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Evaluation Of Subgingival Fusobacterium Nucleatum Levels After Srp With Adjunctive Aloe Vera As Ldd In Chronic Periodontitis – A Randomized Controlled Trial

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Abstract:
Context: Periodontitis is the inflammatory disease results from the interaction between microorganisms and immune response in a susceptible host. Porphyromonas gingivalis, Tanerella forsythia, Treponema denticola, Fusobacterium nucleatum are most notorious organisms implicated in destruction of periodontal tissues. Scaling and Root planing (SRP) is considered as "Gold Standard" initial treatment of periodontitis. As an adjunct to SRP, Naturopathic medicines as Local drug delivery (LDD) system has become an effective treatment option. Aloe Vera species is extensively used in Periodontology as LDD due to its easy availability and innumerable medicinal properties with minimal or no adverse effects.
Aims: To Compare Subgingival Fusobacterium nucleatum levels and Clinical parameters after SRP with Adjunctive Aloe vera as LDD in Chronic Periodontitis patients. Settings and Design: A Split Mouth, Double Blind, Randomized Controlled Trial.
Methods and Material: 35 subjects were randomized to test (SRP with Aloe vera gel) and control group (SRP alone). Clinical parameters (PPD, CAL, GBI and PI) were recorded and plaque samples were collected for F. nucleatum assessment. All subjects received SRP and test group alone received subgingival delivery of 0.5ml Aloe vera gel. Parameters were reassessed at 1 and 3 months.
Statistical analysis used: Mann Whitney U test, T test & Repeated measures ANOVA. Results: Post-Op 3-months RT-PCR showed statistically significant values (p-value=0.01*) between both groups from baseline till 3 months.
Conclusion: Since Aloe vera gel reduces levels of F. nucleatum and improves clinical parameters, it can be used as LDD.
Key-words: Aloe vera, Fusobacterium nucleatum, Local drug delivery.

Introduction:

Periodontitis is the multifactorial inflammatory disease which results from the interaction between microorganisms and immune response in a susceptible host. Supragingival plaque plays a primary role in the initiation of periodontitis.[1, 2]

Chronic periodontitis is the most prevalent form of periodontal disease characterized by pocket formation, clinical attachment loss, alveolar bone destruction and ultimately leads to tooth loss. Periodontitis can be treated in two ways, non-surgical and surgical methods. Non-surgical treatment (SRP) helps in eliminating the supra and subgingival plaque and calculus.[1, 3] Common bacteria isolated in periodontitis patients include P. gingivalis, P. intermedia, T. forsythia, T. denticola, F. nucleatum, E. corrodens, C. rectus and Aa. comitans.[4] In inaccessible areas such as furcation and deep periodontal pockets, SRP cannot effectively remove the plaque and calculus. Hence, some anaerobic bacterial species like Aa. comitans, P. gingivalis, F. nucleatum cannot be eliminated which potentially leads to treatment failure.[3] Since few periodontal microorganisms are tissue invading, SRP alone is insufficient to eliminate the bacteria, which marks the use of systemic antibiotics. But, use of oral antibiotics may lead to systemic toxicity, adverse effects, and development of super resistant bacteria.[3] In order to combat them, LDD systems emerged as an effective treatment option in adjunct to SRP since they have primary advantage of maintaining consistent and prolonged concentration profiles.[5] Several drugs like Tetracyclines, Triclosan, Doxycycline, Minocycline, Metronidazole, Chlorhexidine, Periochip, Periocol which can act as LDD systems are used in improving periodontal health. Various native plant extracts are under the limelight in periodontal treatment.[1] Due to proven anti-inflammatory, healing properties with very minimal or no side effects, natural herbs as LDD agents in treatment of periodontal conditions has increased multifold. Ayurveda medical system uses over 1200 natural medicinal herbs to cure various diseases since they have antibacterial activity against various microorganisms. Fewer side effects and prolonged usage makes them superior over chemical drugs. Herbs such as green tea, Cordiaverbenacea, Mikanialaevigata,[5] Terminaliachebula, Azadirachta indica, piper betle when used as an adjunctive therapy in combination with SRP showed better results than SRP alone.

One of the most common and widely accepted herbs is the Aloe Vera species (perennial succulent xerophyte) belonging to the Liliaceae family with about 360 species.[5] It has been used in various medical, cosmetic, nutraceutical purposes for many centuries for its therapeutic properties since they contain 75 active ingredients such as vitamins, enzymes, minerals, sugars, lignins, saponins, salicylic acids, amino acids and anthraquinones.[2] In 1500 BC, Alexander the Great used them to help his banner man for healing the battle wounds and named it as "the plant of immortality." Modern usage of aloe vera was reported first by Dr. C. E. Collins in 1934 for radiation burns. Aloe vera has been used for centuries for its laxative, anti-inflammatory, immuno stimulant, antiseptic, anti-diabetic, anti-ulcer, anti-arthritic, cancer and anti-bacterial effects.[6, 7] In dentistry, it is been extensively used in treating various diseases like gingivitis, periodontitis, herpetic stomatitis, oral lichen planus, leukoplakia, oral submucous fibrosis, recurrent aphthous ulcers, chemical burns, abscesses, dry socket, lichen planus, benign pemphigus gingival problems associated with AIDS, leukemia, burning mouth syndrome, denture sore mouth, candidiasis, desquamative gingivitis, vesiculobullous diseases and xerostomia.[6] Fusobacterium nucleatum, one of the most implicated organisms in periodontal disease, is a gram negative, cigar-shaped bacillus. They induce apoptotic cell death in mononuclear and poly-morphonuclear cells and can trigger the release of cytokines, elastase, and oxygen radicals from leukocytes.[3] These organisms co-aggregate with most of the microorganisms and are important bridging organisms between the primary and secondary colonizers. Many authors reported that Fusobacterium nucleatum was most commonly isolated in chronic periodontitis.[1]

To the best of our knowledge, no clinical studies have evaluated the antibacterial effect of aloe vera gel on subgingival Fusobacterium nucleatum. Hence, in this study, effort was taken to clinically evaluate their levels by using locally delivered aloe vera gel.

Methods:

The clearance from Institutional Review Board (Ref: SVDC/IRB/2021/2701/PG/THESIS/05) and Institutional Ethical committee (No:42/SVMCH/IEC-Cert/Feb21) was obtained. Study was registered in Clinical trial database (CTRI/2022/08/044625) and ethical principles as enumerated in Helsinki declaration were meticulously followed during the study.[8] All subjects received the detailed information sheet about the study and gave written informed consent.

Systemically Healthy subjects between 25-50 years of age with Chronic Periodontitis having Bilaterally comparable periodontal defect with Probing Pocket Depth (PPD) of 5-7 mm & Clinical Attachment Level (CAL) of ≥3mm were included in the study. Patients with any history of periodontal or antibiotic therapy for past 6 months, any known disease or intake of medication that affects periodontal status, teeth with poor periodontal prognosis, patients allergic to herbal medications, aggressive periodontitis, current smokers, alcoholics, pregnant or lactating females were excluded from the study.

