"EFFICACY OF *Ocimum sanctum* GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING (SRP) IN THE TREATMENT OF CHRONIC PERIODONTITIS – A SPLIT MOUTH RANDOMISED CONTROLLED TRIAL"

By

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REG. NO. IK0218001

Dissertation

Submitted to

KLE Academy of Higher Education and Research (KLE University),

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In partial fulfilment

of the requirements for the degree of

MASTER OF DENTAL SURGERY

in

PERIODONTICS

(Branch II)

Under the guidance of

Dr. VINAYAK KUMBHOJKAR M.D.S.,

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2018 - 2021

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Dr. Mohit Milind Kulkarni

LIST OF ABBREVIATIONS

CGP	**Chronic Generalised Periodontitis**
BPT	**Basic Periodontal Therapy**
SRP	**Scaling and root planing**
AM	**Antimicrobial agent**
A.a	***Aggregatibacter actinomycetemcomitans***
P.g	***Porphyromonas gingivalis***
P.i	***Prevetolla intermedia***
C.F.U	**Colony forming units**
P.I	**Plaque Index**
G.I	**Gingival Index**
PPD	**Pocket Probing Depth**
CAL	**Clinical Attachment Level**
BL	**Baseline**
MIC	**Minimum Inhibitory Concentration**
MBC	**Minimum Bactericidal Concentration**

ABSTRACT

BACKGROUND: Chronic periodontitis is a destructive disease that affects the supporting structures of the tooth namely the periodontal ligament, cementum and alveolar bone. Oral cavity houses more than 500 different types of bacteria that are reportedly associated with different oral diseases. *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia* have been recognised to be the most common microorganisms responsible for the initiation of periodontitis. Mechanical instrumentation is the first treatment of choice. If plaque/calculus complete removal is not done it results in recurrence of the disease. Complete removal of plaque and calculus is very difficult in deep pockets.

Many studies have addressed the need for the local application of antimicrobial agents to the subgingival area for treatment of periodontitis. For eg, the use of tetracycline group of drugs as local drug delivery has been found to be effective in treatment of chronic periodontitis. The purpose of the present study is to evaluate if *Ocimum sanctum* gel can exert a beneficial therapeutic effect as an adjunct in the treatment of chronic periodontitis patients and to evaluate the Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Ocimum sanctum* gel for *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia.*

AIM: - To determine and compare the antimicrobial activity of *Ocimum sanctum* (tulsi leaves) gel as an adjunct to non-surgical therapy in chronic periodontitis patients.

MATERIALS AND METHODS: This was a split mouth randomised controlled clinical trial study design. The study included thirty four patients in the age group of 17- 45 years with chronic periodontitis and no medical history and dental treatment in the preceding 6 months including oral prophylaxis. The split mouth study included a Control site in which scaling and root planing was carried out in (Group A) and a test site where application of *Ocimum sanctum* gel along with scaling and root planning was done (Group B).The participants were evaluated clinically for the following clinical variables- plaque index, sulcus bleeding index, pocket probing depth, clinical attachment level at baseline (visit 1), 14^{th} day (visit 2) and at the end of 3^{rd} month (Visit 3). The subgingival plaque was also collected prior to scaling and root planing.

Results: There was a reduction in Sulcus Bleeding index, Plaque index at the end of 1 month but there was rise in Plaque index by the end of 3^{rd} month in test group (SRP + application of gel) compared to control group (SRP) alone. Pocket probing depth gradually reduced at an equal rate in both control and test group by end of 1st month but there was rise in pocket probing depth measurements in control group from 1^{st} month to third month.

Conclusion:

1) The MIC and the MBC of *Ocimum sanctum* gel against predominant periodontal pathogens is 10%.
2) 10% *Ocimum sanctum* gel is known to exert a significant antimicrobial effect against *P. gingivalis* and *A.actinomycetamcomitans* and limited antimicrobial effect against *Prevotella intermedia*.

3) 10% *Ocimum sanctum* gel when used as an adjunct to SRP brought about a significant reduction in the clinical parameters such as Bleeding on probing, Pocket probing depth and Clinical attachment loss.
4) The therapeutic benefits observed with application of 10% *Ocimum sanctum* gel as an adjunct to SRP were sustained only for a period of 1 month from baseline and tended to reverse from 1 month to 3 months' time interval. This warrants the application of the gel at every recall visit.

Keywords: - plaque index, gingival index, pocket probing depth, clinical attachment level.

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INTRODUCTION

"Chronic Periodontitis (CP) is an inflammatory disease of the supporting tissues of the tooth caused by specific microorganisms in a susceptible host" [(1)]. "Periodontal disease is triggered by the presence of microbial biofilms that colonize the sulcular region between the tooth surface and the gingival margin". The following periodontal pathogens have been described in the literature as contributing to periodontal disease; "*Aggregatibacter (Actinobacillus) actinomycetemcomitans, Porphyromonasgingivalis, Prevotellaintermedia, Tannerella Forsythia, Peptostreptococcus micros, Camphylobacter rectus, Eikenellacorrodens, Fusobacteriumspp, Treponemadenticola, Selenomonas*spp, beta hemolytic streptococci, a variety of enteric rods and pseudomonas, enterococci, staphylococci and possibly yeasts".[(2)]

"Dental plaque is a type of biofilm, defined as a sessile community of interdependent microorganisms organized within an exopolymer that is attached to solid surfaces or associated with interferences"[(3)].SRP is the standard periodontal treatment. However traditional SRP may fail to eradicate the subgingival bacteria located in inaccessible areas such as multi-rooted teeth, furcation areas, root concavities, interproximal areas and deep periodontal pockets. Additionally conventional SRP does not completely remove periodontal disease causing bacteria as they can persist within the root cementum and dentinal tubules or translocate from oral reservoirs to periodontal disease sites.

After mechanical debridment, supragingival plaque microorganism recolonization can occur within hours or days. Subgingival plaque microorganism recolonization may take several months for those patients who have good oral

hygiene. But for those patients with poor oral hygiene, recolonization can be established within 42-60 days. [7]

Periodontal pockets are not only the reservoirs for periodontal pathogens. The tongue, tonsillar and buccal mucosal areas harbor many of the above mentioned periodontal pathogens [8]. **"**In addition, some periodontal species also have the ability to penetrate into the gingival epithelial cells and subepithelial connective tissue and have high affinity for the crevicular epithelium and dentinal tubules. These periodontal pathogens are *A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, P.micros*, enteric rods". [9]

Since the use of mechanical debridement alone is not sufficient to eliminate periodontal pathogens, there may be a benefit in using chemical antimicrobial agents, such as systemic antibiotics, local antibiotics or local antiseptics. [10]

The use of systemic antibiotics presents some possible adverse effects, such as a chance of developing bacterial resistance, and is subject to lack of patient compliance. [11]

In addition, several topical antibiotics are available in the market such as tetracycline-HCL, minocycline, metronidazole or oflaxacin. The application of these topical antibiotics in patients diagnosed with chronic periodontitis have shown significant pocket depth reduction [12]. However, the use of such topical antibiotics can cause problems with possible resistance of bacteria and adverse host reactions [11]. The majority of these local and systemic antibiotics have a high acquisition cost and some are only available in a few countries. [13]

In comparison to the systemic antibiotics, there are many benefits of local antimicrobial agents :1) local drug delivery can achieve a higher concentration in subgingival sites compared with a systemic antibiotic therapy, 2) local drug delivery have less problems with patient compliance, and 3) they reduce the risk of developing drug- resistance microbial populations.[11]

However, local antimicrobial agents present other difficulties, such as the small entrance and the outflow of crevicular fluid in a periodontal pocket [12]. For the pharmaceutical effect to occur, the local antimicrobial agent should reach the deepest area of the pocket and should be maintained long enough and at a sufficient concentration [14].

The local drug delivery agents can be divided into categories of personally applied (in patients, home self-care) and professionally applied (in dental office). The personally applied agents can be subdivided into non-sustained, subgingival drug delivery (home oral irrigation) and sustained, subgingival drug delivery. The professionally applied agents can be divided into sustained -release devices (drug delivery for less than 24hours) and controlled- delivery devices (drug release exceeding1 day). [13]

Various plants are used for manufacturing of drugs which includes: Morphine (derived from papaversomniferum), Ephedrine (derived from Ephedra vulgaris) Atropine (derived from atropa belladonna) [4]

Ocimum sanctum L. is one such medicinal plant having numerous medicinal properties, belongs to the family Lamiaceae. It is also called as Krishna Tulsi[4]

- "*Ocimum sanctum* is known as queen of herbs"[5]
- "Eugenol – based gels are available for treatment of periodontitis"[6]
- "Free eugenol can cause bone resorption and osteoradionecrosis"[6]

It is touted to be a sacred and highly medicinal plant in Indian indigenous literature and is said to be one of the main pillars of herbal medicine. Alternatively, whole Tulsi extract has been employed in traditional medicine and human clinical trials with good results and insignificant adverse events.

Many literature reviews show several studies on *Ocimum sanctum* as mouthwash as a gel formulations, and as an intracanalirrigant. However there is no study conducted clinically to evaluate the effect of the gel formulation of Tulsi.

Therefore the present study is planned to evaluate if *Ocimum sanctum* gel can exert a beneficial therapeutic effect as an adjunct in the treatment of chronic periodontitis patients

AIM AND OBJECTIVES

AIM

To determine and compare the antimicrobial activity of *Ocimum sanctum* (Tulsi leaves) gel as an adjunct to non-surgical therapy in chronic periodontitis patients.

OBJECTIVES

1) To evaluate the Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Ocimum sanctum* gel for *Aggregatibacteractinomycetemcomitans, Porphyromonasgingivalis, Prevotellaintermedia.*

2) To assess the antimicrobial effect of *Ocimum sanctum* gel on *Aggregatibacteractinomycetemcomitans*.

3) To assess the antimicrobial effect of *Ocimum sanctum* gel on *Porphyromonasgingivalis*

4) To assess the antimicrobial effect of *Ocimum sanctum* gel on *Prevotellaintermedia*

5) To evaluate the effect of application of *Ocimum sanctum* gel as an adjunct to Scaling and Root planing (SRP) on Sulcus Bleeding Index (SBI), Pocket Probing Depth (PPD) and Clinical Attachment Level (CAL) in periodontal pockets.

REVIEW OF LITERATURE

Oral infections are known mixed infections that is they are associated with aerobic and anaerobic organisms. Among the 500 species that colonize the oral cavity," *Porphromonasgingivalis, Aggregatibacteractinomycetemcomitans, Prevotellaintermedia* are established putative perio pathogens which have been associated with initiation and progression of periodontal disease".

These bacteria are often found in the dental plaque that is present in periodontal pockets. "Dental plaque is considered to be the main etiologic agent in the initiation of gingivitis and progression of the lesion to periodontitis".

"Mechanical debridement such as scaling and root planning is considered as treatment of choice for removal of dental plaque. However incomplete removal of the dental biofilm can cause recurrence of the disease. This is seen especially in cases of deep periodontal pockets where remnants of dental plaque and calculus cause failure of periodontal treatment".

To address this issue antibiotic local drug delivery systems have been developed and used as an adjunct to non-surgical periodontal therapy in treatment of periodontitis. These local drug delivery agents have been found effective in reducing periodontal disease burden but the emergence of antibiotic resistance has now shifted focus on developing similar system using indigenous herbal drugs.

These herbal systems decrease the chances of development of microbial drug resistance and lower the cost of the LDD system overall.

So we decided to test Tulsi gel (*Ocimum sanctum*) which is also known as "Holy Basil" known to possess significant medicinal properties. Tulsi has also been

found to be effective in reducing putative periodontal pathogens levels in periodontitis patients.

1) **Hosadurga et al (2015)** conducted study on Wistor Albino rat model having experimental periodontitis with 2% *Ocimum sanctum* gel. It was experimental periodontitis as periodontitis was induced using ligature model. “36 Wistar Albino rats were randomly assigned to 3 groups which were control group, group with plain gel and with 2% gel”. “To induce periodontitis 5-0 silk ligature was used. Anti-inflammatory activity, gingival index and pocket probing depth were assessed and showed 33.6% inhibition of edema and peak activity was noted at 24 hours. Statistically significant reduction in GI and pocket probing depth was noted and concluded that *Ocimum sanctum* gel was effective in treatment of experimental periodontitis”.

2) **Gupta et al (2014)** conducted a randomized controlled clinical trial with triple blinding to evaluate the efficacy of *Ocimum sanctum* mouthwash against chlorhexidine in the removal of dental plaque and reduction of gingival inflammation. The study used normal saline as placebo group and was assessed on volunteered medical students and divided in 3 groups namely *Ocimum sanctum* mouthwash, Chlorhexidine and normal saline having 36 subjects in each group. Results showed that *Ocimum sanctum*mouthrinse is equally effective in reducing plaque and gingivitis as chlorhexidine. Gingival bleeding and plaque indices showed a significant reduction over a period of 15 and 30 days as compared to control group. The study concluded that *Ocimum sanctum* mouthrinse can be an effective mouthwash to decrease overall periodontal indices.

3) **Gaur et al (2015) "**conducted a randomized controlled clinical trial with 4% *Ocimum sanctum* and 0.2% chlorhexidine irrigation as adjunct to scaling and root planing in treatment of chronic periodontitis". The study had 30 chronic periodontitis patients which were randomized into two groups namely SRP + irrigation of *Ocimum sanctum* and other SRP + irrigation of chlorhexidine. Parameters like Plaque index, Gingival index, Pocket probing depth and Clinical attachment level were assessed at baseline and 30 days. Results showed that *Ocimum sanctum* was equally effective in reducing periodontal indices as chlorhexidine.

4) **Jayanti et al (2018) "**conducted an in vitro analysis of antimicrobial activity of *Ocimum sanctum* extract on *Aggregatibacteractinomycetemcomitans*and *Porphromonasgingivalis"*. Different concentrations used in the study were 2, 4, 6 and 8% which were diluted with dimethylformamide. Positive control was 0.2% CHX and negative control was dimethylformamide. Zone of inhibitions were measured each. Results showed 8% concentration of Tulsi extract showed maximum zone of inhibition against *Aggregatibacteractinomycetemcomitans*and *Porphromonasgingivalis.* The study had a concluding remark as 8% concentration of *Ocimum sanctum* extract showed maximum antimicrobial activity against the pathogens.

5) **Eswar et al (2016)** conducted an in vitro study to evaluate the anti-microbial activity of *Ocimum sanctum* on *Aggregatibacteractinomycetemcomitans*present in dental plaque by evaluating Minimum Inhibitory Concentration and zone of inhibition and to compare with 0.2% CHX as positive control and dimethyl sulfoxide as negative control.

Various concentrations ranging from 1% to 10% were obtained by diluting with inert solvent dimethyl sulfoxide. Results showed that at 6% w/v concentration of *Ocimum sanctum* extract widest zone of inhibition in 10 different concentrations ie of 22 mm was obtained. The study was concluded by remark that at 6% concentration maximum antimicrobial potential was observed.

6) **Mallikarjun et al (2016)** conducted an in vitro analysis to evaluate the antimicrobial efficacy of Tulsi leaf (*Ocimum sanctum*) extract on *Aggregatibacteractinomycetemcomitans,Porphromonasgingivalis, Prevotellaintermedia.* Various concentrations of the extract were diluted by inert solvent dimethyl foramide in 5 different concentrations (0.5%, 1%, 2%, 5% and 10%). Ethanolic extract was prepared by cold extraction method. The study had Doxycycline as positive control and dimethyl formamide as negative control. Results showed that at 5% concentration Tulsi extract showed inhibition zones as similar to control but *Porphromonasgingivalis and Prevotellaintermedia*showed resistance to Tulsi extract with smaller inhibition zones. The study was concluded by remark that Tulsi extract can be used as an adjunct to standard periodontal care.

7) **Ramamurthy et al (2018)** published a review on *Ocimum sanctum* and its effect on oral health in which the author stated that due to drug resistance of currently used pharmacological drugs there was a need to search for an alternative safe, efficacious and cost effective treatment options. Herbal drugs can be beneficial as it has anti-inflammatory, antibacterial, antifungal and antiviral effects. The antimicrobial effect of *Ocimum sanctum* has already

been shown against *Aggregatibacteractinomycetemcomitans,Porphromonasgingivalis* and *Prevotellaintermedia*as it contains Civsilinelol, Civsimavatine, Isothymonin, Apigenin, Rosavinic acid, linoleic acid and eugenol. The study concluded that Ocimum sanctum has got antimicrobial, anti-inflammatory properties which can be used in treatment of periodontal diseases.

