

Assessment of the Efficacy of Indigenously Prepared *Azadirachta Indica* Mouthwash on *P. Gingivalis* in Chronic Periodontitis Patients: A Microbiological Study

Research Article

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Abstract

Aim: The present study aimed to compare and evaluate the antimicrobial effect of *Azadirachta Indica* (Neem mouthwash) to chlorhexidine mouthwash on *P. gingivalis*. **Materials and Methods:** The subjects were divided into 2 groups. Group I included 5 subjects who used self-prepared neem mouthwash, and Group II included 5 subjects who used 0.2% chlorhexidine mouthwash. Subgingival plaque samples were taken at baseline and the 15th day after using the mouthwash and subjected to microbiological evaluation. **Results:** The colony forming unit (CFU) of *P. gingivalis* was calculated at baseline and on the 15th day after using mouthwash using the digit colony counter. A comparison of the total CFU of both mouthwashes revealed that the neem mouthwash was effective in reducing the microbial colonies of *P. gingivalis* from baseline to the 15th day. **Conclusion:** It was concluded that the self-prepared neem mouthwash was effective in reducing the colony-forming unit of *P. gingivalis* in the sub-gingival plaque samples and showed better results than the commercially available chemical-based chlorhexidine mouthwash.

Keywords: Chlorhexidine, Neem, Herbal mouthwash, Periodontitis, *Porphyromonas gingivalis*, Anti-bacterial.

Introduction

Periodontitis is a multifactorial disease involving the tooth-supporting structures and if left untreated may lead to the loss of alveolar bone, cementum, and periodontal ligament. (1) Dental plaque is although necessary for the initiation of periodontal disease, but the destruction of the periodontal structures is mainly due to the host's response to this microbial challenge. (2) The human oral cavity harbours hundreds of bacterial species but only a few are responsible for the initiation and progression of periodontal disease. (3)

Porphyromonas gingivalis (*P. gingivalis*) one of the bacteria of the red complex, is the primary pathogen found in the subgingival plaque samples of the periodontitis patient. (4) It is a Gram-negative, rod-shaped, anaerobic, and non-motile bacterium colonising the deep periodontal pockets. (5) Standard approaches for plaque control can be Mechanical or chemical. Mechanical plaque control can be carried out by the patient himself or by a professional dental clinician. The mechanical plaque control carried out by the individual on its own is toothbrushing, using inter-dental aids, etc.

while professional mechanical plaque control measures include scaling and root planing (SRP). (6)

The chemical plaque control can be carried out by the individual himself or by the dental professional. The chemical plaque control carried out by the individual himself includes therapeutic oral rinses or mouthwash. Among all chemical-based mouthwashes, chlorhexidine mouthwash is considered the gold standard in chemical plaque control. However, numerous advantages of chlorhexidine come with some harmful effects too. The adverse effects associated with chlorhexidine include tooth staining, taste alterations, desquamations, and ulcers of the oral mucosa. (7) Recently, a lot of interest has been developed in herbal products made from natural extracts without the use of harmful chemicals. Herbal products for maintaining oral hygiene like mouthwashes, toothpaste, and oral gels are recently gaining popularity since they are natural and safe as compared to chemical-based products. (8)

Azadirachta indica or neem is an indigenous Indian herb known for its medicinal properties since time immemorial. It has antibacterial, anti-inflammatory, anti-oxidant, anti-viral, cytotoxic, and astringent properties. (9) Hence, the study aimed to analyse the antimicrobial activity of *Azadirachta indica* mouthwash against *P. gingivalis* and compare it with commercially available chemical-based chlorhexidine mouthwash.

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Materials and methods

A case-control study was planned on the patients visiting the outpatient department of periodontology and oral implantology diagnosed with mild to moderate chronic periodontitis, systemically healthy aged 30-60 years, with no adverse oral habits (smoking). Verbal Informed consent was taken from the patients.

