The Efficacy of Locally Delivered *Azadirachta indica* (Neem) Extract Gel in Chronic Periodontitis

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Abstract **- The present study aimed to assess the efficacy of locally delivered *Azadirachta indica* (neem) extract gel as an adjunct to nonsurgical periodontal therapy in the management of chronic periodontitis. Plaque control in periodontics range from mechanical debridement of tooth surfaces by professional scaling and root planing and self-performed plaque removal to local and systemic delivery of chemical antimicrobial agents. In recent times, herbal products as novel drugs are tried in local drug delivery. Neem has multi-various actions like anti-microbial, anti-septic, anti-inflammatory properties. This study was conducted at the Department of Periodontology, University of Dental Medicine, Yangon. A total of 44 sites from 44 subjects with chronic periodontitis were included after obtaining a fully-formed consent and were randomly collected by balanced randomization method into two groups. Study group was treated by scaling and root planing with locally delivered neem extract gel whereas control group was treated by scaling and root planing alone. The clinical parameters [Plaque Index (PlI), Gingival Index (GI), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL)] were recorded at baseline and 6th week after treatment. The results showed improvements in clinical parameters at both study and control sites. On comparison of clinical parameters at baseline and 6th week after treatment, a mean change of PPD of 1.79±0.04 in study group and 1.12±0.04 in control group were observed and statistically high significant difference (p<0.05). This study revealed that the adjunctive use of locally delivered neem extract gel has no known side effects and has promising results.**

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Introduction

Periodontal disease is a complex multifactorial disease characterized by destruction of periodontal tissues and loss of connective tissue attachment (Newman *et al*., 2015). Elimination or adequate suppression of putative periodontopathic microorganisms in the subgingival microbiota is essential for periodontal healing (Eley *et al*., 2010). The standard treatment of periodontitis consists of phase I periodontal therapy with the objective of reducing the total bacterial load and changing the environmental conditions of these microbial niches. Although mechanical treatment (scaling and root planing) reduces the level of subgingival bacteria, it does not eliminate all the pathogens which reside deep into the connective tissue and destroy the bone (Jain *et al*., 2012).

Success of any drug delivery system designed to target periodontal infections depends upon its ability to deliver the

antimicrobial agents to the base of pocket, at a bacteriostatic or bactericidal concentration (Ashtaputre & Limaye, 2014). It has been observed that the local route of drug delivery can attain 100-fold higher concentrations of an antimicrobial agent in subgingival sites compared with a systemic drug regimen thereby reducing the total patient dose by over 400 fold avoiding development of drug-resistant at non-oral body sites (Goodson, 1994).

Various locally delivered agents that are successfully used include tetracycline fibers, 10% doxycycline, 2% minocycline, metronidazole and chlorhexidine gluconate, but none are without side effects. These are expensive and easily not available. Recently, research is being conducted for the use of the natural products. One such natural plant which holds the medicinal value is neem (Jain *et al*., 2012). Oil from the leaves, seed and bark possesses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, includeing *Streptococcus mutans, Prevotella intermedia, Capnocytophaga, Fusobacterium nucleatum* and Lactobacillus species (Singh *et al*., 2012).

Neem extract contains (1) Azadirachtin - the active principal compound, (2) glycosides - antimicrobial, (3) sterols, (4) luminols - anti-inflammatory and (5) flavonoids - antioxidant and anti-inflammatory (Moore, 1987). Neem has also shown better efficacy in the treatment of oral infections and plaque growth inhibition in treating periodontal disorders (Pai *et al*., 2004). Based on the assumption of obtaining better efficacy of neem extract in the oral cavity and the properties of ingredients along with the easy availability of neem, with no known adverse reactions and being cost effective, has been selected for this study as a locally delivered agent adjunctive to treatment of periodontitis. Neem leaf extract was formulated in a gel form for the ease of placement and retention at the target site following placement.