8) **VitulAgarwal (2015)** published a short review on the anti-fungal properties of *Ocimum sanctumLinn.* in which the author stated that fluconazole is the most widely used drug in the treatment and prevention of candidiasis but long usage of azole drugs lead to drug resistance in *C.albicans*. Study conducted by **Amber et al (2010)** tested antifungal nature of *Ocimum sanctum* in which methyl chavicol and linalool are two main antifungal components were tested. In the study fluconazole resistant and non-resistant strains were exposed to *Ocimum sanctum* and results showed that there is synergistic action of *Ocimum sanctum* with fluconazole against all Candida species.

9) **Siva M et al (2016)** published a review on the pharmacological properties of *Ocimum sanctum* in which the author stated that Tulsi has been used for thousands of years in Ayurveda for its diverse healing properties. Tulsi is also called as “elixir of life”. The pharmacological properties mentioned in the article are anti-microbial effect, anti-fungal effect, antioxidant effects, antidiabetic effect, anti-carcinogenic property, radio-protective effect, antilipidperoxidative effect, antigenotoxic effect, wound healing effect, anti-fertility activity, anti-ulcer activity, immunologic effect, anti-anaphylactic, antihistaminic and mast cell stabilization activity, anti-stress activity,

hypolipidemic activity, anti-helminthic activity, anticonvulsant activity, cardio-protective activity, memory enhancer activity, anti-arthritic activity, anti-thyroid activity, anti-pyretic activity and antidote activity.

10) **Surender Singh et al (1996) "**conducted a study to evaluate the anti-inflammatory potential of fixed oil of *Ocimum sanctum* (Holybasil) as *Ocimum sanctum* (Labiatae) was found to possess significant anti-inflammatory activity against carrageenan and different other mediator-induced paw edema in rats. Inhibitory effect was also observed in castor oil induced diarrhea in rats". The extract inhibited arachidonic acid and leukotriene- induced paw edema. The article concluded that *Ocimum sanctum* may be a useful anti-inflammatory agent which blocks both the pathways ie Cyclooxygenase and lipoxygenase of arachidonic acid metabolism.

11) **Naveen Srinivas et al (2016)** published a review on therapeutic aspects of Tulsi in which oral implications of Tulsi are stated. Tulsi can be used as an intracanalirrigant, for toothache, for candidiasis, for oral infections and as an anti-cariogenic agent. Tulsi also has antineoplastic mechanism under which it increases apoptosis stimulation, cytotoxic induction, oxidative stress and lipidperoxidation. It decreases free glutathione, angiogenesis, and metastasis.

12) **Phillip et al (2018)** published an article on *Ocimum sanctum* local drug delivery agent in managing periodontal disease in which emphasis was given on periodontal Therapy. Three studies were reviewed in the article in which **Chaurasia et al** reported that massaging with Tulsi powder was highly effective in gingival and periodontal diseases. The concentration used was

capped at 4%. **M Hosamane et al** proved that Holy Basil has antibacterial activity against *Prevotellaintermedia*and*Fusobacteriumnucleatum* in his invitro – vivo study. **Gaur J et al (2015)** did intra-pocket irrigation of *Ocimum sanctum* in 30 chronic periodontitis patients and found that there as reduction in all clinical parameters over a period of four weeks.

13) **Prakash et al (2005)** published a review article on therapeutic uses of *Ocimum sanctum* with special emphasis on Eugenol and its pharmacological actions. Eugenol also known as (1-hydroxy-2-methoxy-4-allylbenzene) is the active constituent present in *Ocimum sanctum*. Eugenol has been known known to reduce raised blood sugar level, triglyceride and cholesterol levels and activities of LDH, GPT, GOT and alkaline phosphatase. Eugenol has been known to be a vasodilator.

14) **Ahmed et al (2017)** published a review article on pharmacological evaluation of *Ocimum sanctum*. The article stated the various uses of different parts of the Tulsi plant ie roots, leaves, seeds and whole plant and the usage of the plant is suggested for treating a wide plethora of diseases like bronchitis, dysentery, malaria, diarrhea, eye ailments, dermatological issues, rheumatoid arthritis. The article concluded that Tulsi also increases stamina and increases efficient use of oxygen in the body, strengthens immune system, reduces inflammation protects from radiation, reduces aging.

MATERIALS AND METHODS

This study was performed as a split mouth randomized, controlled, blinded, clinical trial group according to lottery method of randomization. This research project was approved by the K.L.E's V.K Institiute of Dental Sciences, Belagavi. All the patients were reviewed and written informed consent was obtained prior to the procedure.

Study participants - The study included 34 systemically healthy adults with chronic periodontitis.

Participant selection

- A minimum complement of 20 teeth to be present at the time of evaluation.
- Age – 17 to 45 years of either sex
- No history of dental treatment in the preceding 6 months including oral prophylaxis.
- Bleeding Index Score of ≥ 2
- Pocket Probing Depth $\geq$ 5mm
- Plaque Index Score of ≥ 2

EXCLUSION CRITERIA

- Smokers
- Pregnant women and lactating mothers.
- Immuno-compromised patients.
- Patients who have received antimicrobial therapy or periodontal treatment six months prior to the study.
- Subjects with prosthetic treatment

STUDY DESIGN

Clinical part of the study was performed at the K.L.E's V.K Institiute of Dental Sciences, Belagavi.

The split mouth randomized control clinical trial was conducted by a single blinded examiner.

- All study participants received thorough clinical examination at baseline, day 14, and 1 month and at end of third month.
- The following clinical parameters was assessed in each participant, according to the standard protocol.

- **Sulcus Bleeding Index** (SBI) (Muhlemann H.R and Son.S)
- **Dental Plaque Index** (PI) (Silness and Loe).
- **Periodontal Pocket depth** (PPD) in mm.
- **Clinical Attachment Level** (CAL) in mm.

- The presence or absence of supragingival plaque determined by visual examination without the aid of disclosing solutions.
- Bleeding on probing was noted within 30 seconds after probing till full pocket depth, at facial, lingual, mesiofacial, distofacial, mesiolinugal and distolingual surfaces of individual tooth.
- Probing depth was measured on the same surface of the individual tooth in millimeters using a graduated William periodontal probe.

- **The patients were divided for the split mouth study into two groups:-**

CONTROL SITE (GROUP A) – Patients undergoing scaling and root planing alone.

TEST SITE (GROUP B) – Patient treated with a combination of scaling and root planing (SRP)

- The clinical variables were recorded and collection of subgingival plaque samples was done at all the scheduled recall visits before scaling and root planing.

COLLECTION OF SUBGINGIVAL PLAQUE SAMPLES:-

- "The subjects were asked to rinse their mouth with plain water to reduce the contamination of plaque with soft debris".
- The area of plaque collection was isolated with cotton gauze.
- The subgingival plaque was collected using sterile Gracey curette by inserting it in to the periodontal pocket.
- The plaque samples collected were transported to the microbiological lab in 200 µl peptone water as a transport medium.

First Visit (Baseline) –

- At first visit, the study subjects were given oral hygiene instructions"
- The clinical parameters were recorded and subgingivalplaque were collected from the Control and Test sites.
- The Control sites received conventional scaling and root planing treatment.

- The test sites underwent scaling and root planing followed by the sub-gingival application of *Ocimum sanctum* gel- The gel was injected using the irrigant syringe into deep periodontal pockets with isolation protocol.
- Patients were recalled at visit 2, visit 3, and visit 4. The clinical variables were recorded and subgingival plaque samples was collected before scaling and root planing procedures.

CLINICAL PROCEDURE

Equipments required.

- Mouth mirror.
- Williams graduated periodontal probe.
- Suction tip
- Sterile masks and gloves
- *Ocimum sanctum* gel
- Irrigation syringe.
- Subgingival curette.
- Peptone water

The following clinical parameters will be examined in each subject, according to the standard protocol.

1) General oral examination.
2) Number of teeth.
3) Plaque Index.
4) Pocket Probing Depth.
5) Sulcus Bleeding Index
6) Clinical Attachment Level

MICROBIOLOGICAL PROCEDURE.

- The subgingival plaque samples were transported in peptone water and examined for microbiological analysis at K.L.E's PrabhakarKore's Basic Science Research Centre, Belagavi.
- The following micro-organisms were isolated from the plaque samples-
    1) *Aggregatibacteractinomycetemcomitans*
    2) *Porphyromonasgingivalis*
    3) *Prevotellaintermedia*

Antibacterial susceptibility tests

"The antibacterial effects of raw extract, emulsion and gel of *Ocimum sanctum* were assessed against each of the bacteria using broth dilution assay (Resazurin) for minimum inhibitory concentration (MIC) and agar plate assay for minimum bactericidal concentration (MBC).

Media: Brain Heart Infusion (BHI) agar and Muller Hinton Agar and broth (MHA and MHB)"

Test organisms: Three bacteria were selected for the study i.e. *Aggregatibacteractinomycetemcomitans, Porphyromonasgingivalis and Prevotellaintermedia*.

Inoculum preparation: "Inoculum preparation was carried out in specific broth media. The standard colonies of the same morphological type were selected from an agar culture plate further, each colony was scooped with a sterile loop and the grown bacteria were transferred into a tube containing 4–5 mL of BHI broth. The broth culture was incubated at 37°C for 8–14 h until it achieved the turbidity of the 0.5

McFarland standards. The turbidity of actively growing bacterial culture was adjusted with broth to obtain a final turbidity of 0.5 McFarland standards".

Extract preparation: The supercritical fluid extract of *Ocimum sanctum* was diluted with 1 ml of Tween 80 (surfactant) and 1 ml of water to form an emulsion. Further, the prepared emulsion was serially diluted to required concentrations.

Broth dilution method. Initially ten wells were selected from 96 well plates for broth dilution method. A total of 100 µl of broth was added to all the 10 wells. In the first well 100 µl of emulsion and gel were added and serially diluted to required concentrations up to 10^{th} well. Further, 20 µl bacterial inoculum was added to all the ten wells; separately another ten wells were used as positive and negative controls. The 96 well plates were kept for incubation in McIntosh and Filde's anaerobic jar and resazurin reagent was added after 48 hours and observed after 4 hours for possible colour change. Any colour change from blue/violet to slight pink/pink/magenta was recorded as MIC of emulsion. The results were recorded by taking good quality photographs.

Note: Separate 96 well plates used for emulsion and gel.

CONTENTS OF SUPERCRITICAL FLUID EXTRACT

SAMI LABS LIMITED
Biotechnology
No. 19/3, 20/1 & 20/2, Bhavikere Village, Kasaba Hobli, Nelamangala,
Bangalore Dist 562123. Ph : 91-80-2770 1928, 2770 1952
Email : samibiotech@samilabs.com

FICCI Winner FICCI FOOD 360° AWARDS — Winner of DSIR National Award 2002

CERTIFICATE OF ANALYSIS

Product Name	: TULSI LEAVES SCF EXTRACT		
Product Code	: 2044		
Batch No.	: G180583	Date of Manufacture	: Oct/2018
T R No.	: NL18F0437	Date of Expiry	: Sep/2020
Category	: Intended for Nutraceutical application	Solvent (s) used for extraction	: Super critical carbondioxide
Botanical name	: Ocimum sanctum	Other solvent (s) used in the manufacture	: None
CAS No	: 91845-35-1	Final extract ratio	: 30:1
Plant part	: Leaves	Standardization	: Eugenol, Caryophyllene, Caryophyllene oxide
Preparation type	: SCF Extraction	Excipients used	: Olive oil

Excipients Details

Name	% Used	CAS No.
Olive oil	40% to 50%	-

Parameter	Result	Limit	Reference
Physical			
Description	Complies	Dark greenish brown thick pasty oil with characteristic odour*	Visual and Organoleptic
Identification	Complies	To comply by GC for 1) Eugenol, 2) Caryophyllene, 3) Caryophyllene oxide	SLL/STP-T-007
Solubility			SLL/STP-S-028
-Alcohol solubles (1% w/v solution in 95% v/v alcohol)	88.96 % w/w	Not less than 80.0% w/w	
Moisture content by KF method	1.3 % w/w	Not more than 6.0% w/w	USP <921>
Chemical			
Assay			
-Content of Eugenol by GC	17.7 % w/w	Not less than 12.0% w/w and not more than 20.0% w/w	SLL/STP-T-007
-Content of Caryophyllene by GC	6.64 % w/w	Not less than 3.0% w/w and not more than 8.0% w/w	SLL/STP-T-007
-Content of Caryophyllene oxide by GC	2.97 % w/w	Not less than 0.8% w/w and not more than 3.0% w/w	SLL/STP-T-007
Others			
Total heavy metals	<10 ppm (µg/g)	Not more than 20ppm (µg/g)	USP <231> Method II
Lead	<0.2 ppm (µg/g)	Not more than 3ppm (µg/g)	ICP-OES, SLL/STP-H-006**
Arsenic	<0.2 ppm (µg/g)	Not more than 1ppm (µg/g)	ICP-OES, SLL/STP-H-006**
Cadmium	<0.2 ppm (µg/g)	Not more than 1ppm (µg/g)	ICP-OES, SLL/STP-H-006**
Mercury	<0.02 ppm (µg/g)	Not more than 0.1ppm (µg/g)	ICP-OES, SLL/STP-H-006**
Residual solvents	Not applicable	Not applicable	
Residual pesticides	Complies	To comply as per USP	USP <561>
Microbiological Profile			
Total aerobic microbial count	<100 cfu/g	Not more than 5000cfu/g	USP <2021>
Total yeasts and molds count	<10 cfu/g	Not more than 100cfu/g	USP <2021>
Escherichia coli	Complies	Negative/10g	USP <2022>
Salmonella	Complies	Negative/10g	USP <2022>
Staphylococcus aureus	Complies	Negative/10g	USP <2022>
Pseudomonas aeruginosa	Complies	Negative/10g	USP <62>
Bile tolerant Gram negative bacteria	Complies	Negative/10g	USP <2021>

Corporate Office : 19 /1 & 19/2, I Main, II Phase, Peenya Industrial Area, Bangalore - 560 058. Ph: 91-80-2839 7973-75, 78
Fax: 91-80-2837 3035 Website: www.samilabs.com Email: mail@samilabs.com, CIN : U24249KA1991PLC011880

SAMI LABS LIMITED
Biotechnology
No. 19/3, 20/1 & 20/2, Bhavikere Village, Kasaba Hobli, Sulamangala,
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T R No.	: NL18F0437	**Date of Expiry**	: Sep/2020

Additional Information	
Sanitising treatment	Non-irradiated and not treated with ETO
Certification Status (Kosher/Halal)	Kosher certified, Halal certified
Genetic Modification Status	GMO free
BSE/TSE status	BSE / TSE free
Country of Origin	India
Cultivated or wild crafted	Cultivated
Storage condition	Store at room temperature

***Since it is a herbal product, there is likely to be minor colour variation because of the geographical and seasonal variations of the raw material.**

Remarks : The product complies as per the specification No. FPS/ARD/651, Issue No. 4 Dated: November 16, 2015

Disclaimer: Suitability of this product for its particular use is at the sole discretion of the purchaser.

Date: 22-Oct-2018

Vedamurthy S
Manager - QC

***In-House developed and validated methods*

Corporate Office : 19 /1 & 19/2, I Main, II Phase, Peenya Industrial Area, Bangalore - 560 058 Ph: 91-80-2839 7973-75, 78
Fax: 91-80-2837 3035 Website: www.samilabs.com Email: mail@samilabs.com, CIN : U24249KA1991PLC011880

PREPARATION OF GEL

Formulation table

SL No	Ingredients	Concentration %	Uses
1	Tulsi extract	10	Active ingredient (drug)
2	Carbopol 940	3	Gel forming agent
3	Tween 80	1	Dispersing agent
4	Propylene glycol	3	Humectant and dispersing agent
5	Sodium methyl paraben	0.05	Bactericidal agent
6	Sodium propyl paraben	0.01	Bactericidal agent
7	Sodium benzoate	0.5	Bacteriostatic agents
8	Triethnolamine	0.5	pH adjusting agent to pH7
9	Distilled water qs	100	Solvent

Procedure:

Carbopol 940 was soaked in 60ml of distilled water for 24 hrs with the aid of magnetic stirrer at room temperature. Tulsi extract was triturated in pestle and mortar with Tween 80 and propylene glycol to get uniform distribution of extract. In 30 ml of distilled water, preservative was added and stirred for half an hour for complete solubility using magnetic stirrer for half an hour. In Tulsi extract solution, Carbopol 940 solution was mixed and followed by preservative solution. Total volume was made up to required quantity using distilled water. Final solution pH was adjusted to 7 using Triethnolamine drop by drop with constant stirring, using high speed propeller stirrer for 10 minute to get uniform dispersed Tulsi gel. Final prepared gel was stored in air tight container till further use.

