Innovations

Efficacy of Locally Delivered 2% Grape Seed Extract on Porphyromonas Gingivalis and Fusobacterium Nucleatum: A Randomized Placebo Controlled Clinical Trial

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Abstract

Background and Objectives: Periodontitis is an infection-driven inflammatory disease in tooth-supporting tissues. Mechanical debridement alone won't be sufficient to eliminate the putative pathogens from pockets completely because of the ability of these organisms to invade gingival tissues or deeper areas inaccessible to periodontal instrumentation. Grape seed extract (GSE) has been reported to possess a broad spectrum of pharmacological and therapeutic effects including anti-microbial and anti-inflammatory activity. This study aims to assess the efficacy of grape seed extract in the form of local drug deliveory system for the treatment of chronic periodontitis; clinically and microbiologically. **Methods:** 28 patients (60 sites) with chronic (mild to moderate) periodontitis were divided into two groups as follows: GROUP I -SRP + 2% grape seed extract chip, GROUP II -SRP+ placebo chip. The following clinical parameters were assessed at baseline, and 3 months: Plaque index, Bleeding index and Probing pocket depth. Subgingival plaque samples were collected at baseline and 3 months to assess for the following microorganisms: Fusobacterium nucleatum and Porphyromonas gingivalis. **Results:**A statistically significant reduction was observed in PI scores, BI scores and Probing pocket depth in Group I and group II from baseline to 3-month follow-up. Inter-group comparison between group I and group II showed evident results in group I for all the clinical parameter except PI scores. Intergroup comparison of CFUs of F. nucleatum and P. gingivalis showed statistically significant reduction in Group I (P value<0.05). **Conclusion**: The study demonstrated that placement of 2% grape seed extract chip after SRP in the periodontal pocket is effective and beneficial in patients with chronic periodontitis and hence can be recommended as a treatment option for chronic periodontitis when used as adjunct to non-surgical periodontal therapy.

KEY WORDS: *Chronic Periodontitis, Scaling and root planing, Local drug delivery, Grape seed extract chip*

Introduction:

An infection-driven inflammatory disease of tooth-supporting tissues (i.e., the periodontium) is defined as periodontitis. Genetics and environmental and behavioural factors aid in the development of the disease, the

exposure of susceptible individuals to its initiation, and the speed of progression.[1]Periodontium has a diverse structure; it is composed of the gingiva, the underlying connective tissue, cement on the root surface, alveolar bone, and the periodontal ligament between the cementum and alveolar bone.[1] The junctional epithelium of the gingiva is located at the bottom of the gingival sulcus, which controls the constant presence of bacteria at this site and is a unique structure.[1]Chronic inflammation is a complex biological process that occurs in response to infection and/or other triggers and leads to tissue injury.[2] Chronic periodontitis is a periodontal disease which is characterized by both dysbiosis of oral microbiota and proinflammatory events involving both cells and mediators from innate and adaptive immunity.[3]

Porphyromonas gingivalis (P. gingivalis) and *Fusobacterium nucleatum (F. nucleatum)* are Gram-negative anaerobic bacteria possessing several virulence factors that make them potential pathogens associated with periodontal disease.[4]

The periodontopathogenic bacteria initiate periodontal tissue destruction by releasing specific bacterial products like lipopolysaccharides (LPS) and gingipain that can cause oxidative stress from the activity of excessive reactive oxygen species (ROS) or a deficiency of antioxidants or from activating transcription factors which may be the most pertinent factor relating to periodontal tissue damage.[5,6]

Scaling and root planing along with patient education is the mainstay for treating periodontal disease. But mechanical debridement alone won't be sufficient to eliminate the putative pathogens from pockets completely because of the ability of these organisms to invade gingival tissues or deeper areas inaccessible to periodontal instrumentation.

