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## **Clinical evaluation of 10% azadirachta indica mouth rinse as a subgingival irrigant along with ultrasonic scaling for the treatment of chronic gingivitis and chronic periodontitis**

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**Abstract**--The aim of the present study is to evaluate the clinical efficacy of Azadirachta Indica (A. Indica)/ neem mouth rinse in conjunction with scaling and root planning in the treatment of chronic gingivitis and chronic periodontitis. Total of 50 patients who were

diagnosed with generalized chronic gingivitis and chronic periodontitis were divided equally into 2 groups. In first group subgingival ultrasonic instrumentation was performed along with the 0.2% chlorhexidine mouth rinse and in the other group 10% A. indica-mouth rinse was used. Clinical parameters were recorded on the baseline, 7<sup>th</sup> day, 30<sup>th</sup> day and 90<sup>th</sup>-day post-therapy. The intra-group comparison showed a statistically significant difference in clinical parameters at different intervals post-therapy whereas in the inter-group comparison the difference in clinical parameters was statistically non-significant at various time intervals. Neem mouthwash as an irrigant during ultrasonic scaling enhances the benefits of scaling and root planing in the treatment of chronic gingivitis and chronic periodontitis and can be used as an effective alternative for chlorhexidine.

**Keywords**---chlorhexidine, irrigation, neem, root planing, scaling.

## **Introduction**

Gingivitis simply states inflammation of the gingiva. If the inflammatory process involves the gingiva and the periodontium and loss of periodontal attachment have occurred, then according to this definition, the condition should be called *periodontitis*<sup>1</sup>. Dental biofilm is considered the main etiologic factor of periodontal disease<sup>2,3</sup>. Mechanical and chemical plaque control measures have been employed as the primary means to eliminate or reduce gingivitis<sup>4</sup>.

The rationale for using anti-plaque agents as adjunct to mechanical cleaning methods is based on two principles<sup>5</sup>. Firstly, plaque is the main primary etiological factor for gingivitis<sup>6</sup> and the prevalence of gingivitis<sup>7,8,9</sup> and studies of tooth cleaning suggest that mechanical cleaning methods alone are inadequate<sup>10,11,12,13</sup>. Current opinion favors that the concept of periodontitis is always preceded by gingivitis,. However, not all gingivitis proceeds to periodontitis.<sup>14</sup> For individuals with an existing disease with frank periodontal pocketing, the use of vehicles such mouth rinse or toothpaste to deliver anti-microbial and antiplaque agents has only limited or no effects on the subgingival flora<sup>15,16</sup>. In these cases, chemical agents such as chlorhexidine need to be administered directly into the subgingival environment by subgingival irrigation or by some alternative drug release device<sup>17</sup>. Chlorhexidine, by virtue of its substantivity<sup>18</sup>, remains the gold standard of chemical antiplaque agents and the positive control to compare other agents. Mechanical methods of plaque removal can be supplemented by antimicrobial agents and in this respect one particular agent, chlorhexidine<sup>19</sup> in the form of chlorhexidine gluconate, has been identified as an appropriate chemotherapeutic agent at a concentration of 0.2%<sup>20</sup>. In order to be effective, however, it must be applied to the subgingival flora in deep periodontal pockets<sup>21</sup>. Pulsated jet oral irrigators have been useful in applying such antimicrobial agents subgingivally and have produced statistically significant changes in periodontitis parameters<sup>22</sup>.