Materials and Methods

This randomized control clinical trial was conducted from October, 2015 to September, 2016 at the Department of Periodontology, University of Dental Medicine, Yangon. Subjects were collected from the volunteer patients attending at the Department of Periodontology, University of Dental Medicine, Yangon. Informed consent was obtained from the subjects prior to the study. Neem extract was prepared from the dried leaves of neem collected from the medicinal garden of FAME Pharmaceuticals Industry Co., Ltd, Yangon, Myanmar.

In neem oral gel contained neem extract 25% as active ingredient, carbomer 0.6% as gel base thickener, sorbitol 20% as sweetener, peppermint oil <0.1% as flavor, methylparaben 0.1% as preservative and triethanolamine (TEA) as pH adjuster and water as base. Carbomer was sprinkled in water until thoroughly spread and stirred. The mixture was swelled for 1 hour. TEA was added until pH 6.5 and then gel formed. Sorbitol was added and then, neem extract was also added. Peppermint oil and methylparaben for long shelf-life was added and stirred homogenously. The dosage used was 25 mg to be delivered at the target site.

Systemically healthy 44 subjects including both males and females in the age group of 35-59 years old were selected. Subjects diagnosed to have chronic moderate periodontitis with probing pocket depth 3-5 mm without furcation involvement. Subjects were excluded if they had taken antibiotics two weeks before the study or if they had

received any periodontal treatment in the previous 3 months. Pregnant women, lactating mothers, subjects who had smoking and betel quid chewing habits, subjects with systemic diseases which have adverse effect on periodontal healing such as diabetes mellitus or bleeding disorders and subjects allergic to neem were the other criteria for exclusion.

A total of 44 sites from 44 subjects were randomly collected by balanced randomizing method into two groups, control group and study group. At baseline, thorough history taking and clinical periodontal examination (clinical parameters) were recorded. All study subjects were given scaling and root planing. And then, study group was treated with locally delivered neem extract gel and the treated area was given periodontal pack. The pack was removed after 7 days. On 3rd week follow up, oral hygiene status was checked and oral hygiene measures were reinforced as necessary. And then, on the 6th week follow up, clinical parameters of the selected target sites were recorded.

The following clinical parameters were used to assess the periodontal status; Plaque Index (PlI) (Silness & Loe, 1964), Gingival Index (GI) (Loe & Silness, 1963), Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL). This study was approved by Research and Ethical Committee of University of Dental Medicine, Yangon.

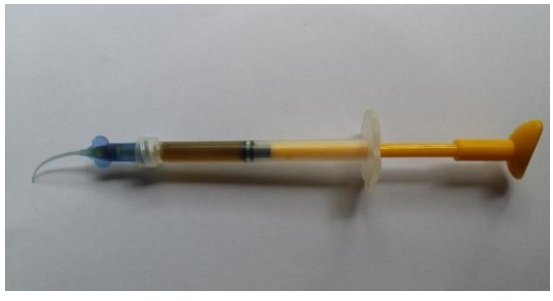

Figure 1. Diaflex Syringe with NEEM Extract Gel

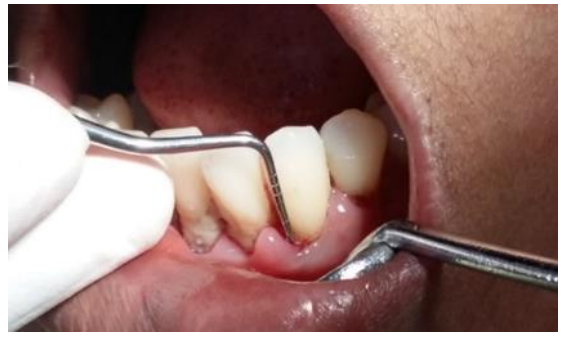

Figure 2. Baseline examination

Figure 3. Delivery of Neem extract gel

Statistical Analysis

The data was entered into the computer using Microsoft excel 2007 and statistical analysis was carried out using computer analysis software SPSS (Statistical Package for Social Science) version 20.0. Descriptive and summary statistics were carried out. For the categorical data, frequency and percentage were calculated and for the continuous data, mean and standard deviation were calculated by Chi-Square tests. Statistical significance of differences in two groups were determined by independent t-test. Statistical significance of differences of before and after treatment for each group were determined by pair t-test. Two tailed $p<0.05$ was considered as statistically significant.

