The Clinical Efficacy of A Probiotic Miswak Oral Spray in Patients with Gingivitis

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Abstract

Aim: to compare the use of Miswak probiotic spray against using each individually in gingivitis patients. **Methods and Results:** A total of 80 patients with chronic gingivitis were randomly divided into four groups. Group A received an oral spray bottle containing a mixture of Miswak and probiotic, group B received a Miswak spray bottle, group C a probiotic spray bottle and group D a placebo spray bottle. All Patients were instructed to administer 12 puffs of the spray, two puffs per sextant, one on the vestibular side and one on the oral side of the tooth surfaces, twice daily and then swallow. Plaque index (PI), gingival index (GI), and Stain index (SI) were assessed at baseline, after 3 days, one week and two weeks. The Group A showed the highest mean percentage of change in PI, GI and SI with the least change for group D. No intergroup statistical significant difference was found at all time intervals. **Conclusion:** Combining Miswak and probiotic was more efficient in reducing plaque and gingivitis then using each component individually, however this reduction was not statistically significant.

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Introduction

Maintaining oral hygiene is directly related to plaque control in the oral cavity. There are different methods of plaque control, where mechanical plaque removal is essential for plaque control and chemotherapeutics play a vital role as adjuncts to mechanical plaque-control methods.[1] Chemotherapeutics includes antibiotics, enzymes, bisguanides, metallic salts, etc. Althoughmany of these methods have been proved effective, their continuous application can cause harm and may fail in preventing further microbial colonization. [2]

In recent years the use of beneficial bacteria for therapeutic purposes has been strongly advocated. The concept of restoring and maintaining oral health has shifted from eliminating causative bacteria to alteration of the bacterial ecology by using probiotics.[3] Probiotics are living microorganisms which when administered in proper amounts provide beneficial effects for the host .[4]

The understanding and use of such microorganisms in different fields of healthcare has witnessed great development in the last years, and has encouraged their introduction in oral healthcare. Clinically, Probiotics have demonstrated their effectiveness in different oral diseases and conditions such as tooth decay, halitosis, and oral candidiasis. [5]

Lactobacilli is a probiotic that plays an important role in oral health maintenance by stimulating the natural immunity and interacting with the other members of the flora thus contributing to the balance of the microflora. [2]

Probiotics can be beneficial in these diseases by interfering in the growth and colonization of periodontal pathogens and the replacement of pathogenic microorganisms by beneficial bacteria.[6,7] Clinically research on probiotic therapy have focused on studying the effect of probiotic therapy on bacterial numbers, however few studies have used disease indicators to

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prove their efficacy in gingivitis or periodontitis.[8] A recent clinical trial advocated the use of herbal and probiotic mouthwashes as alternatives to chlorhexidine mouthwashes.[9]

On the other hand Miswak or Salvadora Persica extract has been shown to be effective against oral bacteria and plaque formation[10] and people in the Middle East and Africa have been using Miswak to maintain their oral hygiene for centuries,[11,12] where the most common sources of Miswak are the roots and branches of Arak (Salvadora persica).[13] In particular Miswak was shown to have strong antibacterial effect against microorganisms associated with periodontitis and caries such as on P.gingivalis, A. Actinomycetomcomitans, and H influenzae.[10]

The use of a probiotic in combination with plant extract is relatively a new field, and data regarding the effectiveness of this combination in oral diseases are scarce. Therefore, the aim of this study is to assess the effect of probiotic Miswak oral spray on clinical parameters in gingivitis patients.

Materials and methods

Study protocol

The study was designed as a double-blind, randomized controlled clinical study. 80 patients were recruited from the Periodontology, Diagnosis and Oral Medicine department, Faculty of Dentistry, Cairo University. Inclusion criteria were patients diagnosed with plaque induced gingivitis, with plaque index (PI) ≥ 2 according to modified Quigley and Hein index,[14] age range18-60 years, subject should at least 20 teeth with a minimum of five teeth per quadrant, and no pocket probing depth or attachment loss > 4 mm.

Exclusion criteria were smokers, pregnant and breastfeeding females, fixed orthodontic appliances, removable partial dentures, or use of antibiotics or anti-inflammatory drugs in the 6 months prior to the study. Furthermore, subjects were selected provided they had no medical or pharmacological history or parafunctional habits that could compromisethe conduct or outcome of the study.

