EFFECT OF NEEM EXTRACT ON PERIODONTAL STATUS & AST LEVELS IN CHRONIC PERIODONTITIS : A CLINICAL & BIOCHEMICAL STUDY

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The present clinical study was conducted to assess the effect of neem extract on periodontal status & estimation of AST levels before and after scaling and root planing. 20 patients were selected in the age group of 30-55 years with chronic generalized periodontitis having pocket probing depth e" 5mm.The split mouth design in which test sites were treated with scaling and root planing along with subgingival irrigation of neem extract as a local application on the 0 day, 7th day and 14th day while the control sites were treated with scaling and root planing alone. The clinical parameters of the selected patients were undertaken for both sites at 0 day, 15th day, 30th day and estimation of aspartate transaminase level was done at baseline & 30th day. This clinical study observed significant improvement in periodontal status in both the sites. Test sites showed significant improvement in gingival scores, reduction in pocket depth, gain in clinical attachment level, and reduction in aspartate transaminase level when compared to control sites. This suggests that neem extract is beneficial in improving periodontal status & reducing the AST levels in chronic periodontitis as compared to scaling and root planing alone.

Key words: *Azadirachta indica*, Periodontal pocket, Gingival crevicular fluid, Aspartate aminotransferase.

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions affecting supporting structures of the dentition[1]. Many studies have shown that dental plaque plays a major role in the etiology of periodontal diseases.[3] However, it is the paradoxical impact of the susceptible host's inflammatory response to the microbial challenge that ultimately leads to the destruction of periodontal tissues.

The most effective method of prevention & maintenance of periodontal diseases is mechanical oral hygiene combined with proper professional maintenance. However, in reality the degree of motivation & dexterity required for an optimal oral hygiene level may be beyond the ability of majority of patients.[3]

Herbal medicines history can be rooted from ancient civilization wherein their role as a primary source of medication is evident. Herbs with medicinal properties are a useful and effective source of treatment for various disease processes. They are reported for use in gum and tooth problems and act as a powerful healing agent. Most herbalists and natural healers recommend using warming, astringent, connective-tissue-healing herbs to enhance and maintain oral health.[4]

Azadirachta Indica (Neem) has been used in India throughout southern Asia for many years as a preferred tool for keeping teeth & gums healthy. In dentistry, *Azadirachta indica* has demonstrated a good efficacy in the treatment of periodontal disorders. Recent investigations have shown that *Azadirachta indica* elaborates a vast array of biologically active compounds that are chemically diverse. Various components are responsible for the activity of neem or its extract against plaque development. The antibacterial and anti-inflammatory components make it useful in the treatment of oral infections and it has been used as an extract, infusion of leaves to alleviate toothache.[2] current data have suggested that by-products of *Azadirachta indica* possess several biological properties, such as

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antiviral and oral antimicrobial. Therefore, *Azadirachta indica* can be regarded as a valuable plant source for the rationalization of its use in traditional medicine and for modern drug development as well.[3]

Asparate transaminase (AST) previously termed glutamic oxaloacetate transferase (GOT), is an enzyme used clinically as an indicator of cell injury in pathological conditions in myocardial infarction and liver disease.[5] Significant AST levels have also been found in gingival epithelial cells, human gingival fibroblasts and human periodontal ligament fibroblasts. Cells release AST into the extracellular space during periods of tissue necrosis or trauma, resulting in detectable enzyme quantity in the peripheral circulation. Higher levels of AST are found in the GCF of diseased sites than in healthy sites, and have been reported as a possible marker of periodontally active sites.[6]

Aim of the study

The purpose of this clinical study was to assess the effect of neem extract (*Azadirachta indica)* as subgingival irrigation on periodontal status and quantitative estimation of AST levels in chronic periodontitis.

MATERIALS & METHODS

Source of Data: This clinical study was conducted in Department of Periodontics and Department of Biochemistry, I.T.S.-CDSR, Muradnagar, Ghaziabad, Uttar Pradesh

Inclusion criteria

20 patients with age group of 30 to 55 years with chronic generalized periodontitis having probing pocket depth e" 5mm and patients free from systemic illness.

Exclusion criteria

Patients received antibiotic treatment 6 months prior to study, Inability to comply with the follow-up visit requirements and Pregnant & nursing patients.

Study Design

The nature and design of the clinical trial was explained to the patients and consent was obtained from the patients.