Sample size calculation using the Gpower computer program (Faul&Erdfelder, 2007) indicated a total sample of 70 (35 in each group) would be needed to detect large effects (d=0.70) with 10% attrition. Hence, 35 patients (Split mouth - 70 sites) fulfilling the inclusion criteria who reported to Department of Periodontology, Sri Venkateshwaraa Dental College, Puducherry, were included in this study. They were divided into two groups. Control (SRP only), and Test (SRP followed by Adjunct Aloe vera as LDD). Randomization was done using a computer-generated sequence. Allocation concealment was done with SNOSE (Sequentially Numbered Opaque Sealed Envelope) method.

Individual acrylic stents were prepared for standardization of clinical measurements. All the clinical measurements, Probing pocket depth (PPD), Clinical Attachment Level (CAL) **(Fig 1)**, Gingival Bleeding Index (GBI - Ainamo and Bay in 1975) and Plaque Index (PI- Silness and Loe in 1964) were carried out by a single masked examiner at baseline and post-op 1 and 3 months. For estimation of F.n levels, Subgingival Plaque sample was collected by placing the sterile 30s paper points into the periodontal pockets of Control and Test sites for 20 seconds at baseline and Post-op 3 months **(Fig 2)**. These paper points were then transferred to Eppendorf tubes containing buffer solution and stored in -80°C. F.n level was estimated using RT- PCR assay.

Subjects from both groups underwent SRP. The parenchyma of aloe vera was squeezed well to obtain fresh aloe vera gel **(Fig 3)**. 0.5ml of freshly prepared aloe vera gel was loaded and delivered subgingivally using blunt cannula **(Fig 4)**. Periodontal pack was placed to ensure the stability of injected aloe vera gel**.** The patients were advised not to brush or disturb the site. The subjects were recalled after 7 days. Periodontal dressings were removed and site was irrigated with saline. All the treated sites healed uneventfully. Oral hygiene instructions were reinforced. The study subjects were followed up at 1st and 3rd month **(Fig 5)**. Clinical parameters were recorded during follow-up visits.

Results:

1) Comparison of Fusobacterium nucleatum (F.n) levels at baseline and 3 months:

RT-PCR assay have been used to detect F.n levels. In this, positive reaction is detected by accumulation of a fluorescent signal. The number of cycles required for the fluorescent signal to exceed background levels is the Cycle threshold (Ct) value. Ct score are inversely proportional to the qualitative amount of target nucleic acid present in the sample. [i.e., Lesser the Ct values, more the (target nucleic acid) organisms present in the sample and vice versa].[9] Every 3-point increase in Ct value is roughly equivalent to a 10-fold decrease in the quantity of viral genetic material.[10] The Ct values of F.n levels has been represented as mean values. For intra and intergroup comparisons of F. nucleatum, Mann Whitney U test have been used. Inter and Intragroup Post-op 3 months RT-PCR values showed statistically significant values (p-value=0.01*) between the groups. Aloe vera group has higher Ct values of F. nucleatum, whereas Control group shows lesser Ct values of F. nucleatum at 3 months. This reveals that Aloe vera group showed increased reduction in the Subgingival F.n comparing the control group **(Table 1)**.

2) Comparison of Pocket Depth (PD) values at baseline, 1 month and 3 months:

Intergroup comparisons of PD values showed statistically significant values at 3 months in Mesiobuccal (pvalue=0.01), Midbuccal (pvalue=0.04) and Distobuccal sites (pvalue=0.04). Intragroup comparisons of PD values in Aloe vera group showed statistically significant values at two different intervals in Disto-buccal site, (1 Month Vs 3 Months: pvalue=0.02) and (Baseline Vs 3 Months: pvalue=0.01). Similarly, Control group showed statistically significant values at two different intervals, (1 Month Vs 3 Months: pvalue=0.03) in Mid-buccal site, and (Baseline Vs 3 Months: pvalue=0.03) in Disto-buccal site **(Table 2)**. Both Aloe vera and control groups show gradual decrease in pocket depth, But there was

higher reduction of PD mean values in aloe vera group.
3) Comparison of Clinical Attachment Level (CAL) values at baseline, 1 month and 3 months:
Intergroup comparisons of CAL values showed statistically significant values at 3 months only in Disto-buccal site (p-value = 0.02). Intragroup comparisons of CAL values in both groups showed statistically significant values at a same interval (Baseline Vs 3 Months) in Disto-buccal site, where Aloe vera showed (pvalue=0.01) and Control group showed (pvalue=0.02) **(Table 3)**. Both the groups show gradual increase in Clinical attachment level from baseline to 1 month and 3 months in all 3 sites. But comparing the control groups, there was higher increase of CAL mean values in aloe vera group.
4) Comparison of Gingival Bleeding Index (GBI) values at baseline, 1 month and 3 months:
Intergroup group comparisons showed statistically significant values at 3 months only in Disto-buccal site (p-value=0.02). Intragroup comparisons showed statistically significant values only in Aloe vera group at (Baseline Vs 3 months: pvalue=0.02) **(Table 4)**. Comparatively there was higher reduction of GBI mean values in aloe vera group than control group from baseline till 3 months.
5) Comparison of Plaque Index (PI) values at baseline, 1 month and 3 months:
Intergroup group comparisons showed statistically significant values at 3 months only in Disto-buccal site (p-value=0.01). Intragroup comparisons of PI values showed statistically significant values only in Aloe vera group at (Baseline Vs 3 months: pvalue=0.01) **(Table 5)**. Comparatively there was higher reduction of plaque index mean values in aloe vera group than control group from baseline till 3 months.
Discussion:

Periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth.[3] Though SRP is considered as Gold Standard for periodontal treatment,[5] adjunct use of antibiotics may be indicated as bacteria can invade periodontal tissues. But they may cause hypersensitivity reactions. Aloe vera is widely used as LDD in periodontal treatment due to its inherent healing and antimicrobial property.[11] The effective anti-bacterial activity of aloe vera against various pathogens have been previously reported, except Fusobacterium nucleatum. Hence, effort was taken to clinically evaluate the Subgingival F.n levels by using locally delivered aloe vera gel.

Bashir A et al,[12] compared the antimicrobial activities of aloe vera extracts against skin infections and found 100% efficacy against gram negative isolates pseudomonas aeruginosa and 75.3% efficacy against gram positive pathogens S. aureus, S. epidermidis, S. pyogenes. Whereas, Fani M et al,[13] estimated the inhibitory activity of Aloe vera gel on isolated cariogenic and periodontopathic bacteria and stated that S. mutans was most sensitive to Aloe vera gel. Jain J et al,[14] proved that aloe vera gel showed antibacterial effect at higher concentrations (100% and 50%, p-value <0.001). Active ingredients in the Aloe vera gel such as Acemannan, aloin, aloemodin, aloemannanaloeride, naftoquinones, methylchromones, flavonoids help provides antibacterial action against potential pathogens thereby, subsequently reducing the plaque formation. Similar to these studies, results from this trial demonstrated that application of fresh Aloe vera gel was effective not only in reducing the subgingival F.n levels, but also helped in achieving improved clinical parameters (PPD, CAL, BI, PI). The mean reduction of subgingival F. nucleatum in aloe vera group was (4.49) and in control group was (-1.03) during 3rd month review. This implies the reduced counts of aloe vera species in test sites comparing control group. To the best our knowledge, this is the first study to evaluate the effect of aloe vera gel against subgingival F. n levels both clinically and microbiologically.

