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Clinical and microbiological efficacy of systemic ayurvedic immunomodulator in the treatment of chronic periodontitis-- A randomized double blind placebo controlled clinical trial

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ABSTRACT

OBJECTIVE: The present clinical trial was designed to investigate the clinical and microbiological effectiveness of systemic ayurvedic immunomodulator Septilin as an adjunct to scaling and root planing (SRP) in the treatment of chronic periodontitis (CP).

MATERIAL AND METHODS: Sixty five subjects presenting with moderate - severe CP were selected. 33 subjects were randomly assigned to full-mouth SRP + Septilin (group 1) and 32 subjects were assigned to full-mouth SRP + Placebo (group 2). The clinical outcomes evaluated were plaque index (PI), gingival index (GI), clinical attachment level (CAL), probing depth (PD) and % of sites with bleeding on probing (%BOP) at baseline (B/L), 7 days, 1, 3 and 6 months interval and percentage of sites positive for *A. actinomycetemcomitans (Aa), P. gingivalis (Pg)* and *T. forsythia (Tf)* were recorded at B/L, 3 and 6 months using polymerase chain reaction (PCR).

RESULTS: Sixty subjects could be evaluated up to 6 months. At 6 months, group 1 resulted in greater mean reduction (2.67 ± 0.68 mm) in PD as compared to group 2 (1.05 ± 0.46 mm) ($p<0.05$) and also a greater mean CAL gain (2.56 ± 0.71 mm) in group 1 as compared to group 2 (1.58 ± 0.46) ($p<0.05$). There was reduction in percentage of sites positive for *P. gingivalis* and *T. forsythia* periodontopathic bacteria over the duration of the study in both groups and a statistically significant reduction in the number of sites positive for *A. actinomycetemcomitans* in group 1 ($p<0.05$).

CONCLUSION: Septilin was found to significantly improve the clinical and microbiological parameters in CP individuals.

Keywords: ayurvedic immunomodulator, chronic periodontitis, microbiology

INTRODUCTION

Chronic periodontitis (CP) comprises a group of multifactorial diseases, which is a microbial plaque infection-driven chronic inflammatory disease that triggers host inflammatory and immune responses against the integrity of tooth-supporting periodontal tissues.[1] Oral microflora has periopathogens including the "red complex" bacteria, *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*, as well as other organisms, such as *Aggregatibacter actinomycetmcomitans*, *Prevotella intermedia*, and *Eikenella corrodens.*[2,3] These microorganisms in the subgingival dental plaque biofilm, have been implicated in the initiation and progression of the disease. Along with periodontal bacteria, the host susceptibility, as determined by various genetic, environmental, and

other risk factors, is also implicated in the etiopathogenesis of periodontitis.[4, 5] In fact, the tissue damage is primarily mediated by the host inflammatory reaction to uncontrolled bacterial challenge.[6- 8]

To reduce the numbers of periodontal pathogens, mechanical approaches such as scaling and root planing (SRP), are important for periodontal therapy although often inefficient on their own.[9, 10] The determination that host immune response plays a primary role in periodontal tissue destruction have led to the introduction of several host modulation therapies that have thus been proposed and tested experimentally or clinically.[11] Another approach to modulating the host response in periodontitis involves systemic or topical administration of antibiotics like tetracyclines (subantimicrobial dose doxycycline, minocycline),[12] and non-steroidal anti-inflammatory drugs.[13,14] Because of emergence of several antibiotic resistant strains of microorganisms in plaque biofilm[15] there is need for some alternative methods to tackle with these microorganisms such as going for some of natural plant extracts that have both antimicrobial efficacy and biocompatibility that have become an important part of research in the field of ayurvedic medicine.[16]

Since ancient times natures extract of herbal drugs have been used as remedies for the treatment of a variety of diseases. Although there is advance in modern medicine, plants still make an important contribution to health care.[17] Numerous biological activities of different compounds that are herbal extracts or essential oils prepared from medicinal plants have been confirmed by in vitro and in vivo studies, such as antibacterial, antiviral, anti-inflammatory, antioxidant properties.[18-20] Some compounds are used for the treatment of diabetes, nervous system disorders such as Alzheimer's disease, dental diseases, male infertility and erectile dysfunction [21] among other therapeutic properties.