Neem has been astronomically used in ayurveda, unani well as in homoeopathic medicine and has become a cynosure of modern medicine. The tree has been considered as 'village dispensary' in India. The neem tree has been reported as *Azadirachta Indica* as early as 1830 by De Jussieu<sup>23</sup>. Neem has been long considered to have an astringent, antiseptic, insecticidal, anti-ulcer and for medical purpose<sup>24</sup>. The antibacterial activity of neem has been evaluated and known from ancient times<sup>25,26</sup>. Other than this, the leaf extract of neem has also shown superior antiviral and anti-hyperglycemic activity *in vitro* and *in vivo* on animals.<sup>27</sup>Neem has been used in India and South Asia for thousands of years as a perfect tool for maintaining healthy periodontium<sup>24</sup>. It is used for periodontitis and other dental diseases.<sup>24</sup>Neem leaves have been used in the treatment of gingivitis and periodontitis. The possible mechanism of anti-inflammatory action of neem is by inhibiting prostaglandin E and 5-HT and thus reducing the inflammation<sup>24</sup>. The antibacterial action can be explained by "Azadiachtin" that is known to destroy bacterial cell wall and thus inevitably inhibit the growth of bacteria<sup>24</sup>, also the breakdown of cell wall which disturb osmotic pressure and leads to cell death<sup>24</sup>.

It would appear that further work should attempt to combine the antimicrobial action of a chemical agent with mechanical plaque removal. In this respect, a recently introduced ultrasonic scaling instrument, which allows the irrigating solution to be delivered through a specially designed insert to the scaling tip, may prove to be a suitable vehicle for the subgingival delivery of antimicrobial agents, enhancing mechanical subgingival debridement.<sup>19</sup> Based on the assumption of obtaining better efficacy of neem extract in the oral cavity this clinical study was planned to evaluate the effect of neem as an irrigating solution in conjunction with scaling and root planing in the treatment of chronic gingivitis and chronic periodontitis.

### **Aim and Objective**

1. To evaluate the clinical efficacy of 0.2% chlorohexidine as subgingival irrigant in conjunction with scaling and root planing clinically after 7 days, 30 days and 90 days.
2. To evaluate the clinical efficacy of 10% *Azadirachta Indica* as subgingival irrigant in conjunction with scaling after 7 days, 30 days and 90 days.
3. Comparison between the 0.2% chlorhexidine and 10% *Azadirachta Indica* (neem) clinically as a subgingival irrigant during ultrasonic scaling after 7 days, 30 days and 90 days.

### **Materials and Method**

#### **Inclusion criteria**

- 1) Healthy patients diagnosed as having chronic gingivitis or chronic periodontitis, and aged 25- 60years old.
- 2) At least one site with probing depth of 5mm or 6mm.
- 3) Presence of at least 15 teeth in the oral cavity.
- 4) Patients willing to participate in the study and maintain regular appointments.

### **Exclusion Criteria**

- 1) Patients who have received any topical or systemic antimicrobial treatment in the past 6 months, including the use of mouthwash.
- 2) Patients who had undergone periodontal treatment in the past 6 months.
- 3) Smokers (former smoker and current smoker).
- 4) Patients otherwise not systemically healthy.
- 5) Pregnant and lactating females.
- 6) Patients unwilling to complete the treatment protocol.

50 patients who were diagnosed with generalized chronic gingivitis and chronic periodontitis, aged between 25 to 60 years were selected. All the selected subjects received non-surgical periodontal therapy (supra and subgingival ultrasonic scaling). Periodontal examination of this phase was considered as the baseline examination.

Subjects were divided randomly into two groups:

- *Group 1*- included 25 patients and subgingival ultrasonic instrumentation performed with the 0.2% chlorhexidine mouthrinse.
- *Group 2*- included 25 patients and subgingival ultrasonic instrumentation performed with the 10% *A. indica* mouthrinse.

This study was approved by the ethical committee and informed written consent was received from all the patients before their enrollment in the study.

The clinical parameters recorded were:

- Plaque index – (Silness P and Loe H 1964)
- Gingival index (Loe H and Silness P 1963)
- Sulcular bleeding index – (Muhlemann H.R and Son.S 1971)
- Probing pocket depth (PPD)
- Relative distance between the base of the pocket and the fixed reference point on the stent for assessing Clinical Attachment Gain or Loss.

All the clinical parameters were recorded at the baseline, 7<sup>th</sup> day, 30<sup>th</sup> day and 90<sup>th</sup> day.