Calculations of Tulsi extract strength in gel formulation.

Tulsi extract(5gm) was dispersed in Tween 80 (1gm) and 1ml of distilled water triturated in pestle and mortar to get uniform emulsion. From this stock solution, different concentrations were added to the three microorganisms for evaluation of MIC and MBC. Results of MIC and MBC were found to be 0.166 and 0.25 MIC and 0.25, 0.5MBC for respective microorganisms form 0.2% Tulsi extract emulsion.

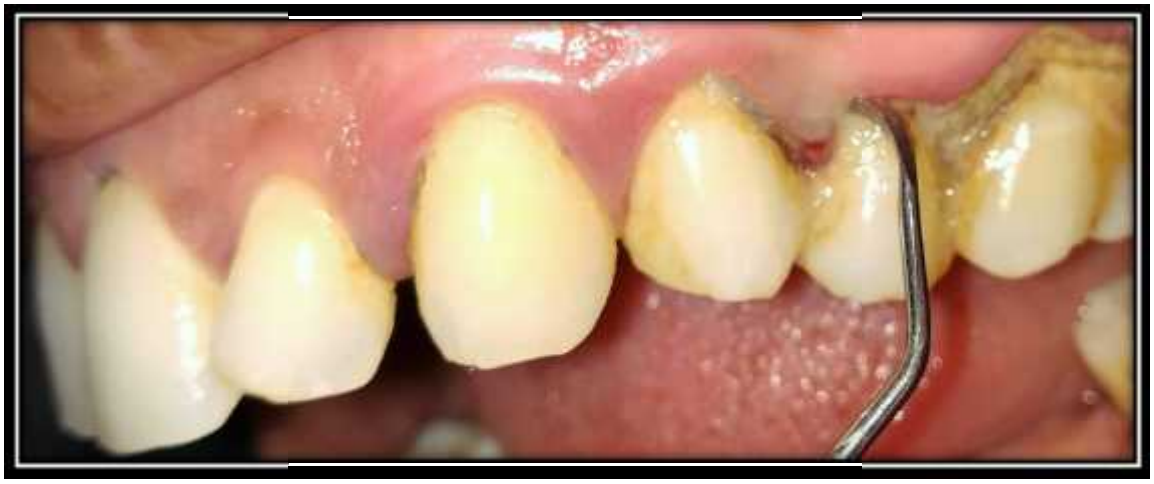
The concentration of Tulsi extract was determined to be as = 0.2% Tulsi extract= 200mg in 100ml.

From Tulsi extract gel of 5% the MIC and MBC were 200 µg/ml and 250 µg/ml respectively. Thus, 5% Tulsi extract had limited exposure to the specific microorganisms. But for the *invivo* study the percentage of Tulsi extract was increased to 10% in view of other microorganisms prevailing in the oral cavity.

PHOTOGRAPHS

1. CLINICAL ARMAMENTARIUM

2. CHRONIC PERIODONTITIS PATIENT



3. COLLECTION OF SUBGINGIVAL PLAQUE SAMPLES

4. TRANSFER OF SUBGINGIVAL PLAQUE SAMPLE IN RTF MEDIA

5 .INCUBATOR

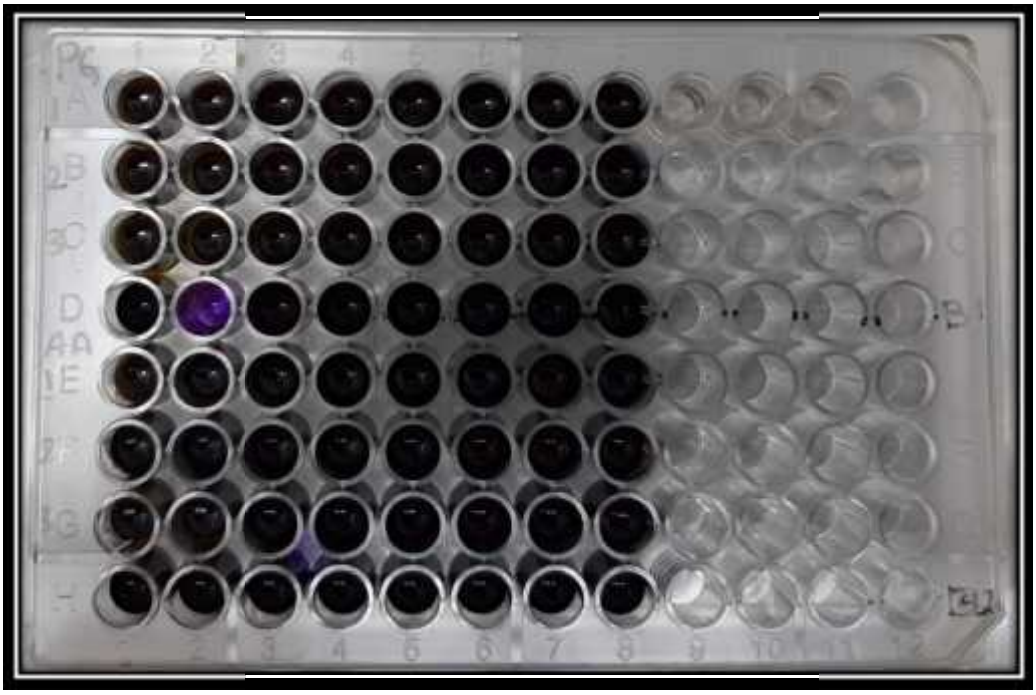
6. MCINTOSH ANAEROBIC JAR

7. LAMINAR AIR FLOW UNIT USED TO TRANSFER PREPARED TRANSPORT MEDIA INTO PETRI PLATES UNDER STERILE CONDITIONS

8 .BRUCELLA AGAR BASE

9. MUELLER HINTON BROTH

10. SPATULA



11. MIC OF PURE EXTRACT

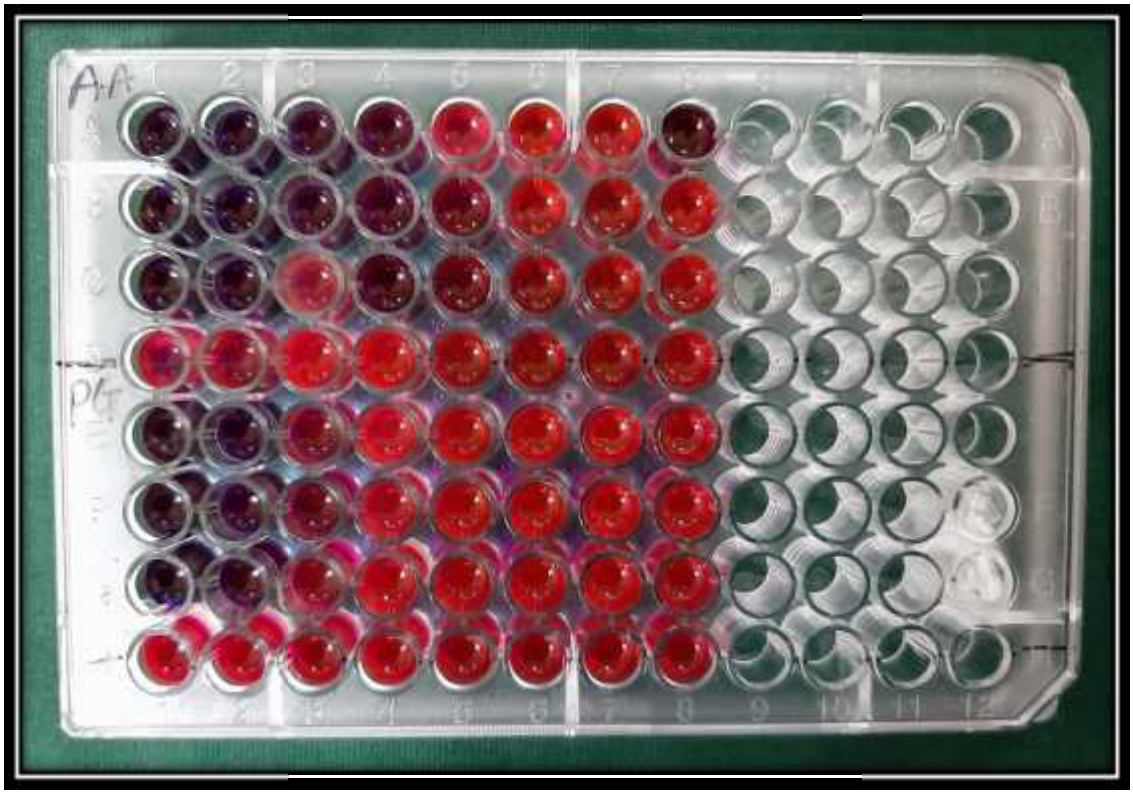
Organisms Tested A – B (*Aggregatibacteractinomycetemcomitans*)

D – E (*Porphyromonasgingivalis*)

G – H (*Prevotellaintermedia*)

