



## The Effect of Miswak versus Chlorhexidin Gel on Subgingival Microbiota (Clinical and Microbiological Study)

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### KEYWORDS

Antimicrobial, CHX., Chronic  
Miwak, periodontitis, P.gingivalis,

### ABSTRACT

**Aim:** This study evaluates the clinical and microbiological effect of miswak and chlorhexidine gel on subgingival microbiota. **Subjects and Methods:** A full mouth randomized controlled clinical trial that carried out on 45 patients of both sex with mild to moderate chronic periodontitis. Selected patients were classified randomly into three equal group. After receiving the basic periodontal therapy group I used miswak 5 time a day (in accordance with the religious tradition), group II used CHX gel with tooth brush twice time daily, group III used tooth brush alone twice time daily. They evaluated clinically by Gingival Index, Plaque Index, Propping Depth and Attachment level at base line, after scalling and root blanning, one week, one month and finally after three month of using of each method, and microbiologically for detection and assessment of P.gingivalis at base line , after scalling and root blanning, one week, one month and finally after three month. **Resultes:** The present study exhibit that miswak more effective than tooth brush, as antimicrobial aginist p. gingivalis. There was no significant difference in the efficacy of miswak and CHX. **Conclusion:** Miswak more effective than tooth brush, as antimicrobial agonist.

### INTRODUCTION

Periodontitis is a biofilm-induced chronic inflammatory disease that leads to the destruction of the periodontium. Dental plaque is required but not sufficient to induce periodontitis, because the host inflammatory response to this microbial challenge that ultimately can cause destruction of the periodontium<sup>1</sup>. Diagnosis of chronic periodontitis is mostly based on an array of clinical measurements that include clinical attachment level, bleeding on probing, probing depth and radiographic findings<sup>2</sup>. plaque develops and matures over a period of several weeks, initially developing supragingivally with mainly aerobic bacteria. Over time, the flora changes from predominantly gram-positive to gram-negative, from facultative aerobes to strictly anaerobic species, with more motile forms present.

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Mature subgingival biofilm takes up to 12 weeks to develop<sup>3</sup>. *Porphyromonas gingivalis* is a Gram-negative, immobile, saccharolytic, obligate anaerobic rod, found mainly immersed in the subgingival microflora<sup>4</sup>. Because of its ability to produce a large quantity of virulence factors, it is considered to be a major pathogen in the onset and development of chronic periodontitis<sup>5</sup>. Personal oral hygiene maintenance is the key factor in the long-term preservation of periodontal health. Since chronic form of periodontitis is plaque oriented There are different methods available for maintenance of oral health; mainly mechanical and chemical. Tooth brush and dentifrices are widely used for cleaning the teeth, miswak has been used since ancient history<sup>6</sup>. The conventional meaning of miswak is stick used on tooth and gums to clean them<sup>1</sup>. The root of *Salvadora persica* contains a steam-distillable oil composed of 90% Benzyl isothiocyanate (BIT) and 10% benzyl nitrate. It has been proposed that; these chewing sticks have anti-plaque effects and postulated that they may also affect the pathogenesis of periodontal disease by reducing the virulence of periodontopathogenic bacteria<sup>7</sup>. as an antimicrobial agent, chlorhexidine is 2 effective in vitro against both Gram-positive and Gram-negative bacteria including aerobes and anaerobes and yeasts and fungi<sup>8</sup>, also chlorhexidine can reduce the adherence of *Porphyromonas gingivalis* to epithelial cells<sup>9</sup>. In this respect, it is very important to evaluate the efficacy of variable methods on the subgingival microbiota and oral hygiene improving.

## SUBJECTS AND METHODS

This study was designed as a full mouth randomized controlled clinical trial that carried out on 45 patients of both sex (24 females and 21 males ranged in age from 28-45 years) with mild to moderate chronic periodontitis. All patients were free from any systemic diseases, all patients showing probing pocket depth not more than

5mm and clinical attachment level (CAL) less than 5mm. Selected patients were classified randomly into three equal groups after receiving the basic periodontal therapy group I used miswak 5 times a day [in accordance with the religious tradition], group II used CHX gel with tooth brush twice a day, group III used tooth brush alone twice a day.

### Periodontal treatment:

For each group, thorough scaling and root planning was performed using hand instruments<sup>1</sup> Gracey curettes and scalers and ultrasonic device<sup>2</sup>.

### After scaling and root planing:

Group I instructed to use fresh miswak, its colour is light brown, 15 cm length, and 0.7-1 cm diameter, the functional end of the miswak is striped off followed by chewing to give brush like appearance that helps in cleaning the teeth and massaging the gums. Time of brushing 5 min<sup>10</sup>.

Group II instructed to use CHX gel (D.G care tooth paste)<sup>3</sup> twice daily and brush with Bass method technique, for 2 min. group III instructed to use tooth brush with soft bristles by using bass method for 2 min as recommended by ADA<sup>11</sup>.