Therefore, antibacterial agents have been used along with mechanical debridement in the management of periodontal infection. Most of the problems associated with systemic therapy can be avoided with the concept of local drug delivery by limiting the drug to its target site and hence achieving a much higher concentration.[7]

Literatures has been shown that the harmful effects of oxidative processes in living organisms can be reduced by the dietary intake of flavan-3-ols and procyanidins. Grape seed extract (GSE) has been reported to possess a broad spectrum of pharmacological and therapeutic effects including anti-microbial and anti-inflammatory activity.[8] The most abundant phenolic compounds isolated from grape seeds are catechin, epicatechin, and procyanidins.[6] Possible mechanisms by which these phenolic compounds might exert their protective effects include antioxidative properties, oxygen and nitrogen scavenging abilities and inhibitory effect`s on both arachidonic acid cascade and i-NOS modulation.[5] Certain studies have shown that catechin type compounds from grape seed extract inhibit lipoxygenase and induce antioxidant or pro-bacterial action.[6] They inhibit attachment of microorganisms to the periodontal tissue and reduce bacterial biofilm formation, collagenase activity and invasion by neutralizing periodontopathogen proteinases and cytotoxicity.[6]

Hence, this study aims to assess the efficacy of grape seed extract in the form of local drug delivery system for the treatment of chronic periodontitis; clinically and microbiologically.

Material and Methods

This randomized double blinded placebo-controlled trial was carried out in Department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore. 28 patients (18 males and 10 females) in the age group of 28-57 years diagnosed with localized periodontitis were enrolled for the study.

Inclusion criteria:

- A clinical diagnosis of chronic periodontitis with pocket depth 5-6 mm with evident radiographic horizontal bone loss.
- Systemically healthy patients
- Non-smokers.
- Patients who have not undergone any periodontal therapy in the previous 6 months.
- Willingness to provide informed consent and to comply with the OHI given.

Exclusion criteria

- Pregnant women and breastfeeding mothers.
- Patients taking dietary supplements.
- Patients allergic to grape seed extract.
- Patients who have undergone periodontal therapy in the previous 6 months.
- Patients under systemic or topical antibiotic treatments in the past 3 months.
- Patients taking any medications that could interact with grape seed extract.

The nature and design of the clinical trial were explained to the patients in the local language, and written consent was obtained for their participation. Oral hygiene instructions for supragingival plaque control were given. Patients were asked to brush twice daily using a soft toothbrush and paste according to Bass method.

Pooled subgingival plaque samples were collected using sterile Gracey curette into the Eppendorf tubes and transported in fluid thyoglycolate medium to be processed immediately.

A full mouth probing and charting were done to assess the suitability of the subjects for the study. The treatment in each of the patient included full mouth scaling with ultra-sonic scaler and root planing with Gracey curettes. After SRP the study sites were randomly assigned to either grape seed extract group or control groups using fair coin tossing method and subjected to double-blinded evaluation. Groups were divided as follows:

GROUP I: SRP along with administration of grape seed extract chip (Test group)

GROUP II: SRP along with administration of placebo chip (Control group)

Screening of Patients:

All potential participants were selected randomly and were explained the need and objective of the study. Only those subjects who consented for the study were included after obtaining an informed consent. The subjects were screened for their suitability with the use of mouth mirror and UNC 15 periodontal probe. The subjects also underwent full mouth periodontal probing to assess the periodontal status. Mild to moderate periodontitis patients were diagnosed based on criteria presented and discussed at the 1999 International Workshop for the Classification of Periodontal Diseases organized by the American Academy of Periodontology.

Clinical parameters recorded at baseline (T0) and 3 months (T1)

- Plaque index (Silness P and Loe H-1964)
- Bleeding index (BI) (Ainamo and Bay-1975)
- Probing pocket depth (PPD)

Microbiological analysis:

Pooled subgingival plaque samples were collected for microbiological analysis from each quadrant at baseline and 3 months. The microbiota which was taken into consideration for the present study was Fusobacterium nucleatum and Porphyromonas gingivalis. To obtain the plaque sample, Area specific Gracey curette was inserted to the depth of the pocket and then transferred immediately to a sterile Eppendorf tube containing transported in fluid thyoglycolate. This was then transported immediately to the laboratory for microbiological analysis. 20µl of suspension was cultured in selective brucella blood agar and incubated anaerobically using Gas pak system.

Statistical Analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive statistics

Descriptive analysis of all the explanatory and outcome parameters was done using frequency and proportions for categorical variables, whereas in Mean & SD for continuous variables. **Inferential Statistics**

Repeated measures of ANOVA test were used to compare the mean GI, PI scores & PPD levels between baseline and 3 months. ANOVA Test followed by Wilcoxon sign rank test was used to compare the mean PPD values between 2 groups during Baseline and 3 months period. Mann Whitney test was used to compare the mean CFUs of different organism's b/w 2 groups at Baseline and 3 months period. The level of significance was set at $P<0.05$.