12. PREPARATION OF *Ocimum sanctum* EMULSION

13. MIC OF *Ocimum sanctum* EMULSION

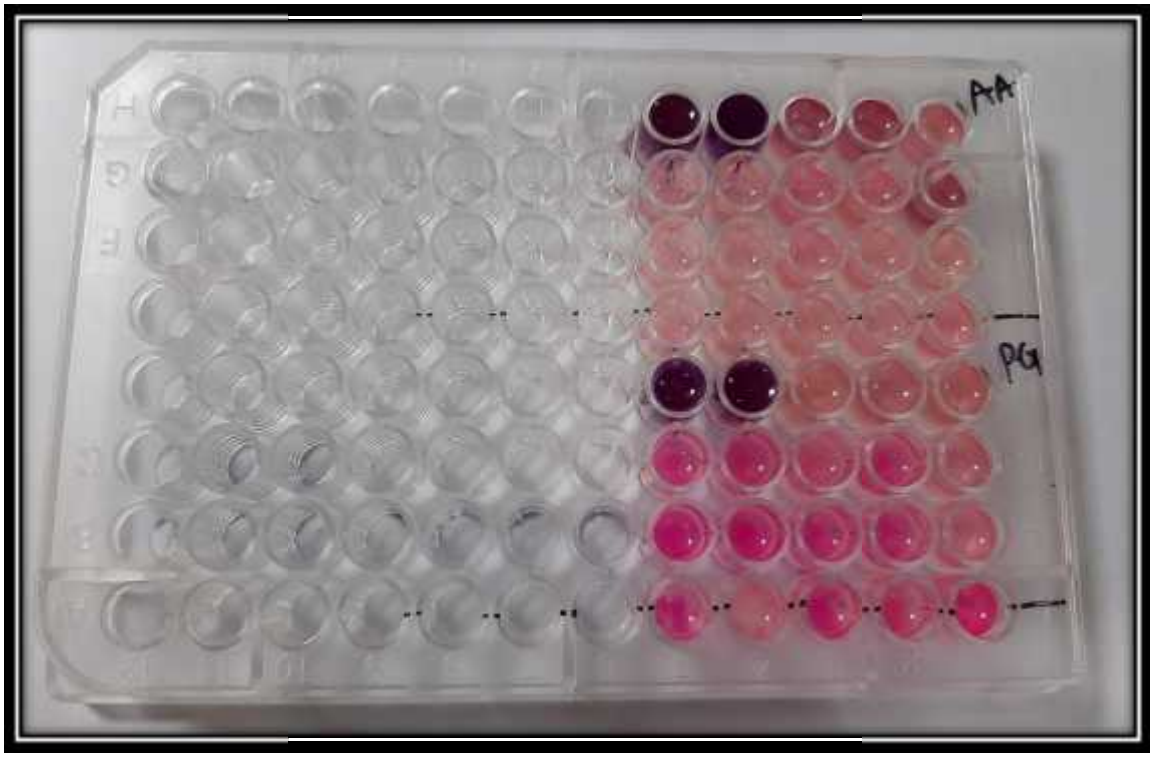


Aggregatibacteractinomycetemcomitans

Porphyromonasgingivalis

Prevotellaintermedia

14. MIC OF *Ocimum sanctum* GEL

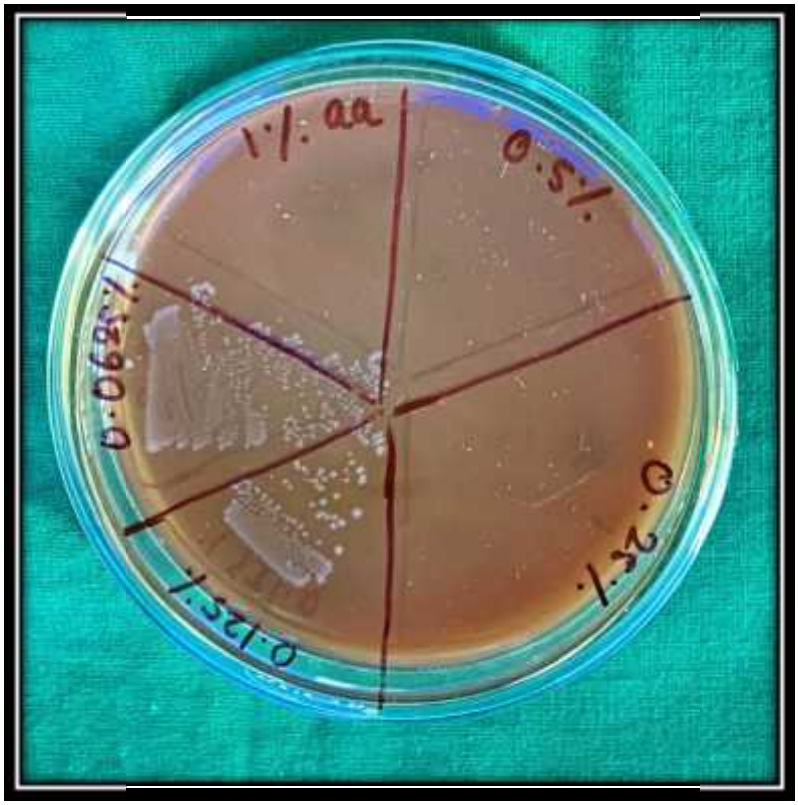


Aggregatibacteractinomycetemcomitans

Porphyromonasgingivalis

Prevotellaintermedia

15. MBC OF *Ocimum sanctum* EMULSION



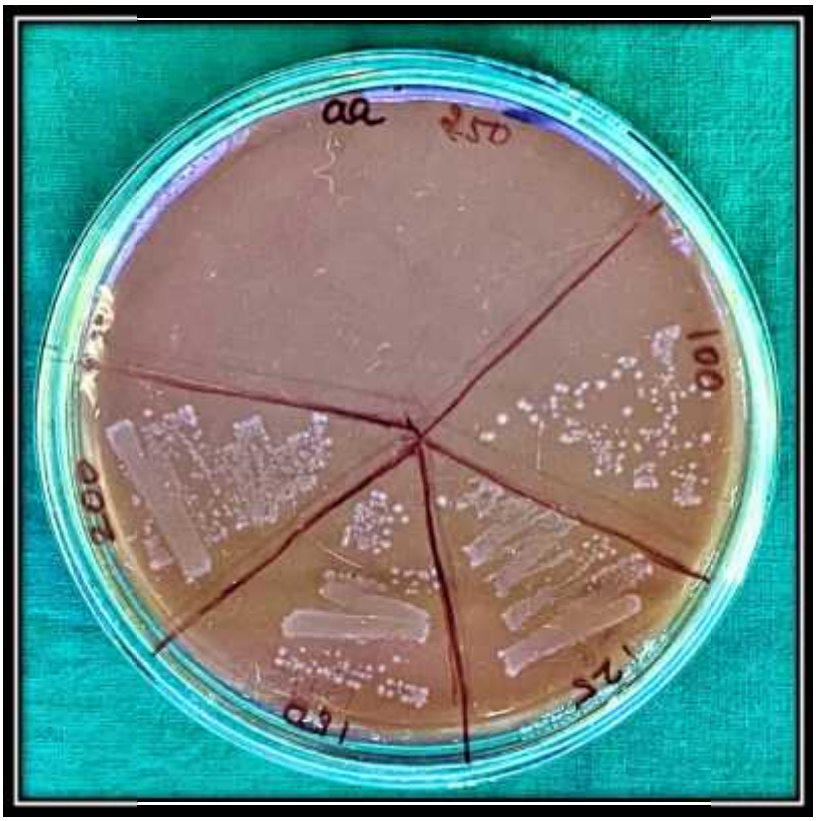
Aggregatibacteractinomycetemcomitans

Porphyromonasgingivalis

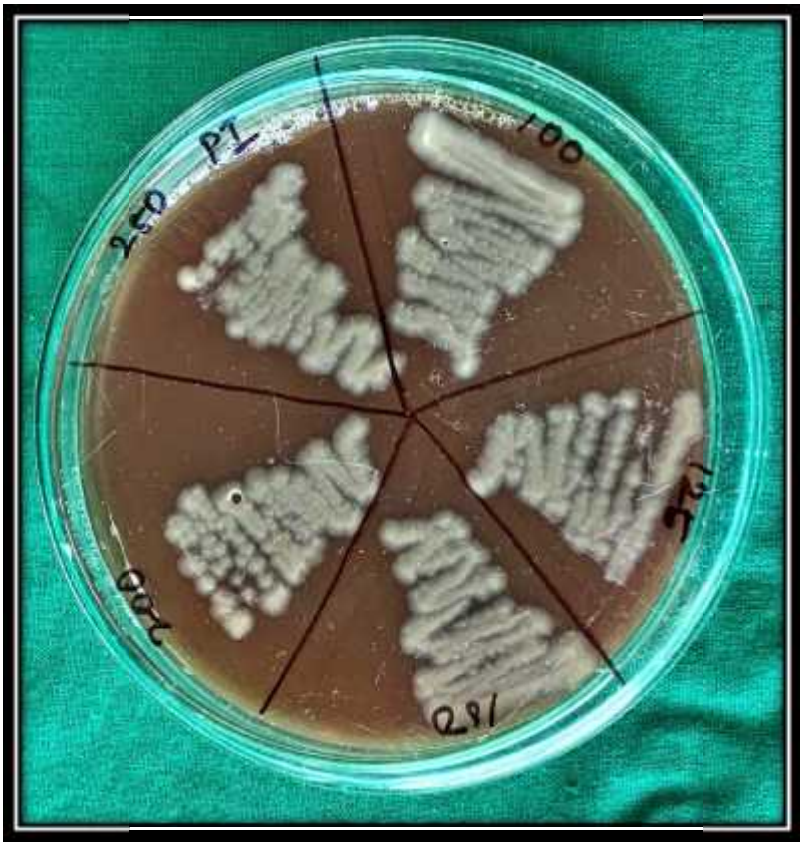
Prevotellaintermedia

16. MBC OF *Ocimum sanctum* GEL

Aggregatibacteractinomycetemcomitans

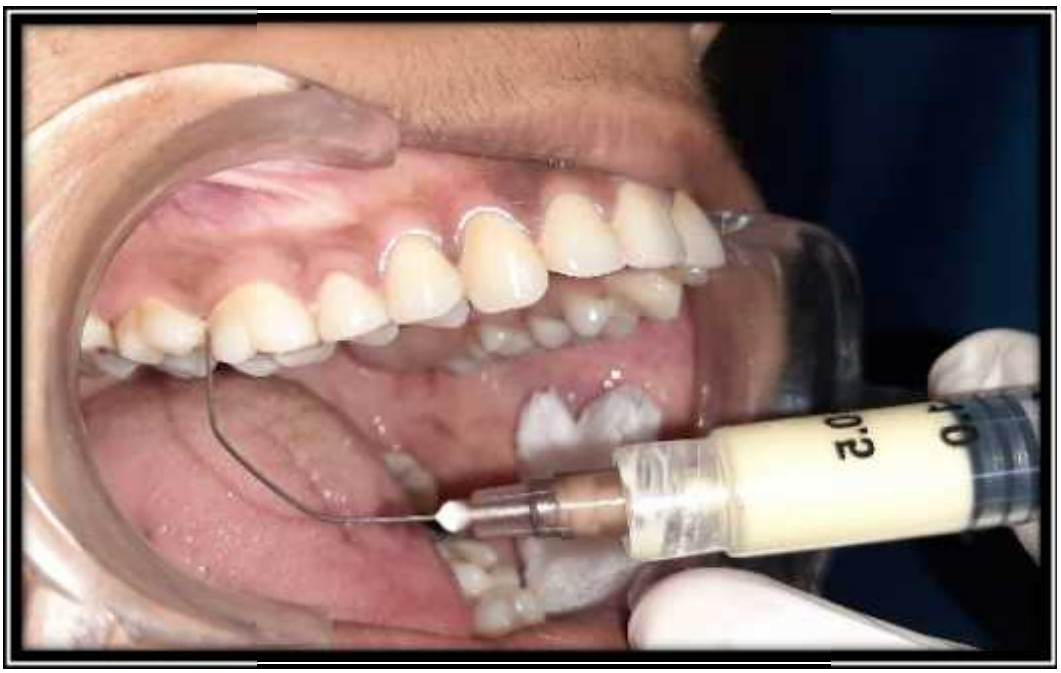


Porphyromonasgingivalis

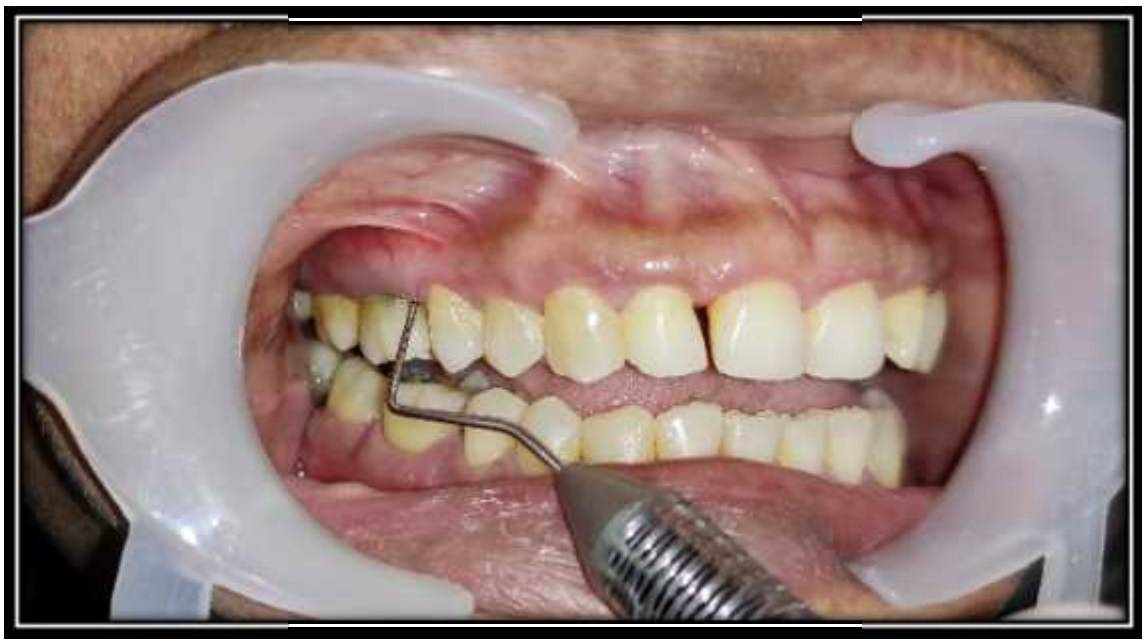


Prevotellaintermedia

17. PATIENT APPLICATION OF GEL IN TEST GROUP



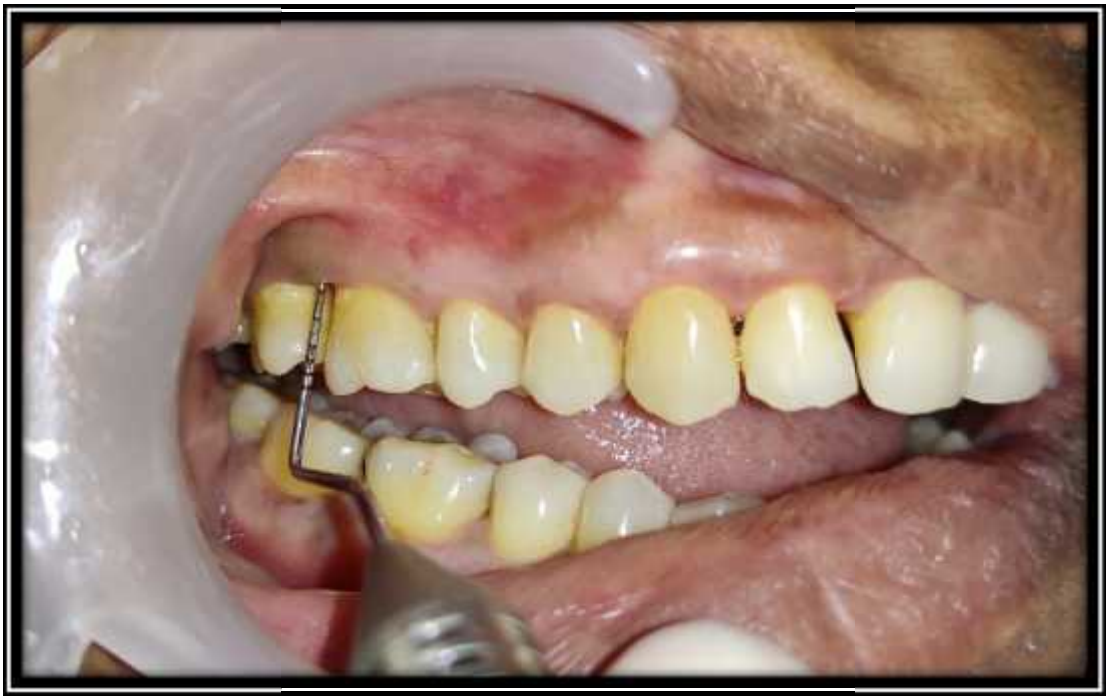
18. FOLLOWUP AT 14TH DAY



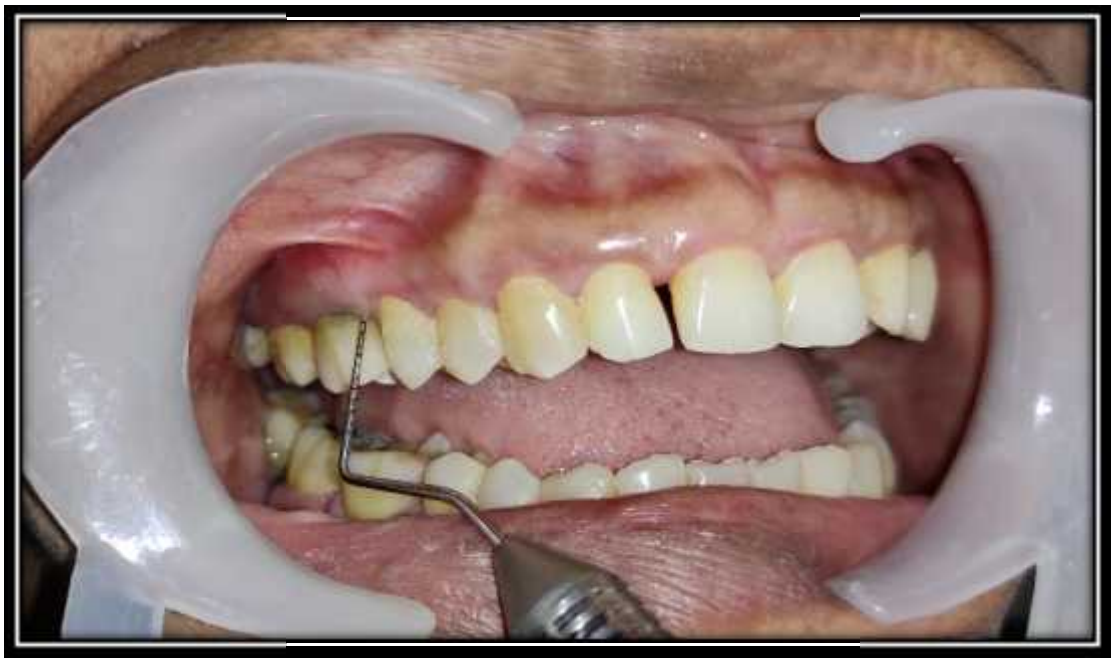
CONTROL

TEST

19. FOLLOWUP AT 1 MONTH



CONTROL



TEST

20. FOLLOWUP AT 3 MONTHS

CONTROL

TEST

RESULTS AND OBSERVATIONS

A total of 34 patients with chronic periodontitis were treated with scaling and root planing (Control) and Scaling and root planing along with application of *Ocimum sanctum* gel (Test)

All the patients were called for followup at 14^{th} day, 1 month and 3 months.

Considering the current COVID 19 pandemic, 10 patients dropped out of follow-up (which was considered during sample size calculation). Therefore statistical analysis was carried out for 34 patients who reported at subsequent follow-ups.

The data was entered in Microsoft excel and subjected to statistical analysis using SPSS software version 20.0

Baseline data for both the groups was almost the same as the study was carried out with a split mouth design.

Out of 34 patients, 12 were male and 22 were female with a mean age of 33.235 ± 7.612.

Table 1: Gender wise distribution of respondents with mean age and SD age

Sex	Number	Percent	Mean age	SD age
Male	12	35.29	32.750	6.824
Female	22	64.71	33.500	8.152
Total	34	100.00	33.235	7.612

Figure 1: Gender wise distribution of respondents

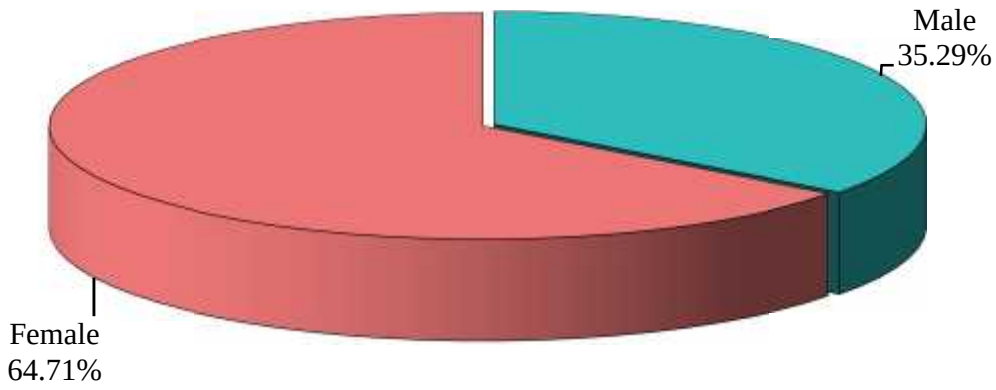


Table 2: Region of application wise distribution of respondents

Region of application	Number	Percent
15	2	5.88
16	4	11.76
17	3	8.82
26	3	8.82
27	1	2.94
33	1	2.94
34	1	2.94
35	3	8.82
36	6	17.65
37	2	5.88
43	1	2.94
45	2	5.88
46	5	14.71
Total	34	100.00

4 Clinical Parameters were assessed ie Sulcus Bleeding Index, Plaque Index, Pocket Probing Depth, Clinical Attachment Level for which Mann Whitney U test and Wilcoxon Matched Paired T tests were applied.

Table 3 shows normality of all parameters ie Sulcus bleeding index, plaque index, pocket probing depth and clinical attachment loss in two study groups at different time points by using Kolmogorov Smirnov test which shows statistically significant with both control and test groups at different time points with p value of less than 0.05 ($p < 0.05$).

