

Comparative Clinical Effects of *Salvadora Persica* Oral Rinse and A Phenolic Commercial Mouth Wash on Human Oral Health; An In vivo Randomized Trial



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OBJECTIVE: To compare the clinical effects of *Salvadora persica* oral rinse and commercial Phenolic mouth wash on oral health status of socially deprived madrasa girls after six months of a triple blind randomized clinical trial.

METHODOLOGY: Girls aged 18-22 years living permanently in a madrasa of Multan city were recruited. After determining the sample size and trial duration participants were randomized into group A and Group B and were provided with freshly *Salvadora persica* oral rinse and commercial Phenolic mouth washes respectively. Pre, mid and post-interventional examinations were executed by a single, blind and calibrated examiner using Turesky Quigley Hein Plaque and Loe and Silness Gingival indices. Statistical analysis was carried out by descriptive statistics, two sample independent t-tests and ANOVA. The p-value of <0.05 was considered significant at 95 % confidence level and 80% power.

RESULTS: Sixty subjects were enrolled for the present trial. The mean age of the participants was found out to be 21.5±0.76 years. No statistically significant difference was identified between the mean Plaque and Gingival scores of the two interventional groups at any of the examination phase.

CONCLUSION: *Salvadora persica* oral rinse is suggested to be equally effective as the commercial Phenolic mouth wash for the control of plaque deposition and prevention of gingival inflammation.

KEYWORDS: Dental plaque, Gingivitis, Miswak, Mouthwash

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INTRODUCTION

Dental plaque, particularly its supra-gingival deposits has an established etiology for different periopathies.¹ Increasing global trends of oral

diseases has now imposed the use of both the mechanical and chemical plaque controlling therapies.² The mechanical oral cleansing practices involve toothbrushes with dentifrices, however, chewing sticks or miswak has now proven its significant competence against effective plaque removal and gingival health.³⁻⁸

Miswak is now in its recompensing phase. Laboratory examinations have established that many favorable natural ingredients are present in almost all types of miswak sticks which provide both systemic and local oral curative effects.

It has been reviewed that miswak particularly contains ascorbic and tannic acids which have verified effectiveness in healing inflamed and bleeding gums. Trimethylamine generate stimulatory effect on gingiva and Fluoride (1.0ug/ml) have a recognized dental hard tissue

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remineralization function.⁹⁻¹² Literature reports the anti-
periopathic and anti- carious property of miswak by reducing
plaque formation;¹³⁻¹⁴ also it has an established antimicrobial
effect against Streptococcus Mutans and Fecalis which are
the most vulnerable microorganisms of oral cavity.^{12&15}

Moreover, different local chemotherapeutic agents are
also used as an adjunct to mechanical cleansing
predominantly where gingival inflammation is more
prevalent.¹ The widely known and recommended oral rinse
includes Phenolic containing salicylic acid and Chlorhexidine
gluconate mouth washes, which have evidently reported
their anti-bacterial and anti-plaque efficacy.^{1,16-17}

In the current study Listerine mouth rinse brand was
chosen because of its affordability and easy on counter
availability in Pakistan. Listerine mouth rinse enclose a
blend of phenolic compounds which is reported to have
high bactericidal properties and has great ability to restrict
the inflammatory diseased processes of oral soft tissues. A
nine month clinical study also demonstrated that Phenolic
mouth wash has a dramatic effect on plaque toxic activity.¹⁷

Keeping in mind the limited awareness and affordability
of people in developing countries to use Phenolic and similar
products which are prescribed frequently as treatment for
highly prevalent periopathies, importance should be given
to the comparison of these products to natural and cheaper
alternatives whose affordability and availability are never
argued. Also, to date, the reported in-vivo data on such
comparison is really sparse. Hence, the testing null hypothesis
of this study was "no difference will be observed in the oral
health status of subjects using *Salvadora persica* oral rinse
or Phenolic mouth wash". The objective of this experimental
study was to compare the clinical effects of *Salvadora persica*
oral rinse and commercial Phenolic mouth wash on oral
health status of socially deprived madrasa girls after
six months of a triple blind randomized clinical trial.

