Efficacy of 10% whole *Azadirachta indica* (neem) chip as an adjunct to scaling and root planning in chronic periodontitis: A clinical and microbiological study

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ABSTRACT

Introduction: Anti-microbial therapy is essential along with conventional therapy in the management of periodontal disease. Instead of systemic chemical agents, herbal products could be used as antimicrobial agents. Herbal local drug delivery systems are effective alternative for systemic therapy in managing the chronic periodontal disease. In this study, 10% neem oil chip was used as a local drug delivery system to evaluate the efficacy in the periodontal disease management.
Materials and Methods: Twenty otherwise healthy patients with the bilateral periodontal probing depth of 5–6 mm were included in the study. After scaling and root planning (SRP), 10% nonabsorbable neem chip was placed in the pocket in one side of the arch. Other side was done with SRP only. Clinical parameters were recorded on the baseline, 7th day, and 21st day. Plaque samples were obtained for a microbiological study on the baseline and 21st day. *Porphyromonas gingivalis* strains were seen using quantitative and qualitative polymerase chain reaction assay. All results were statistically evaluated.
Results: Clinical parameters showed statistically improved on the neem chip sites and presence of *P. gingivalis* strains were significantly reduced on the neem chip sites.
Conclusion: Hence, 10% neem oil local delivery system delivers desired effects on *P. gingivalis*. Further research is needed to evaluate the neem oil efficacy on other periodontal pathogens.

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Periodontitis has been proposed as having an etiological or modulating role in cardiovascular, cerebrovascular disease, diabetes, respiratory disease, and adverse pregnancy outcome; several mechanisms have been proposed to explain or support such theories.[1] The goal of periodontal therapy is to prevent, arrest, control or eliminate periodontitis and to restore the lost form, function, esthetics, and comfort.[2] The standard periodontal therapy is with the objective of reducing the total bacterial load and changing the environmental conditions of the microbial niches.[3] Although mechanical treatment (scaling and root planning [SRP]) reduces the level of subgingival bacteria, it does not eliminate all the pathogens which reside deep into connective tissue could be responsible for tissue destruction.[3] Systemic antibiotics are used to eliminate the subgingival microbial flora. However, side effects are not uncommon in systemic therapy.

To overcome the shortcomings of systemic antimicrobial therapy, a different approach has been introduced using local delivery systems that contain antibiotic or antiseptic agents.[4] These systems allow the therapeutic agents to be delivered directly to the disease site with no appreciable systemic effects. Various locally delivered agents that

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are successfully used include tetracycline fibers,[5] 10% doxycycline,[6,7] 2% minocycline,[8] metronidazole,[9] and chlorhexidine gluconate,[10] but none is without side effects.

To overcome the side effects of these drugs, research is being conducted for the use of the natural products. With the growing interest and increasing knowledge about the medicinal value of natural products, various formulations have been made commercially available. Natural products lead to a pathway of true and healthy healing. One such natural plant which holds the medicinal value is neem (*Azadirachta indica*).[11]

Neem, a native tree of India is an incredible plant that has been declared the "Tree of the 21st century" by the United Nations. In India, it is variously known as "Divine Tree," "Life-giving tree," "Nature's Drugstore," "Village Pharmacy," and "Panacea for all diseases."[12]

The neem leaves, flowers, seeds, roots, bark, and fruits are utilized to treat inflammation, infections, and skin diseases and have been proved to be useful in dental care also. Neem has been used as the preferred tool to maintain healthy gums and teeth. Various compounds such as nimbin, nimbidin, ninbidol, sodium nimbidate, and azadirachtin are also found in neem which acts as anti-inflammatory, antipyretic, antihistamine, antifungal, antimalarial, vasodilator, analgesic, antibacterial, and antiulcer agents.[13,14]

Based on the assumption of obtaining better efficacy of neem extract in the oral cavity when delivered in the local delivery form of a chip, this clinical study was planned to evaluate the effect of neem chip when inserted into the periodontal pocket as an adjunct to SRP.

Aims and objectives

- To evaluate the efficacy of 10% *A. indica* (neem) chip as local drug delivery system in periodontitis
- To compare the effectiveness of neem chip with conventional mechanical periodontal therapy
- To investigate qualitative and quantitative changes of *Porphyromonas gingivalis* in response to neem local drug delivery system.