Table 3: Normality of all parameters scores at different time points in two study groups (Control and Test) by Kolmogorov Smirnov test

Variables	Treatment times	Control group		Test group	
		Z-value	P-value	Z-value	P-value
Sulcus bleeding index	Baseline	1.8350	0.0020*	1.8350	0.0020*
	14^{th} day	1.9030	0.0010*	1.8290	0.0020*
	1 month	2.0070	0.0010*	2.0960	0.0001*
	3 months	3.0490	0.0001*	3.0490	0.0001*
	Baseline -14^{th} day	2.5090	0.0001*	2.3780	0.0001*
	Baseline - 1month	1.6450	0.0090*	1.9430	0.0010*
	Baseline -3months	1.8670	0.0020*	1.6140	0.0110*
	14^{th} day -1 month	1.7540	0.0040*	2.7660	0.0001*
	14^{th} day -3 months	1.8290	0.0020*	1.8290	0.0020*
	1 month -3months	1.9930	0.0010*	2.0820	0.0001*
Plaque index	Baseline	2.8350	0.0001*	2.8350	0.0001*
	14^{th} day	2.1890	0.0001*	2.9120	0.0001*
	1 month	2.9840	0.0001*	2.5860	0.0001*
	3 months	2.5860	0.0001*	3.1030	0.0001*
	Baseline -14^{th} day	2.9120	0.0001*	2.8000	0.0001*
	Baseline - 1month	3.1400	0.0001*	2.7660	0.0001*
	Baseline -3months	1.8730	0.0020*	2.4540	0.0001*

	14^{th} day -1 month	3.0490	0.0001*	3.0490	0.0001*
	14^{th} day -3 months	1.8320	0.0020*	2.5090	0.0001*
	1 month -3months	1.9280	0.0010*	2.2410	0.0001*
Pocket probing depth (in mm)	Baseline	1.7100	0.0060*	1.7100	0.0060*
	14^{th} day	2.1360	0.0001*	1.6620	0.0080*
	1 month	2.6710	0.0001*	1.6790	0.0070*
	3 months	1.9030	0.0010*	2.3970	0.0001*
	Baseline -14^{th} day	2.4120	0.0001*	2.5860	0.0001*
	Baseline - 1month	1.9280	0.0010*	1.7260	0.0050*
	Baseline -3months	1.4700	0.0260*	1.3920	0.0410
	14^{th} day -1 month	2.5140	0.0001*	2.4120	0.0001*
	14^{th} day -3 months	2.2410	0.0001*	1.5730	0.0140*
	1 month -3months	2.2350	0.0001*	1.7540	0.0040*
Clinical attachment level (in mm)	Baseline	1.7100	0.0060*	1.7100	0.0060*
	14^{th} day	2.1360	0.0001*	1.6620	0.0080*
	1 month	2.6710	0.0001*	1.6790	0.0070*
	3 months	1.9030	0.0010*	2.3970	0.0001*
	Baseline -14^{th} day	2.4120	0.0001*	2.5860	0.0001*
	Baseline - 1month	1.9280	0.0010*	1.7260	0.0050*
	Baseline -3months	1.4700	0.0260*	1.3920	0.0410*
	14^{th} day -1 month	2.5140	0.0001*	2.4120	0.0001*
	14^{th} day -3 months	2.2410	0.0001*	1.5730	0.0140*
	1 month -3months	2.2350	0.0001*	1.7540	0.0040*

Note: All parameters scores at different time points in two study groups (Control and test) not follow a normal distribution. Therefore, the non-parametric tests were applied.

SULCUS BLEEDING INDEX

Table 4: Comparison of two study groups (Control and Test) with Sulcus bleeding index scores at different treatment times by Mann-Whitney U test

Treatment times	Control group				Test group				U-value	Z-value	p-value
	Mean	SD	Median	IQR	Mean	SD	Median	IQR			
Baseline	2.59	0.66	2.50	0.50	2.59	0.66	2.50	0.50	578.00	0.0000	1.0000
14th day	1.68	0.59	2.00	0.50	1.56	0.56	2.00	0.50	519.50	-0.8160	0.4150
1 month	0.62	0.55	1.00	0.50	0.65	0.54	1.00	0.50	561.50	-0.2340	0.8150
3 months	0.12	0.33	0.00	0.00	0.12	0.33	0.00	0.00	578.00	0.0000	1.0000
Baseline -14th day	0.91	0.45	1.00	0.00	1.03	0.46	1.00	0.00	517.00	-1.0610	0.2890
Baseline - 1 month	1.97	0.67	2.00	0.13	1.94	0.60	2.00	0.00	565.50	-0.1750	0.8610
Baseline-3 months	2.47	0.66	2.00	0.50	2.47	0.71	2.00	0.50	572.00	-0.0820	0.9350
14th day -1 month	1.06	0.65	1.00	0.13	0.91	0.38	1.00	0.00	506.00	-1.1210	0.2620
14th day -3 months	1.56	0.56	2.00	0.50	1.44	0.56	1.00	0.50	528.00	-0.6980	0.4850
1 month -3 months	0.50	0.56	1.00	0.50	0.53	0.56	1.00	0.50	561.50	-0.2320	0.8170

Table 4 shows comparison of two study groups with sulcus bleeding index scores by Mann Whitney U test showing Z value of -0.81 for Baseline – 14 Days and -0.23 for 14 days – 1 month. P value was found to be less than 0.05 ($p<0.05$)

Figure 2: Comparison of two study groups (Control and Test) with Sulcus bleeding index scores at different treatment times

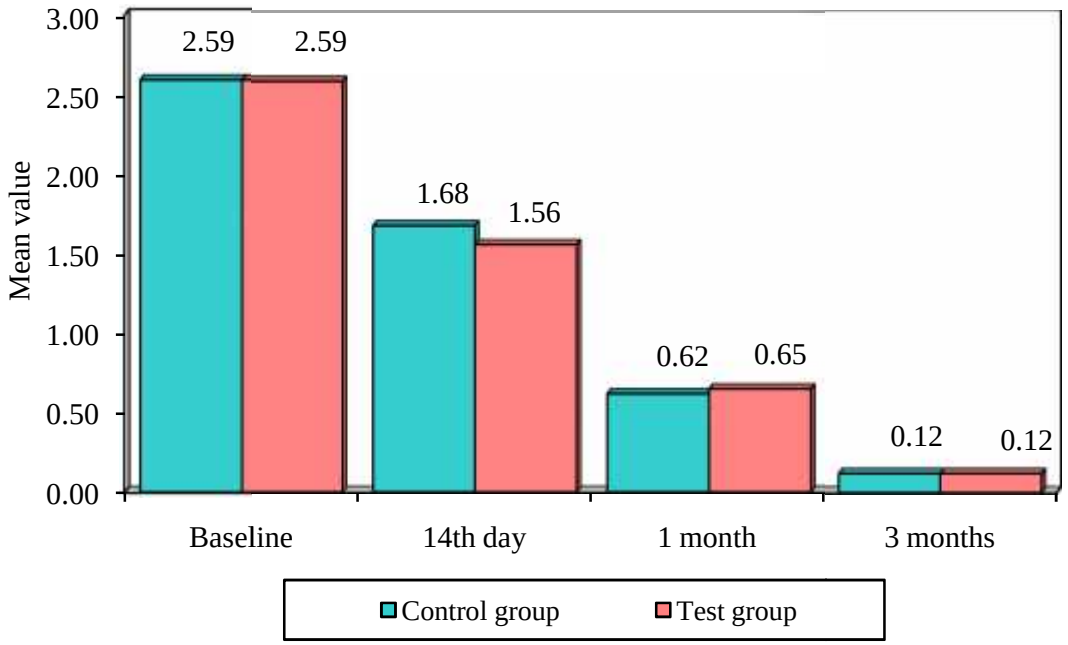
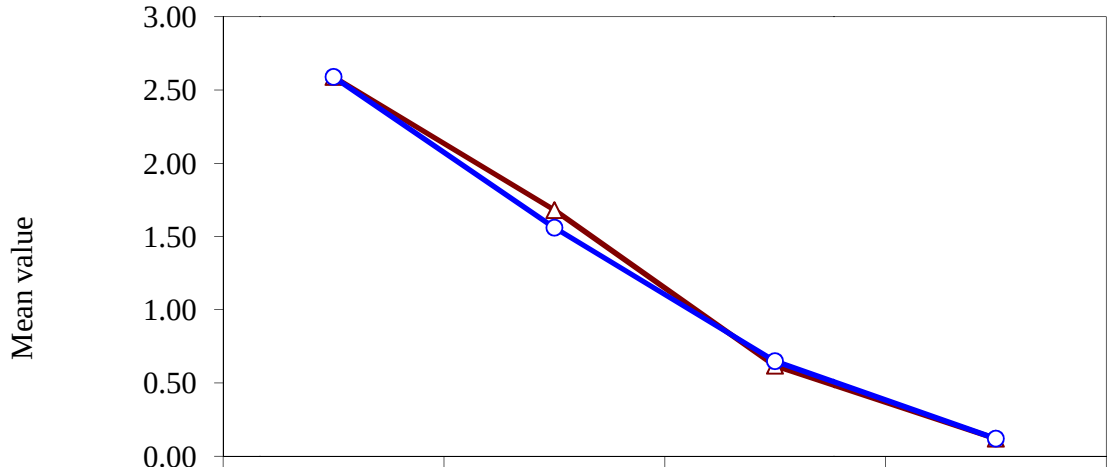


Figure 3: Comparison of two study groups (Control and Test) with Sulcus bleeding index scores at different treatment times



	Baseline	14th day	1 month	3 months
Control group	2.59	1.68	0.62	0.12
Test group	2.59	1.56	0.65	0.12

SULCUS BLEEDING INDEX

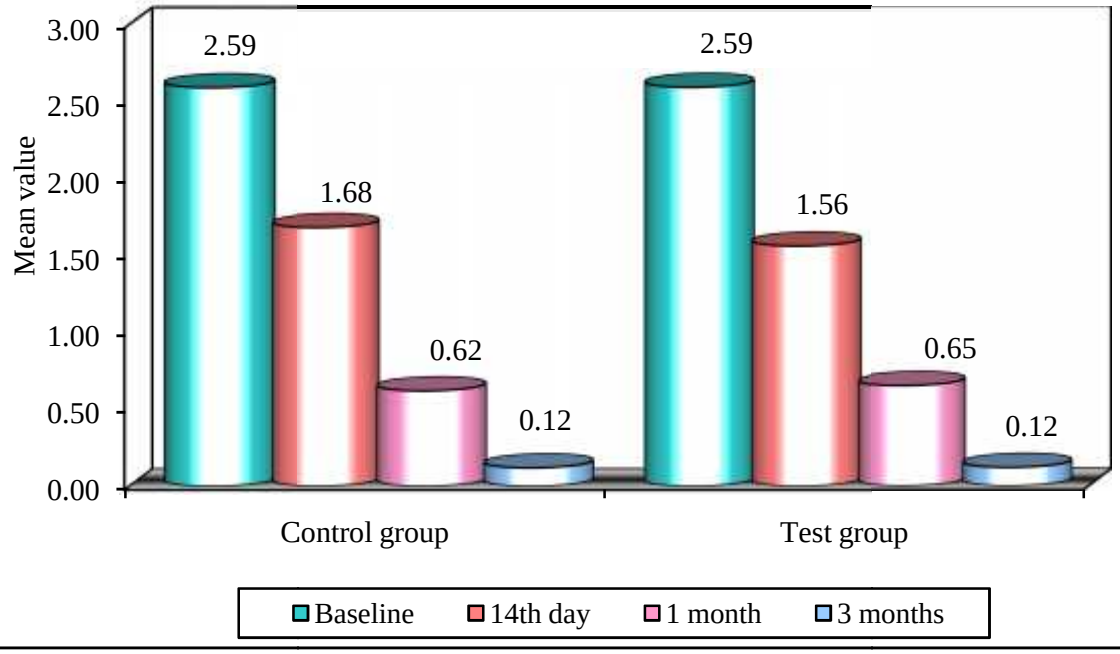
Table 5: Comparison of different treatment times with Sulcus bleeding index scores in two study groups (Control and Test) by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Control group	Baseline -14th day	0.91	0.45	35.23	-5.2310	0.0001*
	Baseline - 1 month	1.97	0.67	76.14	-5.2090	0.0001*
	Baseline -3 months	2.47	0.66	95.46	-5.2150	0.0001*
	14th day -1 month	1.06	0.65	63.16	-4.8500	0.0001*
	14th day -3 months	1.56	0.56	92.98	-5.2310	0.0001*
	1 month -3 months	0.50	0.56	80.96	-3.9000	0.0001*
Test group	Baseline -14th day	1.03	0.46	39.77	-5.2960	0.0001*
	Baseline - 1 month	1.94	0.60	75.00	-5.2670	0.0001*
	Baseline -3 months	2.47	0.71	95.46	-5.1950	0.0001*
	14th day -1 month	0.91	0.38	58.49	-5.3960	0.0001*
	14th day -3 months	1.44	0.56	92.45	-5.1690	0.0001*
	1 month -3 months	0.53	0.56	81.81	-4.0250	0.0001*

*$p<0.05$

Table 5 shows comparison of different times with sulcus bleeding index scores in two study groups (control and test) having Z values of -5.23 from Baseline to 14 Days and – 5.2 from Baseline to 3 months by Wilcoxon matched paired test having p value of less than 0.05 ($p<0.05$)

Figure 4: Comparison of different treatment times with Sulcus bleeding index scores in two study groups (Control and Test)



PLAQUE INDEX

Table 6 : Comparison of two study groups (Control and Test) with plaque index scores at different treatment times by Mann-Whitney U test

Treatment times	Control group				Test group				U-value	Z-value	p-value
	Mean	SD	Median	IQR	Mean	SD	Median	IQR			
Baseline	2.21	0.41	2.00	0.00	2.21	0.41	2.00	0.00	578.00	0.0000	1.0000
14th day	1.03	0.52	1.00	0.00	1.18	0.39	1.00	0.00	505.00	-1.2390	0.2150
1 month	0.15	0.36	0.00	0.00	0.29	0.46	0.00	0.50	493.00	-1.4520	0.1470
3 months	0.29	0.46	0.00	0.50	0.09	0.29	0.00	0.00	459.00	-2.1430	0.0320*
Baseline - 14th day	1.18	0.39	1.00	0.00	1.03	0.30	1.00	0.00	496.00	-1.7120	0.0870
Baseline - 1 month	2.06	0.24	2.00	0.00	1.91	0.38	2.00	0.00	497.00	-1.8850	0.0590
Baseline -3 months	1.91	0.62	2.00	0.13	2.12	0.48	2.00	0.00	478.00	-1.5070	0.1320
14th day - 1 month	0.88	0.33	1.00	0.00	0.88	0.33	1.00	0.00	578.00	0.0000	1.0000
14th day - 3 months	0.74	0.75	1.00	0.50	1.09	0.45	1.00	0.00	429.50	-2.2020	0.0280*
1 month -3 months	-0.15	0.61	0.00	0.50	0.21	0.54	0.00	0.50	410.50	-2.4250	0.0150*

*$p<0.05$

Table 6 shows comparison of two study groups (control and test) with plaque index scores at different treatment times with Z values of -1.23 from Baseline to 14 Days and -2.14 from Baseline to 3 months by Mann Whitney U test having p Value less than 0.05 ($p<0.05$)

Figure 5: Comparison of two study groups (Control and Test) with plaque index scores at different treatment times

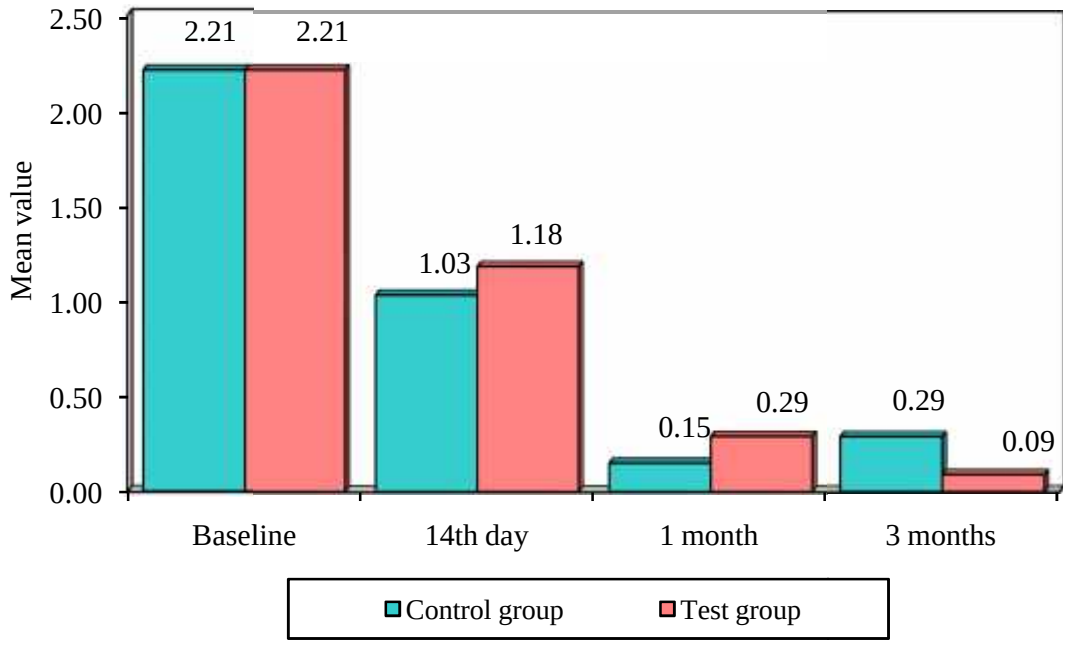
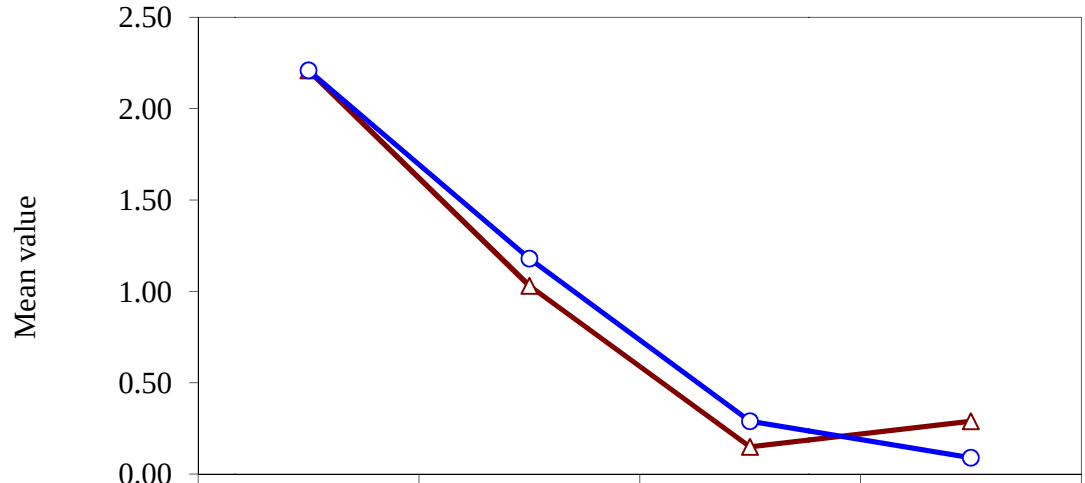