Results

All the subjects in both groups have completed the 6-week clinical

evaluation. In both groups, there was decreased in plaque index after treatment and statistically significant (p<0.05). There were no significant changes between two groups on 6th week after study procedure with a 't' test value of -1.68 and it was not statistically significant (Table 1). The mean values of gingival index were fair rating of score. A statistically highly significant reduction in gingival index was observed at both control group and study group after treatment. There were no significant changes between two groups on 6th week after study procedure with a 't' test value of -0.60 and it was not statistically significant (Table 2).

In the present study, both treatments effectively reduced the probing pocket depth (Table 3). Mean PPD score at baseline (2.98±0.39) was reduced to 1.86±0.43 in control group while mean PPD score at baseline (3.31±0.34) was reduced to 1.52±0.43 in study group. On comparison of baseline and 6th week after treatment, a mean change of PPD of 1.12±0.04 in control group and 1.79±0.04 in study group were observed. With a 't' test value of 2.58, it indicated a statistically high significant difference (p<0.05).

There was statistically high significant gain in clinical attachment level in both groups after treatment (Table 4). On comparison of both groups, although there was no statistically significant difference between two groups after treatment, gain in CAL was greater in study group than control group. No patient reported any discomfort in both groups. No adverse reaction was observed in any subject from study group, without any post-application complications.

Table 1. Comparison of mean plaque index between control group and study group at baseline and 6th week after study procedure

Clinical Parameter	Time	Group	N	Mean	±SD	t-test	*p*-value
PII	Baseline	Control Group	22	1.87	±0.34	-0.22	0.82
		Study Group	22	1.89	±0.26		
	6th week after Study Procedure	Control Group	22	0.10	±0.12	-1.68	0.10
		Study Group	22	0.20	±0.26		

Table 2. Comparison of mean gingival index between control group and study group at baseline and 6th week after study procedure

Clinical Parameter	Time	Group	N	Mean	±SD	t-test	*p*-value
GI	Baseline	Control Group	22	1.78	±0.28	-0.70	0.48
		Study Group	22	1.84	±0.23		
	6th week after Study Procedure	Control Group	22	0.09	±0.11	-0.60	0.54
		Study Group	22	0.11	±0.10		

Table 3. Comparison of mean probing pocket depth between control group and study group at baseline and 6th week after study procedure

Clinical Parameter	Time	Group	N	Mean	±SD	t-test	*p*-value
PPD	Baseline	Control Group	22	2.98	±0.39	-2.96	0.55
		Study Group	22	3.31	±0.34		
	6th week after Study Procedure	Control Group	22	1.86	±0.43	2.58	0.01*
		Study Group	22	1.52	±0.43		

Table 4. Comparison of mean clinical attachment level between control group and study group at baseline and 6th week after study procedure

Clinical Parameter	Time	Group	N	Mean	±SD	t-test	*p*-value
CAL	Baseline	Control Group	22	3.68	±0.67	-2.14	0.96
		Study Group	22	4.12	±0.66		
	6th week after Study Procedure	Control Group	22	2.75	±1.06	-0.20	0.83
		Study Group	22	2.81	±0.75		

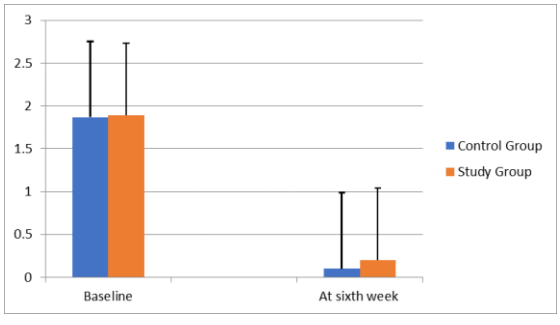

Figure 4. Comparison of mean plaque index between control group and study group at baseline and on 6th week after study procedure

