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Green Tea Extract Gel for Local Drug Delivery System as an Adjunct to Scaling and Root Planing in Chronic Periodontitis

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KEYWORDS

Green tea extract, local drug delivery, chronic periodontitis

ABSTRACT:

Background:

Periodontitis is a chronic inflammatory disease that leads to the destruction of the supporting structures of the teeth due to the interaction between periodontal pathogens and the host immune response. Although scaling and root planing (SRP) is considered the primary treatment modality, complete removal of pathogenic microorganisms from periodontal pockets may be difficult. Herbal agents with antimicrobial and anti-inflammatory properties have gained attention as adjuncts to periodontal therapy. Green tea is rich in polyphenols, particularly catechins, which exhibit antioxidant and antibacterial activity against periodontal pathogens. The present study was undertaken to evaluate the effectiveness of locally delivered green tea extract gel as an adjunct to SRP in the management of chronic periodontitis.

Materials and Methods:

Green tea extract was prepared using ethanol extraction and formulated into a 20% gel with Carbopol 940 as the base. Its antimicrobial activity against Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, and Fusobacterium nucleatum was evaluated using broth dilution and agar well diffusion methods. A randomized split-mouth clinical study was conducted on 30 patients aged 30–55 years presenting with probing pocket depths of 4–6 mm. Test sites received SRP along with locally delivered green tea extract gel, while control sites were treated with SRP alone. Clinical parameters including Plaque Index (PI), Gingival Index (GI), and Probing Pocket Depth (PPD) were recorded at baseline and after 21 days. Data were analyzed statistically using SPSS software with the level of significance set at $p<0.05$.

Results:

Both groups showed significant improvement in clinical parameters from baseline to 21 days. In the control group, reductions of 15.70% in probing pocket depth, 34.98% in gingival index, and 38.25% in plaque index were observed. The test group demonstrated greater improvements, with reductions of 30.29% in probing pocket depth, 52.68% in gingival index, and 54.37% in plaque index. Intergroup comparison indicated that the adjunctive use of green tea extract gel produced better clinical outcomes than SRP alone.

Conclusion:

The findings of the present study suggest that locally delivered green tea extract gel can effectively enhance the outcomes of conventional periodontal therapy. Its use as an adjunct to scaling and root planning resulted in significant improvement in periodontal clinical parameters, indicating its potential as a herbal local drug delivery agent in the management of chronic periodontitis.

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Introduction

One of the primary objectives of Periodontics is to preserve the health and integrity of the periodontium. Periodontitis is the most common and serious widespread health problem because of its high prevalence rate of 25% to 55% worldwide, leading to tooth loss and deteriorating the standard of life[1]. Periodontitis is associated with an increased number of bacteria and a change in the flora towards a richer representation of gram-negative organisms. Inflammation and destruction of periodontal tissues are considered to result from the response of a susceptible host to a microbial biofilm containing gram negative pathogens[2].

The most commonly found bacteria to which periodontal tissue breakdown is attributed are *P. gingivalis*, *A. actinomycetemcomitans*, *F. nucleatum*, *T. forsythia*, *P. intermedia*, *C. rectus*, *E. corrodens*, *Treponema* and *Eubacterium* species. Among these *P. gingivalis* and *A. actinomycetemcomitans* are known to invade host tissues and whose association has been strongly incriminated in a destructive form of periodontitis, while *F. nucleatum* is an intermediate colonizer bridging the attachment of commensals that colonize the tooth and epithelial surface with true pathogens[3].

Scaling and root planing which involves mechanical debridement of plaque and calculus of the affected teeth is considered the "gold standard" in the initial treatment for periodontitis. But it often has reduced effectiveness in the complete debridement in the subgingival environment[4].

Locally delivered anti-infective pharmacological agents in the form of sustained-release delivery systems has numerous clinical, pharmacologic and toxicologic advantages over conventional treatment modalities for periodontal diseases. The anatomy of the pocket makes it an excellent target site for application of a local delivery system. Its size and shape may vary since it is an irregular anatomic site. Local application directly to the pocket can facilitate the administration of a drug that cannot be given orally because of systemic adsorption obstacles[5].