Figure 6: Comparison of two study groups (Control and Test) with plaque index scores at different treatment times



	Baseline	14th day	1 month	3 months
Control group	2.21	1.03	0.15	0.29
Test group	2.21	1.18	0.29	0.09

PLAQUE INDEX

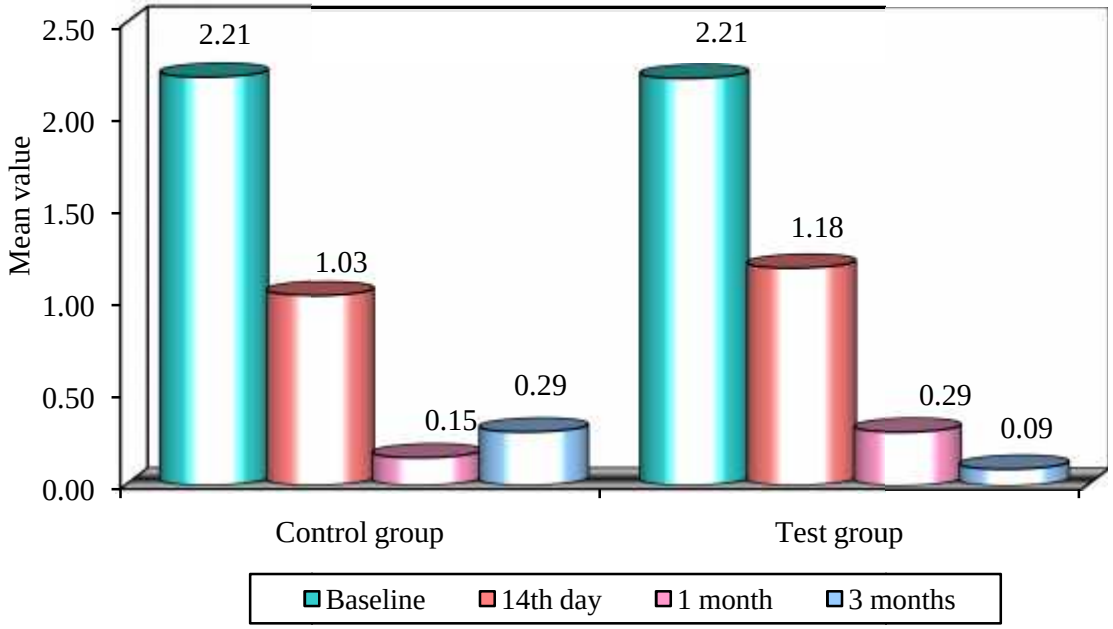
Table 7: Comparison of different treatment times with plaque index scores in two study groups (Control and Test) by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Control group	Baseline -14th day	1.18	0.39	53.33	-5.4680	0.0001*
	Baseline - 1 month	2.06	0.24	93.33	-5.6840	0.0001*
	Baseline -3 months	1.91	0.62	86.67	-5.2460	0.0001*
	14th day -1 month	0.88	0.33	85.71	-5.4770	0.0001*
	14th day -3 months	0.74	0.75	71.43	-4.0770	0.0001*
	1 month -3 months	-0.15	0.61	-99.97	-1.3870	0.1660
Test group	Baseline -14th day	1.03	0.30	46.67	-5.5960	0.0001*
	Baseline - 1 month	1.91	0.38	86.67	-5.5130	0.0001*
	Baseline -3 months	2.12	0.48	96.00	-5.3860	0.0001*
	14th day -1 month	0.88	0.33	75.00	-5.4770	0.0001*
	14th day -3 months	1.09	0.45	92.50	-5.3360	0.0001*
	1 month -3 months	0.21	0.54	70.00	-2.1110	0.0350*

*$p<0.05$

Table 7 shows comparison of different treatment times with plaque Index scores in two study groups (control and test) having Z values -5.46 from Baeline to 14^{th} day and -5.2 from Baseline to 3 months by Wilcoxon matched paired test having p value less than 0.05 ($p<0.05$)

Figure 7: Comparison of different treatment times with plaque index scores in two study groups (Control and Test)



POCKET PROBING DEPTH

Table 8: Comparison of two study groups (Control and Test) with Pocket probing depth (in mm) scores at different treatment times by Mann-Whitney U test

Treatment times	Control group				Test group				U-value	Z-value	p-value
	Mean	SD	Median	IQR	Mean	SD	Median	IQR			
Baseline	6.12	0.81	6.00	0.50	6.12	0.81	6.00	0.50	578.00	0.0000	1.0000
14th day	5.47	0.61	5.00	0.50	4.82	0.72	5.00	0.50	301.00	-3.8070	0.0001*
1 month	4.26	0.45	4.00	0.50	3.47	0.66	3.50	0.50	229.50	-4.8810	0.0001*
3 months	2.68	0.59	3.00	0.50	2.41	0.66	2.00	0.50	428.50	-2.0650	0.0390*
Baseline - 14th day	0.65	0.49	1.00	0.50	1.29	0.46	1.00	0.50	264.00	-4.6640	0.0001*
Baseline - 1 month	1.85	0.61	2.00	0.50	2.65	0.69	3.00	0.50	260.00	-4.3130	0.0001*
Baseline - 3 months	3.44	0.75	3.00	0.50	3.71	0.80	4.00	0.50	485.00	-1.2420	0.2140
14th day - 1 month	1.21	0.48	1.00	0.13	1.35	0.49	1.00	0.50	499.00	-1.2070	0.2270
14th day - 3 months	2.79	0.54	3.00	0.50	2.41	0.70	2.00	0.50	408.00	-2.3590	0.0180*
1 month - 3 months	1.59	0.50	2.00	0.50	1.06	0.65	1.00	0.13	332.00	-3.3640	0.0010*

*$p<0.05$

Table 8 shows comparison of two study groups (control and test) with pocket probing depth scores at different treatment times with Z value of -3.8 from Baseline to 14^{th} day and -2.0 from Baseline to 3 months by Mann – Whitney U test having p value less than 0.05 ($p < 0.05$)

Figure 8: Comparison of two study groups (Control and Test) with Pocket probing depth (in mm) scores at different treatment times

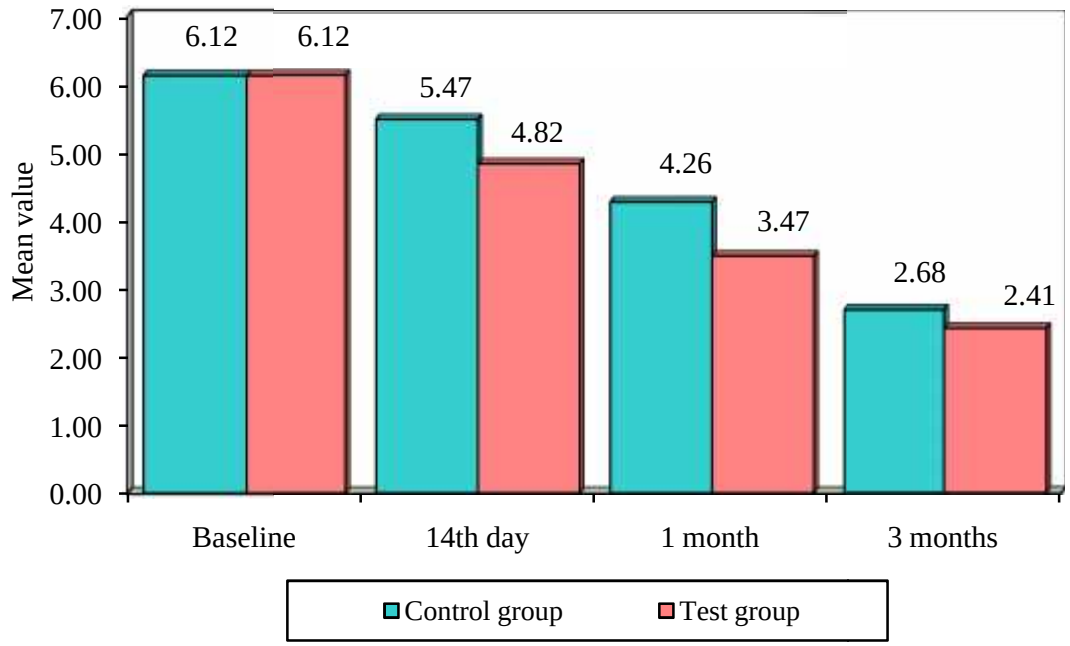
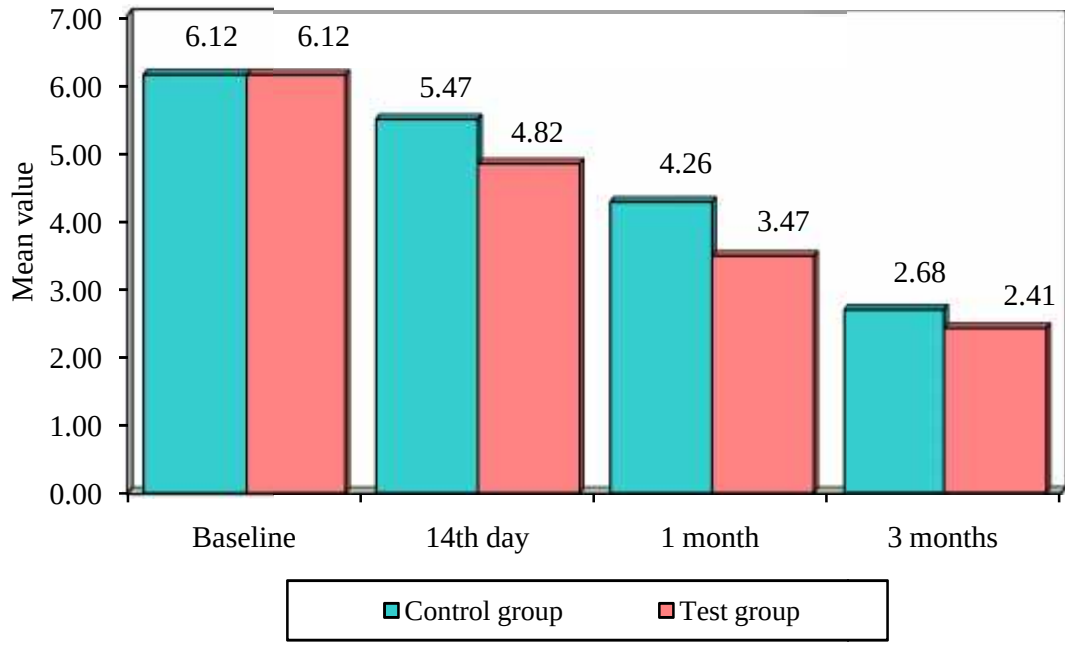


Figure 9: Comparison of two study groups (Control and Test) with Pocket probing depth (in mm) scores at different treatment times



POCKET PROBING DEPTH

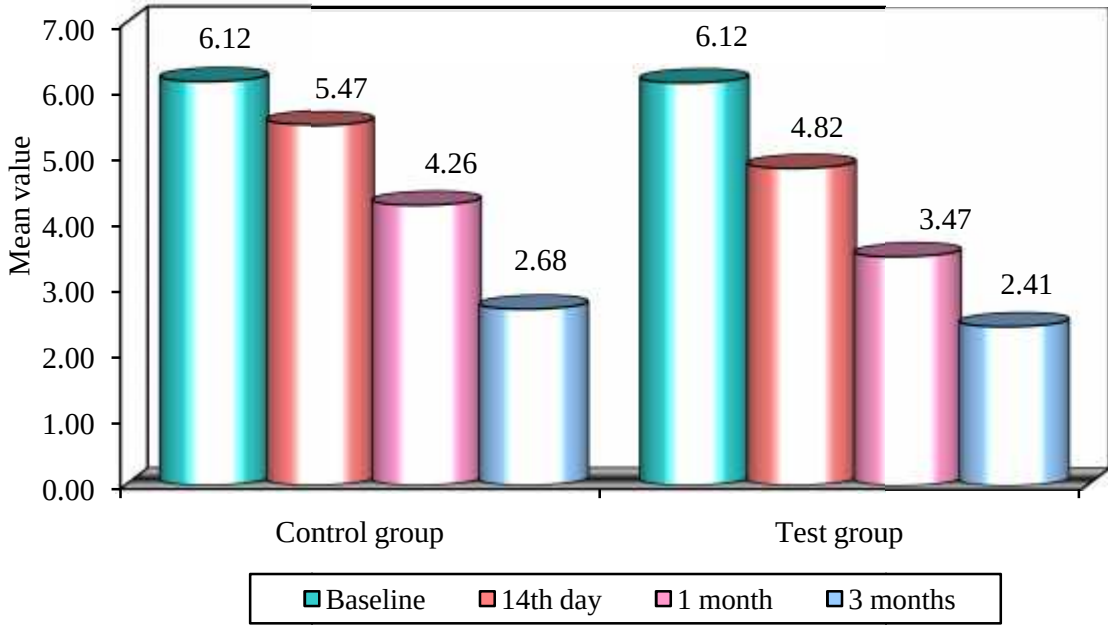
Table 9: Comparison of different treatment times with Pocket probing depth (in mm) scores in two study groups (Control and Test) by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Control group	Baseline -14th day	0.65	0.49	10.58	-4.6900	0.0001*
	Baseline - 1 month	1.85	0.61	30.29	-5.2490	0.0001*
	Baseline -3 months	3.44	0.75	56.25	-5.1840	0.0001*
	14th day -1 month	1.21	0.48	22.04	-5.3040	0.0001*
	14th day -3 months	2.79	0.54	51.08	-5.2980	0.0001*
	1 month -3 months	1.59	0.50	37.24	-5.2610	0.0001*
Test group	Baseline -14th day	1.29	0.46	21.15	-5.3320	0.0001*
	Baseline - 1 month	2.65	0.69	43.27	-5.1960	0.0001*
	Baseline -3 months	3.71	0.80	60.58	-5.1860	0.0001*
	14th day -1 month	1.35	0.49	28.05	-5.2890	0.0001*
	14th day -3 months	2.41	0.70	50.00	-5.1940	0.0001*
	1 month -3 months	1.06	0.65	30.51	-4.8500	0.0001*

*$p<0.05$

Table 9 shows comparison of different treatment times with PPD scores in two study groups (control, and test) with Z Value of -5.33 FROM Baseline to 14^{th} Day and -5.18 from baseline to 3 months by Wilcoxon matched paired test. The values are statistically significant with p values less than 0.05 ($p<0.05$)

Figure 10: Comparison of different treatment times with Pocket probing depth (in mm) scores in two study groups (Control and Test)



