Evaluation of the Therapeutic Efficacy of Green Tea Catechin Strips as an Antimicrobial Agent Local Delivered Drug in the Management of Chronic Periodontitis

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Abstract

Background and objectives: Increased knowledge of anaerobic bacteria in the development of periodontal diseases has led to new treatment strategies aiming at suppression or elimination of periodontal pathogens. Over the last decades, green tea has been subjected to many scientific and medical studies. The aim of the present study is to evaluate the therapeutic efficacy of green tea catechin strips as an antimicrobial agent local delivered drug in the management of chronic periodontitis in pocket of depth of 4-6 treatment mm, when used as an adjunct to scaling and root planning (Test group), as compared to sites that underwent scaling and root planning along with placebo (control group).

Materials & Methods: The present study was a randomized clinical trial with split mouth design in which a total number of 30 sites in patients who were diagnosed with generalized chronic moderate periodontitis, consisting of both genders, aged between 25 to 50 years were selected. These selected sites were randomly divided into test group and control group Test group- included 15 sites that were selected for the placement of Green tea strips (local drug delivery) after scaling and root planning. Control group - included 15 sites that were treated with scaling and root planning along with placebo strips. Clinical parameters taken are the PI, GI, SBI, probing depth (PD), and clinical attachment level (CAL). Anaerobic culture was done to compare the total colony forming unit before and after the treatment. Clinical parameters and the total colony count were assessed at base line, 21 days and 90 days.

Result: Results showed a significant improvement in all the clinical parameters. There was significant decrease in mean probing depth from base line to 90 days, and also there was significant gain in CAL in the test group as compared to control group. There was a significant reduction in the total colony count from base line to 21 days in both the group, but more reduction was observed in the test group.

Conclusion: Within the limits of this study and on the basis of the clinical and microbiological parameters, green tea catechin local drug delivery along with scaling and root planning was more effective than scaling and root planning alone.

Keywords: Chronic Periodontitis, Green tea catechin, Local drug delivery, Root planning, Scaling.

INTRODUCTION

Periodontal diseases are the chronic inflammatory diseases of the periodontium, characterized by inflammatory destruction of gingiva and periodontal ligament. Most of the periodontal diseases are of microbial. The incidence and progression of this disease is related to a substantial increase in Gram-negative anaerobic rods. Among them, Porphyro monas gingivalis and Actino bacillusactinomycetemco mitans are strongly implicated in the etiology of chronic periodontitis. When these pathogens live in local periodontal tissues, an immune response is initiated by fibroblasts and macrophages producing several cytokines (ILs 1 and 6 and TNF-a), as mediators of the inflammatory response and immune reaction. These cytokines were found to play direct and indirect roles in the production and activation of various destructive enzymes, such as matrix metallo proteinases, resulting in progressive periodontal breakdown.2

The scientific rationale for adding locally applied anti- infective agents to SRP is that certain broad-spectrum antimicrobial agents can theoretically reduce the number of subgingival bacteria left behind after SRP. Although mechanical removal or disruption of subgingival biofilms by SRP is usually an effective therapeutic approach for the treatment of chronic periodontitis, it does not sterilize the subgingival environment. Almost immediately after SRP, bacteria left behind begin to re-colonize the subgingival environment to form a new biofilm.3

Many natural products are used for the treatment of periodontitis. Green tea extract is one of among them which has various therapeutic effects. Green tea (Camellia sinesis) is one of the most popular beverages in the world. Recently, it has received considerable attention because of its numerable scientifically proven health benefits attributable to the presence of various polyphenols. Several epidemiologic and experimental observations in the field of medicine and dentistry have suggested that green tea catechins may exert cardio-protective, antioxidant, cholesterol-lowering, anti- obesity, anti-diabetic, anti-cancer, anti-inflammatory, anticaries, anti- bacterial, antifungal, and antiviral effects. These effects have been primarily attributed to the most prevalent polyphenol contained in green tea, (-) - epigallocatechingallate (EGCG).4