The selected patients having two or more sites with pocket probing depth of e" 5mm or more in one quadrant was measured using University of North Carolina (UNC-15) graduated probe.

The treatment sites were divided into two groups by split mouth design in each patient viz. Test group [scaling and root planing plus subgingival irrigation with neem extract (*Azadirachta indica*)] and Control group [scaling and root planing alone] and gingival crevicular fluid was collected from both sites using calibrated volumetric microcapillary pipettes with 0-5µl range for AST estimation.

The test sites was irrigated with neem extract (*Azadirachta indica*) on the 0 day, 7th day and 14th day. (fig.1) and the clinical parameters was recorded on both sites at 0 day, 15 days, 30th day intervals and samples of gingival crevicular fluid will be collected on 0 day and 30th day for AST estimation. (fig.2)

CLINICAL PARAMETERS

(1) Gingival index (Loe & Silness 1963)
(2) Sulcus bleeding index (Mulhemann 1958)
(3) Clinical attachment level, (CAL)
(4) Probing pocket depth.
(5) Estimation of Aspartate aminotransferase (1U/L per site) in gingival crevicular fluid.

Aspartate transaminase estimation procedure

PRINCIPLE (KINETIC METHOD)

$$\alpha\text{-oxoglutarate} + \text{L-aspartate} \longrightarrow \text{L-glutamate} + \text{oxaloacetate}$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{(MDH)}} \text{L-malate} + \text{NAD}^+$$

Collection of gingival crevicular fluid: Samples of gingival crevicular fluid were collected from both test and control sites by placing calibrated volumetric microcapillary pipettes with 0-5µl range ("Sigma chemical company". St. Lowis, Missouri USA.) using extracrevicular method and collected samples was

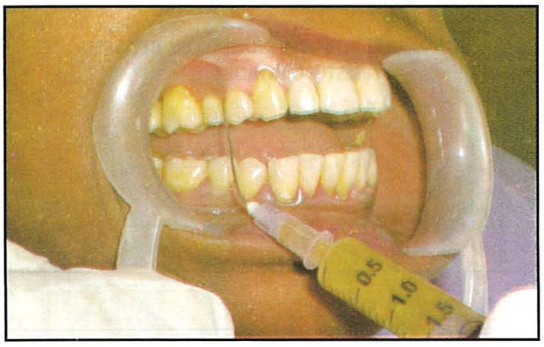


Figure 1. Subgingival irrigation with neem extract **(Azadirachta indica)**

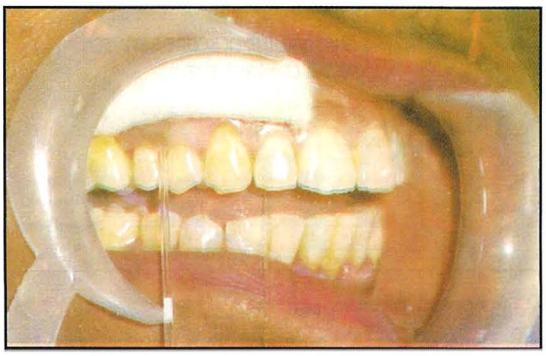


Figure 2. GCF collection using micropipette

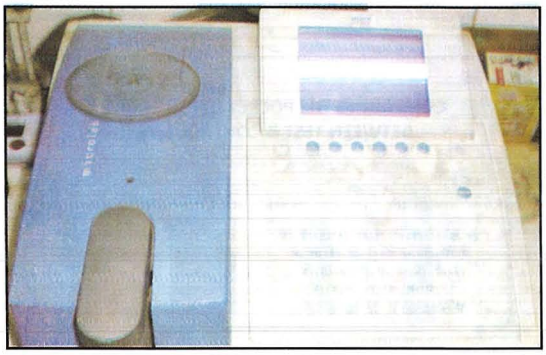


Figure 3. AST semi autoanalyser.

immediately transferred to a sterile bottle containing 100µl of phosphate buffer saline (pH 7, 0.05 M) and sent to the lab for AST analysis.

Determination of Aspartate Transaminase (AST) activity: Aspartate transaminase activity was determined using kinetic method according to the recommendations of the International Federation of Clinical Chemistry (IFCC) using a semi-automated analyzer (Merck).The results obtained are expressed in IU/L. (fig.3)

RESULTS

All 20 patients enrolled in this study reported for schedule post treatment evaluation visits.