### **Preparation of the *A. indica*-mouthrinse**

The preparation of the *A. indica*-mouthrinse was carried out at the Department of Pharmacy of the Rama Dental College, Hospital and Research Centre, Lakhnau, Kanpur. Leaves of *A. indica* were collected from the medicinal garden of the same institution. The leaves were dried under controlled conditions. After drying leaves were powdered by crushing in mixer. 20.0 g of dry powder was mixed with 100 ml of 70% (w/v) ethanol and kept for a week in a round bottom flask with occasional shaking. Flask was kept in dark to avoid effect of light on the active ingredients thereafter, extract was then sieved through a muslin cloth for coarse residue and finally through Whatman no.1 filter paper after that it was measured and kept in an airtight amber colored container. The mouthrinse of *A. indica* used in this

study was prepared in a similar manner as reported in previous study published by Botelho et al. in 2003.<sup>28</sup> Laboratory test of neem mouthwash preparation was done in the laboratory of oil and paints department at Harcourt Butler Technological Institute, Kanpur (HBTI, Kanpur) for finding of the concentration of neem mouthwash.

### **Availability of Chlorhexidine**

A commercially available 0.2% chlorhexidine gluconate mouthrinse (LORHEX-PLUS: KPH COMOS PVT LTD)<sup>®</sup> was used as an antimicrobial oral agent. The results obtained were analyzed statistically using SPSS 17 version software, and comparisons were made within each group using ANOVA.

### **Result**

The plaque index for group 1 was  $2.44 \pm 0.43$  at base line, which was reduced to  $1.57 \pm 0.49$  at 7 days,  $1.04 \pm 0.39$  at 30 days and  $0.86 \pm 0.29$  at 90 days respectively. Whereas, in group 2 it was  $2.42 \pm 0.61$  at baseline, which was reduced to  $1.56 \pm 0.52$  at 7 days and  $1.09 \pm 0.32$  at 30 days and  $0.93 \pm 0.32$  at 90 days respectively, and this intragroup difference in values was statistically significant in both the groups (Table I). The gingival index for group 1 was  $2.37 \pm 0.42$  at baseline, which was reduced to  $1.32 \pm 0.39$  at 7 days,  $0.85 \pm 0.27$  at 30 days and  $0.74 \pm 0.25$  at 90 days respectively. Whereas, in group 2 it was  $2.53 \pm 0.60$  at baseline, which was reduced to  $1.58 \pm 0.55$  at 7 days, and  $1.11 \pm 0.43$  at 30 days and  $0.72 \pm 0.30$  at 90 days respectively, and this intragroup difference in values was statistically significant in both the groups (Table II).

The bleeding index for group 1 was  $2.49 \pm 0.60$  at base line, which was reduced to  $1.35 \pm 0.42$  at 7 days,  $0.67 \pm 0.37$  at 30 days and  $0.52 \pm 0.42$  at 90 days respectively. Whereas, in group 2 it was  $2.47 \pm 0.55$  at baseline, which was reduced to  $1.24 \pm 0.32$  at 7 days, and  $0.63 \pm 0.37$  at 30 days and  $0.34 \pm 0.24$  at 90 days respectively, and this intragroup difference in values was statistically significant in both the groups (Table III). Probing depth for group 1 was  $6.32 \pm 0.87$  at base line, which was reduced to  $5.12 \pm 0.91$  at 7 days,  $3.79 \pm 0.98$  at 30 days and  $3.44 \pm 1.04$  at 90 days respectively. Whereas, in group 2 it was  $6.02 \pm 1.02$  at baseline, which was reduced to  $4.71 \pm 1.2$  at 7 days, and  $3.83 \pm 1.16$  at 30 days and  $3.43 \pm 0.88$  at 90 days respectively, and this intragroup difference in values was statistically significant in both the groups (Table IV).

Clinical attachment level for group 1 was  $6.09 \pm 0.79$  at base line, which was reduced to  $5.03 \pm 0.91$  at 7 days,  $3.98 \pm 1.10$  at 30 days and  $3.45 \pm 1.06$  at 90 days respectively. Whereas, in group 2 it was  $6.04 \pm 1.01$  at baseline, which was reduced to  $4.90 \pm 1.3$  at 7 days, and  $3.84 \pm 1.16$  at 30 days and  $3.57 \pm 0.99$  at 90 days respectively, and this intragroup difference in values was statistically significant in both the groups (Table V). The intergroup comparison of values for the various clinical parameters were non-significant at the baseline as well as on the 7<sup>th</sup> day, 30<sup>th</sup> day and 90<sup>th</sup> day (Table I, II, III, IV, V).