Harjit Kaur Virdi,[15] observed significant reduction of probing depth in SRP and aloe vera group (PPD > 0.1771) in 6 months from baseline comparing SRP alone. Sharmistha Vijay,[16] obtained greater mean reduction of PPD in aloe vera group from Baseline to 3 months (*p*value=0.003), comparing control group in chronic periodontitis patients. Analogous

results were observed in our study, 3 months post operative results showed statistically significant reduction of PPD, in all the sites (Mesiobuccal, Distobuccal: pvalue=0.01*), (Midbuccal: pvalue=0.04) in Aloe vera group comparing control group. Prakash Singh et al,[17] noted significant reduction 34.05% (6.93±0.85 to 5.48±0.87) of CAL in aloe vera group comparing control group 21.34% (56.70±0.73to 5.27±0.91). Sharmistha Vijay[16] found mean reduction of CAL in aloe vera group from Baseline to 3 months (*p*value=0.003) comparing control group. Similarly in our study, the mean reduction of CAL in aloe vera group in all 3 sites (Mesio-buccal, Mid-buccal and Disto-buccal) from baseline till 3 months was noted. Among all the assessed sites, Distobuccal site showed statistically significant CAL reduction (*p*value=0.02*).

Sharmistha Vijay,[16] noted significant reduction of bleeding index in aloe vera group from Baseline to 3 months (pvalue=0.003) comparing control group in chronic periodontitis patients. Similarly in our study, Post-op 3 months results showed significant reduction of bleeding index scores in aloe vera group (*p*value=0.02*). Elsadek et al,[18] mentioned that neoplastic activity of aloe vera is due to the effect of anthraquinone, sterol and lupeols. It reduces oedema of the soft tissues and exhibits strong antiseptic action in periodontal pockets.[2] Sharmistha Vijay,[16] observed mean reduction of plaque index in aloe vera group from Baseline to 3 months (*p*value=0.003) comparing control group in chronic periodontitis patients. explains the anti -bacterial property of aloe vera. Similarly in our study, Post-op 3 months results showed significant reduction of plaque index scores in aloe vera group (*p*value=0.01*). Recently, Hudwekar et al,[19] revealed usage of aloe vera extract was effective in improving the healing scores after periodontal flap surgery. Binding of aloe vera components to insulin-like growth factor receptor, presence of Mannose-6-phosphate accompanied by higher levels of hyaluronic acid and dermatan sulfate, were suggested to stimulate collagen synthesis, and improve healing.

In accordance with previous studies, subgingival application of Aloe vera gel markedly improved the clinical parameters in chronic periodontitis patients as evidenced by decrease in probing depth, gain in CAL, reduced bleeding, and plaque index scores. Most importantly it reduces subgingival levels of Fusobacterium nucleatum, one of the main pathogenic organisms in causing periodontitis. Several studies have utilized commercially available[1] or pre formulated aloe vera gel.[20] Fresh aloe vera extract used in our study has the primary advantage of not containing preservatives or additives and moreover, no adverse effects were observed in our study, implying its safety for clinical use. However, it is possible that the concentration of different active ingredients may vary when harvested at different time points. Since aloe vera is an inexpensive readily available natural plant extract, with minimal or no side effects, this nature's gift can be used as LDD routinely in the treatment of chronic periodontitis.

Parameters	Groups	N	Mean/ Ct values	SD	t value	*p* value
F. Nucleatum **(At Baseline)**	Alovera	35	32.02	3.14	0.646	0.520
	Control	35	35.55	3.76		
F. Nucleatum **(At 3 months)**	Alovera	35	33.50	3.27	2.550	**0.01***
	Control	35	31.06	4.61		

Table 1: Inter-Group Comparison of Fusobacterium nucleatum (Ct) levels between Aloe vera and control groups at baseline and 3 months.

Parameters	Groups	N	Mean/ Ct values	SD	t value	*p* value
F. Nucleatum	Alovera **(At Baseline)**	35	32.02	3.76	-3.727	**0.001***
	Alovera **(At 3 months)**	35	35.50	4.61		
F. Nucleatum	Control **(At Baseline)**	35	32.55	3.14	3.611	**0.001***
	Control **(At 3 months)**	35	31.06	3.27		

Table 2: Intra-Group Comparison of Fusobacterium nucleatum (Ct) levels in Aloe vera and control groups (Baseline Vs 3 months).

	Groups	N	PD at Baseline			PD at 1 Month			PD at 3 Months		
			Mean	SD	*p* value	Mean	SD	*p* value	Mean	SD	*p* value
Mesio-buccal	Alovera	35	6.17	0.74	0.37	4.02	1.22	0.25	3.34	0.83	**0.01***
	Control	35	6.00	0.84		4.37	1.28		3.91	1.06	
Mid-buccal	Alovera	35	4.85	0.91	0.52	2.80	0.99	0.13	2.28	0.71	**0.04***
	Control	35	4.71	0.95		3.25	1.48		2.74	1.12	
Disto-buccal	Alovera	35	6.11	0.79	0.29	3.94	1.32	0.17	3.25	0.91	**0.01***
	Control	35	5.91	0.78		4.37	1.28		3.91	1.06	

Table 3: Inter-Group Comparison of PD values between Aloe vera and Control groups at baseline, 1 month and 3 months.

PPD	Groups	N	Mesio-buccal			Mid-buccal			Disto-buccal		
			Mean	SD	*p* value	Mean	SD	*p* value	Mean	SD	*p* value
Baseline Vs 1 Month	Alovera	35	6.17	0.74	0.00	4.85	0.91	0.00	6.11	0.79	0.00
	Alovera	35	4.02	1.22		2.80	0.99		3.94	1.32	
1 Month Vs 3 Month	Alovera	35	4.02	1.22	0.00	2.80	0.99	0.00	3.94	1.32	**0.02***
	Alovera	35	3.34	0.83		2.28	0.71		3.25	0.91	
	Alovera	35	6.17	0.74	0.00	4.85	0.91	0.00	6.11	0.79	**0.01***

Baseline Vs 3 Month	Alovera	35	3.34	0.83		2.28	0.71		3.25	0.91	

Table 4: Intra-Group Comparison of PD values in Aloe vera group.

PPD	**Groups**	**N**	**Mesio-buccal**			**Mid-buccal**			**Disto-buccal**		
			Mean	**SD**	***p* value**	**Mean**	**SD**	***p* value**	**Mean**	**SD**	***p* value**
Baseline Vs 1 Month	Control	35	6.00	0.84	0.00	4.71	0.95	0.00	5.91	0.78	0.00
	Control	35	4.37	1.28		3.25	1.48		4.37	1.28	
1 Month Vs 3 Month	Control	35	4.37	1.28	0.07	3.25	1.48	**0.03***	4.37	1.28	0.07
	Control	35	3.91	1.06		2.74	1.12		3.91	1.06	
Baseline Vs 3 Month	Control	35	6.00	0.84	0.00	4.71	0.95	0.00	5.91	0.78	**0.03***
	Control	35	3.91	1.06		2.74	1.12		3.91	1.06	

Table 5: Intra-Group Comparison of PD values in Control group.