MATERIALS AND METHODS

Inclusion criteria

Twenty otherwise systemically healthy patients with chronic periodontitis with at least one site 5–6 mm pocket present bilaterally in the age range of 35–55 years seeking dental treatment in the department of periodontology.

Exclusion criteria

- Patients received treatment with antibiotics/ anti-inflammatory drugs in last 3 months
- Smokers
- Patients with the history of systemic diseases
- Pregnant and lactating women
- Drug allergies
- Teeth with traumatic occlusion.

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

Selected sites were randomly divided into control sites and experimental sites, which were treated by split-mouth design.

The motivation for daily plaque control was reinforced at each visit. Education and motivation was followed by full mouth supragingival scaling using hand and ultrasonic scalers. Thorough subgingival scaling was done under local anesthesia in the selected sites using curettes. Forty sites in total with twenty on each side (5 mm) were selected and were divided into two groups.

- Healthy group – It consists of twenty sites, in which no bleeding on probing, no pocket depth (healthy control × group)
- Group I – It consists of twenty sites, in which the only SRP was done (control group)
- Group II – It consists of twenty sites, in which SRP was followed by the placement of the 10% neem chip inside the pocket (SRP + neem chip) (test group).

The neem chip was indigenously prepared with 10% neem drug concentration.

The following parameters were recorded.

Clinical parameters include:

- Plaque index – (Turskey *et al.* modification of Quigley Hein index)
- Gingival index (Loe and Silness 1964)
- Sulcus bleeding index – (Muhlemann and Son, 1971)
- Probing pocket depth (PPD)
- Relative distance between the base of the pocket and fixed reference point on the stent for assessing clinical attachment gain or loss.

Microbiological study was done with the collected plaque samples. Both quantitative and qualitative polymerase chain reaction (PCR) assay carried out with the samples with the use of *P. gingivalis* primer for identifying *P. gingivalis* strain.

The above clinical and microbiological parameters were recorded on the baseline, 7th day, 21st day.

The clinical and microbiological parameters were assessed at the baseline at selected sites, followed by thorough SRP. The local drug delivery system containing 10% neem chip was placed in the periodontal pocket, and Coe-Pak was placed.

Subjects were recalled after 7 days, and postoperative samples were collected on the 7th day after removing the periodontal dressing. Oral hygiene maintenance instructions were given. Clinical parameters were repeated on the 21st day.

RESULTS

Statistical analysis

The results obtained were analyzed statistically, and comparisons were made within each group using paired and unpaired Student's *t*-test using SPSS 19.0 version software (IBM SPSS software, New York, USA). $P<0.05$ was considered the level of significance.

Clinical evaluation

No adverse reaction was observed in any subject, and no patient reported any discomfort. Healing was uneventful. All subjects tolerated the drug very well and without any postoperative complications.

Clinical parameters

Plaque index

The mean plaque index at baseline was 3.294 ± 0.519, significantly reduced to 1.012 ± 0.174 and 0.789 ± 0.277 at 7th day and 21st day respectively as shown in Table 1. When compared between the groups, it was not statistically significant.

Gingival index

At baseline, the mean gingival index score was 1.520 ± 0.196, significantly reduced to 0.566 ± 0.144 and 0.329 ± 0.879 at 7th day and 21st day respectively as shown in Table 2. No statistical difference between the groups.

Sulcus bleeding index

The mean gingival index at baseline was 2.697 ± 0.616, significantly reduced to 0.418 ± 0.163 at 7th day and further reduced to 0.323 ± 0.088 at a 21st day in both groups as shown in Table 3.

Probing pocket depth

At control site, the mean PPD at baseline was 4.90 ± 0.316 significantly reduced to 2.70 ± 0.483 at the 21st day as shown in Table 4. In test site, the mean PPD at baseline was 5.10 ± 0.316, significantly reduced to 2.30 ± 0.483 at 21st day. The values were statistically significant between test and control groups ($P < 0.05$).

Relative attachment level

The mean relative attachment level (RAL) at baseline was 5.00 ± 0.471 and 5.20 ± 0.422 at control and test site, significantly reduced to 2.80 ± 0.422 and 2.40 ± 0.516 at 21st day respectively as shown in Table 5. When compared between test and control groups, the values were statistically significant ($P < 0.05$).

