):**

“When used as chewing sticks the antibacterial efficacy of 0.5% NaF impregnated Miswak sticks on salivary *Streptococcus mutans* and salivary *Lactobacillus* is not different when compared to plain Miswak sticks”.

**MATERIALS AND METHODS**

**Study design:**

Experimental study, Triple blinded, randomized controlled clinical trial, parallel group design.

**Study setting:** Clinical setting

**Sampling methodology:**

Convenience (Non-Probability) sampling technique was used.

**Sample size:**

The sample size of 20 subjects (Group A = 10 and Group B = 10) was selected based upon an earlier study by Almas K et al, where statistically significant difference was observed between the miswak group and the control group with ten individuals in each group.

**Selection of study subjects:**

Study subjects were internees of a dental college and residing in the common hostel who fulfilled the eligibility criteria. Each subject was instructed not to use any oral hygiene aids containing fluoride for one week before the test and not to eat or drink two hours before the collection of saliva sample except for water.

**Inclusion criteria:**

1. Systemically healthy subjects
2. Subjects who consent to participate in the study.

**Exclusion criteria:**

1. Subjects undergoing orthodontic treatment.
2. Subjects under antibiotic therapy for the past two months.
3. Subjects using antimicrobial mouthwash periodically.
4. Smokers and tobacco chewers

**Ethical considerations and Informed Consent:**

Ethical clearance was obtained from the Institutional Review Board to conduct the study and Informed consent was obtained from all the study subjects. Subjects were assured of their anonymity.

**METHOD OF DATA COLLECTION:**

**Collection and preparation of miswak sticks:**

One packet of fresh miswak sticks of ten centimeters length was purchased from the local market. From this packet ten sticks were randomly picked and were made sure that they resemble in their size, diameter and colour. Each miswak stick was cut into two equal parts. One part of each stick was used

as a plain miswak stick and the other part was impregnated with 0.5% sodium fluoride. After cutting, all the miswak sticks were coded by an independent investigator who was not related to this study. Sodium fluoride impregnated miswak sticks were coded as A (A1, A2...A10) while plain miswak sticks were coded as B (B1,B2...B10).

**Fluoride impregnation of Miswak sticks:**

To impregnate fluoride into miswak sticks, they were placed in a sterile bottle containing 500ml of 0.5%NaF solution for a day later Miswak sticks were removed after 24 hours from the bottle using forceps and placed on a filter paper for drying at room temperature.

**Allocation of Study Subjects:**

Concealed random allocation was done. Subjects were instructed to assemble in the morning between 9.00am to 9.30am at the clinic. The study subjects were randomly allocated into miswak stick group (Group B) and 0.5% sodium fluoride impregnated miswak stick group (Group A) using lottery method by an independent investigator.

**Blinding:**

- Primary investigator was unaware about the allocation details of the study subjects.
- Study subjects were unaware to which group they belonged to.
- The microbiologist who tested the coded saliva samples for their antibacterial property was blinded and the person who did statistical analysis was unaware
- about the study details.

**Baseline saliva sample collection:**

Two millilitres of unstimulated saliva was collected in a sterile plastic container from all the study subjects by the primary investigator who was blinded for the groups.

**Collection of saliva sample:**

The bark of the miswak was peeled off before the start of the study. The subjects in each group were asked to chew the stick and to move it around the dentition for six minutes.

**Test sample:**

After chewing the sticks for six minutes, the subjects were instructed not to swallow the saliva but to spit two millilitres of unstimulated saliva in the sterile container. The sterile containers were labeled and coded and the samples were sent for microbiological analysis.

**Microbiological analysis:**

The salivary samples were vortexed, for the uniform mix of the saliva and were transported to the media. The antimicrobial effect of *Miswak* and 0.5% sodium impregnated miswak were assessed by disc diffusion method. MitisSalivarius Bacitracin agar was inoculated with *Streptococcus mutans* at 37 degree Celsius for 48 hours and Rogosa SL agar plates were inoculated by *Lactobacilli* for 72 hours. Colony characteristics formed were studied and the number of colony forming units of *S.mutans* (CFU/ml) and *lactobacilli* (CFU/ml) of saliva were determined using a colony counter.

**Debriefing:**

Group allocation details and results of the study were revealed to the study participants after the trial results were out and analysed.

**Statistical considerations:**

Data obtained was analysed using SPSS software version 17. The significance level was fixed at 5%. Paired t-test was used to compare the mean differences at the baseline and after the intervention within each groups. Unpaired t-test was used for comparison of mean difference in *Streptococcus mutans* and *lactobacilli* counts between the groups (NaF impregnated miswak sticks group and plain miswak sticks group).

**RESULTS****Streptococcus mutans count:**

Significant reduction in the *S.mutans* count was observed after using fluoridated miswak stick compared to baseline count [p=0.006, t=3.565, table 1 (b)]. Although there was a difference in the mean of *S.mutans* counts (CFU/ml) between plain miswak group and fluoridated miswak group, the difference was not statistically significant [table 1 (d)].

**Lactobacillus count:**

Though a reduction in the *lactobacillus* counts was seen after using fluoridated and plain miswak sticks compared to baseline samples, the difference was not statistically significant [table 2 (a,b)].

**DISCUSSION**

The *in-vivo* antibacterial property of plain and 0.5% NaF impregnated miswak sticks against *S.mutans* and *Lactobacilli* was assessed in this study. The study assumes significance in the wake of usage of indigenous oral hygiene aids for oral hygiene maintenance. The main tenet of studying the antimicrobial property of miswak is that it is safe, easily available, culturally acceptable and affordable indigenous oral hygiene aid.