A number of medicinal plants individually or in combinations have been claimed to possess immunomodulatory activity.[22] Septilin[1], a polyherbal preparation is a combination of various medicinal extacts that have proven to enhance body's defence mechanism. Composition of each tablet of septilin (Table 1.) contains: *Maharasnadi quath, Guduchi (Tinospora cordifolia) Manjishtha (Rubia cordifolia), Amalaki (Emblica officinalis), Shigru (Moringa pterygosperma), Yashtimadhu (Gycyrrhiza glabra), Guggulu (Balsamodendron mukul) and Shankh bhasma.*[23]

In the literature, several studies have reported the beneficial immunomodulatory properties of the proposed herbal components of septilin. *Āmalaki* (*Emblica officnalis* Gaertn), one of such plants revalidate the antioxidant, immunomodulatory activities and cytoprotective pharmacologic properties.[22] *Guggul (Balsamodendron mukul*) a medicinal herb have been reported to have antiarthritic and anti-inflammatory activity.[24] *Guduchi (Tinospora cordifolia),* herbal stem has a immunomodulatory protein (ImP), showing lymphoproliferative and macrophage-activating properties[25] and anti-proliferative, differentiation-inducing and anti-migratory/antimetastatic potential in glioma cells.[26] *Yasttmadhu (Glyzerrhaizia glabra)* herbal extract have antimicrobial[27] and antibacterial[28] properties, its anti-inflammatory activity has proved a definite role in Conjunctivitis.[29]

Till date no study has ever evaluated the clinical and microbiological efficacy of Septilin in treatment of CP. Hence the present study aimed to investigate the clinical and microbiological effectiveness of systemically administered, ayurvedic polyherbal immunomodulator Septilin, as an adjunct to SRP in the treatment of CP.

MATERIAL AND METHODS:

Source of Data

Sixty five subjects (32 males and 33 females) aged 30 to 50 years with a history of CP, diagnosed on basis of the current classification of American Academy of Periodontology were selected from the outpatient section of the Department of Periodontics, Government Dental College and Research Institute, Bangalore, India, between August 2013 to January 2014. It was made clear to the potential subjects that participation was voluntary. The nature, potential risks and benefits of their participation in the study were thoroughly informed. Written informed consent was obtained from patients, and ethical clearance for the study was received from the Institutional Ethical

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Committee and Review Board, Government Dental College and Research Institute, Bangalore.

Inclusion and exclusion criteria:

Sixty five systemically healthy subjects with untreated moderate to severe chronic periodontitis were recruited into the study based on the following criteria: a) age 30 to 50 years, b) atleast 20 natural teeth (not including third molars and teeth with orthodontic appliances, bridges, crowns, or implants) and c) radiographic evidence of bone loss.

Exclusion criteria included a) pregnancy or lactation, b) smokers c) systemic diseases like diabetes mellitus and immunocompromised patients d) systemic antibiotics taken within the previous 6 months e) use of anti-inflammatory drugs f) sub-gingival SRP or surgical periodontal therapy in the previous year.

The clinical parameters to be recorded included: plaque index (PI),[30] gingival index (GI),[31] % of sites positive for bleeding on probing (%BOP), clinical attachment level (CAL) measured to the nearest mm from the cemento-enamel junction (CEJ) to the deepest probeable point[31] using a standardized periodontal probe[2] and PD taken from the gingival margin to the bottom of the pocket.

Treatment protocol:

The subjects were randomly allocated to one of the two groups using computer generated table of random numbers to receive one of the two treatments. The allocation of subjects were done by the chief investigator (ARP) based on computerized generation of random allocation sequence. Group 1: 33 subjects and Group 2: 32 subjects. In all the subjects 1 week before the baseline (B/L) visit supra-gingival scaling was performed. Strict oral hygiene instructions (OHI) were instituted upon all subjects at the same time by the operator/clinical examiner (SK). Subjects were also instructed to use 0.2% chlorhexidine rinse twice daily during this period. Clinical examiner calibration was performed on 20 patients and the intra-examiner agreement was 95.2 %.