CLINICAL ATTACHMENT LEVEL

Table 10: Comparison of two study groups (Control and Test) with Clinical attachment levels (in mm) at different treatment times by Mann-Whitney U test

Treatment times	Control group				Test group				U-value	Z-value	p-value
	Mean	SD	Median	IQR	Mean	SD	Median	IQR			
Baseline	6.12	0.81	6.00	0.50	6.12	0.81	6.00	0.50	578.00	0.0000	1.0000
14th day	5.47	0.61	5.00	0.50	4.82	0.72	5.00	0.50	301.00	-3.8070	0.0001*
1 month	4.26	0.45	4.00	0.50	3.47	0.66	3.50	0.50	229.50	-4.8810	0.0001*
3 months	2.68	0.59	3.00	0.50	2.41	0.66	2.00	0.50	428.50	-2.0650	0.0390*
Baseline - 14th day	0.65	0.49	1.00	0.50	1.29	0.46	1.00	0.50	264.00	-4.6640	0.0001*
Baseline - 1 month	1.85	0.61	2.00	0.50	2.65	0.69	3.00	0.50	260.00	-4.3130	0.0001*
Baseline -3 months	3.44	0.75	3.00	0.50	3.71	0.80	4.00	0.50	485.00	-1.2420	0.2140
14th day -1 month	1.21	0.48	1.00	0.13	1.35	0.49	1.00	0.50	499.00	-1.2070	0.2270
14th day -3 months	2.79	0.54	3.00	0.50	2.41	0.70	2.00	0.50	408.00	-2.3590	0.0180*
1 month -3 months	1.59	0.50	2.00	0.50	1.06	0.65	1.00	0.13	332.00	-3.3640	0.0010*

*$p<0.05$

Table 10 shows comparison of two study groups (control and test) with CAL at different times with z values of -3.880 from Baseline to 14 days and -2.0 from Baseline to 3 months by Mann – Whitney U test. The values are statistically significant with p values less than 0.05 ($p< 0.05$)

Figure 11: Comparison of two study groups (Control and Test) with Clinical attachment levels (in mm) at different treatment times

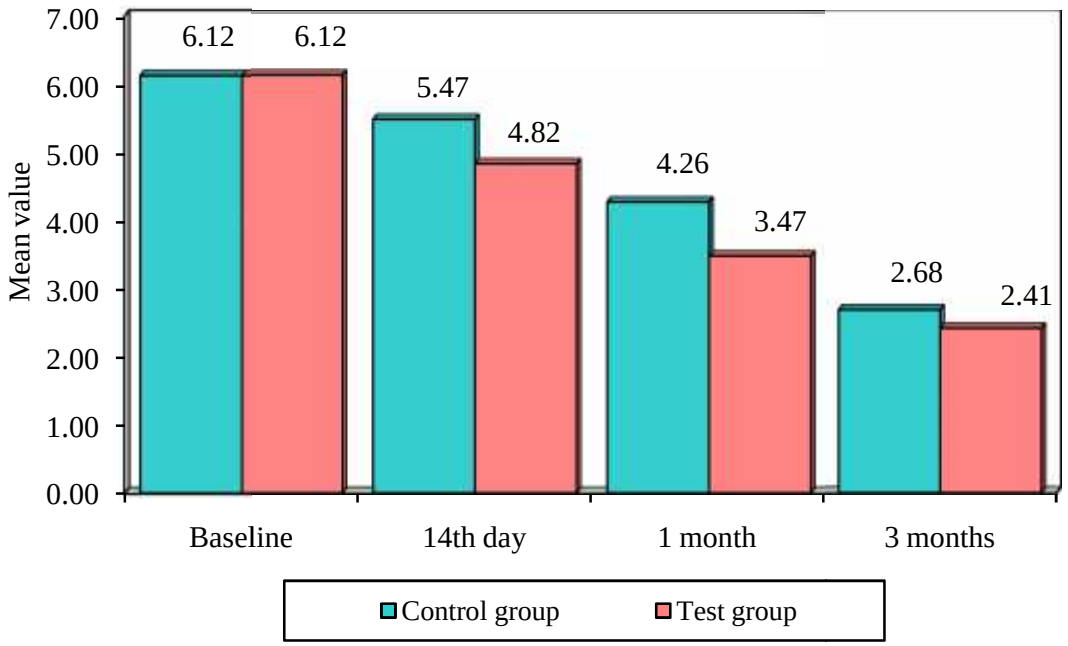
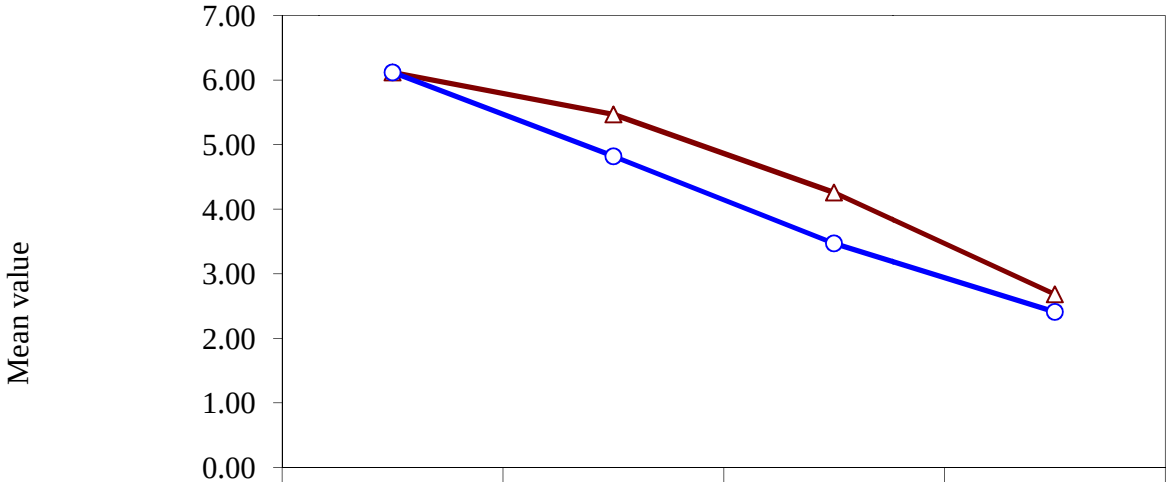


Figure 12: Comparison of two study groups (Control and Test) with Clinical attachment levels (in mm) at different treatment times



	Baseline	14th day	1 month	3 months
Control group	6.12	5.47	4.26	2.68
Test group	6.12	4.82	3.47	2.41

CLINICAL ATTACHMENT LEVEL

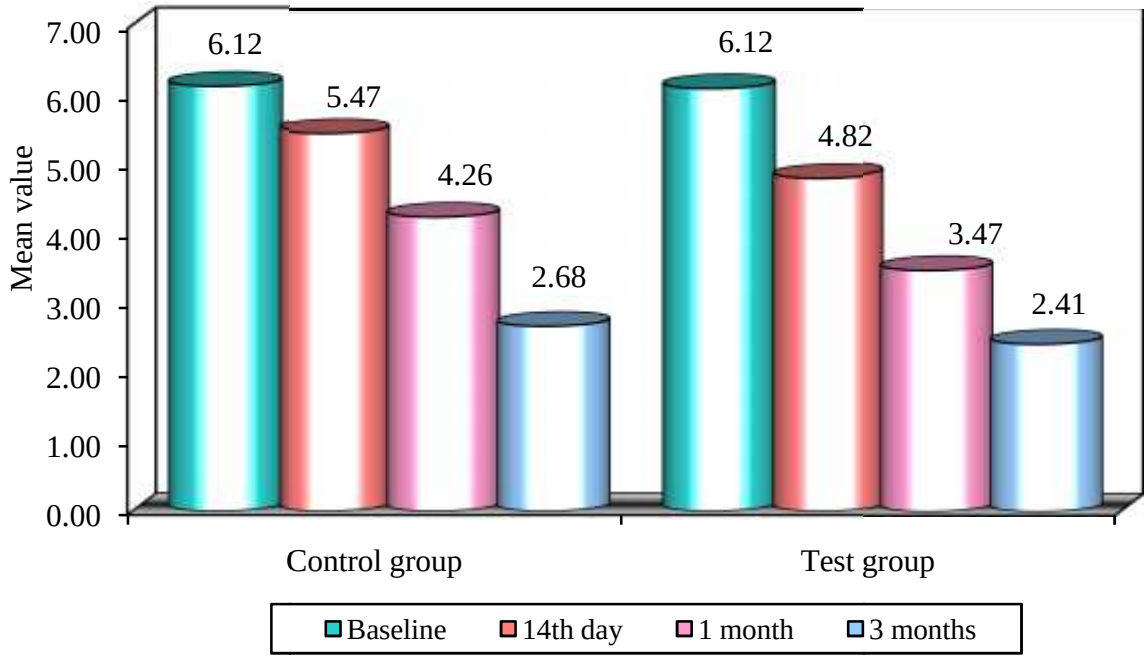
Table 11: Comparison of different treatment times with Clinical attachment levels (in mm) in two study groups (Control and Test) by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	SD Diff.	% of change	Z-value	P-value
Control group	Baseline -14th day	0.65	0.49	10.58	-4.6900	0.0001*
	Baseline - 1 month	1.85	0.61	30.29	-5.2490	0.0001*
	Baseline -3 months	3.44	0.75	56.25	-5.1840	0.0001*
	14th day -1 month	1.21	0.48	22.04	-5.3040	0.0001*
	14th day -3 months	2.79	0.54	51.08	-5.2980	0.0001*
	1 month -3 months	1.59	0.50	37.24	-5.2610	0.0001*
Test group	Baseline -14th day	1.29	0.46	21.15	-5.3320	0.0001*
	Baseline - 1 month	2.65	0.69	43.27	-5.1960	0.0001*
	Baseline -3 months	3.71	0.80	60.58	-5.1860	0.0001*
	14th day -1 month	1.35	0.49	28.05	-5.2890	0.0001*
	14th day -3 months	2.41	0.70	50.00	-5.1940	0.0001*
	1 month -3 months	1.06	0.65	30.51	-4.8500	0.0001*

*$p<0.05$

Table 11 shows comparison of different treatment times with CAL in two study groups with Z value of -5.2 from baseline to 14 days and -5.18 from Baseline to 3 months with Wilcoxon matched pairs test with statistically significant values with p value less than 0.05 ($p<0.05$).

Figure 13: Comparison of different treatment times with Clinical attachment levels (in mm) in two study groups (Control and Test)



Microbiological Report of Minimum Inhibitory Concentration and Minimum Bactericidal concentration.

Results:

MIC of Raw extract

The raw extract yielded no results as there was no growth of bacteria in the 96 well plate so, emulsion was prepared. It was deduced by no colour change at tested concentrations in wells.

Determination of MIC of emulsion

Table. 1. Minimum inhibitory concentration of *Ocimum sanctum* emulsion

Sr.No.	Samples	Minimum inhibitory concentration (MIC) in percentage					
		A.a. comitans	*Average*	*P.gingivalis*	*Average*	*P.intermedia*	*Average*
1	Emulsion	0.125%		0.25%			
2	(Ocimum	0.125%	0.166%	0.25%	0.25%	No effect	No effect
3	sanctum)	0.25%		0.25%			

Table. 2. Minimum bactericidal concentration of *Ocimum sanctum* emulsion

Sr.No.	Samples	Minimum bactericidal concentration (MBC) in percentage					
		A.a. comitans	*Average*	*P.gingivalis*	*Average*	*P.intermedia*	*Average*
1	Emulsion	0.25%		0.5%			
2	(Ocimum	0.25%	0.25%	0.5%	0.5%	No effect	No effect
3	sanctum)	0.25%		0.5%			

Determination of MIC and MBC of Ocimum sanctum gel

Table. 1. Minimum inhibitory concentration of *Ocimum sanctum* gel

Sr.No.	Samples	Minimum inhibitory concentration (MIC) in µg/ml					
		A.a. comitans	*Average*	*P.gingivalis*	*Average*	*P.intermedia*	*Average*
1	Emulsion	200		200			
2	(Ocimum	200	200	200	200	No effect	No effect
3	sanctum)	200		200			

Note: MIC in increasing concentration of gel

Table. 2. Minimum bactericidal concentration of *Ocimum sanctum* gel

Sr.No.	Samples	Minimum bactericidal concentration (MBC) in µg/ml					
		A.a. comitans	*Average*	*P.gingivalis*	*Average*	*P.intermedia*	*Average*
1	Emulsion	250		250			
2	(Ocimum	250	250	250	250	No effect	No effect
3	sanctum)	250		250			

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DISCUSSION

Periodontal diseases are a cluster of ailments that have plagued mankind since mankind came into living. They tend to begin right at the onset of eruption of the tooth into the oral cavity and persists with its very existence in the mouth till the end.

There has been vast research that has focused on understanding the etiopathogenesis of this polymicrobial dental pathology. A similar effort has also looked upon therapeutic and curative remedies available to a clinician ranging from modern science to alternate or traditional medicine.

Application of the vast treasure of Ayurveda has found favor off late particularly in application of antimicrobial, anti-inflammatory and healing properties of a number of herbs available in the Indian subcontinent.

Ocimum sanctum (Tulsi) is a sacred and traditional medicinal plant that has found a significant place in the Vedas as well as the daily rituals of majority of Indian population. It belongs to the family of Lamiaceae and has a wide array of proven health benefits [(15)].

Antimicrobial properties of *Ocimum sanctum* have been well documented and have prompted its application in treating infections caused by *E. coli abs, S. aureus* as well as *Kleibsiella* and *C. albicans*. This coupled with an anti-inflammatory effect via blockade of both cyclooxygenase and lipoxygenase pathways exerts a synergistic effect on the healing of tissues affected by the disease. The application of *Ocimum sanctum* in the treatment of the periodontal disease is a relatively unexplored domain. There have been very few studies that have experimented with the application of this plant in treating active periodontal disease or its efficacy against known periodontal

pathogens. We therefore decided to establish a scientific protocol for evaluation and application of this herb in the in vivo treatment of periodontal disease.

The supercritical fluid extract of *Ocimum sanctum* was obtained from SAMI LABS LIMITED, BANGALORE. The MIC and MBC was evaluated and found to be effective at a concentration of 10% against key periodontal pathogens namely *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Prevotella intermedia*.

The sample size for the study was selected across the mean age group of 35 years with random distribution among the sexes. The application of the gel was done following SRP at the Test site. Reevaluation for clinical parameters was done at end of 14^{th} day, 1 month and 3 months respectively. We observed a significant reduction ($p < 0.05$) in the scores of Sulcus bleeding index beginning at the time of assessment on the 14^{th} day and persistent at 1 month as well as at end of 3 months in the test group as compared to the control (Table 3). A similar observation was seen with regards to the pocket probing depth only upto the end of the 1 month period (Table 3). This can be attributed to the ability of the *Ocimum sanctum* gel to bring about a reduction in the tissue inflammation by alteration of cyclooxygenase and lipoxygenase pathways induced by periodontal pathogens owing to the linolenic acid present in the extract of *Ocimum sanctum*[(15)]

Our observations were in concurrence with the studies carried out by Eswar et al (2016) [(19)] and Mallikarjun et al (2015) [(20)] Eugenol and methyl eugenol present in the extract is known to possess 97 % cyclooxygenase inhibitory activity. Furthermore the reduction in pocket depth and bleeding on probing was effected by a reduction in the periodontal pathogens namely *P.gingivalis and A.a.comitans*.

This phenomenon however was not observed at the 3 month interval which can be probably explained on the basis of a quantum of reduction of the gel at the site of application over a period of time from baseline and thereby reducing the ability of the active ingredient to persistently offer the therapeutic benefits on a continual basis. Also we have observed that the periodontal pathogens *Prevotella intermedia* tended to have a resistance to the antibacterial properties of *Ocimum sanctum*.

This could have led to an initial suppression of *Prevotella intermedia* and subsequent recolonization of the sites at the end of 3 month period leading to relative increase in Bleeding index scores as well as pocket depth scores at the end of 3 month period. A similar phenomenon was observed with regards to the clinical attachment levels also. These observations probably warrant a repeated application of the *Ocimum sanctum* gel on a sequential basis at the end of 14^{th} day, 1 month as well as 3 months respectively. This led to an increase in the plaque scores from the 1 month to 3 months period and reflect the importance of appropriate mechanical plaque control by the individuals along with application of the gel.