Site	**Groups**	**N**	**CAL at Baseline**			**CAL at 1 Month**			**CAL at 3 Months**		
			Mean	**SD**	***p* value**	**Mean**	**SD**	***p* value**	**Mean**	**SD**	***p* value**
Mesio-buccal	Alovera	35	7.11	1.79	0.48	4.94	1.79	0.48	4.14	1.26	0.76
	Control	35	6.80	1.90		5.22	1.59		4.68	1.25	
Mid-buccal	Alovera	35	5.60	1.37	0.48	3.37	1.39	0.25	2.82	0.92	0.81
	Control	35	5.34	1.64		3.37	1.53		3.25	1.09	
Disto-buccal	Alovera	35	6.85	1.37	0.24	4.85	1.91	0.67	3.88	1.23	**0.02***
	Control	35	6.45	1.46		5.02	1.40		4.51	1.03	

Table 6: Inter-group Comparison of CAL values between Aloe vera and Control groups at baseline, 1 month and 3 months.

CAL	**Groups**	**N**	**Mesio-buccal**	**Mid-buccal**	**Disto-buccal**

			Mean	SD	*p* value	Mean	SD	*p* value	Mean	SD	*p* value
Baseline Vs 1 Month	Alovera	35	7.11	1.79	0.00	5.60	1.37	0.00	6.85	1.37	0.00
	Alovera	35	4.94	1.79		3.37	1.39		4.85	1.91	
1 Month Vs 3 Month	Alovera	35	4.94	1.79	0.00	3.37	1.39	0.00	4.85	1.91	0.00
	Alovera	35	4.14	1.26		2.82	0.92		3.88	1.23	
Baseline Vs 3 Month	Alovera	35	7.11	1.79	0.00	5.60	1.37	0.00	6.85	1.37	**0.01***
	Alovera	35	4.14	1.26		2.82	0.92		3.88	1.23	

Table 7: Intra-Group Comparison of Clinical Attachment Level (CAL) in Aloe Vera Group

PPD	Groups	N	Mesio-buccal			Mid-buccal			Disto-buccal		
			Mean	SD	*p* value	Mean	SD	*p* value	Mean	SD	*p* value
Baseline Vs 1 Month	Control	35	6.80	1.90	0.00	5.34	1.64	0.00	6.45	1.46	0.00
	Control	35	5.22	1.59		3.77	1.53		5.02	1.40	
1 Month Vs 3 Month	Control	35	5.22	1.59	0.00	3.77	1.53	0.00	5.02	1.40	0.00
	Control	35	4.68	1.25		3.25	1.09		4.51	1.03	
Baseline Vs 3 Month	Control	35	6.80	1.90	0.00	5.34	1.64	0.00	6.45	1.46	**0.02***
	Control	35	4.68	1.25		3.25	1.09		4.51	1.03	

Table 8: Intra-group Comparison of CAL values in Control group.

Parameters	Groups	N	Mean	SD	t value	p value
GBI at Baseline	Alovera	35	2.05	0.59	1.61	0.11
	Control	35	1.85	0.42		
GBI at 1 Month	Alovera	35	0.82	0.61	1.30	0.19
	Control	35	1.02	0.66		
GBI at 3 Months	Alovera	35	0.14	0.35	2.31	**0.02***
	Control	35	0.14	0.55		

Table 9: Inter-Group Comparison of GBI values between Aloe vera and Control groups at baseline, 1 month and 3 months.

GBI	Groups	N	Mean	SD	*p* value
Baseline Vs 1 Month	Alovera	35	2.05	0.59	0.00
	Alovera	35	0.82	0.61	
1 Month Vs 3 Month	Alovera	35	0.82	0.61	0.00
	Alovera	35	0.14	0.35	
Baseline Vs 3 Month	Alovera	35	2.05	0.59	**0.02***
	Alovera	35	0.14	0.35	

Table 10: Intra-Group Comparison of GBI values in Aloe vera group.

GBI	Groups	N	Mean	SD	*p* value
Baseline Vs 1 Month	Control	35	1.85	0.42	0.00
	Control	35	1.02	0.66	
1 Month Vs 3 Month	Control	35	1.02	0.66	0.00
	Control	35	0.40	0.55	
Baseline Vs 3 Month	Control	35	1.85	0.42	0.00
	Control	35	0.40	0.55	

Table 11: Intra-Group Comparison of GBI values in Control group.

Parameters	Groups	N	Mean	SD	t value	p value
PI at Baseline	Alovera	35	1.62	0.68	0.19	0.84
	Control	35	1.60	0.55		

PI at 1 Month	Alovera	35	0.57	0.50	1.42	0.15
	Control	35	0.74	0.50		
PI at 3 Months	Alovera	35	0.17	0.38	2.40	**0.01***
	Control	35	0.42	0.50		

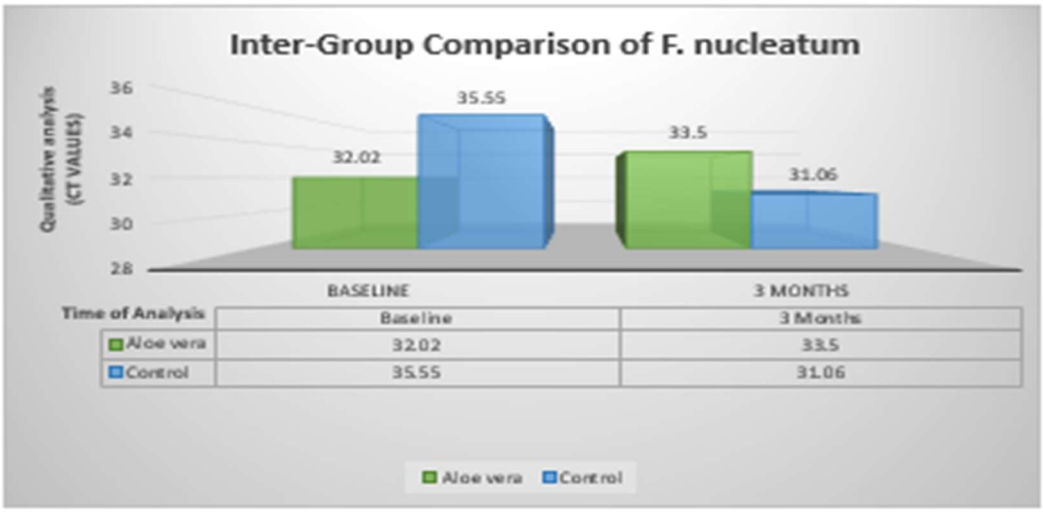
Table 12: Inter-Group Comparison of PI values between Aloe vera and Control groups at baseline, 1 month and 3 months.