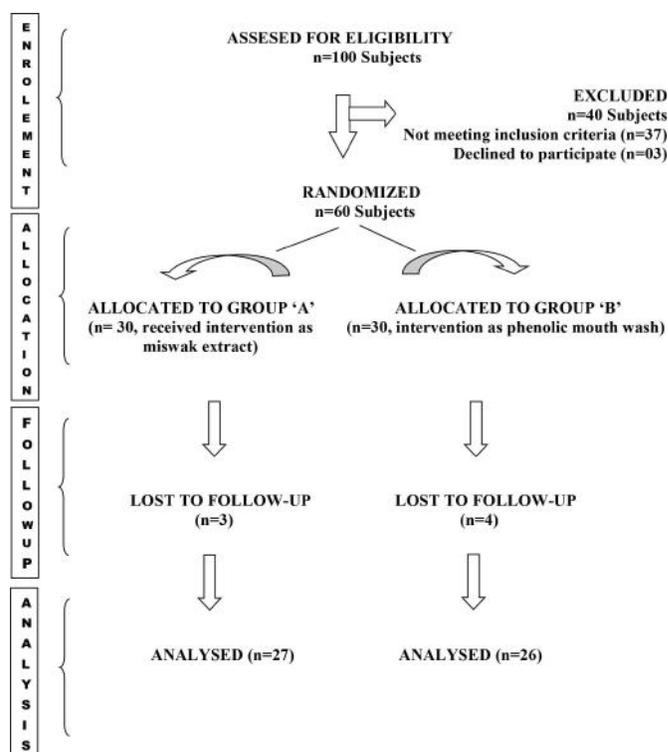
METHODOLOGY

Approval to conduct this trial was attained from the official
Institutional Review Board of Multan Medical & Dental
College, Multan (IRB/MDC/005-24-07-2019). This triple
blind randomized controlled trial over a span of six months
(August 2019 - January 2020) was conducted in the Madrasa
for girls, Multan city. Present trial is based upon the guidelines
of CONSORT (Consolidated Standards of Reporting Trials)
and has strickly followed the checklist provided by the
CONSORT.¹⁸ Also, "The ethical standards of World Medical
Association for human experimentation 2008 version of the
Helsinki Declaration" has been followed in the current
study.¹⁹ The sample size and trial duration were considered
using "The American Dental Association, Acceptance

Program Guidelines for Chemotherapeutic Products for
Control of Gingivitis, 2009".²⁰⁻²²

Written permission was obtained from the madrasa
administration and verbal consent after notifying about the
intention and purpose of the study was attained from all the
girls present in the madrasa. Participants/girls with any
medical or dental disease, dental prosthesis, those taken
antibiotics in last 6 months, those received any periodontal
therapy in last 3 months were excluded, while, participants
with at least 10 sites of chronic mild gingivitis with bleeding
on probing but not having periodontal pockets of greater
than 3mm were included in the trial. (Figure 1)

Figure 1: Flow Diagram of the Phases of the
Randomized Trial, According to the Consolidated Guidelines
for Reporting Trials (CONSORT)



PRE-TRIAL PHASE

Earlier the commencement of this trial, a pre-trial workshop
was led where the participants of the two groups were given
certain instructions to standardize each activity of the trial.
This was done by inculcating all subjects to uniformly use
similar quantity (15 ml) of the given mouthwash for one
minute twice daily (after breakfast and before going to bed).
They were instructed to store the given bottles in refrigerators
having temperature around 40C. They were also advised to
brush their teeth with the same brushing technique to control
the plaque deposition during the trial period. For this the

most appropriate and recommended brushing technique (Bass method) was demonstrated individually to every participant on tripodents. Participants were also shown the related video of Bass method on multimedia. Later the participants were asked to reproduce the taught brushing technique on the own teeth in front of a mirror and in presence of the same single examiner/instructor. All the participants were asked to apply toothpaste on full length of toothbrush and brush teeth two times a day (after breakfast and before going to bed) for 2 minutes. All these standards were strictly reinforced through regular telephonic calls. Feed backs were also obtained from the administration and supervisors of the Madrassa.