At B/L visit, the clinical parameters mentioned earlier were recorded in 4 teeth with the most severe destruction both clinically and radiographically with 6 sites/tooth (mesio-buccal, mid-buccal, disto-buccal, mesio-palatal/lingual, mid-palatal/lingual and disto-palatal/lingual) in each subject of both groups by the same person (SK). Next, the operator treated the periodontally diseased sites with thorough SRP to the depth of the pocket under local anaesthesia. After this each subject received a bottle containing 60 Septilin tablets or placebo medication; all bottles were identical in appearance and were marked only with the subject number. Subjects in group 1 received Septilin tablets, 500 mg of 2 tablets to be taken thrice daily for 7 days while subjects in group 2 received similarly looking tablets in a similar regimen. The treatment group was concealed from the patient, clinical examiner, operator, and statistician.

Clinical monitoring:

After the B/Lvisit, all the subjects in both groups were recalled at 7 days, 1 month, 3 months and 6 months. At these time intervals, parameters including PI, GI, PD, CAL and %BOP were recorded. OHI were checked and reinforced at each point. One session of SRP was performed in all subjects at every interval.

Microbiological analysis:

The microbial analysis was done using polymerase chain reaction (PCR) at baseline, 3 and 6 months.

Plaque sample collection:

Supragingival plaque was removed with a sterile curette[11], subgingival plaque was then collected with a second sterile curette, starting at the most apical extent of the deepest periodontal pocket. The samples were not pooled and only the plaque sample from the single deepest pocket was analysed. Collected samples were placed in airtight plastic vials with 500 ml of Tris EDTA (TE) buffer and were immediately transferred to the laboratory for microbiological analysis using PCR technology.

DNA extraction and polymerase chain reaction:

The samples received were stored at -20^{0}C to be processed immediately. The samples were thawed to room temperature and centrifuged (10,000 rpm, for 3–4 min), the supernatant was discarded and this process was repeated 3 times. To this, (same as mentioned above) about 500 ml of Lysil buffer 1 was

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added and centrifuged. Then, 50 ml of Lysil buffer 2 and 5 ml of proteinase K were added. This mixture was kept in a water bath at 75^0C for 2 h. It was then kept in a boiling water bath for 10 min then was stored at -20^0C for analysis.

PCR Detection:

The samples received were stored at -20°C to be processed immediately. Samples collected at B/L, 3 and 6 months were analyzed by PCR. Species-specific PCRs were performed to detect *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia.*

For *A. actinomycetemcomitans* a denaturation step at 95^0C for 2 minutes was followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, an extension at 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes. [32]

For *P. gingivalis* a denaturation step at 94^0C for 5 minutes was followed by 30 cycles of denaturation at 94^0C for 1 minute, annealing at 70^0C for 1 minute, an extension at 72^0C for 1 minute, and a final elongation step at 72^0C for 2 minutes.[33]

For *T. forsythia* a denaturation step at 95^0C for 2 minutes was followed by 36 cycles of denaturation at 95^0C for 30 seconds, annealing at 60^0C for 1 minute, an extension at 72^0C for 1 minute, and a final elongation step at 72^0C for 2 minutes.[34]

Primers:

Species-specific PCRs were performed to detect and *A.actinomycetemcomitans, P.gingivalis and T.forsythia.*

Forward (F) (sense) primers (16S rRNA genes, short DNA fragments) and reverse (C11R) (antisense) primers were used for detection containing sequences complementary to the target region along with a DNA polymerase are key components to enable selective and repeated amplification. Primers used for *A.actinomycetemcomitans*: 5' AAA CCC ATCTCT GAG TTC TTCTTC 3' 5' ATG CCA ACT TGA CGT TAA AT 3' [32] *P.gingivalis* : 5' AAT CGT AAC GGG CGA CAC AC 3' 5' GGG TTG CTC CTT CAT CAC AC 3' [33] and *T.forsythia*: 5' GCG TAT GTA ACC TGC CCG CA 3' 5' TGC TTC AGT GTC AGT TAT ACC T 3' [34] Conserved reverse primers (C11R) 5'- ACG TCA TCC CCA CCT TCC TC-3' were used in the study. Only one reverse primer was used (C11R) for all three organisms detected. As the reverse primers can hybridize to any bacterial 16S rDNA and can be combine with each species specific forward primer to produce amplicons of different sizes that can be subsequently resolved on an agarose gel.