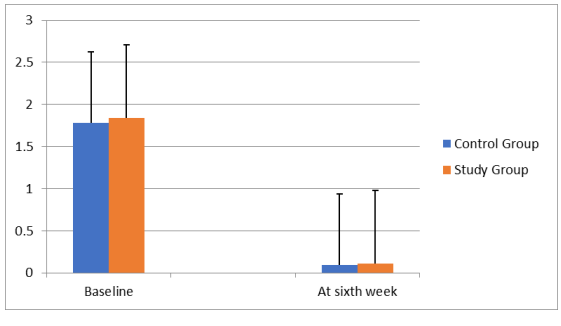

Figure 5. Comparison of mean gingival index between control group and study group at baseline and 6th week after study procedure

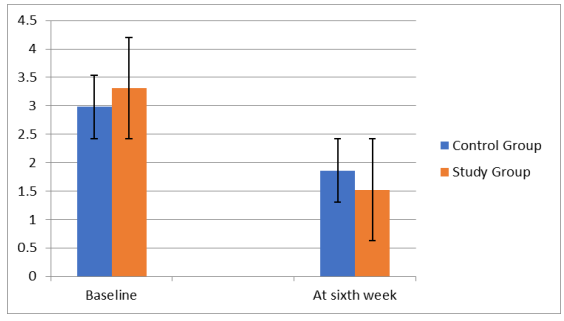

Figure 6. Comparison of mean probing pocket depth between control group and study group at baseline and 6th week after study procedure

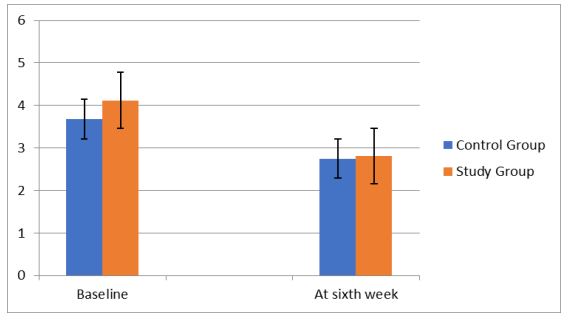

Figure 7. Comparison of mean clinical attachment level between control group and study group at baseline and 6th week after study procedure

Discussion

Extracts of neem, often called "Nature's drugstore", have been used in medicine for over 2500 years and perhaps much longer (Puri, 1999). Neem has multi-various actions like antibacterial, astringent, antiseptic, anti-inflammatory, antiviral and antimicrobial properties. It is easily available and has no known adverse reactions (Jain *et al*., 2012). Locally, it may also have an effect in enhancing healing (Mehta *et al*., 2015). Neem has been used as a locally delivered gel in this study to explore the efficacy in the treatment of patients with chronic periodontitis. Totally in 44 subjects, there

were 18 male and 26 female subjects. Total mean age was 47.91±8.09 years old, minimum was 35 and maximum was 59 years old.

In a 6-week clinical study performed by Pai and coworkers (2004) to evaluate the efficacy of neem extract gel with commercially available chlorhexidine (0.2% w/v) mouthwash as positive control, it was suggested that neem extract gel has significantly (p<0.05) reduced the plaque index (1.588±0.33 at baseline to 0.423 ±0.48) and bacterial count than that of the control group after 6 weeks use of the products.

Jain *et al*., (2012) reported that the clinical evaluation of neem chip showed a statistically significant reduction in probing pocket depth (5.60±0.81 mm, 3.67±0.96 mm, 3.20±0.89 mm at baseline, 6 weeks and 3 months respectively), and a significant reduction in gingival index score was also found at (1.87±0.21 mm from baseline to 1.19±0.30 mm at 3 months) as compared to scaling and root planing alone. There was decrease in plaque index scores and gain in the clinical attachment levels (4.23±0.56 mm at baseline to 3.56±0.67 mm at 3 months) and the results were comparable in both groups at 6 weeks and 3 months evaluation. Though there was gain in the clinical attachment level in both groups but the results were not significant when the two groups were compared at 6th week and at 3rd month after treatment.