Green tea catechin (EGCG) significantly reduced the expression of matrix metalloproteinase-9 in osteoblasts and also inhibits the formation of osteoclasts. Thus, EGCG may prevent alveolar bone resorption that occurs in periodontal disease.5

In relation to osteoclasts, EGCG has been shown to inhibit formation of bone marrow cells and primary osteoblastic cells and induce apoptosis in osteoclasts etiology with environmental, systemic, and other factors playing a secondary role. The destruction seen in periodontal disease is due to microorganisms as well as host inflammatory response.1 differentiated from a macrophage like cell line. Moreover, EGCG has been demonstrated to reduce stimulated chemokine expression and IL-6 production in human gingival fibroblasts (HGFs). EGCG also downregulated MMP-2 and MMP-9 production in cancer cells and weakened IL- 1b–induced MMP-2 production in human synovial fibroblasts.6

Tea polyphenols may act as antioxidants by scavenging reactive oxygen and nitrogen species and by chelating redox- active transition metal ions, and may also act indirectly as antioxidants through, among other mechanisms, the inhibition of "pro-oxidant" enzymes and induction of antioxidant enzymes. The antioxidant properties of flavonoids may therefore, protect tissues, cells and plasma constituents against oxidative damage.7

Since there is limited microbiological study conducted to evaluate the efficacy of green tea chips as a local drug therapy. Hence, in the present study an attempt is made to evaluate the efficacy of locally delivered green tea (HPC strips) as an antimicrobial agent, adjunct to scaling and root planning in the treatment of chronic periodontitis patients. The aim of the study is to evaluate the therapeutic efficacy of green tea catechin as an antimicrobial local delivered drug when used as an adjunct to scaling and root planning in the management of chronic periodontitis.

MATERIALS & METHOD

In Vitro Study

Preparation of Green tea strips: 2.5 grams of green tea leaves were brewed in 200 ml of water.05% of hydroxy-propyl-cellulose (HPC), 200 mg of poly ethylene glycol 4000 and 200 mg of carboxy methyl cellulose was added to the above mixture and stirred on magnetic stirrer for 24 hours. Whole solution was poured in pre fabricated petri dishes with silicon oil and kept for drying for 2 days at room temperature covered with perforated aluminium foil. After the film is peeled off and cut into rectangular strips with diameter of 2mm in width, 3 mm in length and 0.3 mm in thickness. The manufactured strips were brown rectangular in shape (size-2mm in width, length 3mm, 0.3 mm in thickness) with catechin used from green tea powder and hydroxypropyl methylcellulose as carrier. The strips were packed in a medical grade pouch and sterilized by gamma irradiation.

Preparation of Placebo Strips

The manufactured strips were transparent and of similar dimensions. They are prepared in a similar manner without adding the green tea extract powder to hydroxypropyl cellulose (HPC) powder.

In Vivo Study - Clinical Study Design

A randomized, placebo-controlled, parallel-group, with split-mouth design was conducted for 3 months. The sample size of 30 sites were selected from both sex with age limit of 20-65 years from the outpatient Department of Periodontology, The Hazaribag College of Dental Sciences and Hospital, Jharkhand. The inclusion criteria were- Patients diagnosed as generalized chronic periodontitis, Patients having two contra-lateral sites with 4- 6 mm periodontal pocket with active lesion, and radiographic evidence of bone loss with Clinical attachment loss 3 – 5 mm at the base line, Patients who had not undergone any surgical or nonsurgical periodontal therapy in the past 6 months, and patient who are not willing for surgical therapy, Patients with a good state of health without any systemic disorders.

The exclusion criteria were - Patients who had taken antibiotic therapy in the past 6 months, Patients who have received any received any topical or systemic antimicrobial treatment for the past six months, Patients having history of allergy to green tea, Pregnant woman and lactating mothers, Patients with periodontal pockets in which the depth of the pockets corresponded to the apex of the tooth as in probable endodontic-periodontic conditions, Medically compromised patients, Teeth with furcation involvements, Known smokers and smokeless tobacco.