TRIAL PHASE

For this trial the participants were the cohort of young girls between age 18-22 years living permanently in the same madrasa. A total of 100 girls were enrolled in the madrasa and all of them were assessed for eligibility. However, 40 girls did not meet the inclusion criteria and were therefore excluded from the trail. Ultimately, 60 participants were included for the present trial. These 60 girls were then randomly allocated to two interventional groups (n=30 in Group A and n=30 in Group B) by means of Simple Random Sampling using random number table.

The principal investigator (AM) who was not going to be concealed or blind to the group assignment, enrolled and randomized the participants. Particulars of the participants who were randomized was sealed off in sequentially numbered, opaque, sealed envelopes (SNOSE). The participants, examiners and the data analysts (outcome assessor) were blind to the intervention allocation (Figure 1). Each participant of Group A and B was weekly provided with a coloured coded bottle. The rinses in all the bottles had same color so that none of the participant may not recognize the type of mouthwash and remain blinded to the intervention. Each coded bottle contained 500 ml of mouth wash. Freshly prepared *Salvadora Persica* oral rinse was then given to Group A, while Group B was provided with Commercial Phenolic mouthwash.

EXAMINATION PHASE

Dental examination was later conducted at baseline, then after 3 and 6 months, that is, Pre-Interventional, Mid Intervention and Post-Interventional phases respectively. It was executed on inclined chairs in day light using a sterilized dental mirror and CPITN (Community Periodontal Index of Treatment Need) probe. This probe has a ball ended tip with a diameter of 0.5mm. This probe have two coloured bands

of 2mm graduations each, one at 3.5 to 5.5mm and second at 8.5 to 11.5mm. These bands determine the bleeding on probing which correspond to respective gingival score as per Loe and Silness Gingival index (1962).^{25,26} This index which was used as a tool for examination which is universally accepted to determine the oral gingival status. Dental plaque was identified and measured using commercially available plaque disclosing lozenges. These lozenges were bio-compatible and had Iodine as basic chemical compound. The participants were asked to chews this lozenge, swishes it all over the teeth for 30-60 seconds and then rinse it completely. The coloured plaque was then scored according to Turesky Quigley Hein Plaque index (1962).²⁴

All these dental examinations were executed by a single, blind, trained and calibrated examiner. The examiner was trained in the Out Patient Department of Multan Dental College by the Professor of Periodontology by undergoing multiple exercising of using the trial diagnostics, indices and instruments on selected patients. These selected patients were the cohorts having both normal and inflamed gingiva. After multiple exercises, the examiner was calibrated for the two indices to be measured. Later, the intra-examiner reliability of the single examiner was achieved on 10% of total trial sample in the madrasa campus. These 10% of the trial participants were evaluated just to obtain the examiner's reliability and were not included in the final trial. The mean Kappa value of intra-examiner reliability determined on these 10% of the participants was identified as 0.95.

LABORATORY PHASE

The *Salvadora Persica* oral rinse was prepared in the Environmental Research Department of Bahauddin Zikria University, Multan city. One senior person nominated by this department was contacted and provided with all the necessary documents of the current study including the referenced methodology (Standard Operating Procedures) to be strictly followed. Samples of fresh vacuum packed *Salvadora Persica* (Peelu miswak) sticks were collected from local market. These sticks were then being cut into small pieces and allowed to dry at room temperature for two days. Then these were ground to powder. Successive 10 g quantity was put into a sterile screw capped bottle to which 100 ml of sterile deionized distilled water was added. The extract was allowed to soak for 48 hours at 4°C and then centrifuged at 2000 rpm for 15 minutes. The supernatant was passed through filter paper (0.45 µm pore size) and the extract was prepared at 50% concentration.²³ This procedure was performed several times by the same person each time during the study period of 6 months. The miswak extract was suggested by the laboratory to be consumed within one

week, the provision was made on weekly basis for 6 months.

