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 drug 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

**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.

**Key words:** 10% neem oil, chronic periodontitis, local drug delivery system, Porphyromonas gingivalis
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 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...
Efficacy of 10% neem oil chip on chronic periodontitis

Vennila, et al.

Subgingival plaque. *P. gingivalis* and *Aggregatibacter actinomycescomitans* are the main periodontal pathogens responsible for the periodontal destruction. 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. Nonsurgical periodontal therapy may not completely eradicate these periodontal pathogens, which leads into adjunct systemic antibiotic therapy for complete elimination of the periodontal pathogens.

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. 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. 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.

Tetracycline fibers, chlorhexidine chips 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.

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.

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

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**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 | 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 | 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**
The antibacterial activity of neem has been evaluated and known since ancient times. 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. Rao et al. proved its efficacy in reducing plaque cultures and Gram-negative bacteria compared to the commercially available dentifrice. Hence, neem has possessing the antibacterial activity; neem oil extract was used in this study.

According to Morillo JM, et 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.

According to Jervøe-storm et al., 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.

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 (10³ cells 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.

REFERENCES

1. Saini R, Saini S, Saini SR. Periodontal diseases: A risk factor to cardiovascular disease. Ann Card Anaesth 2010;13:159-61.
2. Slots J, Ting M. Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis in human periodontal disease: Occurrence and treatment. Periodontol 2000 1999;20:82-121.
3. Newman MG, Taki HH, Klokkevold PR, Carranza KA. Carranza’s Clinical Periodontology: 10th ed. Los Angeles: Elsevier; 2006. p. 228.
4. Walker CR. The acquisition of antibiotic resistance in the periodontal microflora. Periodontol 2000 1996;10:79-88.
5. Greenstein G, Polson A. The role of local drug delivery in the management of periodontal diseases: A comprehensive review. J Periodontol 1998;69:507-20.
6. Goodson JM, Tanner A. Antibiotic resistance of the subgingival microbiota following local tetracycline therapy. Oral Microbiol Immunol 1992;7:113-7.
7. Larsen T. Occurrence of doxycycline resistant bacteria in the oral cavity after local administration of doxycycline in patients with periodontal disease. Scand J Infect Dis 1991;23:89-95.
8. Javali MA, Vandana KL. A comparative evaluation of atrigel delivery system (10% doxycycline hydrochloride) in treatment of scaling and root planing in patients with chronic periodontitis. A clinical study. J Indian Soc Periodontol 2012;16:43-8.
9. Steenbergen JD, Bercy P, Koh J. Sub gingival minocycline hydrochloride ointment in moderate to severe chronic adult periodontitis: A random, double blind, vehicle controlled, multicenter study. J Periodontol 1993;64:637.
10. Stetzel M, Floré-de-Jacoby L. Topical metronidazole application compared with subgingival scaling. A clinical and microbiological study on recall patients. J Clin Periodontol 1996;23:24-9.
11. Soskolne WA, Heasman PA, Stabholz A, Smart GJ, Palmer M, Flashner M, et al. Sustained local delivery of chlorhexidine in the treatment of periodontitis: A multi-center study. J Periodontol 1997;68:32-8.
12. Sharma P, Tomar L, Bachwani M, Bansal VM. Review of neem (Azadirachta indica): Thousand problems one solution. Int Res J Pharm 2011;2:97-102.
13. Girish K, Bhat SS. Neem – A green treasure. Electron J Biol 2008;4:102-11.
14. Biswas K, Chattopadhyay I, Banerjee RK, Bandopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Curr Sci 2002;82:1336-45.