Results

In the present study a total of 28 subjects with 60 sites between the age range of 28 – 57 years were enrolled. The study subjects were in the age range of 28-57 years with a mean age of 38.78. The gender distribution of the study subjects were males (n=34) making up 56.7% while females (n=26) making up 43.3%.

The mean PI score at baseline was 1.64 ± 0.31, which consistently reduced to a score of 1.07 ± 2.56 at the 3-month follow up in group I and in group II it reduced to 1.06 ± 0.30 at 3 monthfollow-up from 1.56 ± 0.41(baseline). The reduction in PI scores were statistically significant at 3-month follow up visit ($P < 0.05$). Inter-group comparison was done by Mann whitney test and on comparing the mean difference there was no statistically significant difference found between the groups (Figure 1).

There was a statistically significant reduction in the BI from baseline (60.53 ± 9.61) to the 3-month recall visit (51.33 ± 8.06) in group I. In group II BI from baseline (57.17 ± 7.03) had also reduced significantly in the 3-month recall visit (49.58 ± 5.81). Inter-group comparison showed better results in group I compared to group II.

At baseline, the mean probing depth was 5.40 ± .498 and 5.43 ± .504 for Group I and II respectively. The PPD reduction within the groups was statistically significant at 3 month interval with mean value 4.0 ± .743 and 4.43 ± .568 for Group I and II respectively. (Figure 2)

Inter-group comparison between group I and group II showed statistically significant reduction in PPD at 3 months recall in group I compared to group II (P value=0.007).

Microbiological Findings

Intergroup comparison of Colony forming units (CFU) between two groups at different time intervals was done by Mann whitney test and between different time intervals in each group was done by Repeated Measures of ANOVA followed by Wilcoxon signed rank test.

The mean CFUs with respect to P. gingivalis was $1.25 \times 10^5 \pm 2.98 \times 10^5$ in group I and $1.64 \times 10^5 \pm 3.35 \times 10^5$ in baseline which reduced significantly to $0.016 \times 10^5 \pm 0.029 \times 10^5$ in group I and $0.48 \times 10^5 \pm 1.82 \times 10^5$ in group II at 3-month evaluation.

Similarly, the mean CFUs at baseline with respect to F. nucleatum was $0.88 \times 10^5 \pm 2.5 \times 10^5$ and $1.32 \times 10^5 \pm 2.97 \times 10^5$ in group I and group II respectively, which was significantly reduced to $0.012 \times 10^5 \pm 0.024 \times 10^5$ and $0.14 \times 10^5 \pm 0.29 \times 10^5$ at 3 months in group I and group II respectively. (Table 1)

Inter-comparison of both P. gingivalis and F. nucleatum CFU units showed evident statistical significance in Group I compared to Group II.

Discussion

Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation, recession, or both.[43] P. gingivalis and F. nucleatum, Gram-negative, anaerobic bacteria, have been studied extensively as member of the microbiota involved in periodontal disease progression and subsequent bone and tissue destruction (Holt, Kesavalu et al. 1999).[4]

Effective treatment of periodontitis requires the control of subgingival plaque by scaling and root planing. As the pockets deepen, mechanical debridement measures become less effective. Retention of plaque in inaccessible sites can be a nidus for reinfection, which may allow return of microflora with recurrence of disease.[10]

Long-term adjunctive use of systemic and topical chemotherapeutic agents to scaling and root planing in the treatment of periodontitis is contraindicated because of adverse effects and development of bacterial resistance. Local administration of antimicrobial agents offers a 'site-specific' approach to periodontal therapy having several benefits.[10]

The grape (Vitis vinifera), a member of the Vitaceae family has got various kinds like wine grapes, table grapes, seedless, edible seed, and raisin grapes. GSE has anti-inflammatory, anti-apoptotic, anti-necrotic, cardiovascular, and anti-carcinogenic properties, making it useful in the treatment of a variety of ailments.[11]

Many compounds from the vast family of substances known as flavonoids, which is further subdivided into smaller substances like catechins and pro-anthocyanidins, are abundant in grapes(de la Iglesia, Milagro et al. 2010).[11]

GSE produces its anti-inflammatory impact by controlling the production and gene expression of cytokines, which delicately balance the ratio of pro-inflammatory to anti-inflammatory cytokines. GSE was demonstrated to protect against collagen breakdown and had a bacteriostatic effect on the anaerobes that may significantly decrease the maturation of dental biofilm and therefore may be used in the prevention of periodontal disease.[12]

Our clinical trial was undertaken to assess the clinical and anti-microbial efficacy of 2% grape seed extract as a local drug delivery system adjunct to scaling and root planing in the treatment of periodontal disease. All the clinical (PPD, PI, and BI) and microbiological parameters were recorded at baseline and 3 months after therapy.