Preparation of Neem mouthwash

Ingredients-

- Sundried neem leaves powder- 150 grams
- Ethanol-250 ml
- Distilled water- 250 ml
- Stevia
- Aspartame powder
- Mint oil

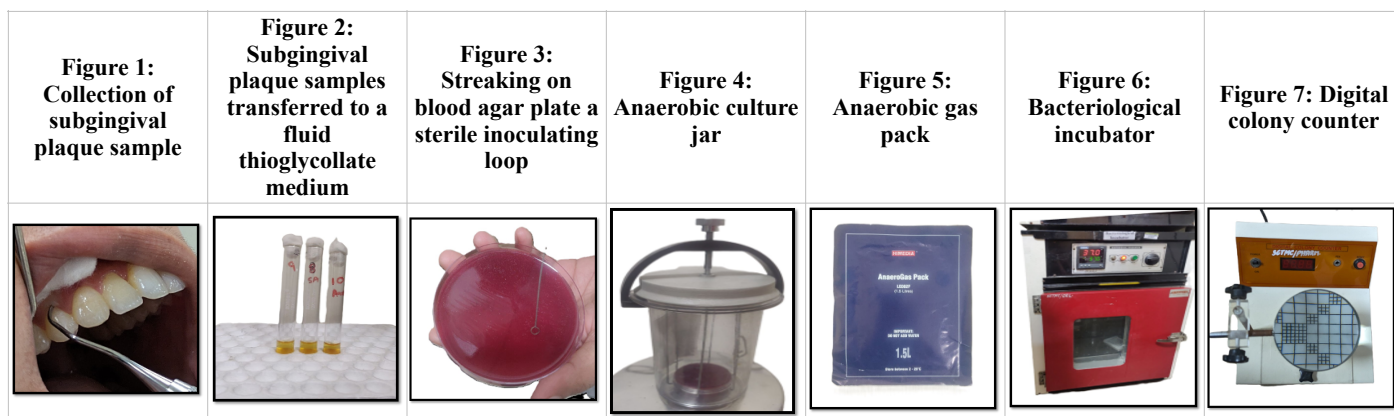
Neem leaves are sundried for two days to dry, crisp, and then ground into a fine powder. To prepare neem extract, 50% distilled water and 50% ethanol are added to 150g of the neem powder. The mixture is soaked for two days, and the macerated content is filtered through a double-layered Muslin Cloth. The pre-filtered macerate is then processed through a vacuum filter to remove particulate matter, resulting in concentrated extract for mouth rinse formulation. The

neem extract is then subjected to a water bath at 60 degrees Celsius to remove excess solvent. The extract thus obtained is utilised for making mouthwash.

Patients with a periodontal probing depth of ≥ 5 mm were included in the study and were divided into 2 groups.

- Group I- Self-prepared Neem mouthwash (10 ml undiluted to be used twice daily for 30 seconds)
- Group II- Commercially available 0.2% Chlorhexidine di gluconate mouthwash (10 ml undiluted to be used twice daily for 30 seconds)

In both groups, the Subgingival plaque samples were collected after proper isolation using sterile cotton rolls at the baseline and on the 15th day (Figure 1). The subgingival plaque samples were transferred to a fluid thioglycolate medium (Figure 2) and bacterial streaking was done with a sterile inoculating loop on a blood agar plate (Figure 3). The plates were transferred to an anaerobic culture jar (Figure 4) along with an anaerobic gas pack (Figure 5). The jar was then placed in a bacteriological incubator (Figure 6) and incubated at 37 degrees Celsius for 48 hours. *P. gingivalis* colony counts were done using a digital colony counter (Figure 7) for both the groups at baseline and on the 15th day of using the mouthwash.



Statistical Analysis

The data in this study is expressed in terms of mean and standard deviation. The Inter-group comparison was done using the Mann Whitney u test, while the Intra-group comparison was done using the Wilcoxon signed rank test. A p-value of less than 0.05% was considered significant.