The three major steps in a PCR were repeated for 30 or 40 cycles. This is done on an automated cycler. Denaturation at 94^0C, annealing at 54^0C and extension at 72^0C were performed. As PCR progressed, the DNA generated was itself used as a template for replication. The master mix contained all the necessary components to generate new strands of DNA for *Aa, Pg, Tf* setting in motion a chain reaction in which the DNA template is exponentially amplified.

Primary and secondary outcome measures:

The primary outcome of the study was gain in CAL. The differences in PI, GI, PD, %BOP between two groups and reduction in number of sites positive for each microbial species from B/L to subsequent intervals were taken as the secondary outcomes.

STATISTICAL ANALYSIS:

Power analysis calculations were performed before the study was initiated. To achieve 90% power and detect mean differences between groups, 30 subjects in each group were required. Normality assumption was tested using Shapiro-Wilk's W test. Continuous variable (PI, GI, PD and CAL) were expressed as mean ± standard deviation (SD) and intergroup comparison between test and control groups was done using Unpaired t test. To compare BOP data between both groups Mann-Whitney U test was employed. Chi-Square test was used to compare microbiological findings between test and control groups. Repeated measures ANOVA was used to assess the change for assessed parameters at all time intervals with the within subject effect. Statistical significance was defined as $p<0.05$. Statistical analysis was performed with statistical software¶.

RESULTS:

Consort flowchart (Figure 1) exhibits the number of subjects finally analyzed and those dropping out. 3

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subjects from group 1 and 2 from group 2 failed to follow up till the end of the study. In all 60 subjects (30 males, 30 females) were finally analyzed.

PI, GI and %BOP:

There was reduction in PI, GI and %BOP from B/L to various time intervals but no significant difference was found between the two groups at any point of time ($p>0.05$) as shown in table 2. But the within-subjects effects and between-subjects effects of PI, GI and %BOP were statistically significant ($p<0.05$) at all time intervals as described in table 5.

PD and CAL:

Table 2 describes the mean values of PD and CAL in the 2 groups. Table 3 describes the mean reduction in PD and mean gain in CAL from baseline to various intervals. There was reduction in PD and CAL from B/L at 10 days but the difference in the 2 groups was not significant. The difference in mean PD values between the two groups at months 1, 3 and 6 was statistically significant ($p<0.05$) as shown in tables 2 and 3. Mean PD values at 1, 3 and 6 months was 4.26 $\pm$ 0.88 mm, 3.81 $\pm$ 0.74 mm and 3.47 $\pm$ 0.60 mm for test group and 5.16 $\pm$ 0.83 mm, 4.89 $\pm$ 0.86 mm and 4.90 $\pm$ 0.82 mm for control group respectively as shown in tables 2. The mean CAL value was also significantly different at 1 month ($p = 0.04$), 3 month ($p <0.0001$) and 6 months ($p = <0.0001$) as shown in table 2. As shown in table 5 within-subjects effects and between-subjects effects of PD and CAL values were also statistically significant ($p<0.05$) at all time intervals.

Microbial analysis:

Table 4 presents percentage of subjects positive by PCR for each microbial species.The PCR analysis showed mean reduction in number of subjects positive for *Aa, Pg* and *Tf* from B/L to 6 months. There was statistically significant difference in subjects positive for *Aa* between test and control group at 6 months interval ($p<0.05$). Subjects positive for *Aa* were decreased from 15 at B/L to 1 at 6 months in test group while in control group number decreased from 16 at B/L to 9 at 6 months. The differences between the percentage of positive subjects in test and control group were not significant at any time interval for *Pg* and *Tf*. However, there was mean reduction in number of positive subjects for *Pg* and *Tf* from B/L to 3 months and 6 months.