Figure 1. Absolute alcohol (ethanol) and Neem leaves powder



Figure 2. 0.2% chlorhexidine bottle connected to dental chair and subgingival irrigation with 0.2% chlorhexidine



Figure 3. Neem mouthwash bottle connected to dental chair and subgingival irrigation with 10% neem mouthwash



Figure 4. At baseline (chx group) and at 90 days (chx group)



Figure 5. At baseline (neem group) and at 90 days (neem group)

Table I. Intra & Inter-group comparison of mean values for plaque index

	Group 1		Group 2		Inter-group <i>p</i> value
	Mean	SD	Mean	SD	
Baseline	2.44	0.43	2.42	0.61	0.89
7 <sup>th</sup> day	1.57	0.49	1.56	0.52	0.94
30 <sup>th</sup> day	1.04	0.39	1.09	0.32	0.62
90 <sup>th</sup> day	0.86	0.29	0.93	0.32	0.42
Intra-group <i>p</i> value	<0.001; Sig		<0.001; Sig		

Table II. Intra & Inter-group comparison of mean values for gingival index

	Group 1		Group 2		Inter-group <i>p</i> value
	Mean	SD	Mean	SD	
Baseline	2.37	0.42	2.53	0.60	0.28
7 <sup>th</sup> day	1.32	0.39	1.58	0.55	0.05

30 <sup>th</sup> day	0.85	0.27	1.1	0.43	0.01
90 <sup>th</sup> day	0.74	0.25	0.72	0.30	0.79
Intra-group <i>p</i> value	<0.001; Sig		<0.001; Sig		

Table III. Intra &amp; Inter-group comparison of mean values for bleeding index

	Group 1		Group 2		Inter-group <i>p</i> value
	Mean	SD	Mean	SD	
Baseline	2.49	0.60	2.47	0.55	0.90
7 <sup>th</sup> day	1.35	0.42	1.24	0.32	0.30
30 <sup>th</sup> day	0.67	0.37	0.63	0.37	0.70
90 <sup>th</sup> day	0.52	0.42	0.34	0.24	0.06
Intra-group <i>p</i> value	<0.001; Sig		<0.001; Sig		

Table IV. Intra &amp; Inter-group comparison of mean values for probing depth

	Group 1		Group 2		Inter-group <i>p</i> value
	Mean	SD	Mean	SD	
Baseline	6.32	0.87	6.02	1.02	0.26
7 <sup>th</sup> day	5.12	0.91	4.71	1.2	0.17
30 <sup>th</sup> day	3.79	0.98	3.83	1.16	0.89
90 <sup>th</sup> day	3.44	1.04	3.43	0.88	0.97
Intra-group <i>p</i> value	<0.001; Sig		<0.001; Sig		

Table V. Intra &amp; Inter-group comparison of mean values for clinical attachment level

	Group 1		Group 2		Inter-group <i>p</i> value
	Mean	SD	Mean	SD	
Baseline	6.09	0.79	6.04	1.01	0.84
7 <sup>th</sup> day	5.03	0.91	4.90	1.3	0.68
30 <sup>th</sup> day	3.98	1.10	3.84	1.16	0.66
90 <sup>th</sup> day	3.45	1.06	3.57	0.99	0.68
Intra-group <i>p</i> value	<0.001; Sig		<0.001; Sig		

## Discussion

The beginning of the periodontal disease occurs through the accumulation of thin film of bacteria on the surface of the teeth, called plaque. Novel approaches have been tried to deliver the drug in different ways in treating such human ailments<sup>1</sup>. Among the various antimicrobials tried as mouthrinse to control the oral infection, chlorhexidine is proved to be dependable in reducing gingivitis and plaque formation<sup>2,3</sup>.