Microbiological parameters

Qualitative PCR assay showed the presence of *P. gingivalis* strains in the Group I (control site) as shown in Figure 1 and near complete absence of *P. gingivalis* in Group II (test site) as shown in Figure 2 at the 7th day as shown in Table 6.

In quantitative PCR assay, *P. gingivalis* count was significantly reduced in the Group II (test sites) when compared to Group I (control sites). 10^2 cells present in 28 thermocycles, 10^5 cells in 20 cycles and 10^6 cells in 18 cycles. Both these results showed the reduction of *P. gingivalis* in areas using the neem chip.

DISCUSSION

The destructive periodontal disease is a multifactorial disease caused by microbial flora which is present in the

Table 1: Mean plaque index scores at baseline, 7 days and 21 days

Interval	Mean±SD	Difference from baseline	*t*	*P*
Baseline	3.294±0.519			
7 days	1.012±0.174	2.912	16.583	0.000
21 days	0.789±0.277	2.505	12.852	0.000

SD=Standard deviation

Table 2: Mean gingival index scores at baseline, 7 days and 21 days

Interval	Mean±SD	Difference from baseline	*t*	*P*
Baseline	1.520±0.196			
7 days	0.566±0.144	0.954	10.064	0.000
21 days	0.329±0.879	1.191	16.970	0.000

SD=Standard deviation

Table 3: Mean sulcus bleeding index scores at baseline, 7 days and 21 days

Interval	Mean±SD	Difference from baseline	*t*	*P*
Baseline	2.697±0.616			
7 days	0.418±0.163	2.279	12.482	0.000
21 days	0.323±0.088	2.374	12.327	0.000

SD=Standard deviation

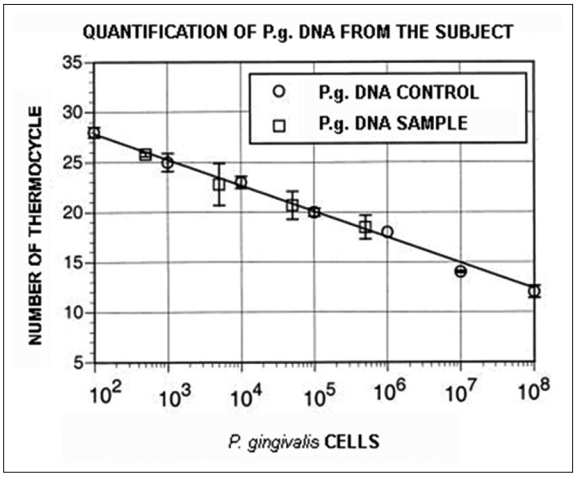

Figure 1: Quantification of *Porphyromonas gingivalis* DNA from the subject

Table 4: Difference in mean probing pocket depth between control and test site at baseline and 21 days

Interval	Control				Test			
	Mean±SD	Difference from baseline	*t*	*P*	Mean±SD	Difference from baseline	*t*	*P*
Baseline	4.90±0.316				5.10±0.316			
7 days	2.70±0.483	2.20	1.50	0.00	2.30±0.483	2.80	2.45	0.00

SD=Standard deviation

Table 5: Difference in mean relative attachment level between control and test site at baseline and 21 days

Interval	Control				Test			
	Mean±SD	Difference from baseline	*t*	*P*	Mean±SD	Difference from baseline	*t*	*P*
Baseline	5.00±0.471				5.20±0.422			
7 days	2.80±0.422	2.20	1.50	0.00	2.40±0.516	2.80	2.45	0.00

SD=Standard deviation

Table 6: Qualitative changes of *Porphyromonas gingivalis* at baseline and 7th day

Baseline		7th day	
Control group	Test group	Control group	Test group
++	++	+	-
++	++	-	-
+	+	-	-
++	++	+	-
++	++	+	-
++	++	+	-
+	++	-	-
++	++	+	-
+	+	-	-
++	++	+	-