The new treatment modalities for periodontitis are aimed at early intervention, causing reduction of the local irritants involved in the initiation of gingivitis and its progression to periodontal disease, resulting in increased resistance to host. Various plant extracts, co-enzymes and vitamins have been used to enhance the firmness and stability of the tissues maintaining the integrity of oral cavity[6].

Green tea is one of the most consumed beverages worldwide with many health benefits. As it is prepared from unfermented leaves, it incorporates highest concentration of powerful antioxidants such as polyphenols and a number of bioactive chemicals, particularly flavonoids (catechins) such as epicatechin (EC), epicatechin 3 gallate (ECG), epigallocatechin (EGC), epigallocatechin 3 gallate (EGCG), carotenoids, ascorbic acid and phytochemical compounds. Catechins possess antioxidant, anti-inflammatory, antibacterial and antiviral properties[7].

Green tea antioxidant activity is related to polyphenols; it directly scavenges reactive oxygen and nitrogen species and chelates redox active transition of metal ions. Indirectly, it inhibits pro-oxidant enzymes, redox sensitive transcription factors, and induction of anti-oxidant enzymes. Green tea extract has been used in the form of chewing gums, mouth rinses and local drug delivery as a part of preventive periodontal maintenance regimen and has demonstrated antimicrobial activity against methicillin resistant staphylococcus aureus, Helicobacter pylori and α Hemolytic streptococcus[8].

Green tea is shown to be effective against gram negative anaerobic rods and black pigmented bacteria. Its catechin inhibits collagenase activity, limiting the tissue destruction and decreases expression of MMP 9 in osteoblasts inhibiting the formation of osteoclasts. It prevents the attachment of *Porphyromonas gingivalis* onto buccal epithelial cell[9]

Thus, the present clinical trial has been undertaken to assess the efficacy of locally delivered green tea extract gel as an adjunct to scaling and root planing in chronic periodontitis patients

Materials and Methods:

Extract preparation:

Green tea leaves were collected and authenticated from KAHER's Shri B M Kankanwadi Ayurveda Mahavidyalaya, Belagavi, and subsequently stored in an airtight container. Following this, the leaves were dried

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using hot air oven at 70ºC for 2 hours before being powdered. About 40g of green tea extract powder was then immersed in a solution containing 160 ml of 90% ethanol and 40 ml of water, left to soak for 72 hours at room temperature. Subsequently, the filtrate was concentrated by evaporation using the "New Brunswick Scientific Excella E24 Incubator Shaker Series" until it reached the desired concentration. The extract was then filtered through Whatman No.1 filter paper. The extract was then evaporated using hot water bath. Following this, the extract underwent sterilization overnight through UV irradiation and was stored at 4ºC.

Inoculum preparation: BHI broth and ATCC strains of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum* were utilized to prepare the inoculum. Colonies were picked using a sterile loop and transferred into a tube containing 5 mL of BHI broth. This stock culture was then incubated at 37°C for 8–14 hours. The turbidity of the actively growing bacterial culture with broth was adjusted to match the 0.5 McFarland standard guidelines.

Broth dilution method with Resazurin test for determining Minimum Inhibitory Concentration: To prepare the broth, 5.5 grams of BHI powder was dissolved in 150 ml of water and thoroughly stirred. Subsequently, it was autoclaved at 120 ºC and 15 psi pressure. The broth was then cooled at room temperature in an aseptic condition under laminar air flow. Then 20 mg/ml of erythromycin was added to the broth. Broth dilution was performed in a sterilized 96-well plate, with the procedure being conducted in triplicates. Initially 10 wells were selected. A total of 100 µl of broth was added to all 10 wells in triplicates. In the first well, 100 µl of green tea extract was added and serially diluted to the required concentrations up to the tenth well. A similar procedure was carried out in the other two rows of the well plates. The 96-well plates were then placed for incubation in a McIntosh and Fildes' anaerobic jar for 48 hours. Following incubation, 30 µl of Resazurin reagent per 100 µl of extract was added to the wells and observed after 4 hours for any potential color change. The color change from blue/violet to slight pink/pink/magenta was noted as MIC. The results were recorded by capturing high-quality photographs.