PI	Groups	N	Mean	SD	*p* value
Baseline Vs 1 Month	Alovera	35	1.62	0.68	0.00
	Alovera	35	0.57	0.50	
1 Month Vs 3 Month	Alovera	35	0.57	0.50	0.00
	Alovera	35	0.17	0.38	
Baseline Vs 3 Month	Alovera	35	1.62	0.68	**0.01***
	Alovera	35	0.17	0.38	

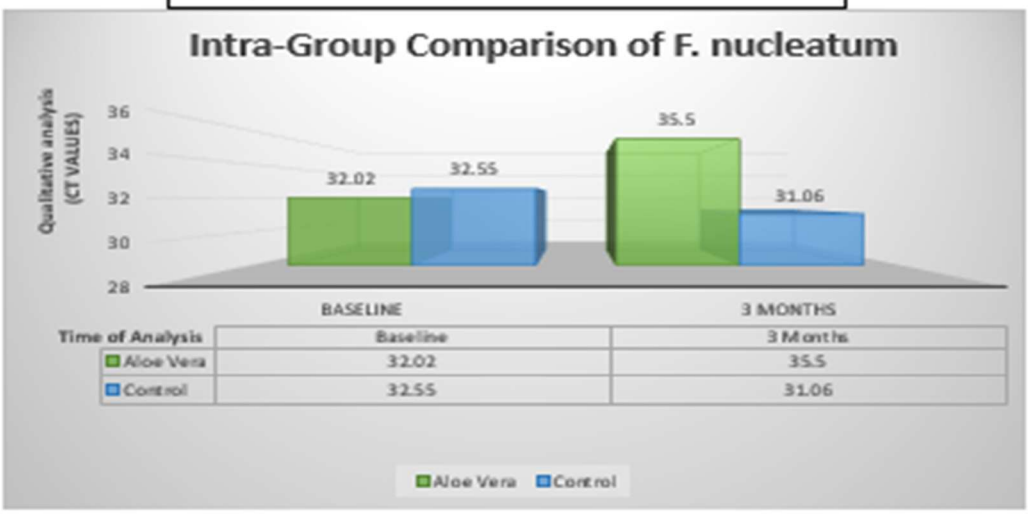
Table 13: Intra-Group Comparison of PI values in Aloe vera group.

PI	Groups	N	Mean	SD	*p* value
Baseline Vs 1 Month	Control	35	1.60	0.55	0.00
	Control	35	0.74	0.50	
1 Month Vs 3 Month	Control	35	0.74	0.50	0.00
	Control	35	0.42	0.50	
Baseline Vs 3 Month	Control	35	1.60	0.55	0.00
	Control	35	0.42	0.50	

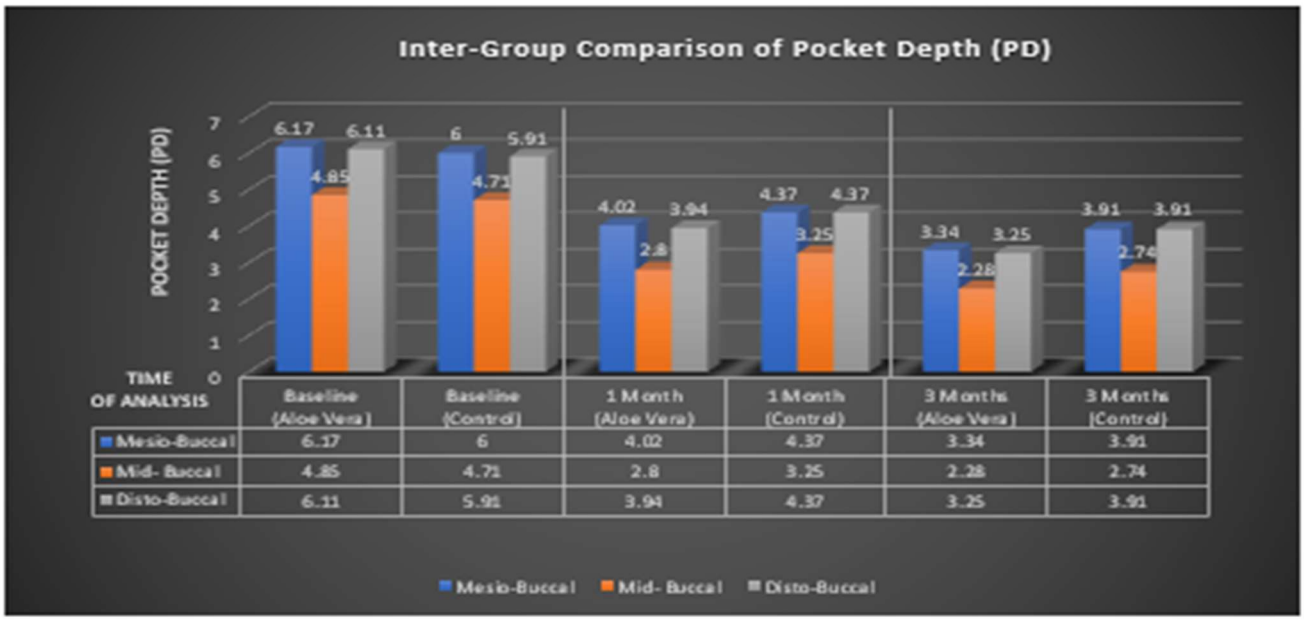
Table 14: Intra-Group Comparison of PI values in Control group.



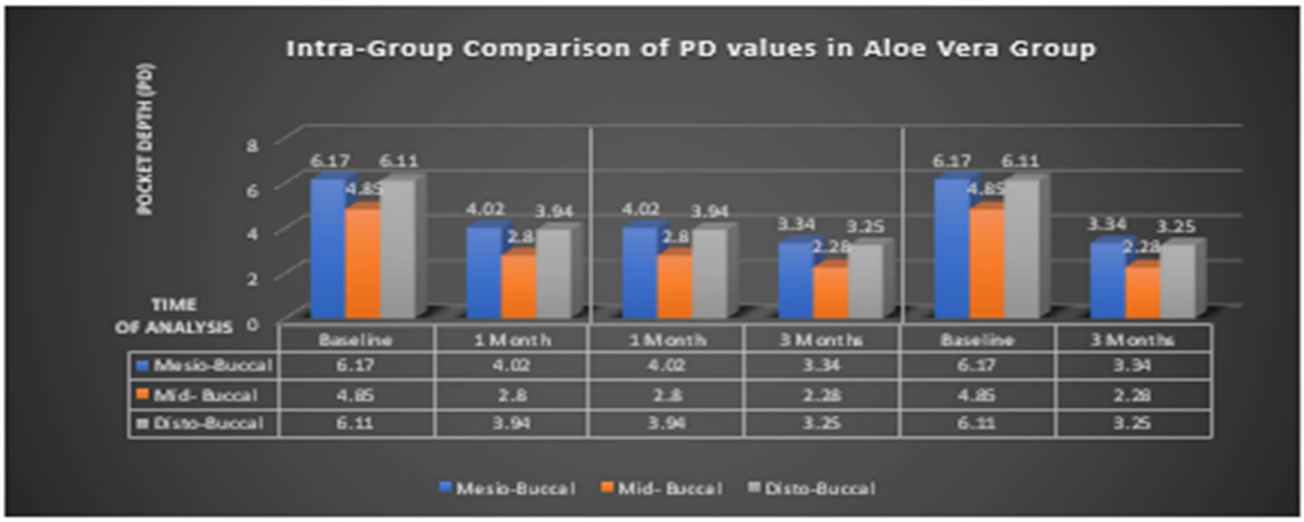
Graph 1: Inter-Group Comparison of F. nucleatum