Adverse drug reactions:

None of the patients in the study reported any form of allergies or adverse reactions throughout the study period for 6 months regime.

DISCUSSION:

Periodontitis being a multifactorial disease[1] its progression is closely related to the colonization of microorganisms, including *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*), *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola.*[35] A combined therapy directed against microbial pathogens and immune modulation will surely have a better treatment outcomes. Systemic medications as an adjunct to SRP, have a number of periodontal benefits including reductions in PD, gain in CAL, long-term reduction of periodontal pathogens, elimination of invasive pathogens in periodontal tissues and a decrease in the extent and severity of periodontal surgery.[36]

Immune modulation is an effective as well as protective approach against emerging infectious diseases.[37] Several studies have quoted the systemic or topical administration of antibiotics like tetracyclines (subantimicrobial dose doxycycline, minocycline),[12] and non-steroidal anti-inflammatory drugs.[13,14] in modulating host response. But there is an incoming tide of concern about the problems of antimicrobial resistance.[37] With the emergence of strains resistant to conventional antibiotics, there is need to carry out studies using alternative methods to control these microorganisms, such as the use of products of plant origin that has demonstrated effective antimicrobial activity besides biocompatibility.[16]

Ayurveda stresses the need to enhance the body immunity while following specific therapeutic regimen indicated for the condition. Ayurveda has a complete solution in form of *Rasayana* Drugs (rejuvenating drugs) which was established in ancient era in order to enhance self-defense mechanism of the body.[37] Septilin, is one of such potent herbal immunomodulator having a combination of several herbs whose pharmacological actions have been proven in literatue. Our present study aims to evaluate the clinical and microbiological efficacy of polyherbal immunomodulator Septilin. The findings

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of the present clinical trial demonstrated that in patients with chronic periodontal disease, systemic administration of Septilin resulted in reduction of PI, GI and %BOP from B/L to various time intervals but no significant difference was found between the two groups at any point of time. The reductions in PD and CAL at various time intervals were statistically significant in group 1. Within-subjects effect and between-subjects effects of PI, GI, %BOP, PD and CAL values were also statistically significant ($p<0.05$) at all time intervals in group 1. Microbial analysis using PCR showed mean reduction in number of sites positive for *Aa, Pg* and *Tf* from B/L to 3 and 6 months but there was statistically significant difference in subjects positive for *Aa* between test and control group at 6 months interval ($p<0.05$) indicating an improvement of the periodontal conditions. The combined mechanical and systemic antimicrobial immunomodulator Septilin therapy (group 1) was more effective than mechanical therapy alone (group 2) in terms of improvement of clinical microbiological features of periodontal disease.

In a study, clinical evaluation of Rasayana drugs of *Amalaki* (*Emblica officinalis* Gaertn.), *Guduchi* (*Tinospora cordifolia* willd.), *Yastimadhu* (*Glycyrrhiza glabra* Linn.) which are also contents of septilin were used as an adjuvant therapy with anti-Koch's treatment against *Mycobacterium tubercle* and results revealed that *Rasayana* compound was found to decrease cough (83%), fever (93%), dyspnea (71.3%), hemoptysis (87%) and increase body weight (7.7%) which was statistically highly significant ($P<0.001$).[38] Studies have demonstrated the antimicrobial[27] and antibacterial[28] activity of *Glycyrrhiza glabra L.* and its extract inhibited the growth of all bacterial strains studied at a concentration of 100 mg/mL.[16] This compound also showed inhibitory effect on the growth of *S. aureus* strains sensitive and resistant to methicillin, showing MIC at 12.5 mg/ mL. [39]

Statti et al. collected *G. glabra L.* from different regions of Calabria, Italy, and verified an inhibitory effect on bacteria and fungi, and this biological activity varied due to differences in the chemical composition of this plant obtained from different sites.[40]

Rosas-Piñón et al. studying the medicinal plants from a Mexico region used by local people to treat dental diseases such as toothache, dental caries, periodontal disease and gingivitis, found the antimicrobial activity of 47 plant species on *Porphyromonas gingivalis* and *S. mutans*.[41] The finding that SRP combined with administration of systemic herbal immunomodulator Septilin was more effective than mechanical therapy alone in terms of eliminating microbial pathogens in deep pockets (≥6 mm) and promoting CAL gain at such sites is in agreement with results reporting *Amalaki* (*Emblica officinalis* Gaertn.), *Guduchi* (*Tinospora cordifolia* willd.),[38] *Yastimadhu* (*Glycyrrhiza glabra*) [38, 27] have antimicrobial and antibacterial [28] activity.