Minimum Bactericidal Concentration (MBC):

MBC was determined using the MIC values of green tea extract with the help of agar plates. BHI agar plates were prepared by dissolving 52 grams of BHI powder in 1000 ml of distilled water, followed by autoclaving at 120 ºC and 15 psi pressure. It was then cooled to room temperature in an aseptic condition under laminar air flow for 10-15 minutes; following which 20 mg/ml of erythromycin was added to the agar, which was then poured and allowed to solidify. The minimum concentration at which the bacteria showed no growth was considered as the MBC value.

Gel preparation:

The green tea extract gel was prepared at KAHER's KLE College of Pharmacy, Belagavi.

Preparation of Carbopol 940 gel base:

Weighed quantity of 1% Carbopol 940 was added in about 50ml of distilled water ensuring Carbopol 940 is added gradually to prevent clumping and promote uniform distribution. Then, it was stirred continuously on a magnetic stirrer for three hours and kept 24 hours for complete hydration.

Preparation of Extract Dispersion:

20% w/w of green tea extract was triturated in a mortar and pestle.

0.06% of Tween 80 which is a dispersing agent and 2% of Propylene glycol which is a plasticizer and humectant was added to the triturated extract to ensure uniform dispersion.

30 ml of distilled water was added to the above triturated extract along with preservatives like 0.033 % sodium methyl paraben, 0.066 % sodium propyl paraben and 0.03% sodium benzoate. The solution was then stirred with a magnetic stirrer for 30 mins at 700 rpm.

Gel Formation:

The extract dispersion was added to the Carbopol 940 gel base and the volume was adjusted with distilled water to achieve the final weight of 100 gm of gel.

0.5% of triethanolamine was added dropwise to the above mixture and stirred using high speed propeller stirrer at 1200rpm for 30 mins.

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The gel was then passed through UV irradiation for 20-30 minutes and was transferred into an airtight container (Figure 1). The gel was stored at ambient temperature for future use.

Figure 1: Prepared Green tea extract gel.

Agar well diffusion assay

The agar well diffusion assay was conducted on bacteriological agar plates. For *A. actinomycetemcomitans*, *P. gingivalis*, and *F. nucleatum*. Mueller Hinton agar plates were prepared by adding 38 grams of Mueller Hinton agar powder to 1000 ml of distilled water and sterilized in a steam sterilizer. After cooling at room temperature for 10-15 minutes, the agar plates were poured and allowed to solidify. Bacterial broth cultures (100 µL) with a turbidity equivalent to 0.5 McFarland's standard were spread evenly over the prepared agar plates using a sterile cotton spreader. Aseptic wells were then created uniformly using a sterile cork borer. Sample reagents (100 µL saline, 100 µL green tea extract gel, and 100 µL Chlorhexidine gel) were added to these wells and placed in anaerobic incubator at 37°C. The plates were observed for diffusion over 24-72 hours of incubation. Growth patterns were observed, and the zone of inhibition was measured for each sample reagent on the plates, with results compared against Chlorhexidine as the standard. The diffusion assay was performed in triplicates for all the three micro-organisms.

SOURCE OF DATA: 30 participants, both male and female with age range of 30-55 years were selected from patients who reported at the outpatient Department of Periodontics, KAHER's KLE V K Institute of Dental Sciences, Belagavi.

METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA

1. Patients with pocket probing depth of 4-6mm in contra lateral quadrants.
2. Patients with a good state of health without any systemic disorders.
3. Patients who had not undergone any form of non- surgical or surgical periodontal therapy in the last 6 months.

EXCLUSION CRITERIA

1. Patients with history of antibiotics and non-steroidal anti-inflammatory drug use in the past 3 months.
2. Pregnant and lactating females.
3. Smokers and patients consuming tobacco.