Addy M (1986)<sup>4</sup> reported chlorhexidine as the most effective and safe antiplaque agent available.

But the long-term use of chlorhexidine is restricted due to its side effects like altering taste<sup>5</sup> and staining of teeth<sup>4</sup> along with supragingival calculus formation<sup>6</sup>. Therefore, new formulations with similar or superior efficacy needs to be investigated. Neem has been considered to have an astringent, antiseptic, insecticidal, anti-ulcer and medical properties<sup>7,8</sup>. The possible mechanism of anti-inflammatory action of neem is by impeding prostaglandin-E and 5-HT and thus reducing the inflammation. Antibacterial action is explained by "Azadiachtin" that is known to destroy bacterial cell wall and thus inhibit the growth of bacteria. Also the breakdown of cell wall eventually disturbs osmotic pressure and leads to cell death<sup>8</sup>. The present study was designed to evaluate the clinical efficacy of 10% *Azadirachta Indica* (neem) mouthwash in comparison to 0.2% chlorhexidine when used as a subgingival irrigant along with scaling in the treatment of chronic gingivitis and chronic periodontitis cases. The changes in clinical parameters were studied over a period of 3 months.

In the present study, highly statistically significant reduction in plaque index, gingival index and bleeding index score was observed from baseline to 3 months for both the treatment groups ( $p < 0.001$ ). While comparing the plaque index, gingival index and bleeding index score between groups at baseline and 3 months, the difference was statistically non-significant at both the points of time. Similar observations were made by Botelho MA (2008)<sup>9</sup> and Chatterjee et al (2011)<sup>7</sup>. In the study by Botelho et al., the effect of neem leaf mouthwash was compared with that of 0.12% chlorhexidine in 54 subjects with chronic gingivitis. PI, gingival index, gingival bleeding index, and *S. mutans* counts in saliva were measured before and after the 1st and 4th week of treatment. No significant differences between the two groups of subjects were noted. Similarly, Chatterjee et al. reported no significant differences in gingival bleeding and PI between subjects using neem leaf extract (0.19%) and those using chlorhexidine (0.2%) mouthwashes. However, both studies stated that neem leaf extract had no side effects, whereas chlorhexidine was associated with an unpleasant taste in the mouth and tooth staining. The results of this study were in accordance with the results of a study conducted by Wolinsky et al<sup>10</sup> who stated that there was a marked reduction in the bacterial aggregation, growth, adhesion to hydroxyapatite, and production of insoluble glucan that affects the formation of *in vitro* plaque by the use of aqueous extracts of neem, derived from the bark-containing sticks (neem stick) of *A. indica*.

There is also a study by Vibha Singh *et al*<sup>11</sup> where neem and chlorhexidine gel was compared on gingivitis patients and found that though the chlorhexidine gluconate as well as neem gel can be effectively used as an adjunct to mechanical plaque control in prevention of plaque and gingivitis, chlorhexidine gluconate gel is more effective when antiplaque and anti-inflammatory properties were considered. Our study showed a highly statistical significant ( $p < 0.001$ ) reduction in probing depth (PD) and gain in clinical attachment level (CAL) from baseline to 3 months in both the groups. On inter-group comparison the difference in the values of clinical parameters was non-significant ( $p > 0.05$ ). In comparison to the present study where 0.2% chlorhexidine and 10% neem mouthwash were used as

subgingival irrigant during ultrasonic scaling at baseline, most of the other studies either used mouth rinses or topical gel application.

Vennila K, Elanchezhiyan S, Sugumari Ilavarasu<sup>41</sup> use 10% neem in form of chip as adjunct to scaling and root planing as a local drug delivery, Clinical parameters were recorded on the baseline, 7<sup>th</sup> day, and 21<sup>st</sup> day. Plaque samples were acquired for a microbiological study on the baseline and 21<sup>st</sup> day. *Porphyromonas gingivalis* strains were analysed using quantitative and qualitative polymerase chain reaction assay. Clinical parameters showed statistically improvement and the presence of *Porphyromonas gingivalis* strains were significantly reduced on the neem chip site.