Results

Table 1: Comparison of CFU among two groups at various intervals of time (Inter-group)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P value
						Lower Bound	Upper Bound			
AT BASELINE	GROUP I	5.000	0.826	0.621	0.278	0.055	1.597	0.227	1.832	0.361
	GROUP II	5.000	1.407	1.188	0.531	-0.069	2.882	0.176	3.263	
ON 15 TH DAY	GROUP I	5.000	0.063	0.060	0.027	-0.011	0.137	0.004	0.151	0.049*
	GROUP II	5.000	0.973	0.970	0.434	-0.231	2.177	0.322	2.655	

* Level of Significance P < 0.05

Table 1 and Graph 1 illustrate the Inter-group comparison of colony-forming units of *P. gingivalis* among two groups at various time intervals. Mann Whitney U test was employed for the Inter group comparison. No significant difference was seen between the two groups at the baseline. A significant difference was seen among the two groups on the 15th day with lower CFU of *P. gingivalis* seen in the neem mouthwash group compared to the chlorhexidine group.

Table 2: Comparison of CFU among two groups at various intervals of time (Intra-group)

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	Mean difference
						Lower Bound	Upper Bound			P value
GROUP I	AT BASELINE	5.000	0.826	0.621	0.278	0.055	1.597	0.227	1.832	0.7631
	ON 15 TH DAY	5.000	0.063	0.060	0.027	-0.011	0.137	0.004	0.151	0.028*
GROUP II	AT BASELINE	5.000	1.407	1.188	0.531	-0.069	2.882	0.176	3.263	0.4334
	ON 15 TH DAY	5.000	0.973	0.970	0.434	-0.231	2.177	0.322	2.655	0.080

* Level of Significance P < 0.05

Table 3: Comparison of absolute reduction in the CFU among 2 groups at various intervals of time (Inter- group)

FROM		N	Mean	Std. Deviation	P value
BASELINE-15 TH DAY	GROUP I	5.000	0.7631	0.6023	0.049*
	GROUP II	5.000	0.3115	0.1638	

* Level of Significance P < 0.05

Table 4: Comparison of Percentage reduction in the CFU among 2 groups at various intervals of time (Inter- group)

FROM		N	Mean	Std. Deviation	P value
BASELINE-15 TH DAY	GROUP I	5.000	0.9164	0.1025	0.008*
	GROUP II	5.000	0.2797	0.0964	

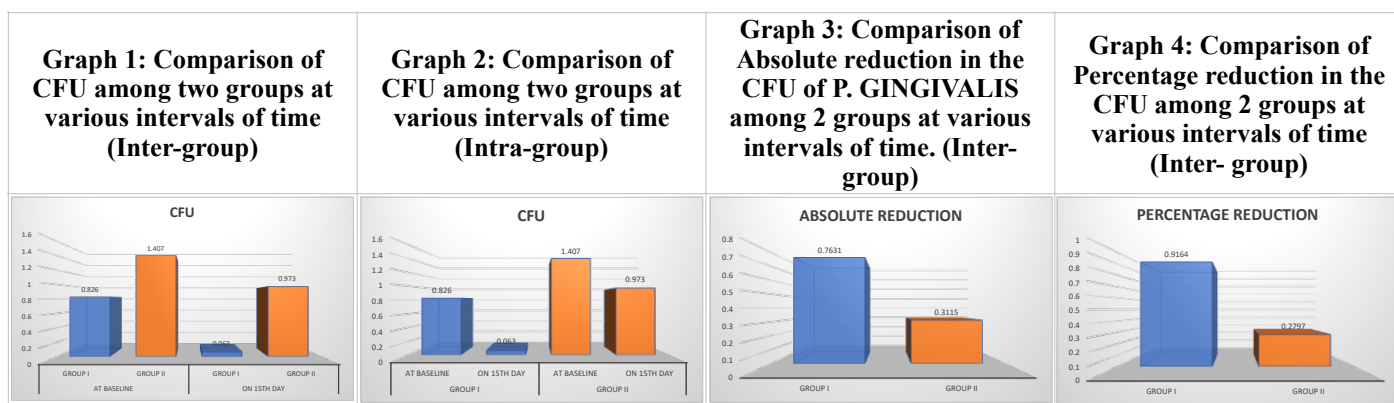


Table 2 and Graph 2 illustrate the Intra-group comparison of colony-forming units among two groups at various time intervals. Wilcoxon Signed Rank test was employed for the Intra- group comparison. The neem mouthwash group showed a significant reduction in the CFU of P. gingivalis at baseline and 15th day of using the mouthwash while no significant difference was seen among the chlorhexidine group at baseline and 15th day.