PROCEDURE:

An informed consent was obtained from all the participants who enrolled for the study. A split mouth design was followed, where two sites in the contra lateral quadrants having pocket depth of 4-6mm in at least 2 teeth were chosen. The subjects were divided randomly by the flip of a coin, into two sites:

Test sites- Probing pocket depth (PPD) was measured at baseline. Included scaling and root planing along with green tea extract gel placement followed by placement of periodontal dressing (Figure 2-4)

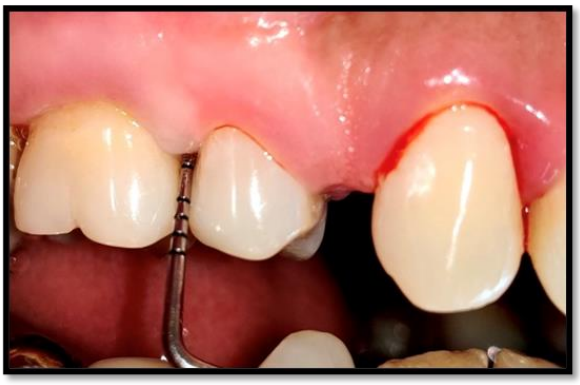

Figure 2: Preoperative measurement of PPD > 4mm

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Figure 3: Placement of Green tea extract gel.

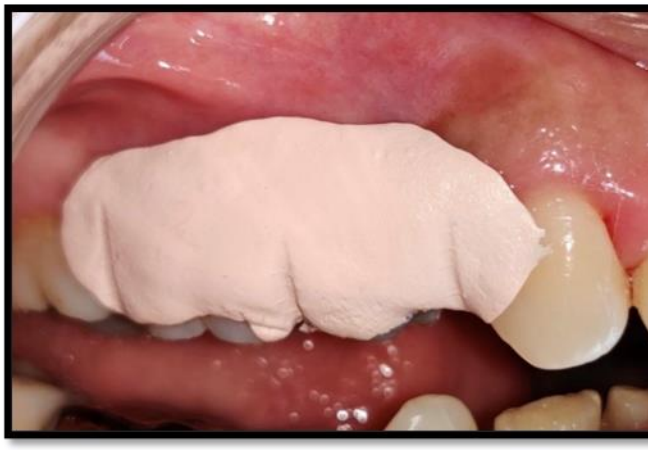

Figure 4: Placement of periodontal pack (Coe-pack).

Control sites- Included scaling and root planing alone.

Patients in the test group were recalled after two weeks for removal of periodontal dressing.

Both test and control sites were assessed for plaque index, gingival index and probing pocket depth which were measured using William's periodontal probe at baseline and 21 days.

Statistical analysis: The mean and standard deviation was calculated for the clinical parameters (gingival index, plaque index and probing pocket depth) of the test and control groups.

The level of significance was 0.05. The SPSS software was used to perform the data analysis.

Results:

Day 0 and Day 21 scores of Probing Pocket Depth, Gingival Index and Plaque Index in control and test group did not follow normal distribution. Therefore, the non-parametric tests were applied. A statistically significant difference was seen between Day 0 and Day 21 treatment time points with Probing Pocket Depth, Gingival index and Plaque index scores of 15.70%, 34.98%, 38.25% respectively in the control group. A significant difference between Day 0 and Day 21 treatment time points with Probing Pocket Depth, Gingival index and Plaque index scores of 30.29%, 52.68%, 54.37% in test group respectively (Table 1-6) (Graph 1-3).

Test group showed better improvement in Probing Pocket Depth, Gingival index and Plaque index after 21 days when compared to control group at the end of 21 days.

Table 1: Comparison of Control group and Test group with Pocket Probing Depth scores at Day 0 and Day 21 treatment time points by Mann-Whitney U test

Time	Control group			Test group			U-value	Z-value	p-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Day 0	5.73	0.45	29.00	5.83	0.38	32.00	405.00	-0.6579	0.5106
Day 21	4.83	0.59	37.52	4.	0.91	23.48	239.50	3.1047	0.0019*

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Difference	0.90	0.61	22.45	1.77	0.86	38.55	208.50	-3.5630	0.0004*

*p<0.05

Table 2: Comparison of Day 0 and Day 21 treatment time points with Pocket Probing Depth scores in Control group and Test group by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	% of effect	Z-value	p-value
Control group	Day 0 to Day 21	0.90	15.70	4.1973	0.0001*
Test group	Day 0 to Day 21	1.77	30.29	4.7030	0.0001*

*p<0.05

Table 3: Comparison of Control group and Test group with gingival index scores at Day 0 and Day 21 treatment time points by Mann-Whitney U test