Various in vitro studies were also carried out to see antibacterial efficacy of neem on different microorganism, Sistla D P *et al*<sup>42</sup> carried out a study to evaluate the antimicrobial efficacy of alcoholic neem and Aloe vera leaf extract against *Enterococcus faecalis* and *Candida albicans* in comparison with 3% sodium hypochlorite (3% NaOCl) and 2% chlorhexidine (2% CHX). It was found that antimicrobial efficacy of the extracts was well signified in agar well diffusion method whereas, Amit R K *et al*<sup>43</sup> evaluated the qualitative and quantitative effect of different concentrations of water soluble azadirachtin (neem metabolite) on *Streptococcus mutans* against chlorhexidine, the results show that there was no statistically significant difference in the inhibition of *Streptococcus mutans* between 40% concentration of water soluble azadirachtin and chlorhexidine. Leali H *et al*<sup>44</sup> evaluated the antibacterial effects of Neem leaf extract on the periodontopathic bacteria *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, and its antioxidant capacities alone and in combination with bacteria and polycationic peptides that may present at the site of inflammation. Broth micro-dilution test was used to evaluate the Minimal Inhibitory Concentration (MIC) of neem leaf extract against each bacterial strain and red blood cells or the polycationic peptides chlorhexidine and lysozyme, were determined using a chemiluminescence assay. Neem leaf extract showed prominent antibacterial activity against *Porphyromonas gingivalis*, but had no effect on the growth of *F. nucleatum* nor on coaggregation of the two bacteria. Yet, it showed enormous antioxidant activity. Rabi, R.A *et al*<sup>45</sup> evaluated the activities of neem stem bark extract against *Streptococcus mutans* and *Escherichia coli* using disc diffusion method. The results demonstrate that the methanolic extracts of neem stem bark have strong antibacterial activities and suggested that it has potentials to be used in the treatment of dental caries. The neem stem bark extract do not have antibacterial activity against *E. coli*. Pranjali D *et al*<sup>46</sup> assessed the efficacy of neem and turmeric as storage media in maintaining periodontal ligament (PDL) cell viability. Thus, the reduction in probing depth and gain in CAL can be attributed to the fact that irrigation with neem mouthwash during ultrasonic scaling causes the arrest of disease progression by altering the sub-gingival microbiota and to create a healthier sub-gingival environment. This concept is supported by the results of Morozumi T *et al*.<sup>47</sup>

In the present study chlorhexidine and neem mouth rinse were used as a subgingival irrigant during ultrasonic scaling. Fine J B *et al*<sup>40</sup>, Wennstrom JL *et al*<sup>21</sup>, Chaves ES *et al*<sup>48</sup> and David L *et al*<sup>49</sup> used chlorhexidine as subgingival

irrigant with different type of mode of application of irrigant except ultrasonic scaler and they found similar results.

All the subjects who received session of irrigation at each follow-up visit, were asked about possible adverse events (such as a burning sensation on the tongue, sensitivity, aphthous lesions, etc.). The examiner also carry out a complete oral examination to verify the presence of oral lesions. With regard to the incidence of adverse events, in present study some patients of neem group complained of uneasy smell of neem, mild headache, nausea and altered taste, few minutes after treatment. This adverse event was probably associated with uneasy smell for long time (Suryasa et al., 2021). In the present study we used 70% alcohol (ethanol) to prepare the neem mouthwash. Haq MW (2009)<sup>50</sup> reported alcohol (Ethanol) in the mouthwashes does not contribute to any therapeutic action. Thus, the therapeutic effect of the solution could be totally attributed to neem.

### **Conclusion**

Within the limits of this study and on the basis of the clinical parameters, it can be concluded that neem mouthwash as an irrigant during ultrasonic scaling enhances the benefits of non- surgical therapy in the treatment of chronic gingivitis and chronic periodontitis. Neem mouthwash can provide dental professionals an additional means to maintain significantly improved clinical health in periodontal diseases. Future studies are anticipated to find out connection between bacterial and clinical data. This will provide better information for the use of the present combination therapy.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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