Therefore, to the best of our knowledge, this is the first study evaluating clinical and microbiological effectiveness of polyherbal immunomodulator Septiin as an adjunct to SRP in patients with CP. Although, significant clinical and improved microbiological outcomes were attained in the group 1 using adjunctive Septilin, limited and qualitative microbiological analysis was the major limitation. Another limitation of this study was that, the individuals were not followed after 6 months where the effects of Septilin could have declined. Thus further long-term trials evaluating the clinical and microbiological effects should be carried out to confirm the effectiveness of Septilin in treatment of CP.

CONCLUSION:

To conclude, systemic use of this polyherbal immunomodulator Septilin, has a wide range of therapeutic applications and particularly in patients allergic to conventional antibiotics. Hence when used along with initial periodontal treatment consisting of SRP in adult subjects with periodontitis, achieves significantly better clinical and microbiological results than initial periodontal treatment alone. Further long term, multicenter longitudinal trials may be carried out to assess and establish the efficacy of systemic septilin in the management of CP and also to compare the same with other established herbal medicine of this group.

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TABLES

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TABLE 1: Composition of each Septilin tablet

EXTRACTS	**CONCENTRATION**
Maharasnadi quath	65 mg
Guduchi (Tinospora cordifolia)	49 mg
Manjishtha (Rubia cordifolia)	32 mg
Amalaki (Emblica officinalis)	16 mg
Shigru (Moringa pterygosperma)	16 mg
Yashtimadhu (Gycyrrhiza glabra)	6 mg
POWDERS	
Guggulu (Balsamodendron mukul)	162 mg
Shankh bhasma	32 mg

TABLE 2 : Mean, mean difference ± SD and p values of PI, GI, PD, CAL and %BOP of the groups 1 and 2 at various time intervals.

Parameter	**Visits**	**Group 1**	**Group 2**	**p Value**
	Baseline	3.54 ± 0.61	3.39 ± 0.54	0.31
	7 days	2.36 ± 0.43	2.29 ± 0.49	0.51
PI	1 month	2.20 ± 0.42	2.15 ± 0.39	0.64
	3 months	2.22 ± 0.38	2.14 ± 0.34	0.39
	6 months	2.20 ± 0.36	2.23 ± 0.35	0.67
	Baseline	2.15 ± 0.34	2.07 ± 0.29	0.32
	7 days	1.44 ± 0.37	1.44 ± 0.40	0.98
GI	1 month	1.25 ± 0.27	1.24 ± 0.37	0.86
	3 months	1.22 ± 0.25	1.21 ± 0.31	0.93
	6 months	1.19 ± 0.20	1.22 ± 0.28	0.58
	Baseline	6.14 ± 1.15	5.95 ± 0.86	0.45
	7 days	5.27 ± 1.01	5.34 ± 0.74	0.77
PD	1 month	4.26 ± 0.88	5.16 ± 0.83	<0.001*
	3 months	3.81 ± 0.74	4.89 ± 0.86	<0.001*
	6 months	3.47 ± 0.60	4.90 ± 0.82	<0.001*

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	Baseline	6.44 ± 1.17	6.57 ± 0.84	0.60
	7 days	5.68 ± 1.05	5.76 ± 0.72	0.70
CAL	1 month	5.00 ± 0.97	5.41 ± 0.64	<0.04*
	3 months	4.28 ± 0.84	5.17 ± 0.66	<0.001*
	6 months	3.88 ± 0.75	4.99 ± 0.71	<0.001*
	Baseline	64.4 ± 12.2	64.3 ± 13.7	0.97
	7 days	28.4 ± 7.75	29.0 ± 8.63	0.76
%BOP	1 month	15.4 ± 4.87	16.7 ± 5.22	0.31
	3 months	17.1 ± 4.79	19.1 ± 5.46	0.11
	6 months	19.3 ± 4.41	20.6 ± 5.47	0.26

* Statistically significant at 5% level of significance ($p < 0.05$).