Eligible subjects were informed regarding the nature of the study and signed a written consent before participating. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and the study protocol was approved by the Ethical Committee of the National Research Centre (Ethical Committee approval registration no: 16-468), Cairo, Egypt.

At baseline, all subjects received a thorough supragingival scaling and polishing to remove all plaque, stain, and calculus. Complete plaque removal was verified by asecond disclosing agent and any remaining plaque was removed. Finally, all subjects received a professional interdental cleaning using dental floss.

On day zero of the experiment (baseline); plaque index (PI) [14,15], gingival index, [16] and stain index (modification of Lobene index 1968) [17,18] were assessed. The previous parameters were recorded again after 3 days, one week and two weeks. A calibrated and trained examiner who was blinded to the treatment assignment recorded all clinical parameters. Before the study calibration of the examiner was done to ensure the measurements are reproducible. The examiner performed the clinical measurements of six gingivitis subjects that were not included in the study and intra-class correlation values of 0.89 was achieved by the examiner.

All spray bottles were coded and had similar color for subject blinding. Coded bottles were given to the study coordinator, all other personnel were blinded regarding allocation to the study test and control groups. Randomization was done using a computer- generated table and subjects were assigned into four groups; Group A: Miswak and probiotic spray, Group B: Miswak spray, Group C: probiotic spray and Group D a placebo oral spray.

All Patients were instructed to administer 12 puffs of the spray, (two puffs per sextant, one on the vestibular side and one on the oral side of the tooth surfaces), twice daily and then swallow. Rinsing after any of these procedures was not allowed for 30 minutes. [19]

Subjects were instructed not to use any other form of oral hygiene method for the whole experimental period, except for their assigned spray. To check for compliance, subjects were asked to mark the time at which they sprayed on a given calendar. At the end of the experimental period, subjects returned to their habitual oral hygiene procedures.

Preparation of the plant material

A commonly used chewing sticks from Miswak trees (Salvadora Persica) were collected from the local market. The fresh Miswak chewing sticks were cut into small pieces and allowed to dry at room temperature for a couple of days, after that, it was ground to powder. 10 g of powder was added to 100 ml of sterile deionized water in a screw capped bottle and soaked for 48hrs at 4°C, then centrifuged at 2000 rpm for 15 minutes. The supernatant was passed through a filter paper (0.45 µm pore size) and the Miswak extract was stored at 4°C.

Bacterial strain and media preparation

The probiotic strain; Lactobacillus rhamnosus was isolated and serologically identified by Probiotics Lab., National Research Centre., Egypt. The selected Probiotic strain was cultivated in De Man, Rogosa and Sharpe broth (Fluka and catalogue no.69966 MRS broth, Sigma-Aldrich) for 48 hours at 37°C inCo2. The cultured bacteria were centrifuged at 5000 rpm for 20 minutes to obtain pure cells (pellet). The total live cell numbers per one gram of pellet were calculated using the followingformula: Live cells (CFUs/g) = number of colonies in the agar plate x dilution factor. [20] In the present study one gram of the previously prepared pellet was found to be contained 1 $x10^8$ cells.

Oral spray formulations

Four oral spray bottles were prepared. For the probiotic spray: one drop of mint was added to 10 ml of sterile distal water then the bottle was inoculated with 5gm of L. rhamnosus (1 $x10^8$ cfu/ml) For the Miswak spray:one drop of mint was added to 10ml of Miswak extract and for the Miswak probiotic spray: one drop of mint was added to 10ml of Miswak extract then the bottle was inoculated with 5gm of L. rhamnosus (1 $x10^8$ cfu/ml). The placebo control bottle was prepared by adding one drop of mint to 10 ml of sterile distilled water.

Statistical analysis:

The codes on the bottles were broken before sending the data to the statistician in order to group the patients.Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics Version 24 for Windows. Data were presented as means, standard deviation (SD).

$$\% \text{ of change} = \frac{Time\ (base\ line) - Time1 * 100}{Time\ (Base\ line)}$$

The Minus values indicates a decrease in the value compared to baseline; positive values indicate increase in the relevant values compared to Baseline values. All tested parameters showed non-parametric distribution; so Kruskal Wallis tests were used to compare between the tested groups. Wilcoxon singed rank test used for comparison between follow up-periods for each group.

Results

Plaque index (PI):

The mean and Standard deviation (SD) values of percentage of change plaque index (PI) are presented in Table (1) and figure (1).