Time	Control group			Test group			U-value	Z-value	p-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Day 0	1.69	0.35	30.37	1.71	0.34	30.63	446.00	-0.0517	0.9587
Day 21	1.10	0.33	35.53	0.81	0.44	25.47	299.00	2.2251	0.0261*
Difference	0.59	0.40	26.12	0.90	0.54	34.88	318.50	-1.9368	0.0528

*p<0.05

Table 4: Comparison of Day 0 and Day 21 treatment time points with gingival index scores in Control group and Test group by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	% of effect	Z-value	p-value
Control group	Day 0 to Day 21	0.59	34.98	4.3835	0.0001*
Test group	Day 0 to Day 21	0.90	52.68	4.7821	0.0001*

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*p<0.05

Table 5: Comparison of Control group and Test group with plaque index scores at Day 0 and Day 21 treatment time points by Mann-Whitney U test

Time	Control group			Test group			U-value	Z-value	p-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Day 0	2.09	0.54	28.03	2.19	0.40	32.97	376.00	-1.0867	0.2772
Day 21	1.29	0.42	36.43	1.00	0.42	24.57	272.00	2.6242	0.0087*
Difference	0.80	0.51	24.03	1.19	0.49	36.97	256.00	-2.8608	0.0042*

*p<0.05

Table 6: Comparison of Day 0 and Day 21 treatment time points with plaque index scores in Control group and Test group by Wilcoxon matched pairs test

Groups	Changes from	Mean Diff.	% of effect	Z-value	p-value
Control group	Day 0 to Day 21	0.80	38.25	4.6226	0.0001*
Test group	Day 0 to Day 21	1.19	54.37	4.7821	0.0001*

*p<0.05

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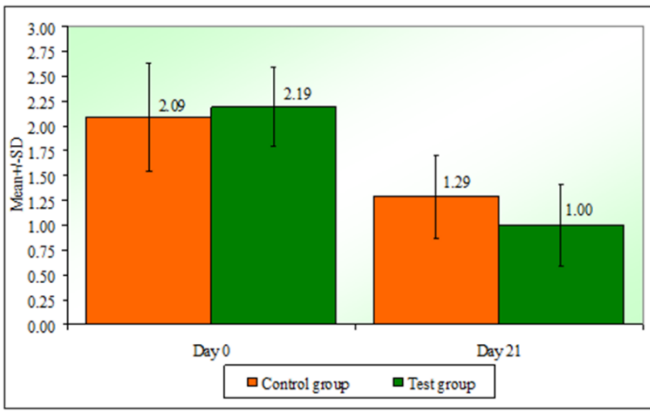
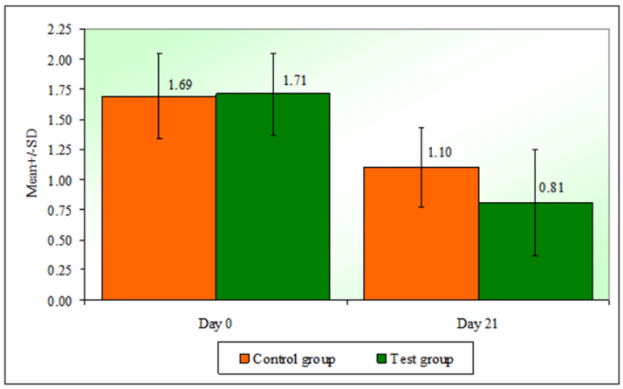
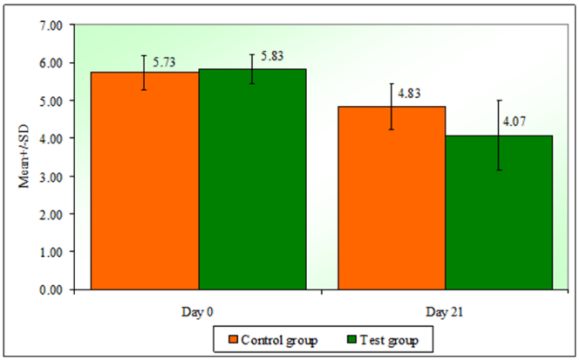


Graph 1: Comparison of Control group and Test group with plaque index scores at Day 0 and Day 21 treatment time points



Graph 2: Comparison of Control group and Test group with gingival index scores at Day 0 and Day 21 treatment time points



Graph 3: Comparison of Control group and Test group with Probing pocket depth scores at Day 0 and Day 21 treatment time points.