In this study, both the groups showed a significant improvement in the clinical parameters in chronic periodontitis patients. Among the two groups, SRP with placement of 2% grape seed extract chip demonstrated better result when compared with the placebo group.[13]

Bleeding index (BI) in the present study has been significantly decreased in both the groups from baseline to 3 months. On comparing the groups, group I showed better results with a P value<0.05. This is in consistence with a study by M. Das et al (2021) who evaluated the clinical efficacy of GSE in adjunct to scaling and root planing (SRP) in healing of periodontal pockets, in which bleeding on probing reduction was significant in intra-group comparisons in baseline and 3-month evaluations.[14]

On the contrary another study (Rayyan et al, 2018) which evaluated the effectiveness of applying grape seed extract (GSE) gel in periodontal pockets for the treatment of chronic periodontitis showed no significant improvement in bleeding on probing during 4 weeks and 6 months evaluation.[13]

A statistically significant reduction was observed in PI scores from baseline (1.64 ± .32) to the 3-months (1.07 ± 2.56) (P < 0.05) in group I and PI scores from baseline (1.57 ± .41) to the 3-months (1.06 ± .30) (P < 0.05) in group II suggesting that treatment offered were effective in reducing inflammatory components. But there was no statistically significant difference when both groups were compared.

An in vitro study by A. Furiga et al,2013 investigated the preventive effects of an original combination of a grape seed extract (GSE) with an amine fluoride (Fluorinol®) on dental plaque formation and oxidative damage caused by oral bacteria using the Trolox equivalent antioxidant capacity assay (TEAC). The GSE/Fluorinol® combination showed both a significant antiplaque activity and an important antioxidant capacity *in vitro*, without any bactericidal effects.[15]

The present study showed statistically significantly higher reduction in probing pocket depth at the end of the 3month recall visit for the sites treated with scaling and root planing along with placement of grape seed extract chip (P value< 0.05).

A network of interacting molecular pathways involving proinflammatory mediators and reactive oxygen species (ROS) are involved in the progression of periodontal disease.[16] The presence of antioxidant properties in GSE has been shown to have a much stronger scavenging effect on oxygen free radicals. The availability of phenolic-hydrogens as singlet oxygen quenchers and hydrogen donating radical scavengers leads to proanthocyanidin's (component of GSE) antioxidant properties.[11] It can then regulate oxidative stress by acting as an inflammatory reaction regulator (related to oxidative stress) (Gargari et al. 2011).[17]The use of GSE reduces oxidative stress and assists in anti-inflammatory reduction (Hossein zadeh 2017).[18]

The GSE have also shown an effective antimicrobial property; they are efficiently used against Gram positive bacteria but are more effective against Gram negative bacteria like P. ginigivalis, F. nucleatum or Pseudomonas aeruginosa (Jayaprakasha et al. 2003).[19]

A study by Vanessa Houde et al(2006) investigated the effects of a grape seed proanthocyanidin extract (GSE) on the production of ROS and RNS and on the protein expression of inducible nitric oxide synthase (iNOS) by murine macrophages stimulated with lipopolysaccharides (LPS) of periodontopathogens. Their findings demonstrated that proanthocyanidins have potent antioxidant properties and should be considered a potential agent in the prevention of periodontal diseases.[5]

We assessed the effect of grape seed extract chip on microbiological parameters. F. nucleatum and P. gingivalis were the microorganisms chosen, as they are the common periodontal pathogens. Microbiological analysis showed statistically significant difference in CFUs in both the groups when compared at baseline and 3 months.

When intergroup comparison was done, the CFUs of F. nucleatum and P. gingivalis showed Statistically significant difference in the group with placement of grape seed extract chip compared to the group with placebo chip.

Similar results as our study was seen in a study undertaken by Aurelie Furiga et al,2009) to investigate the effect of a grape seed extract (GSE) on two oral anaerobes (F. nucleatum and P. gingivalis).[20]

We found statistically significant improvement in both clinical and microbiological parameters in both the groups. But on comparing both the groups, grape seed extract chip group showed evident and significant results than the placebo chip group.