In comparative evaluation of the efficacy of neem when incorporated in a local drug delivery system when used as an adjunct to scaling and root planing: a clinico-microbiological study conducted by Mehta and coworkers (2015), PlI, GI and PPD were assessed at baseline, 1 month and 3 months. The results showed reduction in the PPD, GI and PlI as well as the counts of the bacteria in both groups. Mean PPD decreased from 5.81±0.7 at baseline to 3.19±0.3 at 3 months (p<0.05) in neem group.

In the evaluation of the efficacy of neem extract gel as a local drug delivery in the treatment of patients with chronic periodontitis – a double blind randomized clinical trial, Antony *et al*., (2013) recorded the clinical parameters at baseline, 1 month, 3 months and 6 months respectively. The plaque index score showed a statistically highly significant (p<0.000) improvement over a duration of six months. The mean change in PlI at the experimental sites was 0.900±0.25 which was greater than that observed at the control sites (1.350±0.46). A statistically highly significant reduction in GI was observed by a mean change of 0.737±0.24 at experimental site indicated a higher reduction in the GI compared to the control site which showed a mean change of 1.325±0.23 over six months.

On comparison of PPD score of baseline and one month after treatment, it was statistically significant with a 't' test value of 18.38. A statistically highly significant reduction (p<0.000) in PPD and CAL from baseline to 6 months was also noted.

In the present study, it showed that almost all patients maintained a good level of oral hygiene in term of self-performed oral hygiene control measure throughout the study period. This indicated that gingival inflammation and bleeding on probing were improved after treatment. The findings of PlI and GI was consistent with the studies reported by Pai *et al*., (2004), Jain *et al*., (2012) and Antony *et al*., (2013).

The reduction in PPD is more obvious in study group. When the groups were compared to each other at baseline and at 6th week after study procedure, it indicated a statistically high significant difference (p<0.05) between two groups. It can be

assumed that the treatment outcome of study group using neem extract gel showed better efficacy when compared to control group using SRP alone. There was statistically high significant gain in CAL in both groups after treatment. On comparison of both groups, there was no statistically significant difference between two groups after treatment. According to baseline and 6th week measurements, the amount of gain in clinical attachment level in study group was greater than that of gain in clinical attachment level of control group. These results reached consensus with the study conducted by Jain *et al*., (2012) and Antony *et al*., (2013).

Conclusion

The present study cannot be directly compared with the previous studies due to various formulations, difference in composition of neem, different study designs used for evaluation, racial variation and the relatively short duration of the present study involving a small number of subjects. But, the results of all clinical parameters obtained in the present study were in accordance with those of the previous studies where neem extract accelerated healing process through its broad range antibacterial activity.

Within limits superimposed by a relatively smaller sample size, the results demonstrated that both SRP and SRP with neem extract gel produced significant improvements in each group between baseline and follow-up data. The improved clinical resolution of inflammation and destruction in the study group can be explained by the effectiveness of neem extract gel as an anti-inflammatory and antimicrobial agent against periodontal pathogens, which might have prevented microbial recolonization of periodontal pockets.

On comparison between two groups, PlI and GI scores are not significantly different. This may be due to the short term follow up of six weeks and the results showed that there was no difference in plaque deposits and all of the study populations also had similar oral hygiene. The efficacy of neem extract may become better as it was maintained for a longer period of time. This phenomenon helps in maintaining a healthy micro flora for a longer period. Therefore, in future, studies need to be carried out for longer durations with large sample size and also require microbiological analysis for an evidently beneficial clinical outcome. Further standardization of gel formulations should be done and their efficacy should be compared with the gold standard local drug delivery therapeutic agent, chlorhexidine, to evaluate its definitive role in periodontal therapy.

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The authors declare there is no potential conflict of interest.

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