STATISTICAL ANALYSIS

Data collected was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) Version 21 software and has been described in terms of Mean and standard deviations. As the data was normally distributed, two sample independent t-test was employed to see the difference of the mean plaque scores as well as the gingival scores of the two groups at baseline, 3 months and 6 months of the examination. Considering the comparisons at three intervals Analysis of Variance (ANOVA) test was also conducted. Intra-examiner reliability was calculated using Cohen's Kappa. The p-value of <0.05 was considered as statistically significant at the 95% confidence level as the power level was kept 80%.

RESULTS

Figure 1 demonstrates the total number of participants assessed for eligibility (n=100) and those who were excluded (n=40). Therefore, 60 subjects were enrolled for the present trial, 30 in each of the two interventional group. The follow-up attrition was 12% as 7 subjects lost to follow-up during the total trial period of 6 months.

The mean age of the participants was found to be 21.5 ± 0.76 years. Comparative mean plaque scores and mean gingival scores for both the interventional groups at pre-, mid- and post-examination phases are demonstrated in Table 1. The same table illustrates that no statistically significant difference was identified between the mean plaque and gingival scores of two interventional groups at any of the examination phase.

Table 1: Comparative Mean Plaque and Gingival Scores of Two Groups at Pre, Mid and Post Examination Phases

Examination Phases	Intervention Groups	Plaque Index Scores		Gingival Index Scores	
		Mean±SD	p-value*	Mean±SD	p-value*
Pre- Interventional	Group A	3.41 ± 0.62	0.240	2.40 ± 0.33	0.556
	Group B	3.09 ± 0.57		2.72 ± 0.40	
Mid- Interventional	Group A	1.04 ± 0.59	0.681	2.03 ± 0.23	0.228
	Group B	1.59 ± 0.45		1.56 ± 0.33	
Post- Interventional	Group A	0.69 ± 0.53	0.974	0.41 ± 0.32	0.866
	Group B	0.51 ± 0.39		0.45 ± 0.29	

*p-value = Level of significance of change in mean plaque and gingival scores obtained employing independent t-test.

Table 2 describes the findings of ANOVA which suggest that no statistical difference was found between the the two

interventional groups when their plaque and gingival scores were compared at three different examination phases.

Table 2: Analysis of Variance between the Two Groups at Pre, Mid and Post Examination Phases

Indices	Intervention Groups	Examination Phases	F*	p-value
Plaque Index	Group A	Pre-Interventional	1.582	0.215
		Mid-Interventional		
	Group B	Post-Interventional		
Gingival Index	Group A	Pre-Interventional	0.154	0.696
		Mid-Interventional		
	Group B	Post-Interventional		

*F = variance between mean plaque and gingival scores obtained employing ANOVA test.

DISCUSSION

This experimental study was conducted to evaluate the comparative effectiveness of the *Salvadora persica* oral rinse and commercial Phenolic mouth wash on oral health status of selected socially deprived population and has identified no difference in the clinical efficacies of the two interventions in the given trial duration of 6 months.

The present trial has followed "the American Dental Association (ADA) guidelines to conduct clinical studies for the assessment of chemotherapeutic agents". ADA suggests that the clinical advantage of plaque control may be demonstrated by a significant reduction in gingivitis. It also explains that products meant to achieve their anti-gingivitis effectiveness through plaque reduction should determine significant reductions in both plaque and gingival scores at the end of the study.²⁰⁻²² This trial has accomplished all these given commendations when mean plaque and gingival scores were compared from baseline to 6 months.