Difference between tested groups:

Group A (-54.26±10.47 %) showed the highest mean % of change in PI followed by Group B (-51.37±10.06 %) followed by Group C (-47.84±8.3 %) with the least change for Group D (-42.63±10.47 %) with an insignificant difference between tested groups at p=0.442 for 3 days.

Group A (-24.2±10.41 %) showed the highest mean % of change in PI followed by Group C (-24.02±10.41 %) followed by Group B (-22.74±3.06 %) with the least change for Group D (-22.63±3.19 %) with an insignificant difference between tested groups at *P*=0.865 for 1 Week.

Group A (-19.71±9.07 %) showed the highest mean % of change in PI followed by Group B (-16.49±11.51 %) followed by Group C (-11.92±3.39 %) with the least change for Group D (-11.2±8.17 %) with an insignificant difference between tested groups at *P*=0.244 for 2 Weeks.

Gingival index (GI):

The mean and Standard deviation (SD) values of percentage of change Gingival index (GI) are presented in Table (2) and figure (2).

Difference between tested groups:

Group A (-39.81±9.85 %) showed the highest mean % of change in GI followed by Group B (-39.04±8.43 %) followed by Group C (-30.86±8.7 %) with the least change for Group D (-28.35±13.62 %) with an insignificant difference between tested groups at *P*=0.176 for 3 days. Group A (-22.65±3.21 %) showed the highest mean % of change in GI followed by Group C (-18.75±3.93 %) followed by Group B (-20.78±5.81 %) with the least change for Group D (-18.04±1.53 %) with an insignificant difference between tested groups at *P*=0.226 for 1 Week.

Group A (-15.94±3.5 %) showed the highest mean % of change in GI followed by Group B (-11.35±5.02 %) followed by Group C (-7.77±3.35 %) with the least change for Group D (-7.65±2.1 %) with an insignificant difference between tested groups at p=0.08 for 2 Weeks.

Assessment of mean percentage of change in staining index for Difference between groups:

The mean and Standard deviation (SD) values of percentage of change staining index (SI) are presented in table (3) and figure (3).

Group A (-74.85±6.75 %) showed the highest mean % of change in SI followed by Group B (-69.44±7.58 %) followed by Group C (-63.09±9.63 %) with the least change for Group D (-63.17±8.99 %) with an insignificant difference between tested groups at p=0.140 for 3 days.

Group A (59.12±11.11 %) showed the highest mean % of change in SI followed by Group B (49.87±15.01 %) followed by Group C (48.88±12.64 %) with the least change for Group D (47.53±11.68 %) with an insignificant difference between tested groups at p=0.881 for 1 Week.

Group A (55.96±15.95 %) showed the highest mean % of change in SI followed by Group B (52.76±13.41 %) followed by Group C (48.61±14.11 %) with the least change for Group D (42.01±13.01 %) with an insignificant difference between tested groups at p=0.341 for 2 Weeks.

		Groups								*P*-value*
		Group A		Group B		Group C		Group D		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
% of change in PI	3 Days	-54.26[a]	10.47	-51.37[a]	10.06	-47.84[a]	8.3	-42.63[a]	11.18	0.442 NS**
	1 Week	-24.02[b]	10.41	-24.1[b]	7.32	-25.63[b]	3.19	-25.74[ab]	3.06	0.865 NS**
	2 Weeks	-11.2[b]	8.17	-11.92[c]	3.39	-16.49[b]	11.51	-19.71[b]	9.07	0.244 NS**
P-value*		≤0.001**		≤0.001**		≤0.001**		≤0.001**		

Table 1. Mean and standard deviation (SD) values of percentage of change plaque index (PI).
* Kruskal Wallis test to compare groups. Statistically significant difference $P < .05$.
**Means with same letter within each column indicates significant difference. NS= Non-Significant