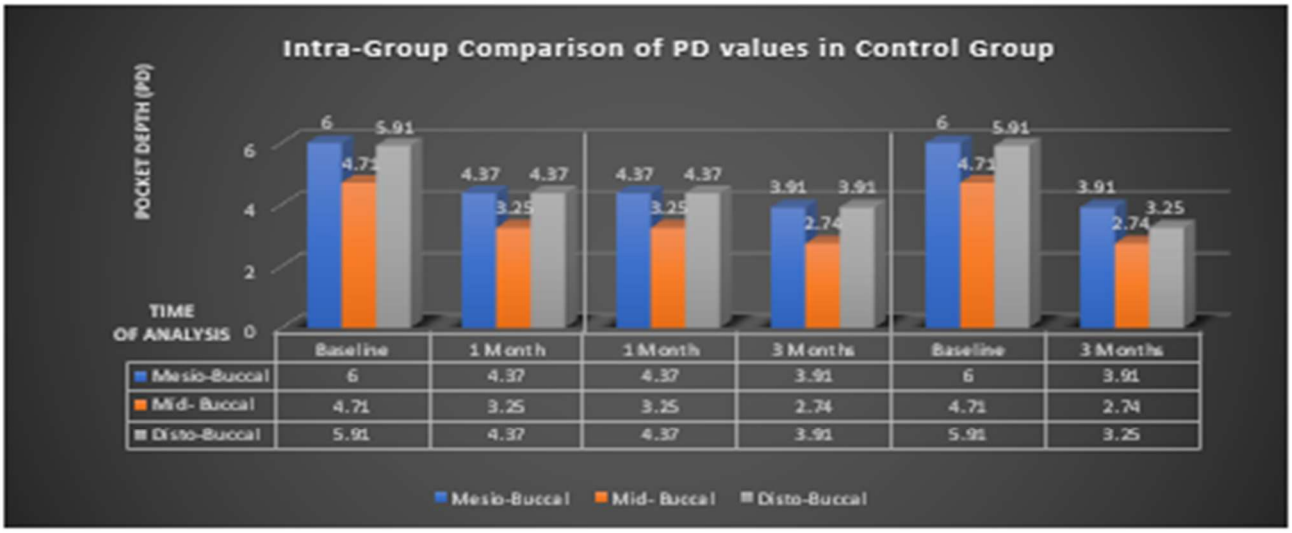
Graph 2: Intra-Group Comparison of F. nucleatum



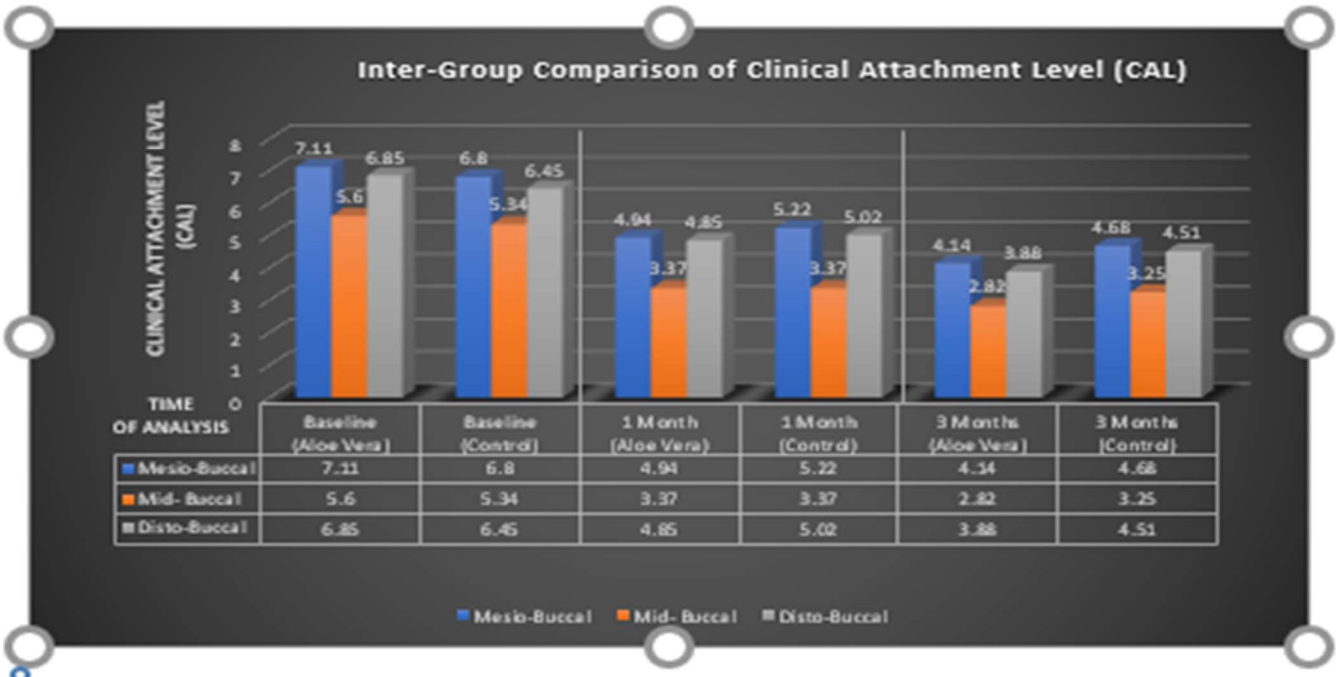
Graph 3: Inter-Group Comparison of Pocket Depth (PD) Values



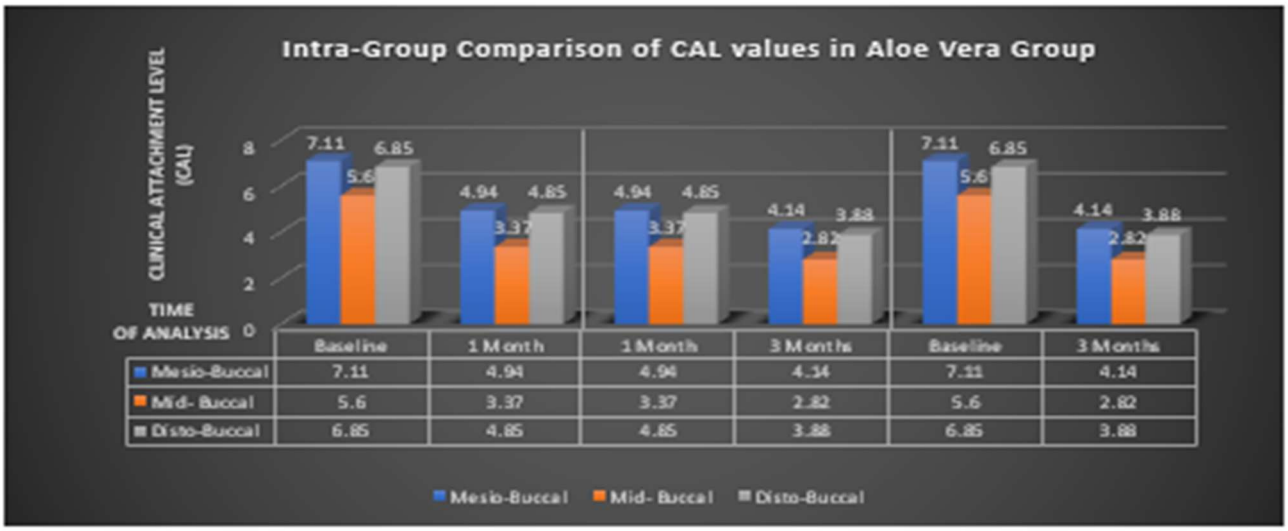
Graph 4: Intra-Group Comparison of Pocket Depth (PD) in Aloe Vera Group