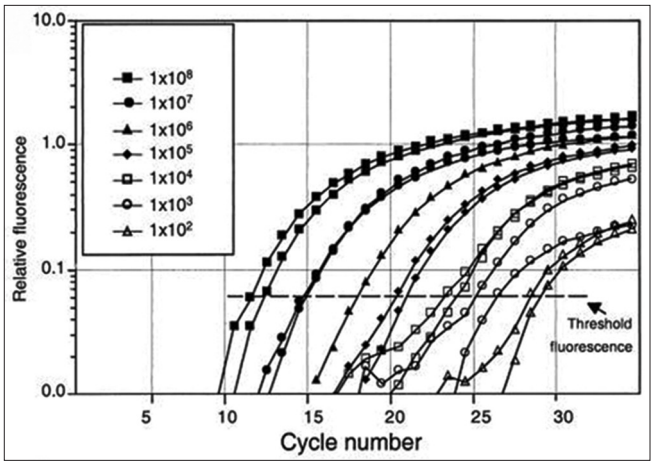

Figure 2: Quantitative analysis of *Porphyromonas gingivalis* DNA by qualitative polymerase chain reaction

subgingival plaque. *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* are the main periodontal pathogens responsible for the periodontal destruction.[2] Chronic periodontitis is more prevalent in periodontal sites exposed to these pathogens. It also assumes that future tissue destruction takes place more frequently in periodontal sites exposed to these organisms than nonexposed sites.

Traditional periodontal therapy has involved in the reduction or elimination of these pathogens.[3] Nonsurgical periodontal therapy may not completely eradicate these periodontal pathogens, which leads into adjunct systemic antibiotic therapy for complete elimination of the periodontal pathogens.[15]

Systemic antibiotic therapy has the action of eliminating all periodontal pathogens, but few shortcomings are present. Some disadvantages such as the inability of systemic drugs to achieve high gingival crevicular fluid (GCF) concentration, increased the risk of adverse drug reactions, increase multiple antibiotic resistant microorganisms and uncertain patient compliance.[16] These shortcomings led the invention of other possibilities as local drug delivery or sustained drug release system.

Local drug delivery systems as local irrigation may not be more effective in periodontal disease management due to inadequate drug penetration into the deep pockets.[17] In sustained release system, higher drug concentrations are achieved at the disease site which reduces side and toxic effects. This system is considered to have excellent potential as an adjunct to traditional periodontal therapy.[18]

Tetracycline fibers,[6] chlorhexidine chips[11] are the few successfully used sustained release system in periodontal therapy. Their sustained release, increases many fold GCF drug concentration than systemic therapy, with nil or few side effects make the systems more successful. Chemical agents used in these systems are relatively expensive.

Instead of chemical agents, natural products could be used in the sustained release systems. With the growing interest and increasing knowledge about medicinal value of natural products, various formulations such as *Eucalyptus* extract, bloodroot, chamomile, turmeric, green tea catechin, *Aloe vera*, tulsi, and neem (*A. indica*) are available in the drug delivery systems.[19-31]

Neem, an Indian native tree considered as "divine tree" and village pharmacy. Parts of neem trees such as leaf, kernel oil, bark, flower, fruit, twig, gum, and seed pulp are used for medicinal purposes.[14]

Nimbidin, sodium nimbidate, nimbolide, gedunin, azadirachtin, gallic acid, epicatechin, catechin, margolone,

margolonone, and isomargolonone are the few principle contents of neem, and they possess antibacterial, antifungal, antiviral, antipyretic, anti-inflammatory, antimalarial, antitumor, and diuretic effects.[13,15]

The antibacterial activity of neem has been evaluated and known since ancient times.[24,26] Oil from seed, leaves and bark possesses a wide spectrum of antibacterial action against Gram-positive and Gram-negative microorganisms. The neem extract possesses potent immune stimulant activity as evidenced by both humoral and cell-mediated immune response. Three weeks administration of leaf extract makes higher immunoglobulin M and immunoglobulin G levels.[23,28] Rao *et al.* proved its efficacy in reducing plaque cultures and Gram-negative bacteria compared to the commercially available dentifrice.[23] Hence, neem has possessing the antibacterial activity; neem oil extract was used in this study.

Around 5% of neem extract will eradicate all the Gram-positive and Gram-negative microorganisms in the culture.[31] Hence, 10% neem oil extract was used for better overall result.