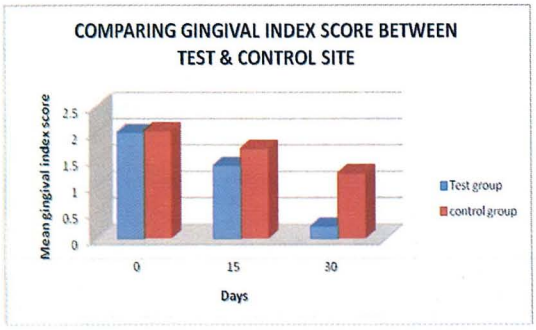
Gingival Index. No statistically significant difference in the mean values for the gingival index was found between the test and control groups at baseline (p =0.8305), but a significant difference was observed at 15 days and at 30 days (p <0.05) (**Table 1 &Graph 1**).

Bleeding Index. No statistically significant difference in the mean values for the gingival index was found between the test and control groups at baseline (p =0.8792), but a statistically significant difference was observed at 15 days and at 30 days (p < 0.05) (**Table 1 & Graph 2**).

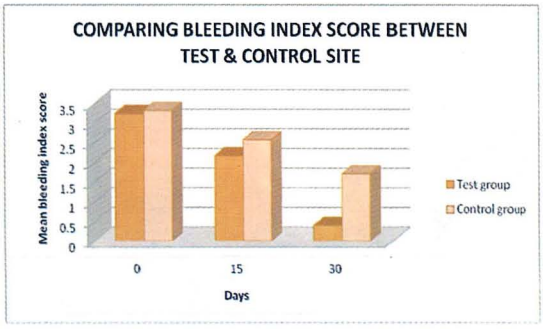
Probing Pocket Depth. No statistically significant difference in the mean values for pocket depth was found between the test and control groups at baseline (p =0.5412). However, the mean value at 15 days and at 30 days (p <0.05) were statistically significant (**Table 2 & Graph no.3**).

Clinical Attachment Level The difference between test and control groups in mean values for levels of clinical attachment at baseline (p =0.3629) and at 15 days (p =1.5415) was not significant. However, the mean values of clinical attachment level at 30 days (p < 0.05) were statistically significant (**Table 2 & Graph no.4**).

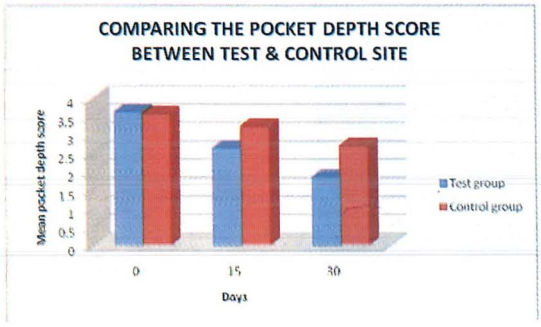
Aspartate transaminase level. No statistically significant difference in the mean values for aspartate transaminase level was found between the test and control groups at baseline



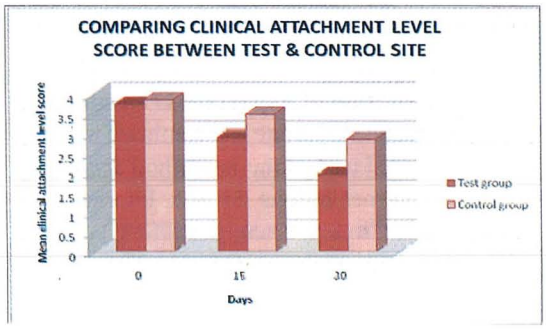
GRAPH 1



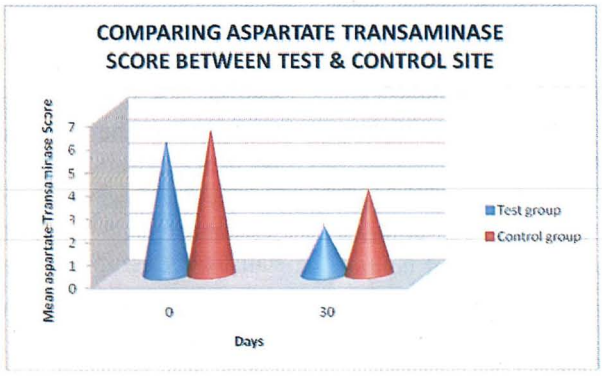
GRAPH 2



GRAPH 3



GRAPH 4



GRAPH 5

TABLE–III

	TIME	TEST		CONTROL		
		Mean ± SD	% Change from Baseline	Mean ± SD	% Change from Baseline	p-value
ASPARTATE TRANSAMINASE (AST)	BASELINE	5.682±2.4483	-	6.1385±3.4559	-	0.4820
	30 DAYS	2.0305±1.3545	64.26%	3.5895±1.9245	41.52%	p < 0 . 0 5