Conclusion

In the present study, 2% grape seed extract chip was found to be effective both clinically and microbiologically as a local drug delivery system adjunct to scaling and root planing for treating chronic periodontitis. The experimental material was well accepted by the patients. Neither complications nor allergic reactions were observed.

Limitations of the present study is possibly the duration for which it was followed up. Follow up for longer duration could possibly indicate whether there will be recurrence of chronic periodontitis. Future trials are needed for better extrapolation of the results.

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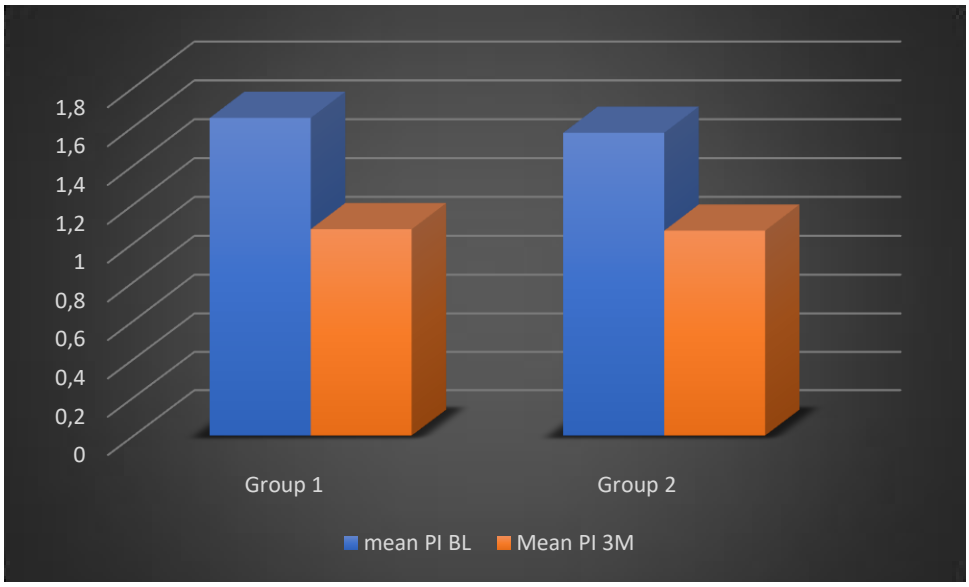