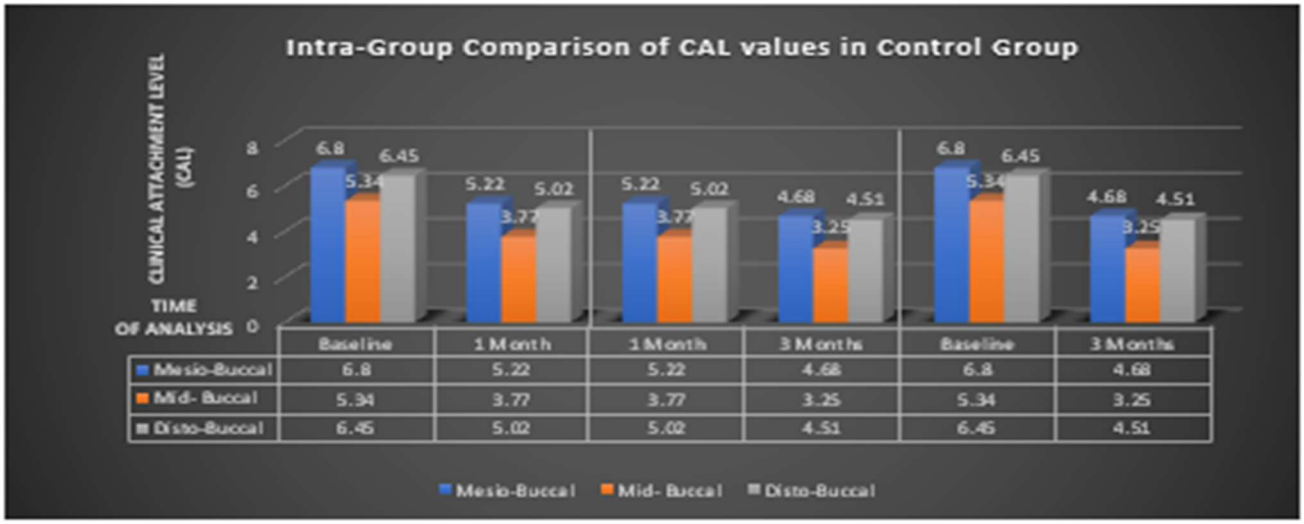
Graph 5: Intra-Group Comparison of Pocket Depth (PD) in Control Group



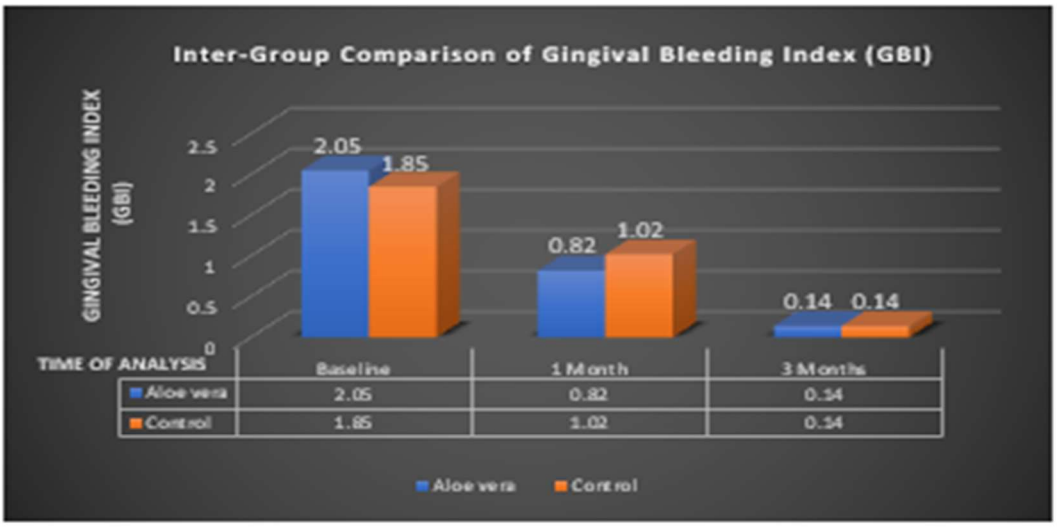
Graph 6: Inter-Group Comparison of Clinical Attachment Level (CAL) Scores



Graph 7: Intra-Group Comparison of Clinical Attachment Level (CAL) in Aloe Vera Group

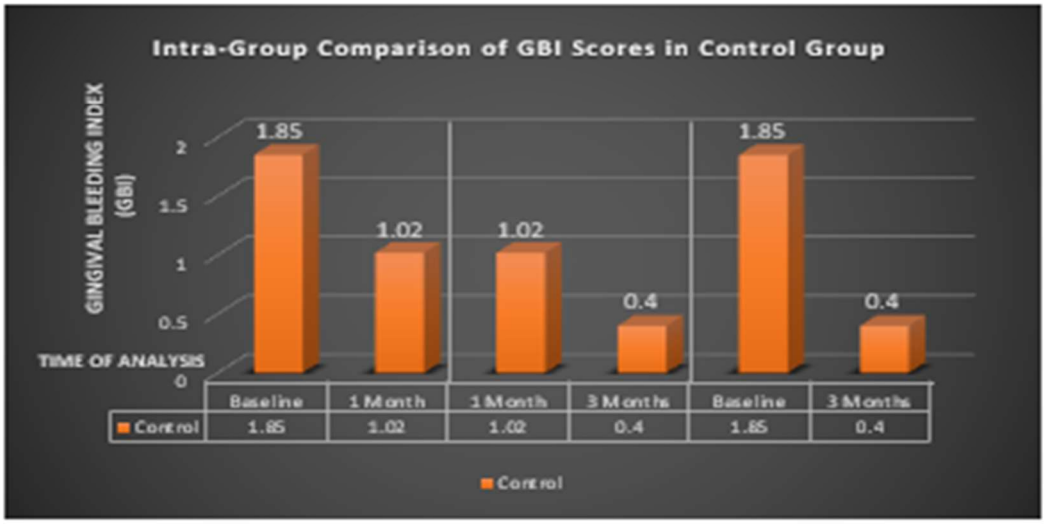


Graph 8: Intra-Group Comparison of Clinical Attachment Level (CAL) in Control Group