Phenolic mouth wash was chosen for this trial as other commercial mouthwashes like chlorhexidine formulations, which has more proven anti-gingivitis efficacy is only recommended for short periods.^{27,28} However, phenolic antiseptic rinses can be used for long terms. They destroys and constrains the growth of both gram-positive and gram-negative colonies of bacteria. Phenolic group of mouth washes are also reported to have efficient antimicrobial activity which not only abolishes bacterial cell surface and prevents the growth of oral bacterial biofilm but also increases the time of bacterial regrowth.¹⁷ *Salvadora persica* miswak was selected in this trial to study its comparative antimicrobial activity with phenolic compounds because of its easy availability and affordability in Pakistan. Also,

Salvadora persica miswak has an established oral therapeutic properties, acceptable taste and odor and significant anti-plaque efficacy.^{3-8,29&30}

The results of the present study are in line with the study of Patel who has reported beneficial anti-gingivitis effect of miswak juice.³¹ This study is also in accordance with the study of Khalid Almas about the effects of Chlorhexidine and miswak extract on healthy and periodontally involved human dentin and concluded that Chlorhexidine 0.2% and miswak extract 50% had a similar effect on dentin specimens.³² Another double-blind clinical trial by Khalessi had studied the comparative antimicrobial activity of Persica and Chlorhexidine against certain pathogens in periodontal tissues such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans in vitro. The study reported superior antimicrobial activity of Persica which is in accordance with our study.³³

Another study of Babol studied the effect of chewing gum containing Persica extract on periodontal health in a double-masked, randomized trial and reported significant reduction of gingival and bleeding scores of subjects using chewing gums having miswak extract compared with other placebo groups.³⁴ This is significant to be reported to support the current study as it explains the healthy outcomes of miswak extracts on oral tissues and therefore validate the effect of miswak extract.

Miswak use may also be promoted in stick form as the previously published literature supports both the mechanical and chemical efficacy of miswak.^{31,32,34} However, mechanical cleansing efficacy of miswak has reported high plaque and gingival scores in few of the subjects using miswak stick when matched with those using toothbrushes. In this context, few investigations have likewise discovered that those subjects who were using chewing sticks and were found to have poor oral hygiene scores, was probably a consequence of improper cleansing techniques in using chewing sticks by the subjects in that particular study.³⁵ Therefore, it may be suggested that if miswak stick is used with appropriate technique, it can efficiently provide anti-plaque and anti-gingivitis effects both mechanically and chemically. Currently, several over the counter mouthwashes are available in Pakistan, and none of them contain miswak extract which has an established therapeutic remedy for the control of plaque and gingivitis.³⁻⁸ This study strongly recommends the incorporation of miswak extract in local mouth washes not only because of its natural pharmaceutical oral health benefits, high fluoride content but for being a cheaper product for the population of any developing country having financial constraints and limited oral health care amenities. However, more controlled trials are needed to establish miswak as a therapeutic medicament both mechanically

and chemically for the prevention and control of periodontal diseases.

CONCLUSION

This trial has revealed no statistically significant difference in the oral health status of the two comparative groups, suggesting that Salvadora persica (miswak) oral rinse may be equally effective as the commercial Phenolic mouth wash for controlling dental plaque and preventing gingival inflammation.

FUTURE RECOMMENDATIONS

Some of the confounding variables responsible for the development of plaque and gingivitis such as few systemic undiagnosed diseases, disturbed hormones, genetics, drugs, malnutrition etc. which may influence the signs and symptoms of the disease must also be evaluated in future studies. Also some sort of objective assessment of the effectiveness of brushing amongst the study participants in all groups may be included in the future trials. Using a third group of true controls (not using any mouth wash but only brushing in the recommended way) in future will also increase the credibility of the study.

CONFLICT OF INTEREST

No conflict of interest was identified among the authors that may profit or loss in the publication of this paper.

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