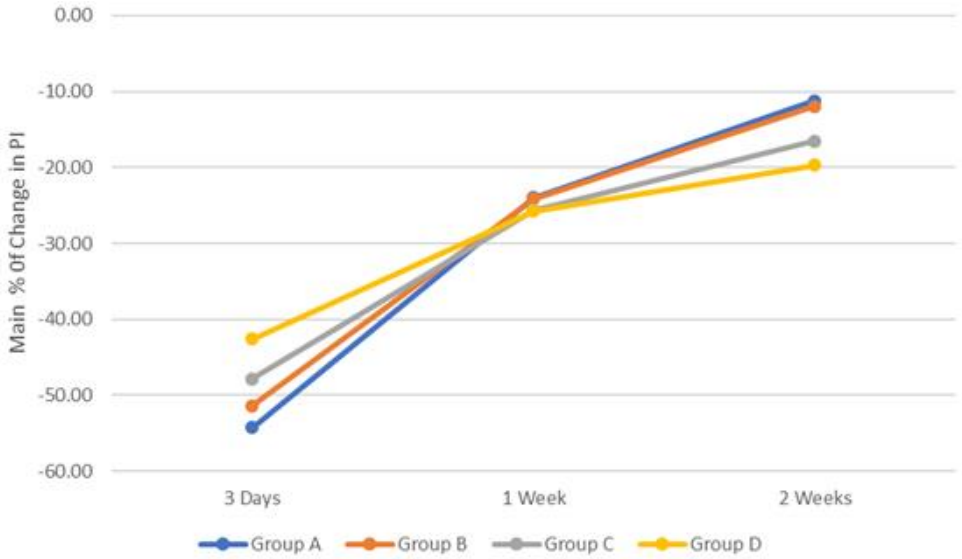

Figure 1. Histogram showing the mean percentage of change plaque index (PI) showing the increase in mean values for each group with increase in time.

		Groups								*P*-value*
		Group A		Group B		Group C		Group D		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
% of change in GI	3 Days	-39.81[a]	9.85	-39.04[a]	8.43	-30.86[a]	8.7	-28.35[a]	13.62	0.176 NS**
	1 Week	-18.04[b]	1.53	-18.75[b]	3.93	-20.78[b]	5.81	-22.65[a]	3.21	0.226 NS**
	2 Weeks	-15.94[b]	3.5	-11.35[b]	5.02	-7.77[c]	3.35	-7.65[b]	2.1	0.08 NS**
P-value*		≤0.001**		≤0.001**		≤0.001**		≤0.001**		

Table 2. Mean and standard deviation (SD) values of percentage of change Gingival index (GI).
* Kruskal Wallis test to compare groups. Statistically significant difference $P < .05$.
**Means with same letter within each column indicates significant difference. NS= Non-Significant

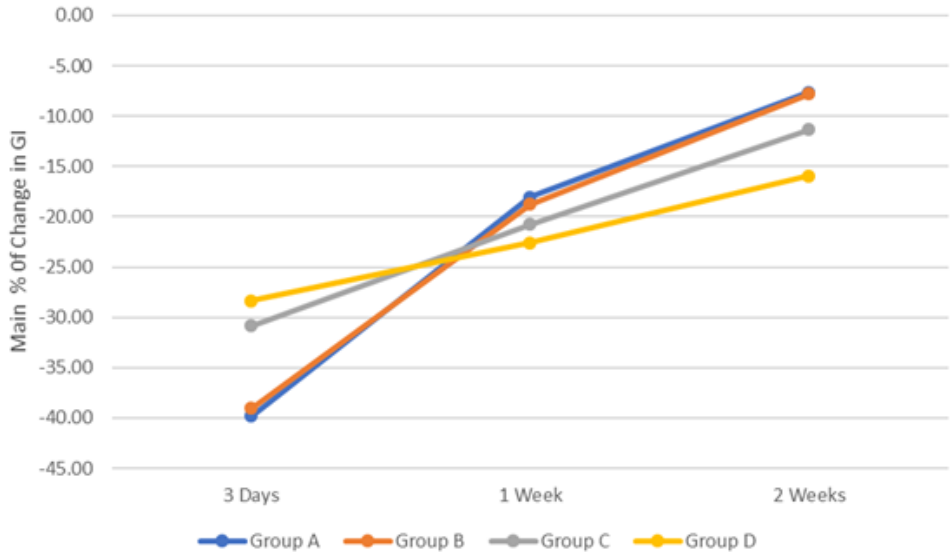

Figure 2. Histogram showing the mean percentage of change Gingival index (GI) showing the increase in mean values for each group with increase in time.