Table 3 and Graph 3 illustrate the comparison of absolute reduction in the CFU of P. gingivalis among 2 groups at various intervals of time. Mann Whitney U Test was employed for this Inter group comparison.

Table 4 and Graph 4 illustrate the comparison of Percentage reduction in the CFU of P. GINGIVALIS among 2 groups at various intervals of time. Mann Whitney U Test was employed for this inter-group comparison.

Discussion

The present study aimed to compare and evaluate the antimicrobial efficiency of self-prepared neem mouthwash with the commercially available 0.2% chlorhexidine mouthwash based on reduction in P. gingivalis colony forming units.

Over the years chlorhexidine has been the gold standard in chemical plaque control because of its high substantivity and high potency against microbials. However, much evidence from the literature has reported adverse effects after using chlorhexidine mouthwash such as taste alterations, sore throat, mouth ulcers and mucosa desquamations, staining of teeth and tongue, numbness in mouth and tongue, and xerostomia. Some of the rare side effects associated with chlorhexidine usage as oral paraesthesia, glossodynia, swelling of the parotid gland, swelling of the face, wheezing or difficulty in breathing, etc. (10)

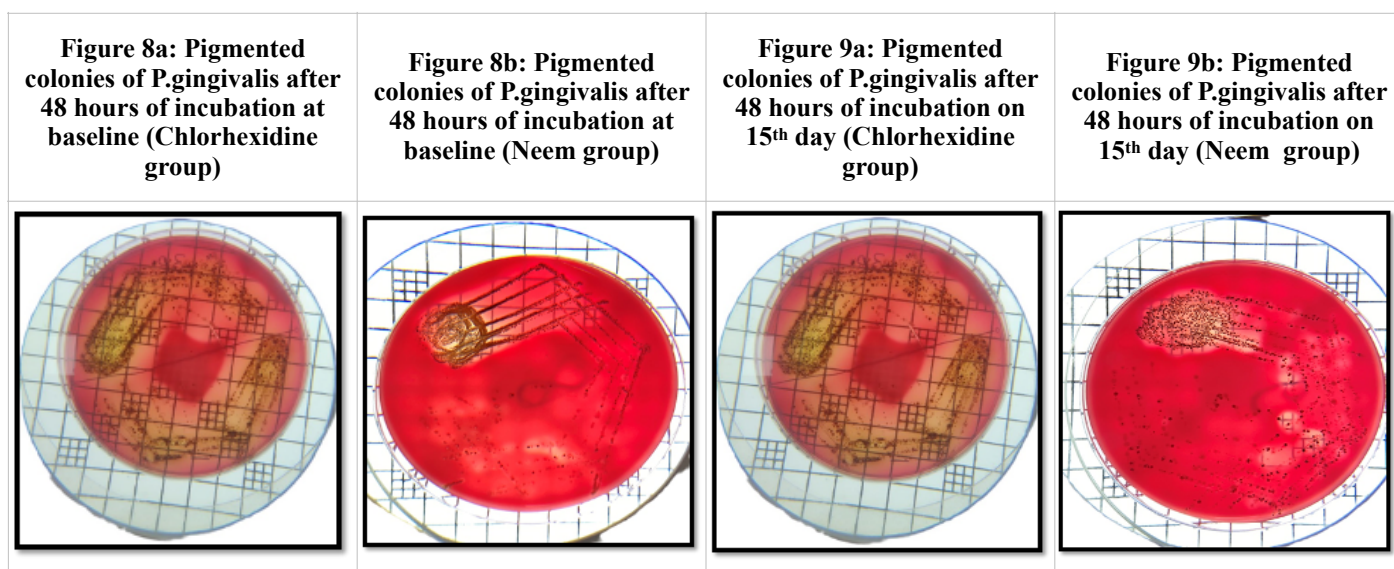
In recent years, a lot of interest has been generated in using herbal extracts in mouthwashes and other oral hygiene products. These herbal products offer