TABLE 3: Decrease in PD and CAL gain from baseline (mean ± SD) at different time intervals for groups 1 and 2

Parameter	Visits	Group 1	Group 2	p value
	7 days	0.86 ± 0.37	0.61 ± 0.36	0.008
PD(mm)	1 month	1.87 ± 0.43	0.79 ± 0.42	<0.001*
	3 months	2.33 ± 0.56	1.06 ± 0.46	<0.001*
	6 months	2.67 ± 0.68	1.05 ± 0.46	<0.001*
	7 days	0.76 ± 0.42	0.80 ± 0.38	0.65
CAL(mm)	1 month	1.44 ± 0.60	1.15 ± 0.37	0.27
	3 months	2.15 ± 0.65	1.40 ± 0.34	<0.001*
	6 months	2.56 ± 0.71	1.58 ± 0.46	<0.001*

* Statistically significant at 5% level of significance ($p < 0.05$).

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TABLE 4 : Number and Percentage of subjects positive by PCR for each microbial species.

	Time interval	Group 1	Group 2	p value
Aa	Baseline	15 (45.5%)	16 (50.0%)	NS
	3 months	3 (9.1%)	7 (21.9%)	NS
	6 months	1 (3.0%)	9 (28.1%)	0.005*
Pg	Baseline	31 (93.9%)	32 (100.0%)	NS
	3 months	21 (63.6%)	20 (62.5%)	NS
	6 months	14 (42.4%)	17 (53.1%)	NS
Tf	Baseline	27 (81.8%)	26 (81.3%)	NS
	3 months	18 (54.5%)	20 (62.5%)	NS
	6 months	13 (39.4%)	14 (43.8%)	NS

NS- Not Significant,

* Statistically significant at 5% level of significance ($p < 0.05$).

TABLE 5 : Repeated Measures ANOVA assessing Within-Subjects and Between-Subjects Effects for parameters PI, GI, PD, CAL and %BOP.

Parameter		Source	Type III Sum of Squares	df	Mean Square	F	Sig.
	Tests of	factor1	81.18	4	20.29	416.51	<0.001*
	Within -Subjects	factor1 * Group	0.28	4	0.07	1.46	0.214
PI	**Effects**	Error(factor1)	12.27	252	0.04		

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	Tests of Between Subjects Effects		1991.69	1	1991.69	2535.57	<0.001*
	Tests of	factor1	38.14	4	9.53	499.43	<0.001*
	Within -Subjects	factor1 * Group	0.11	4	0.02	1.45	0.216
GI	**Effects**	Error(factor1)	4.81	252	0.19		
	Tests of Between Subjects Effects		681.17	1	681.17	1592.41	<0.001*
	Tests of	factor1	151.54	4	37.88	392.20	<0.001*
	Within -Subjects	factor1 * Group	30.85	4	7.71	79.86	<0.001*
PD	**Effects**	Error(factor1)	24.34	252	0.097		
	Tests of Between Subjects Effects		7877.42	1	7877.42	2338.61	<0.001*
	Tests of	factor1	176.70	4	44.17	511.73	<0.001*
	Within -Subjects	factor1 * Group	13.44	4	3.36	38.93	<0.001*
CAL	**Effects**	Error(factor1)	21.75	252	0.86		
	Tests of Between Subjects Effects		9201.57	1	9201.57	2748.65	<0.001*
	Tests of	factor1	105228.72	4	26307.18	1173.60	<0.001*
	Within -Subjects	factor1 * Group	43.37	4	10.84	0.48	0.748
%BOP	**Effects**	Error(factor1)	5648.77	252	22.41		
	Tests of Between Subjects Effects		282533.8	1	282533.8	1265.19	<0.0001*

* Statistically significant at 5% level of significance ($p < 0.05$).