		Groups								*P*-value*
		Group A		Group B		Group C		Group D		
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
% of change in staining index	3 Days	-74.9[a]	6.75	-69.4[a]	7.58	-63.1[a]	9.63	-63.2[a]	8.99	0.140 NS**
	1 Week	47.5[b]	11.68	49.9[b]	15.01	48.9[b]	12.64	59.1[b]	11.11	0.881 NS**
	2 Weeks	42[b]	13.07	48.6[b]	14.11	52.8[b]	13.41	56[b]	15.95	0.341 NS**
P-value*		≤0.001**		≤0.001**		≤0.001**		≤0.001**		

Table 3. Mean and standard deviation (SD) values of percentage of change in staining index.
* Kruskal Wallis test to compare groups. Statistically significant difference $P < .05$.
**Means with same letter within each column indicates significant difference. NS= Non-Significant

Discussion

The onset of dental disease can be prevented by regular control of plaque formation, that is why plaque control should be an important chore of every person. Previous studies have demonstrated that tooth brushing alone does not adequately remove plaque, and therefore the use of antimicrobial mouth rinses as an adjunctive aid in plaque control has been advocated.[9] Natural herbs when used as mouthwashes have shown significant advantage over chemical ones.[21] On the other hand the use of oral sprays is thought to overcome some shortcomings of mouthwashes and oral ointments as they don't need to be spit out and do not require finger insertion for their application.[22] The present study was designed to correlate the efficacy of three types of oral spray in reducing plaque accumulation and gingivitis, namely a mixture of probiotic and Miswak (group A), Miswak (group B) and probiotic (group C). To the authors' knowledge no studies have compared these three types of oral spray so results of the present study cannot be compared to directly to previous studies and is originally focused on the present results. A meaningful power analysis was difficult to perform before beginning the study because no randomized control trials were previously conducted on those products and hence we considered our work to be pilot in nature.

Miswak, prepared from the roots, twigs and stems of Salvadora persicais used in many developing countries as a traditional mean for oral hygiene and was also recommended by the World Health Organization (WHO) as an effective tool for oral hygiene. On the other hand, probiotic bacteria were suggested to play a role in the maintenance of oral health, and confers benefits to the host when administered in adequate amounts (FAO/WHO 2001).[23]

Oral prophylaxis was done for all subjects to have a homogenous baseline data between the compared groups.[9]

All three oral sprays showed reduction in plaque, gingival and stain indices in comparison to control group, although group C (probiotic) displayed the lowest mean percentage of change of all indices at all time intervals with insignificant difference between tested groups.

The antibacterial property of Miswak might have caused the reduction in plaque and improvement in gingival health in subjects that used the Miswak spray (group B). Present results are in agreement with previous studies that have reported that the use of Miswak inhibit the formation of dental plaque chemically and exert antimicrobial effect against many oral bacteria.[24] Moreover, *in vitro* studies have demonstrated that aqueous extracts of Miswak have growth-inhibitory effects on several micro-organisms.[25] This property has been attributed to the release of tannins and thiocyanate that disrupt early colonization of bacteria. More over thiocyanate by activation the peroxidase/thiocyanate system potentiate the antimicrobial effect.[26]

Probiotic therapy was found to positively affect indicators of gingival inflammation as they can enhance the commensal flora preventing the microbial shift and pathogens colonization when administrated in adequate amounts.[27,28] The ability to adhere to oral tissue as well as to co-aggregate is a very important criterion of probiotic, that might explain their superior results.[28] Although not statistically significant, the Miswak probiotic strain containing oral spray

(Group A) resulted in lower mean change in plaque, gingival and stain index when compared to groups B and C. This may be explained by the fact that this combination could have augmented the antimicrobial activity of each compound in the mixture. This explanation was drawn from previously related research which showed that a mixture of propolis extract, miswak extract and probiotic Lactobacillus rhamnosus demonstrated better antibacterial activity then each used individually.[20] The addition of lactobacillus to Miswak extract has also shown to augment its anti-cariogenic property. [30]

As for the insignificant difference between the groups that used the oral sprays and placebo, this is possibly caused by the Hawthorne effect, where participants tend to have a better oral hygiene control when being supervised irrespective of the treatment modality.[31]

Regarding the mean percentage of change in staining index, SalvadoraPersica or Miswak has shown to have Silica and Sodium bicarbonate which have an abrasive effect and can remove stains from teeth.[32] The effect of probiotic on stain removal is not known but secondary metabolites might that might have been released from the probiotic could have aided in stain removal.

Conclusions

The combination of Miswak and Probiotic resulted better reduction in plaque, gingival and staining indices using each individually, with no statistically significant differences amongst all groups. Additional studies with larger sample size are needed to elucidate the potential of Miswak and probiotic in preventing and/or treating gingivitis.

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Declaration of interest

The authors declared that no conflicts of interest.

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