Discussion:

Periodontitis is an infection of the periodontium caused by group of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation. Although the disease is multifactorial in nature, the role of microbiota in the initiation and progression of periodontal disease is conclusive. Periodontal treatment is thus directed at altering the composition of subgingival microbiota, which facilitates removal of the bacterial deposits from the tooth surface causing a shift from the pathogenic microbiota to one compatible with periodontal health and less conducive to retention of bacterial plaque in the vicinity of gingival tissue.

Therapeutic approaches which include mechanical scaling and root planing, is the first recommended step and is an imperative phase of periodontal therapy which results in reduction of gingival inflammation and pocket probing depth. However, the complex anatomy and the contours of the root may hamper the treatment and prevent sufficient reduction of the bacterial load due to lack of accessibility to microorganisms. Hence, incorporation of adjunctive chemotherapeutic agents, with mechanical instrumentation provides additional microbial effect enhancing outcomes at sites not responsive to conventional therapy.

Over the last decades, local drug delivery systems have been optimized in attempts to treat periodontal diseases. Local application of chemotherapeutic agents into periodontal pocket may be advantageous, as the drug concentration is increased directly at the site of disease activity, extending the time of drug delivery as well as prevention of systemic side effects. Other advantages offered are improved patient compliance, ease of application and lesser tendency for development of bacterial resistance.

Green tea is one of the most consumed beverages worldwide with many health benefits. It has antioxidant activity and effective against gram negative anaerobic rods. Its catechin inhibits collagenase activity, thereby limiting the tissue destruction.

The present study demonstrated that the reduction in plaque index scores in both the test and control group from baseline to day 21 were statistically significant ($p<0.05$) (Table 6). The improvement of plaque index

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scores in the control group might be expected as a result of scaling and root planing which aids in mechanical disruption of a biofilm resulting in improved plaque control efficiency. However, on intergroup comparison, plaque index scores were statistically significant in the test group (1.19±0.49) and control group (0.80±0.51) (p=0.0042) when compared to baseline.

Jain and colleagues in a clinical trial compared gingival index with neem chip plus SRP and SRP alone, and their results were also similar with the current study at four weeks[10], where there was significant reduction in gingival index scores in both test group (0.90±0.54) and control group (0.59±0.40) when compared to baseline. In the present study, on day 21, the results showed statistically significant reduction in probing pocket depth in both the test group (1.77±0.86) and control group (0.90±0.61) when compared to baseline.

The results of the present study are in accordance with previous studies by Makimaru at al 1993, Sakanaka et al 1996, Hirawasa et al 2002 and Kudwa et al 2012 on green tea extracts[11,12,13,14]. The antibacterial action of green tea is attributed to gallo-radical present in EGC, EGCg which possess strong bactericidal activity, inhibitory activity against both the toxic metabolites of *P. gingivalis* and collagenase of eukaryotic and prokaryotic cells. Green tea polyphenols bind with the fimbria of *P. gingivalis* inhibiting its adherence onto the human epithelial cells[11,12].

Hirasawa et al reported that green tea catechin exerts bactericidal activity against black pigmented rods with an MIC of 1 mg/ml[13]. Recently Kudwa et al demonstrated that green tea catechin strips caused significant reduction of *A. actinomycetemcomitans, F. nucleatum, Capnocytophaga* species and *P. intermedia* in the test group[14]. Thus, administration of green tea extract gel as a local drug delivery along with scaling and root planing could be a viable treatment option for treating periodontal pockets with moderate probing depth of 4-6 mm in chronic periodontitis patients. Future prospects can be aimed at conducting clinical trials for longer follow up period to further explore this treatment option.

CONCLUSION

The findings of the present study suggest that local application of green tea gel resulted in significant reduction of gingival index, plaque index scores, thus reducing inflammation and maintaining periodontal tissue integrity. It was also effective in significantly reducing the probing pocket depth in the patients in test group. Hence, it can be concluded that green tea extract gel can be effectively used as a local drug delivery agent and as an adjunct to scaling and root planning in treating patients with chronic periodontitis.

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