**Periodontal Evaluation:** the periodontal conditions were evaluated for all patients at baseline, after scaling and root planing, one week, one month, 3 months after treatment using plaque Index (PI), gingival Index (GI), probing Pocket Depth (PPD), clinical Attachment Level (CAL) Probing depth and attachment Level measured using Williams graduated periodontal probe.

### Microbiological evaluation:

Gingival Crevicular Fluid (GCF) samples were obtained from the site which showed the highest probing depth (range 4-5 mm). selected teeth for isolated with cotton roll, and supragingival plaque was removed without touching the marginal



gingiva. Samples of GCF were obtained before probing into the site by using sterilized paper point<sup>4</sup>. The collected GCF was immediately transferred into an Eppendorf tube containing phosphate buffer saline and transported to the laboratory for DNA extraction. Samples were screened and quantitatively analyzed for *P. gingivalis* by quantitative Real time PCR using SYBR Green technology. GCF samples were collected at baseline, after scaling and root planing, one week, one month, and 3 months after treatment using.

1. Hu-Friedy, Chicago, Finland
2. EMS Mini Piezon Switzerland
3. Al Esraa Pharm. Optima
4. Paper point size# 30

### Statistical analysis:

The data was collected, tabulated, computed and statistically analyzed. Data were explored for normality using Kolmogorov-smirnov and Shapiro-wilk tests, and showed parametric (normal) distribution for all parameters. One-way ANOVA followed by Tukey post-hoc test was used to compare between more than two groups in non-related samples. Repeated measure ANOVA was used compare between more than two groups in related samples. Paired sample t-test was used to compare between two groups in related samples. The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

## RESULTS

A statistically significant difference between means of plaque index in all groups at the different intervals when compared to the baseline. A statistical significant difference between group III and each of

group I, group II, and there is no significant difference between group I and group II at 3 months interval. Table (1)

A statistically significant difference between means of gingival index in the three groups at the different intervals when compared to the baseline. a statistical significant difference in group III when compared with group I and group II, no statistically significant difference was found between group I and group II Table(1). There was a statistically significant difference between means of probing pocket depth measurement in each group at the different intervals when compared to the baseline. a statistical significant difference in group III when compared with group II and group I at 3 months, no statistical significant difference between group I and group II Table (1).

A statistically significant difference between attachment level Measurements in each group at the different intervals when compared to the baseline, a statistical significant difference in group III when compared with group II and group I and no statistical significant difference between group I and group II Table (1).

A statistically significant difference reduction was found between bacterial copy number in all groups at the different intervals compared to baseline a statistical significant difference in group III when compared with group II and group I and no statistical significant difference between group I and group II Table (1).

There was non-significant positive relationship between Microbiological results and plaque index, gingival index, and pocket depth but there was a significant positive relationship between Microbiological results and Attachment level Table (2).

**Table (1)** The p.vale of antibacterial effect, plaque index, gingival index, pocket depth, and attachment level groups

p.vale	Variables				
Attachment Level	Pocket depth	Gingival index	Plaque index	Antibacterial Effect	
Base	0.862ns	0.076ns	0.776ns	0.632ns	0.183ns
After scalling	0.920ns	0.076ns	*0.019	*0.001	0.192ns
* > 0.001	*0.018	*0.011	*>0.001	*0.010	After 1 week
After 1 month	* > 0.001	*0.010	*0.009	* >0.001	*0.003
* > 0.001	* > 0.001	*> 0.001	* >0.001	*0.001	After 3 Months

**Table (2)** Coreltion between Microbiological result with plaque index, gingival index, pocket depth

Microbiological result with	Variables			
Attachment Level	Gingival index	Pocket depth	Plaque index	
0.202	0.106	0.150	0.219	Correlation Coefficient
sign. > 0.05	0.056	0.319	0.157	0.092

## DISCUSSION

This study was deigned to evaluate the clinical and microbiological effect of miswak and chlorhexidine gel on subgingival microbiota. The time period for the present investigation was kept at 3 months. We depend on Real-time PCR to realize the antimicrobial effect of miswak and CHX as Real-PCR offers a sensitive, efficient, and reliable approach to quantitation. Using the Taq Man system able us to determine both the amount of *Porphyromonas gingivalis* and the total number of bacterial cells present in GCF samples as reported by Sharon R et al (2000)12.

The microbiological result of present study showed a statistically significant difference between tooth brush user group and Miswak user group, and

no statistically significant difference was found between Miswak user group and CHX user. These results in accordance to the findings by Alali et al (2004)13, they reported that *Salvadora persica* (Miswaak) stems exhibits potent antibacterial activity against both Gram-positive and Gram-negative bacteria. These results are in consistent with Almas et al (1995)14, and Sofrata H et al (2011)15 they revealed that miswak action is a concentration dependent, and showed that the miswak exhibited stronger antibacterial activity against the Gramnegative bacteria especially *P. gingivalis* than the Gram-positive bacteria evaluated, as evidenced by the pronounced differences in inhibition zones associated. yet the inhibition zones were less pronounced that of Chlorhexidine solution at all concentrations.