Student't'- test, p<0.05 is significant at level of significance 0.05

TABLE–I

	TIME	TEST		CONTROL		
		Mean ± SD	% Change from Baseline	Mean ± SD	% Change from Baseline	p-value
GINGIVAL INDEX	BASELINE	2.0125±0.0559	-	2.0375±0.1223	-	0.8305
	15 DAYS	1.3875±0.1512	31.05%	1.7±0.1739	16.56%	p<0.05
	30 DAYS	0.2375±0.1716	88.19%	1.2375±0.2977	39.26%	p<0.05
SULCUS BLEEDING INDEX	BASELINE	3.25±0.2294	-	3.325±0.3045	-	0.8792
	15 DAYS	2.175±0.3636	33.07%	2.575±0.2821	22.56%	p<0.05
	30 DAYS	0.4±0.3077	87.69%	1.7125±0.4157	48.49%	p<0.05

TABLE–II

	TIME	TEST		CONTROL		
		Mean ± SD	% Change from Basclinc	Mean ± SD	% Change from Baseline	p-value
PROBING POCKET DEPTH	BASELINE	3.6375±0.3292	-	3.575±0.3981	-	0.5412
	15 DAYS	2.65±0.5281	27.14%	3.225±0.4652	9.79%	p<0.05
	30 DAYS	1.8625±0.3670	48.79%	2.7±0.4631	24.47%	p<0.05
CLINICAL ATTACHMENT LEVEL	BASELINE	3.738±0.8326	-	3.8475±1.0615	-	0.3629
	15 DAYS	2.879±1.0153	22.98%	3.4805±1.1419	9.53%	1.5415
	30 DAYS	1.955±0.7613	47.69%	2.8385±0.9570	26.22%	p<0.05

(p =0.4820). However, the mean value at 30 days ($p < 0.05$) were statistically significant (**Table 3 & Graph no.5)**

DISCUSSION

The prevalence and severity of periodontal disease can be reduced by both total plaque control and by selective removal or inhibition of pathogenic microflora using either systemic or locally applied anti-microbial agents. Conventional treatment methods include mechanical (SRP) removal of bacterial plaque to eliminate or reduce the number of periodontal pathogens. However, the microbiologic effects of SRP rarely result in complete removal of periodontal pathogens and appear to be somewhat self-limiting because bacterial pathogens may recolonize after treatment. This lead to the adjunctive use of antibacterial agents either in the form of systemic or local applications to overcome the efficacy of the conventional treatment. [7]

Many researchers have started searching for herbal and alternative medicine as a natural option for treating various conditions. This is due largely to the prohibitive cost of many prescription drugs, and the potential for dangerous side effects that are often associated with this medications.[4]

Neem leaves and stems are used as a preferred method for maintaining healthy teeth and gums. The isolation of bioactive compounds from *Azadirachta indica*, commonly known as the Neem plant, has led to an expanding number of scientific reports on its other interesting biological properties and uses. Neem plant might also contain chemotherapeutic antiplaque agents should not be overlooked.[8] The overall usage of neem either as such or in various commercial preparations has been limited due to its bitter taste.

Nimbidin isolated from neem oil and sodium nimbidinate has shown dose dependent anti-inflammatory activity. Antipyretic activity has also been reported and confirmed in nimbidin. Mahmoodin & Nimbolide, a deoxygedunin isolated from seed oil, has been shown to possess moderate antibacterial action against gram – negative and gram-positive microorganisms.[9]

The gingival index and bleeding index of experimental period i.e. on days 0, 15th and 30th was compared in both the test site and control site. Results showed a highly significant change with regard to improvement in the gingival inflammation and reduced bleeding on probing on both the control and the test site from the baseline but the reduction was more in test site. The definite reductions exhibited could be due to regular follow up visit, reduction in pocket depth and reinforcement of oral hygiene instruction followed by the patient throughout the study period. Similar findings were reported by **Pai MR, Acharya LD et al.,[2], Botelho MA ,Santos RA, Marins JG et al.**[3]

Gains in clinical attachment levels and reduction in probing depth are the most common parameters used to measure clinical improvement. Decrease in **probing depth** in test group between baseline and 30 days was 48.79% as compared to control group which showed a decrease of 24.47%. Our study demonstrated statistically significant reduction in the probing pocket depth in both the test and the control groups. However, the test group demonstrated more reduction of the probing depths compared to the control group at 30 days interval.