an advantage over the harmful side effects of chemical-based products. However, the antimicrobial efficiency of these herbal products is not proven against periopathogenic bacteria such as *P. gingivalis*. Hence this study was undertaken to analyse and compare the effect of self-prepared herbal neem mouthwash with the chemical-based chlorhexidine mouthwash.

Azadirachta indica, commonly known as Neem, has been used to treat and cure various systemic and oral illnesses since time immemorial due to its medicinal properties. (9) Lakshmi et al (11) in their review article focussed on dental aspects of various parts of Neem extract with their chemical constituents and biological activities. The author has highlighted various properties of neem such as anti-bacterial, anti-plaque, and anti-cariogenic, and concluded that the neem tree possesses antiseptic and astringent activity

which could be beneficial to exterminate oral aerobic and anaerobic pathogens present in the oral cavity.

In the present study, the subjects were divided into 2 groups. In group I, the patients were given Self-prepared Neem mouthwash and were asked to use 10 ml undiluted twice daily for 30 seconds, while in group II, chlorhexidine mouthwash was given and was asked to use 10 ml undiluted twice daily for 30 seconds. The subgingival plaque samples were collected at baseline (Figure 8a, 8b) and on the 15th day (Figure 9a, 9b) and microbial analysis was done for *P. gingivalis*. The colony-forming units were calculated using a digital colony counter. The results showed a significant reduction in the colony-forming unit of *P. gingivalis* after using the neem mouthwash for 2 weeks as compared to chlorhexidine mouthwash.



Several studies were undertaken to compare the effect of herbal mouthwash with chemical-based chlorhexidine mouthwash. A study by Dr. Sabyasachi et al (12) aimed to evaluate the efficacy of indigenously prepared neem mouthwash on oral microflora and compare it with chlorhexidine and distilled water. The subjects included in the study were asked to rinse with 15ml of given mouthwash for 30 seconds, twice per day for 15 days. The saliva samples were taken at the baseline and on the 15th day and were assessed for *Streptococcus mutans*, *Lactobacillus acidophilus*, *Actinomyces viscosus*, and *Streptococcus sanguis*. The result of the microbial analysis indicated that the neem mouthwash significantly reduced the pathogenic bacteria responsible for dental caries and periodontal disease.

Another randomized control trial by Aswini et al (13) compared the effectiveness of 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health. All three groups were assessed for plaque accumulation and gingival condition. The plaque, gingival, and oral hygiene index scores were recorded at baseline, after 1st rinse, after 1 week, 2nd week, and 3rd week in all three groups. The antiplaque effectiveness was highest in the tea group, while the neem and tea group showed

proportional effectiveness on gingival health better than the chlorhexidine group. Hence, it was concluded in the study that the effectiveness of 0.5% tea was highest, followed by tea and then chlorhexidine mouthwash.

Conclusion

Neem can be used as an adjuvant to maintaining oral hygiene to prevent as well as the incidence of periodontal disorders due to its antibacterial and antioxidant qualities. India has a rich source of natural plant items with therapeutic values. Neem leaf extracts may benefit periodontal tissues and infections in various ways. The self-made herbal Neem mouthwash significantly lowers the colony-forming units of *P. gingivalis*, which causes periodontal disorders, according to the current study's microbiological analysis. The findings might significantly influence the development of a low-cost, highly effective oral health intervention for low-socioeconomic populations. Hence, the Neem extract mouthwash can be used as an herbal alternative to chlorhexidine which has many adverse effects including staining of the tongue, teeth, dentures or restorations, taste alteration, and an increase in tartar or calculus accumulation.

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