These studies have linked Benzyl isothiocyanate, a component of *Salvadora persica*, to rapid and strong bactericidal effects gramnegative periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* compared to the antibacterial effects of mouthwash with chlorhexidine on *Streptococcus mutans*16, 17, 18. The clinical result of present study which include (the gingival index, plaque index, pocket depth and attachment level) show A statistically significant difference was found in Miswak and CHX user group over the tooth brush user group where ( $p=0.001$ ).



These results are similar to the findings other studies among Ethiopian and Nigerian students and Saudi Arabian dental students, using Miswak and toothbrush was compared. These comparisons showed that Miswak was more effective than toothbrush in removing plaque<sup>19</sup>. Also, the present findings are in accordance to another double-blinded, randomized trial in 72 cases of moderate gingivitis showed that there was a significant reduction in the plaque index (PI), gingival index (GI), and bleeding index (BI) following the use of *Salvadora persica* extract chewing gum<sup>20</sup>. In addition **Al-Otaibi et al (2003)**<sup>21</sup> concluded that the miswak is more effective than tooth brushing for reducing plaque and gingivitis when preceded by professional instruction regarding its correct application. the use of Miswak appeared to be more effective than tooth brushing for removing the plaque from the embrasures; thus, enhancing interproximal oral health this in accordance with the present findings as there is a significant reduction in plaque and gingival index in miswak user group more than found in tooth brush user group. A study by **Malik et al (2014)**<sup>22</sup>. proposed that miswak has comparable or at times greater chemical and mechanical capability in plaque removal. as showed in this study there a significant reduction in plaque index in miswak user group. **Kaur et al (2004)**<sup>23</sup>. indicated that commercially available miswak chewing sticks, in addition to containing high amounts of calcium and chloride, may possibly release phosphate and thiocyanate into the saliva. These findings suggest that the commercially available miswak used as chewing sticks may have the potential to release substances into the saliva that could influence the state of oral health.

## CONCLUSIONS

Miswak has been proven effective as an oral hygiene aid and must be introduced to general population based on a scientific rules. Suggested that miswak must play an important role in periodontal disease prevention.

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## الأزهر مجلة أسبوط لطب الأسنان

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### الملخص العربي

#### الغرض من الدراسة:

أُجريت هذه الدراسة لتقييم التأثير الكلينيكي والميكروبيولوجي لالسواك والكلورهيكسيدين جل على البكتيريا تحت اللثوية.

#### الموضوعات والأساليب:

- اشتملت الدراسة خمسة وأربعون مريضاً تراوحت أعمارهم ما بين 29 إلى 45 عام تم تشخيصهم إكلينيكيًا علي أنهم مرضي التهاب الأنسجة حول السنه المزمن.
- تم أخذ عينات من السائل اللثوي لكل الحالات لمعرفة نسبة البروفايرومونس جينجيفالس الموجودة بها معملياً باستخدام تقنية تفاعل البلمرة المتسلسل و تم عمل كحت للجير و الجيب اللثوية باستخدام كواحت الجير واللثة اليدوية.
- تم تقسيم المرضى إلى ثلاث مجموعات.

المجموعة الأولى: وتتكون من مرضي تم علاجهم باستخدام طرق علاج اللثة التقليدية بالإضافة إلى استخدام المسواك خمس مرات يومياً.

المجموعة الثانية: وتتكون من مرضي تم علاجهم باستخدام طرق علاج اللثة التقليدية واستخدام الكلورهيكسيدين جل مرتين باليوم .

المجموعة الثالثة: وتتكون من مرضي تم علاجهم باستخدام طرق علاج اللثة التقليدية بالإضافة إلى استخدام فرشاة الأسنان فقط مرتين يومياً.

#### النتائج:

أظهرت نتائج هذه الدراسة ما يلي:

أولاً: وجود فروق إحصائية في قياس طبقة البلاك , دليل قياس إتهاب اللثة, قياس الجيوب اللثوية , ومستوى التصاق اللثة بعد العلاج في كل المجموعات مقارنة بالنتائج التي تم الحصول عليها قبل العلاج.

ثانياً: ووجود فروق إحصائية بين المجموعة الثالثة وكلا من المجموعة الأولى والثانية, ولا يوجد فروق إحصائية بين المجموعة الأولى والثانية.

دليل قياس التغيير في عدد النسخ البكتيرية

أولاً: وجود فروق إحصائية في دليل قياس التغيير في عدد النسخ البكتيرية في كل المجموعات خلال فترة الدراسة مقارنة قبل العلاج.

ثانياً: وجود فروق إحصائية بين أفراد المجموعة الثالثة وكلا من المجموعة الأولى والثانية ولا يوجد فروق إحصائية بين المجموعة الأولى والثانية بعد ثلاث اشهر من العلاج.