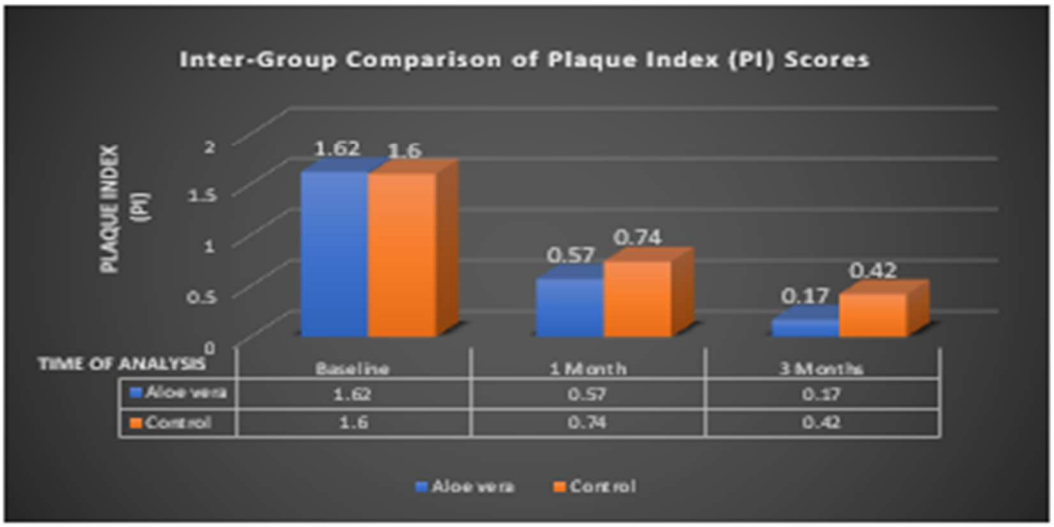


Graph 9: Inter-Group Comparison of Gingival Bleeding Index (GBI) Scores

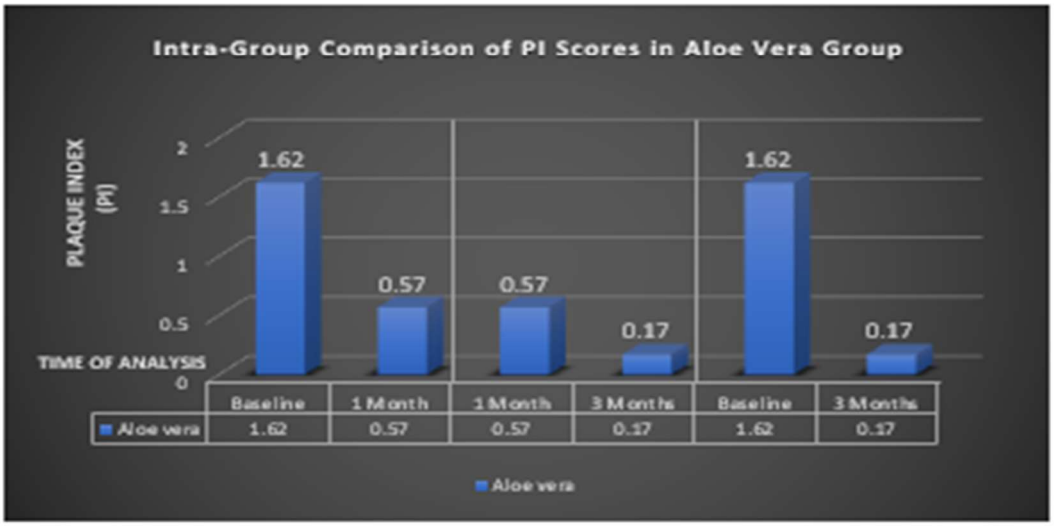
Graph 10: Intra-Group Comparison of Gingival Bleeding Index (GBI) in Aloe Vera Group



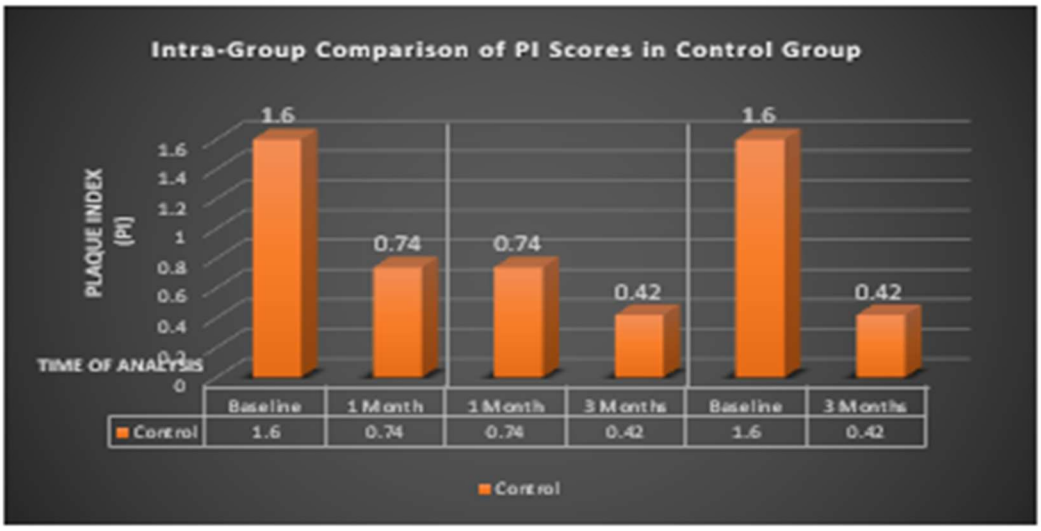
Graph 11: Intra-Group Comparison of Gingival Bleeding Index (GBI) in Control Group



Graph 12: Inter-Group Comparison of Plaque Index (PI) Scores



Graph 13: Intra-Group Comparison of Plaque Index (PI) Scores in Aloe Vera Group



Graph 14: Intra-Group Comparison of Plaque Index (PI) Scores in Control Group

***Fig 2*:** RT-PCR Kit for Fusobacterium nucleatum

***Fig 3*:** Acrylic Stent preparation for standardization

***Fig 4*:** Probing pocket depth (PPD) and Clinical attachment level (CAL) measurement

Fig 5: Plaque sample collection using 30s sterile paper points

Fig 6: Paper points were transferred to Eppendorf tubes containing buffer solution

Fig 7: Eppendorf tubes with plaque samples stored at -80°C

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Fig 8: Fusobacterium nucleatum estimation using RT-PCR

Fig 9: Aloe vera gel obtained from parenchyma

Fig 10: Loading 0.5ml of freshly prepared aloe vera gel

Fig 11: Subgingival delivery of 0.5ml Aloe vera gel using blunt cannula

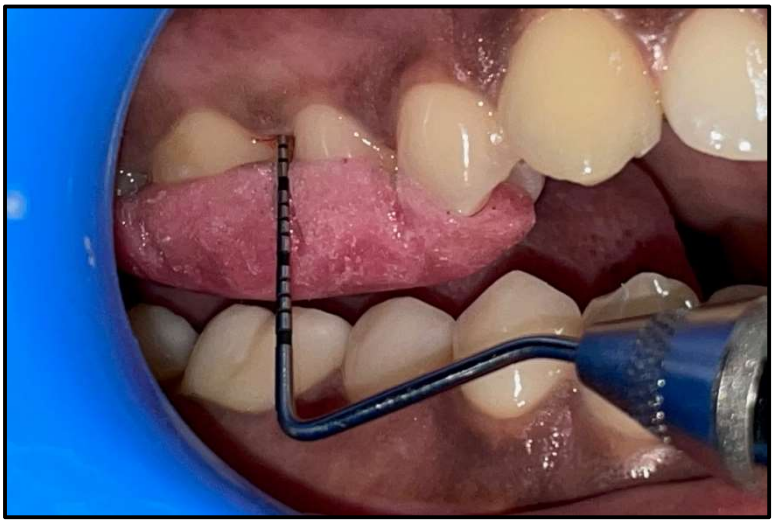

Fig 12: Post-Op 3months (Reduced probing pocket depth and improved Clinical attachment level)