The study demonstrated a statistically significant increased gain in the **attachment levels** in both the test and the control groups. This gain in clinical attachment from baseline to 30 days was 47.69% for test group whereas for control group it was 26.22% only indicating that the gain in clinical attachment level in test group was more than the control group.

The levels of GCF aspartate transaminase level were reduced significantly in both groups after initial periodontal therapy corresponding with improved periodontal status. The reduction in GCF aspartate transaminase level from baseline to 30 days was 64.26% in test group whereas for control group it was 41.52% only indicating greater reduction in test group. This could be attributed to the fact that AST is a non-specific marker for cell death & tissue

destruction, and can increase in both gingivitis and periodontitis; hence the level of GCF aspartate transaminase may have reduced due to reduction in gingival inflammation and probing pocket depth.[10]

As evident from this clinical study, it can be suggested that neem extract along with SRP shows beneficial results in improving periodontal status mainly gingival scores, reduction in probing pocket depth, gain in clinical attachment level and reducing the AST levels in chronic generalized periodontitis as compared to scaling and root planing alone.

However further long term clinical and biological studies should be undertaken to obtain more clinical evidence for regular use of neem extract on clinical parameter for a larger sample.

BIBLIOGRAPHY

1. Giannobile W, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT . Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000, 2009;50:52-64
2. Pai MR, Acharya LD, Udupa N. The effect of two different dental gels and a mouthwash on plaque and gingival scores: a six-week clinical study. Inter Dent J 2004;54:219-223
3. Botelho MA, Santos RA, Martins JG, Carvalho CO, Paz MC, Azenha C et al. Efficacy of a mouthrinse based on leaves of the neem tree (Azadirachta indica) in the treatment of patients with chronic gingivitis: A double-blind, randomized, controlled trial.J Med Plants Res.2008;2:341-346.
4. Flora Parsa Stay. Herbal Therapy in Dentistry. The complete Book of Dental Remedies. Avery Publishing Group 1996.
5. Cohen R.L., Alves M.E.A.F., Crawford J.M., Mc Swiggin and Chambers D.A. Association of Gingival crevicular fluid Aspartate Aminotransferase levels with histopathology during ligature induced periodontitis in the beagle dogs. J Dent Res 1991; 70: 984-987.
6. Barbosa Silva E, Salvador S, Fogo J, Marcantonio A. Use of aspartate aminotransferase in diagnosing periodontal disease: a comparative study of clinical and microbiological parameters. J Oral Science 2003;45:33-38
7. Connie Hastings Drisko. Nonsurgical periodontal therapy. Periodontology 2000. 25, 2001: 77-88.
8. Wolinsky LE, Mania S, Nachnani 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(2):816-822
9. Biswas K, Chattopadhyay I, Banerjee RK, Bandyopadhyay U. Biological activities and medicinal properties of neem (*Azadirachta indica*).Current science 2002;82(11):1336-1345
10. Oringer RJ, Howell TH, Nevins ML, Rosenor DS, Davis GH, Sekler J, Fiorellini JP. Relationship between crevicular Aspartate Aminotransferase levels and periodontal disease progression. J Periodontol 2001; 72:17-24

DR. T. SAMRAJ RECEIVES LIFETIME ACHIEVEMENT AWARD

Dr. T. Samraj, Secretary & Treasurer of PFA was honored with the Life Time Achievement Award by the Tamilnadu Dr.MGR Medical University on Dentists Day, March 06, 2011. This is the first time in the 24 years of its existence that the Tamilnadu Dr.MGR Medical University has honored a Dentists with Life Time Achievement Awards.



Photograph shows the Vice-Chancellor of the Tamilnadu Dr.MGR Medical University, Dr. Mayil Vahanan Natarajan, presenting the Award. Heartiest Congratulations Dr. Samraj.