15. Cobb CM. Non-surgical pocket therapy: Mechanical. Ann Periodontol 1996;1:443-90.
16. Goodson JM. Antimicrobial strategies for treatment of periodontal diseases. Periodontol 2000 1999:5:142-68.
17. Taninoff N, Hock J, Camosci D, Helldén L. Effect of stannous fluoride mouthrinse on dental plaque formation. J Clin Periodontol 1980;7:232-41.
18. Rams TE, Slots J. Local delivery of antimicrobial agents in the periodontal pocket. Periodontol 2000 1996;10:139-59.
19. Kudva P, Tabasum ST, Shekhawat NK. Effect of green tea catechin, a local drug delivery system as an adjunct to scaling and root planing in chronic periodontitis patients: A clinicomicobiological study. Indian J Soc Periodontol 2011;15:39-45.
20. Virdi HK, Jain S, Sharma S. Effect of locally delivered Aloe vera gel as an adjunct to scaling and root planning in treatment of chronic periodontitis: A clinical study. Indian J Oral Sci 2012;3:84-9.
21. Sen P. Therapeutic potentials of tulsi: From experience to facts. Drugs News Views 1993;1:15-21.
22. Ray D, Sharatchandra KH, Thokchom IS. Antipyretic, anti diarrhoeal, hypolycemic and hepatoprotective activities of ethyl acetate extract of Acacia catechu in albino rats. Indian J Pharmacol 2006;38:408-13.
23. Rao DV, Singh I, Chopra P, Chhabra PC, Ramanujaulu G. In vitro antibacterial activity of neem oil. Indian J Med Res 1986;84:314-6.
24. Wolinsky LE, Mania S, Nachmani S, Ling S. The inhibiting effect of aqueous Azadirachta indica (Neem) extract upon bacterial properties influencing in vitro plaque formation. J Dent Res 1996;75:816-22.
25. Almas K. The antimicrobial effects of extracts of Azadirachta indica (Neem) and Salvadoras persica (Arak) chewing sticks. Indian J Dent Res 1999;10:23-6.
26. Subapriya R, Nagini S. Medicinal properties of neem leaves: A review. Curr Med Chem Anticancer Agents 2005;5:149-6.
27. Mahfuzul Hoque MD, Bari ML, Inatsu Y, Juiceki VK, Kawamoto S. Antibacterial activity of guava (Psidium guajava L.) and neem (Azadirachta indica A. Juss.) extracts against foodborne pathogens and spoilage bacteria. Foodborne Pathog Dis 2007;4:481-8.
28. Botelho MA, Santos RA, Martins JG, Carvalho CO, Paz MC, Azenca C, et al. Efficacy of a mouth rinse based on leaves of neem tree (Azadirachta indica) in treatment of patients with chronic gingivitis: A double blind randomized control trial. J Med Plants Res 2008;2:341-6.
29. Kumar S, Rajasekar T, Anandhi A. A comparative study of in vitro anti bacterial activity of neem and Miswak extracts against isolated cariogenic from dental caries patients. J Chem Pharm Res 2011;3:638-45.
30. Jain S, Kaur H, Brar S. To evaluate the efficacy of neem chip as an adjunct to scaling and root planning in patients with chronic periodontitis. Indian J Dent Sci 2012;4:42-5.
31. Chatterjee A, Saluja M, Singh N, Kandwal A. To evaluate the antagonistic and antipalque effect of an Azadirachta indica (neem) mouthrinse on plaque induced gingivitis: A double-blind, randomized, controlled trial. J Indian Soc Periodontol 2011;15:398-401.
32. Baert B, Boozen J, Burvenich C, Roche N, Stillaert F, Blondeel P, et al. A new discriminative criterion for the development of Franz diffusion tests for transdermal pharmaceuticals. J Pharm Pharam Sci 2002;82:1336-45.
33. Imran MF, Rani A, Dar MM. Stannous fluoride mouth rinse as an adjunct to scaling and root planning in chronic periodontal disease. Periodontology 2000 1996;1:443-90.
34. Morillo JM, Lui L, Sanchez M, Herrera D, et al. A quantitative real-time polymerase chain reaction based on single copy gene sequence for detection of periodontal pathogens. J Clin Periodontol 2004;31:1054-60.
35. Suzuki N, Yoshida A, Nakano Y. Quantitative analysis of multi-species oral biofilms by TaqMan Real-Time PCR. Clin Med Res 2005;3:176-85.

36. Boutaga K, van Winkelhoff AJ, Vandenbroucke-Grauls CM, Savelkoul PH. The additional value of real-time PCR in the quantitative detection of periodontal pathogens. J Clin Periodontol 2006;33:427-33.

37. Boutaga K, Savelkoul PH, Winkel EG, van Winkelhoff AJ. Comparison of subgingival bacterial sampling with oral lavage for detection and quantification of periodontal pathogens by real-time polymerase chain reaction. J Periodontol 2007;78:79-86.

38. Jervøe-Storm PM, Koltzscher M, Falk W, Dörfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. J Clin Periodontol 2005;32:778-83.