Thus the clinical benefits obtained by the application of the *Ocimum sanctum* gel were found to remain up to a period of 1 month only and tended to reverse from 1 month to 3 months (Table 4 to 11). It can therefore be inferred from our study that 10% *Ocimum santum* gel is safe and efficacious in the reduction of periodontal disease when used as an adjunct to scaling and root planing. However its ability to maintain the therapeutic benefits achieved beyond a period of 1 month or more via series of recall applications needs to be looked into from a future research perspective employing a larger sample size than that of the present study.

SUMMARY AND CONCLUSION

Periodontal disease is caused by pathogenic bacterial species located sub-gingivally that harbors plaque which is characterized by a high proportion of Gram negative anaerobes such as *Aggregatibacter actinomyctetemcomitans, Porphyromonas gingivalis, Prevotella intermedia*. These bacteria play an important role in the periodontal disease process.

Conventional periodontal therapy aims at removing supra and subgingival biofilms in conjunction with meticulous supra-gingival plaque control by the patient. The use of systemic antimicrobial agents during conventional periodontal therapy is insufficient to achieve complete resolution of infection because of its limitations. T As adjuncts to conventional periodontal therapy, locally administered subgingival antimicrobials should be considered.

Therefore, the aim of our study was to evaluate the efficacy of *Ocimum sanctum* gel as an adjunct to scaling and root planing in the treatment of chronic periodontitis patients. The study was conducted at the Outpatient Department of Periodontics, K.L.E's V.K. Institute of Dental Sciences, Belagavi. The microbiological analysis was carried out in the Dr. Prabhakar Kore's Basic Science and Research Centre, Belagavi.

A total of 34 patients of the age group 17-45 years were included in the study. It was a split mouth randomized controlled trial. A written informed consent was obtained from the patients before their inclusion in the study. Detailed medical history was taken and dental examination consisted of recording the Plaque index, Sulcus Bleeding index, Pocket probing depth and Clinical attachment level. Patients were divided into two groups, Control sites which underwent scaling and root planing

(SRP) and the test site had application of *Ocimum sanctum* gel along with SRP. Data was entered in the proforma.

The study consisted of baseline (visit 1), 14th day (visit 2), end of 1st month (visit 3) and at end of 3rd month (visit 4). Subgingival plaque samples and clinical parameters were recorded at every visit before gel application.

Data obtained from the patients were analyzed statistically using Wilcoxon matched pairs test, Mann – Whitney U test and Kolmogrov – Simrnov test.

In light of the observations of the present study the following conclusions can be drawn.

1) The MIC and the MBC of *Ocimum sanctum* gel against predominant periodontal pathogens is 10%.
2) 10% *Ocimum sanctum* gel is known to exert a significant antimicrobial effect against *P. gingivalis* and *A.a.comitans* and limited antimicrobial effect against *Prevotella intermedia*.
3) 10% *Ocimum sanctum* gel when used as an adjunct to SRP brought about a significant reduction in the clinical parameters such as Bleeding on probing, Pocket probing depth and Clinical attachment loss.
4) The therapeutic benefits observed with application of 10% *Ocimum sanctum* gel as an adjunct to SRP were sustained only for a period of 1 month from baseline and tended to reverse from 1 month to 3 months' time interval. This warrants the application of the gel at every recall visit.

Furthermore its use alone for local drug delivery applications with different vehicles and devices in a larger sample size needs to be explored.

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ANNEXURE-I - ETHICAL CLEARANCE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
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Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No.: 1225

CERTIFICATE

This is to Certify that the synopsis titled

Efficacy of Ocimum sanctum gel as anadjunct To scaling and root planing (SRP) in the treatment of chronic periodontitis - A split mouth randomised controlled trial

Submitted by

Dr. Mohit Milind Kulkarni *P. G. Student /*

Staff, Guided by Dr. Vinayak Kumbhojkar *from Department of* Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 24/06/2019

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE-II - CONSENT FORM

DEPARTMENT OF PERIODONTICS
KAHER'S KLE'S V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI
CONSENT FORM

EFFICACY OF *Ocimum sanctum* GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING (SRP) IN THE TREATMENT OF CHRONIC PERIODONTITIS – A SPLIT MOUTH RANDOMISED CONTROLLED TRIAL

PRINCIPAL INVESTIGATOR: DR. MOHIT KULKARNI

I, _____, aged _____ years have been informed about my involvement in the study.

I agree to give my personal details like Name, Age, Gender, Residential Address, Previous and Present dental history and any other details if required for the study to the best of my knowledge.

I will co-operate with the dentist.

I will follow the instructions given by the dentist during study.

I will visit the dentist as and when required for the study, at the given time and date.

I permit the dentist to utilize the information given and results obtained from this study for presentation and publication without disclosing my identity.

I have understood the nature of the study and permit the dentist to collect subgingival Plaque samples and application of *Ocimum sanctum* gel in periodontal pocket.

I will not claim any returns for co-operation in this study, even if it is being sponsored by any agency. I am participating with my own will and wish.

If for any reason I am unable to participate in the study, for reasons unknown, I can withdraw from the study.

In my full consciousness and presence of mind, after understanding all the procedures and related complications if any, in my vernacular language, I am willing and give my consent to participate in this study.

Date: Address & Ph. No:

Signature:

DEPARTMENT OF PERIODONTICS

KAHER'S KLE'S V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI

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PRINCIPAL INVESTIGATOR: DR. MOHIT KULKARNI

- मी,_____,वय_____वर्षे,मलाहयाअभ्यासाबद्धलपुर्णकल्पनादेण्यातआलीआहे.
- मीमाझीवय्यक्तिकमाहितीजसेकी,नाव,वय,लिंग,पत्ता,मागीलवसध्याचीदंतउपचाराचीमाहितीवअन्यतपशीलदेण्याससहमतआहे.
- मीदंतचिकित्सकहयांनात्यांच्याअभ्यासासाठीपुर्णसहकार्यदेईन.
- दंतचिकित्सकहयांचाअभ्यासचालूअसताना,मीत्यांनीदिलेल्यासर्वसुचनांचेपालनकरीन.
- मीदंतचिकित्सकहयांनीसांगितलेल्यावेळेलावतारखेलात्यांच्याअभ्यासासाठीहजरराहीन.
- दंतचिकित्सकहयांच्याअभ्यासादरम्यानत्यांनीप्राप्तकेलेलीमाझीसर्वमाहितीवअभ्यासाचेपरिणाममाझीओळखनउघडताकुठल्याहीप्रकाशनातसादरकरायलामाझीपरवानगीआहे.
- मीदंतचिकित्सकहयांच्याअभ्यासाचेस्वरूपसमजलेआहेवमीत्यांनाआवश्यकअसेसबजिंजायवलप्लाकचेनमुनेवमाझ्यापेरीओर्डोंटलपॉकेटमध्येजेलचाउपयोगकरण्यासपरवानगीदेतआहे.
- मीदंतचिकित्सकहयांच्याअभ्यासातमाझेसहकार्यदिल्याबद्धलकोणत्याहीपरताव्याचादावाकरणारनाही,त्यांचाअभ्यासकोणत्याहीएजन्सिमार्फतप्रायोजितकेलाअसेलतरीहीमीपरताव्याचादावाकरणारनाही. मीमाझ्यास्वतःच्याइच्छेनेहयाअभ्यासातसहभागीहोतआहे.
- मीकोणत्याहीकारणास्तवअभ्यासातभागघेऊशकतनसीन,तरमीहयाअभ्यासातुनबाहेरपडुशकतो.
- मीपुर्णशुद्धीतवमाझ्यामनाच्याउपस्थितीत,सर्वप्रक्रियावत्यांचेक्वचितहोऊशकणारेदुष्परिणामसमजुन,माझ्यामातृभाषेतहयाअभ्यासातसहभागीहोण्याससम्मतीदेतो.

तारीख:-

पत्तावदूरध्वनीक्रमांक:-

स्वाक्षरी:-

DEPARTMENT OF PERIODONTICS
KAHER'S KLE'S V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI
CONSENT FORM

EFFICACY OF *Ocimum sanctum* GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING (SRP) IN THE TREATMENT OF CHRONIC PERIODONTITIS: A SPLIT MOUTH RANDOMISED CONTROLLED TRIAL

PRINCIPAL INVESTIGATOR: DR. MOHIT KULKARNI

- ನಾನು,_____
  ವಯಸ್ಸು_____ತಮ್ಮಅಧ್ಯನದಲ್ಲಿನನ್ನಮಹಿತೆಯನ್ನುನೀಡುತ್ತೇನ .
- ನನ್ನಹೆಸರು , ವಯಸ್ಸು , ಲಿಂಗ, ವಿಲಾಸ, ಕಿ ಂದಿನಮತ್ತುಸದ್ಯದಹಲ್ಲಿನಇತಿಹಾಸವನ್ನುತಮ್ಮಜ್ಞಾನದಅತ್ಯುತಮಅಧ್ಯಯನಕ್ಕಬಕಾಗುವಎ ಲ್ಲವಿವರಗಳನ್ನು,ಹಾಗೆನನ್ನವಯೆಕ್ತಿಕವಿವರಗಳನ್ನುನೀಡಲುವಪ್ಪುತ್ತೇನ .
- ನಾನುತಮ್ಮಅಧ್ಯನದಸಲ್ಲುವಾಗಿಸಹಾಯಮಾಡುತ್ತೇನೆ.
- ನಾನುತಮ್ಮದಂತಅಧ್ಯನದಲ್ಲಿನೀಡಿದಸೂಚನೆಗಳನ್ನುಅನ್ನುಸರಿಸುತ್ತೇನೆ.
- ನಾನುತಮ್ಮದಂತಅಧ್ಯನದಸಲುವಾಗಿತಾವುಸೂಚಿದದೀನಾ೦ಕಮತ್ತುವೇಳೆಗೆಅನುಸಾರವಾಗಿತ ಪ್ಪದೆಬರುತ್ತೇನೆ.
- ತಮ್ಮದಂತಅಧ್ಯನದಲ್ಲಿನನ್ನಹಸರನ್ನುಅನೂಮೂಡಸದನನ್ನಹಲ್ಲಿನಮಾಹಿತಮತ್ತುವಯಕ್ತಿತಮಾ ಹಿತಿಯನ್ನುಪ್ರಸ್ತುತಪಡಿಸುವೆಲ್ಲಮತ್ತುಪ್ರಕಟ್ಟಸಲುನಾನುವಪ್ಪುತ್ತೇನ .
- ನಾನುತಮ್ಮದಂತಅಧ್ಯನದಬಗ್ಗೆತಿಳಿದಿದ್ದು,ನನ್ನಹಲ್ಲಿನಮೇಲೆನೆಪ್ಪಾಕ್ಕನುತಗದುಕೊಳ್ಳಲುಅನ್ನು ಮತಿಯನ್ನುಕೂಡುತ್ತೇನಮತ್ತುವದ್ಯರೂನೀಡದಜಲನ್ನುಪಾಕಟ್ಟಲ್ಲಿಹಚ್ಚಲುಬೆಡುತ್ತೇನ .
- ನಾನುಯಾವುದಆಮಷಕ್ಕವಳಗಾಗದಆಧ್ಯನಕ್ಕಾಗಯಾವುದಪ್ರಯ್ಯೋಚಕರುಹಣಸಹಾಯಮಾ ಡಿದಾಗಅದರಲ್ಲಿಸಹಕಾರವನ್ನುಯಾವುದೇಆದಾಯದೆನರೀಕ್ಷಯನ್ನುಮಾಡದಸೆಹಕರಿಸುತ್ತೇನ.
- ನಾನುತಮ್ಮದಂತಅಧ್ಯನದಲ್ಲಿಸ್ವೇಯಿಚ್ಚೆಯಿಂದಬಾಗಿಯಾಗುತ್ತೇನೆ.
- ನನ್ನವಯಕ್ತಿಕಕಾರಣಗಳಿಂದತಮ್ಮದಂತಆಧ್ಯನಕ್ಕಸಹಕರಸಲುಸಾಧ್ಯವಾಗದಹೋದೆರನಾನುಅ ಧ್ಯನದಿಂದಹಿಂದಸೆರಯುತ್ತೇನ
- ನನ್ನಪೂರ್ಣಪ್ರಜ್ಞೆಮತ್ತುಸಮಯಪ್ರಜ್ಞೆ,ಅಧ್ಯನದಬಗ್ಗೆನನ್ನಮಾತೃಬಾಷಯಲ್ಲಿತಿಳಿದು,ಅಧ್ಯನದಲ್ಲವ ಳಗಾಗುವಎಲ್ಲವಿದಾನಗಳುಮತ್ತುಸಂಕೀರ್ಣಗಳನ್ನುತಿಳಿದು,ಅಧ್ಯನದಲ್ಲಿನನ್ನನ್ನುನಾನುತೊಡಗಿ ಕೊಳ್ಳಲ್ಲುವಪ್ಪುತ್ತೇನೆ .

ದೀನಾ0ಕ : ಎಳಾಸ&ಪೂೀ.ನ0 :

ಸಹಿ :

PATIENT INFORMATION SHEET

- Pre-treatment intra oral findings and case history will be recorded.
- Treatment protocol will include ultrasonic scaling, root planing using Curettes and local application of tulsi gel.
- Periodontal pack will be placed at the site of gel application for 1 week.
- Oral hygiene instructions will be given.
- Post treatment findings will be recorded at 14th day, 1st month and 3rd month recall visits.

ANNEXURE –III - PROFORMA

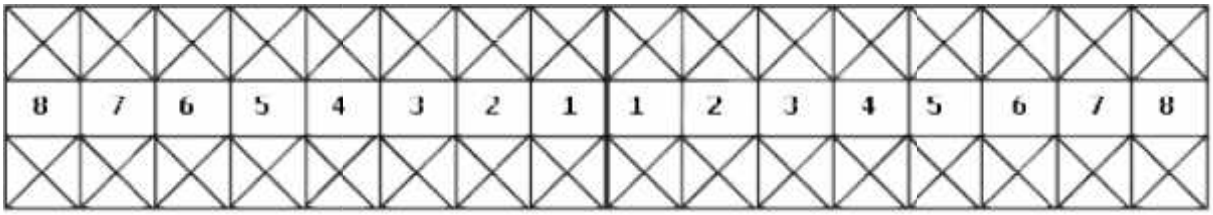
DEPARTMENT OF PERIODONTICS

KLE V.K. INSTITUTE OF DENTAL SCIENCES, BELAGAVI

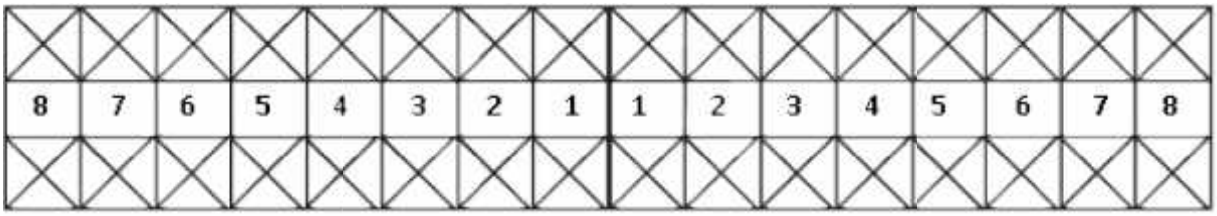
"EFFICACY OF *Ocimum sanctum* GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING (SRP) IN THE TREATMENT OF CHRONIC PERIODONTITIS – A SPLIT MOUTH RANDOMISED CONTROLLED TRIAL"

- Case No: OPD No:
- Name:
- Age: Sex: Occupation:
- Address:
- Chief Complaint:
- Dental history:

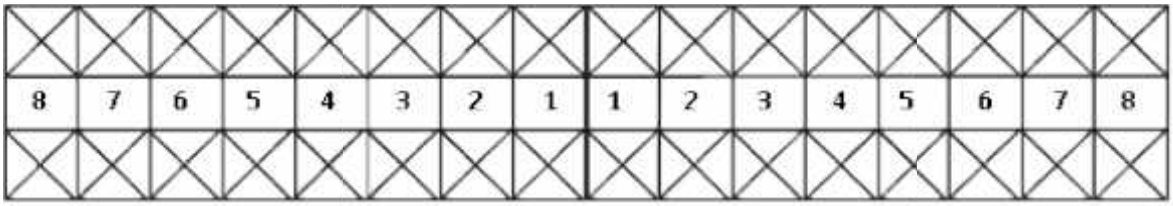
Sulcus Bleeding Index (SBI)



Pocket Probing Depth (PPD)



Clinical Attachment Level (CAL)



Plaque Index (PI)