Figure 1 - Mean Plaque scores between different time intervals in each group

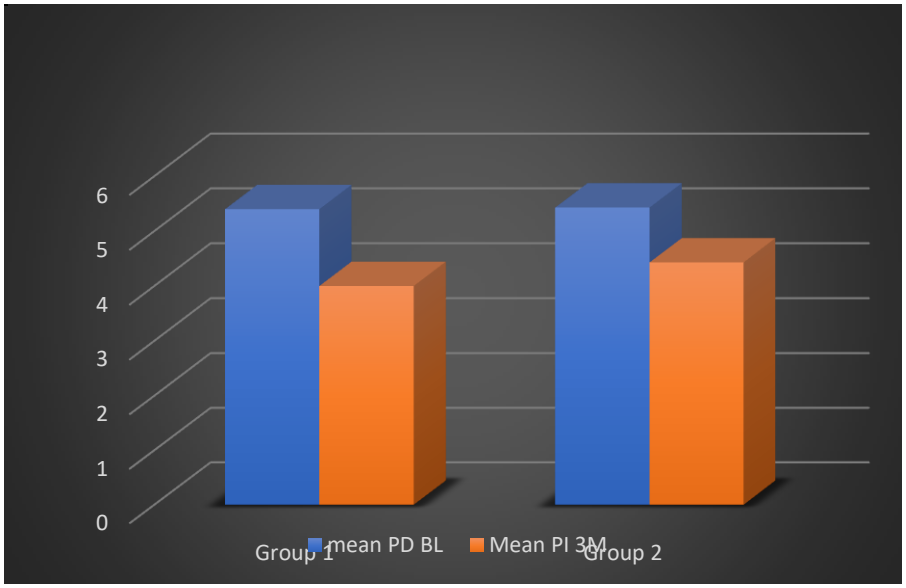

Figure 2 - Mean PPD levels between different time intervals in each group

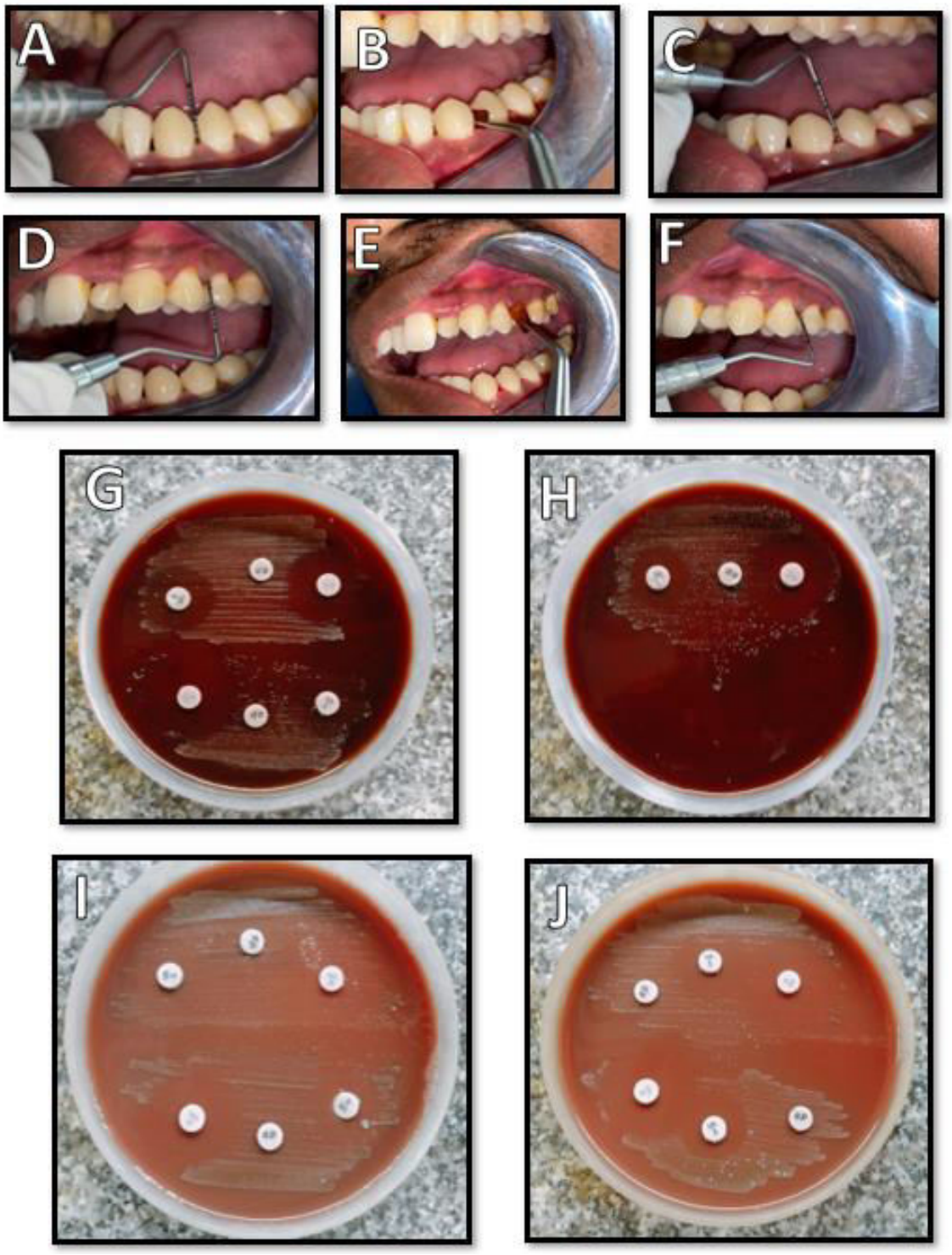

Figure 3 – A. Probing depth at baseline of Group I, B. Placement of grape seed extract chip, C Probing depth at 3 months of Group I, D. Probing depth at baseline of Group II, E. Placement of placebo chip, F. Probing depth at 3 months of Group II. G. CFU of P. gingivalis and F. nucleatum at baseline of Group I, H. CFU of P. gingivalis and F. nucleatum at 3 months of Group I, I. CFU of P. gingivalis and F. nucleatum at baseline of Group II, J. CFU of P. gingivalis and F. nucleatum at 3 months of Group II