As it is an *in vivo* study, it was important to fix the sodium fluoride solution at a safer concentration. In the present study 0.5% sodium fluoride was used to impregnate miswak sticks as it was considered to be safe and effective by a study where different concentrations of sodium fluoride like 0.01%, 0.1%, 0.5%, 1% and 3% NaF were used to impregnate miswak sticks where fluoride clearance and retention in saliva after chewing with NaF sticks were assessed both *in vitro* and *in vivo*<sup>3</sup>.

The subjects selected for the present study were belonging to common hostel who were more or less on a similar institutionalized diet so as to control the influence of the diet on the study results to the extent possible.

There was significant reduction in *S.mutans* count after using fluoridated miswak sticks. This result is in line with that of a previous study where significant reduction in *S.mutans* counts was observed after using fluoridated miswak extract mouthrinse<sup>1</sup>.

There was no significant reduction in the *lactobacillus* counts after using miswak sticks. However contradictory results were observed in previous study where significant reduction in *lactobacilli* counts were observed after using miswak extracts<sup>4</sup>. In the present study, *Lactobacillus* and *S.mutans* strains were considered as they are most commonly implicated microorganisms in the causation of dental caries<sup>12</sup>. Mitis Salivarius Bacitracin agar was used for inoculation of

*S.mutans* while Rogosa SL agar plate was used for inoculation of lactobacilli as they are considered to be standard media to be used for respective strains<sup>13</sup>. The antimicrobial effect of miswak and 0.5% sodium fluoride impregnated miswak were assessed by disc diffusion method as this method is considered to be a standard method of assessing the antimicrobial property<sup>13</sup>.

The results of this study showed that there was no significant difference in *S.mutans* and *Lactobacilli* levels at baseline between plain miswak group and fluoridated miswak group. This allowed for valid post intervention comparability between both the groups. The study has some limitations. The sample size was small and a larger sample might have disclosed more valid results. Also, the influence of oral hygiene practices and dietary habits on the study results cannot be ignored.

Miswaks are commonly used in India, where there is high prevalence of dental caries. Therefore, NaF impregnated miswak may be useful in prevention of dental caries. Miswak stick may prove as an effective fluoride vehicle in those countries where they are commonly used. Further *in vitro* and *in vivo* clinical trials with large sample size should be carried out for evaluating the caries preventive effect of fluoridated miswak.

**CONCLUSIONS**

1. *In vivo* antimicrobial effect of miswak sticks (plain and fluoridated miswak sticks) against streptococcus mutans was more when compared to antimicrobial effect against lactobacilli.
2. *In vivo* antimicrobial effect of 0.5% sodium fluoride impregnated miswak sticks was appreciable against streptococcus mutans but less against lactobacilli.
3. *In vivo* antimicrobial effect of 0.5% sodium fluoride impregnated miswak sticks against streptococcus mutans and lactobacilli was more when compared to plain miswak sticks.

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**Table 1: ANTIMICROBIAL EFFECT OF PLAIN AND 0.5% SODIUM FLUORIDE IMPREGNATED MISWAK STICK AGAINST SALIVARY S.MUTANS LEVELS (CFU/ML×2000).**

	Mean S.mutans count (At Baseline) Mean ± SD	Mean S.mutans count (Post Intervention) Mean ± SD	Mean Difference
Plain Miswak Group (n=10)	103.9 ± 124.3	34.9 ± 6016	69.0+ (a)
NaF impregnated Miswak Group (n=10)	32.3 ± 14.22	14.6 ± 15.84	17.7* (b) (p=0.006)
Mean Difference	71.6+ (c)	20.3+ (d)	

\*Significant difference between the baseline and after chewing miswak stick within the NaF impregnated miswak stick group by using Paired t-test (p<0.05).

(+) Not significant

(SD) = Standard deviation

(a) – Mean difference in salivary S.mutans counts at baseline and after using plain miswak sticks.

(b) – Mean difference in salivary S.mutans count at baseline and after using NaF impregnated miswak sticks.

(c) – Mean difference in salivary S.mutans counts at baseline between plain miswak and NaF impregnated miswak groups.

(d) – Mean difference in salivary S.mutans counts post intervention between plain miswak and NaF impregnated Miswak sticks groups

**Table 2: ANTIMICROBIAL EFFECT OF PLAIN AND 0.5% SODIUM FLUORIDE IMPREGNATED MISWAK STICK AGAINST SALIVARY LACTOBACILLUS LEVELS (CFU/ML×2000)**

	Mean Lactobacillus count (At Baseline) Mean ± SD	Mean Lactobacillus count (Post Intervention) Mean ± SD	Mean Difference
Plain Miswak Group (n=10)	3.8 ± 11.0	8 ± 21.35	4.2+(a)
NaF impregnated Miswak Group (n=10)	2.8 ± 4.87	25.7 ± 45.5	22.9+(b)
Mean Difference	1+(c)	17.7+(d)	

(SD) = Standard deviation

(a) – Mean difference in salivary Lactobacilli counts at baseline and after using plain miswak sticks.

(b) – Mean difference in salivary Lactobacilli count at baseline and after using NaF impregnated miswak sticks.

(c) – Mean difference in salivary Lactobacilli counts at baseline between plain miswak and NaF impregnated miswak groups.

(d) – Mean difference in salivary Lactobacilli counts post intervention between plain miswak and NaF impregnated miswak sticks groups.

(+) – Not significant

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