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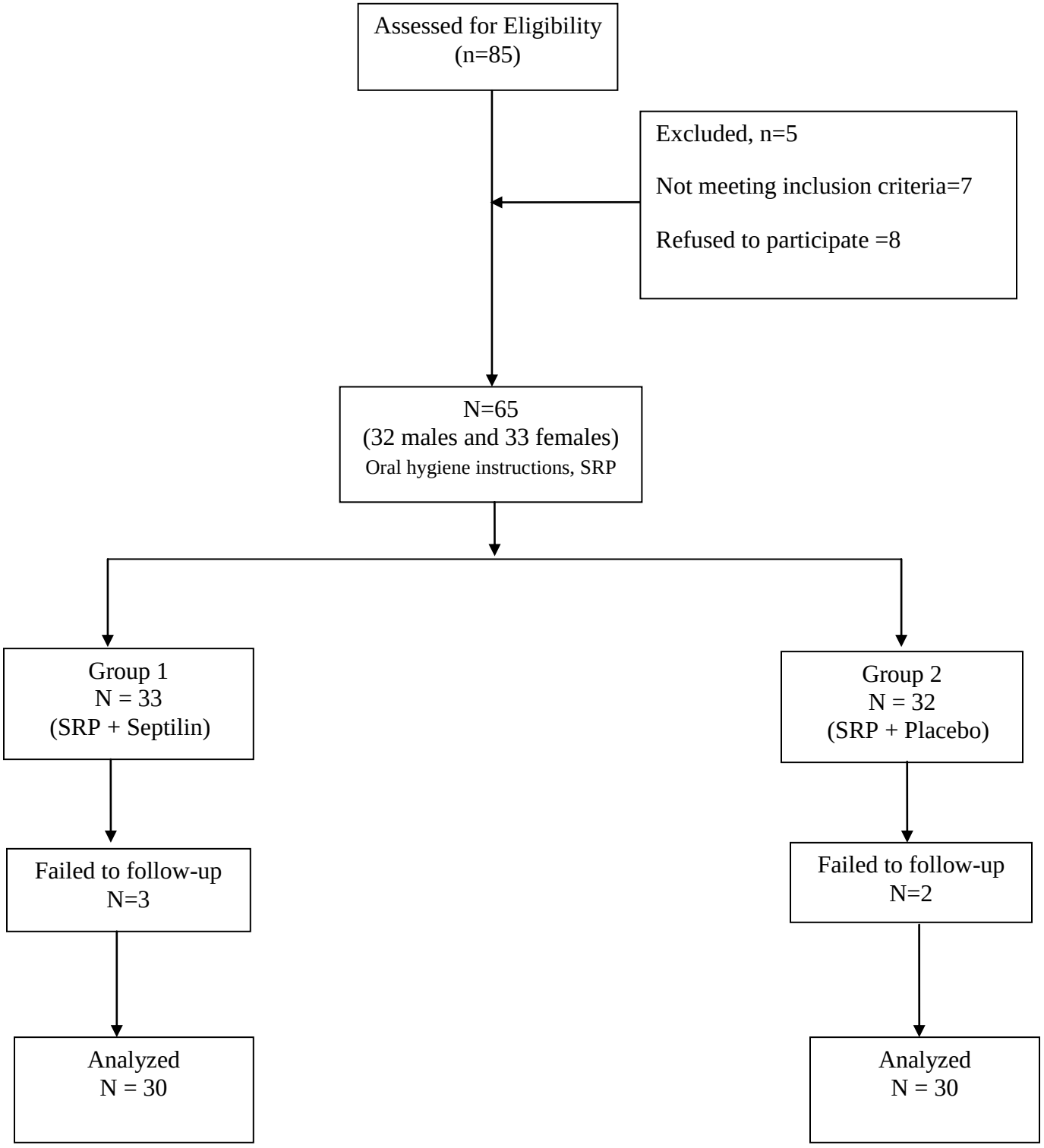
FIGURE 1: STUDY FLOW CHART



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