Efficacy of 10% neem oil in indigenously prepared chips adjunct to SRP was evaluated in the present study. Chip form was used in this study for sustained release of drug for around 7 days. According to Franz diffusion method,[32] maximum release of drug from chip was within 7 days. Hence, the period of 7 days for placement of chip was included in the study. Nondegradable neem chips were prepared for this study with the advantages of therapist control over the removal of the device and, therefore, have greater control over the time of exposure of the drug in the pocket.[33]

The clinical parameters such as plaque index, gingival index, sulcus bleeding index, were recorded at baseline, 7th day and 21st day to witness the immediate changes in the parameters after removing chip at 7th day. Parameters such as PPD, RAL were recorded at baseline and 21st day, because connective tissue healing is completed only after 21 days.

Plaque index was significantly reduced at 7th day and still reduced in the 21st day at test and control site. Plaque index score was 3.294 ± 0.519 at baseline while in the 7th day was 1.012 ± 0.174 and in the 21st day was 0.789 ± 0.277 showed significant changes due to optimal oral hygiene maintenance therapy. Gingival index score was 1.520 ± 0.196 at baseline, reduced to 0.566 ± 0.144 at 7th day and in the 21st day, it was significantly reduced 0.329 ± 0.879. No significant changes between test and control site. In this study at baseline, Sulcus bleeding index score was found to be 2.697 ± 0.616. This score was reduced to 0.418 ± 0.163 at 7th day and further reduced to 0.323 ± 0.088 at 21st day. The changes obtained in index scores at test and control site were not statistically significant. The mean PPD was significantly reduced in the 21st day compared to the baseline in both control and test sites. Mean PPD was 4.90 ± 0.316 at baseline while 2.70 ± 0.483 at 21st day in control sites. Mean PPD was 5.10 ± 0.316 at baseline while 2.30 ± 0.483 at 21st day in test sites showed the significant reduction even compared to the control sites. Mean RAL was 5.00 ± 0.471 at baseline while 2.80 ± 0.422 at 21st day in control sites. Mean RAL was 5.20 ± 0.422 at baseline while 2.40 ± 0.516 at 21st day in test sites showed a significant reduction in test sites compared with the control sites.

Microbiological plaque samples were taken at baseline and after SRP (the 7th day). According to Morillo JM, *el al.*, *P. gingivalis* was most predominant and prevalent among all periodontal pathogens in chronic periodontitis. Hence, the present study was planned to evaluate the *P. gingivalis* level in plaque samples qualitatively and quantitatively by PCR assay.[34-38]

According to Jervøe-storm *et al.*,[38] inability of cultivation methods to distinguish between close related bacteria, and the problems of keeping periopathogenic bacteria viable, which are required for standard cultivation, the real-time PCR was found to be a sensitive and specific identification method, with the additional possibility to perform a quantification of specific bacteria in the subgingival plaque samples.[36-38]

In qualitative PCR assay, the present study showed the complete absence of *P. gingivalis* in almost all the test sites at 7th day, while in control sites *P. gingivalis* was present in some of the samples postoperatively at 7th day. This shows the efficacy of neem chip in reducing the micro-organism level significantly when compared to SRP alone. To substantiate the significance level, quantitative PCR assay was performed.

In quantitative PCR assay, *P. gingivalis* was completely absent in test sites at 7th day like that of healthy groups, while, in the control group, it was present even at small number of thermocycle (10^6cells at 17 thermocycles). The results showed that the significant reduction of *P. gingivalis* in neem chip placed site when compared to SRP alone at 7th day. This assay proves the antibacterial efficacy of neem chip in infected sites.

The changes in clinical and microbiological parameters of the present study were revealed the effectiveness of neem in the treatment of chronic periodontitis. Any form of neem could be used either individually or adjunct to any other modalities in effective management of periodontal diseases.

CONCLUSION

The present study involved a comparative clinical and microbiological evaluation of 10% neem chip and SRP

alone in the treatment of chronic periodontitis. From this randomized, controlled clinical study, the following conclusions have been elucidated,

- Sustained release systems prevent the recolonization of pathogens for long period
- Significant reduction in the clinical parameters compared with conventional therapy
- Significant reduction in qualitative and quantitative counts of *P. gingivalis* in response to neem local drug delivery.

Thus, the present study concluded that neem chip can be effectively used as an adjunct to SRP in the treatment of chronic periodontitis and for reduction of microbial load in the subgingival environment.

Though the study has a positive outcome for a particular pathogen; elaborate studies are needed to prove the efficacy of neem in other periodontal pathogens and also with reference to areas such as proper concentration and release system.

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Conflicts of interest
There are no conflicts of interest.

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