Procedure

Thirty sites diagnosed with generalized chronic periodontitis were selected. These selected 30 sites were randomly &equally (15 each) divided into test group (SRP+ green tea chips) and control group (SRP+ placebo chips). At the selected sites, the clinical parameters – plaque index, gingival index, sulcular bleeding index, probing depth and clinical attachment level were evaluated at base line, 21days and 90 days with the help of acrylic stent after obtaining written consent from each patient who had participated in the study. The ethical clearance was obtained from Ethical Committee of The Hazaribag college of Dental Sciences and Hospital, Jharkhand. The total anaerobic colony counts were recorded at base line, 21 days and 90 days as per the study design.

Plaque Sample Collection

The plaque samples were collected using sterile Gracey curette by inserting it sub-gingivally into the deepest portion of the periodontal pocket and moved coronally by scarping along the root surface. The samples were then transferred to thioglycollate transport media and was sent to Department of Microbiology, The Hazaribag college of Dental Sciences and Hospital, Jharkhand for the assessment of colony forming units.

Application of Green Tea Chips

In the two selected contralateral site the clinical parameters were recorded and plaque samples were taken at base line for microbiological analysis. Full mouth scaling and root planning was performed. Then the area was isolated with cotton rolls and dried. Green tea catechin chip was inserted deep in the periodontal pocket with the help of tweezer. The site was sealed with coe-pak to prevent ingress of oral fluids and to prevent the dislodgement of chips. Same procedure was repeated for the placement of placebo chips. The patients were then instructed not to use any other chemical plaque control methods other than normal brushing and rinsing. The patient

was informed to report to the clinic in case of any irritation and discomfort. The coe-pak was removed after 7 days. Then the patients were recalled after 21 days and 90 days for clinical and microbiological analysis.

Method of Statistical Analysis

All the analysis were done using SPSS version 14. A p-value of <0.05 is considered statistically significant. The CFU values were logarithmically transformed to ensure normality. Comparison of test and control group was done using paired t test. Comparison of values from baseline through 90 days was done using repeated measures ANOVA with post-hoc Bonferroni test. Comparison of mean difference baseline and follow-ups was done using paired t test.

RESULTS

Gingival index: Inter group comparison of gingival index score at various interval is represented in Table 1. At base line plaque score showed no difference between control and test group. After 21 days there was no statistically significant difference in the mean plaque score between test and control group ($p = 0.334$). However at 90 days, there was statistically significant difference between test and control group ($p < 0.001$), with greater improvement in the test group.

Plaque Index

Inter group comparison of plaque score at various interval is represented in Table 2. At base line plaque score showed no statistically difference ($p = 0.334$) between control and test group. After 21 days there was statistically significant difference in the mean plaque score between test and control group ($p <0.001$). However, at 90 days, there was statistically significant difference between test and control group ($p < 0.001$), with greater improvement in the test group.

Sulcular Bleeding Index

Inter group comparison of sulcular bleeding score at various interval is represented in table 3. After 21 days there was not much significant difference in the mean sulcular bleeding score between test and control group ($p = 0.106$). At 90 days, there was a greater significant difference between test and control group ($p < 0.001$), with greater improvement in the test group.

Probing Pocket Depth

Inter group comparison of mean probing depth at various interval is represented in table 4. At baseline mean probing depth showed no difference ($p = 0.001$) between control and test group. After 21 days there was significant reduction in the mean probing depth in the test group in comparison to the control group. At 90 days reduction in the mean probing depth for test group and control group remain same.

Clinical Attachment Level

Inter group comparison of mean clinical attachment level at various interval is represented in table 5. At base line the clinical attachment level showed no statistically significant difference between test and control group ($p =0.334$). The gain in clinical attachment level from base line to 21 day was 1.00 ± 0.38 for control group, whereas the gain in clinical attachment level for the test group was 2.07 ± 0.46, which was statistically significant ($p < 0.001$).

Colony Forming Units

Inter group Comparison of mean values for test and control for colony forming units (log CFU) is represented in table 6. At base line the CFUs showed no statistically significant difference between the control group and test group. However after 21 days there was significant difference in the total anaerobic colony count between test and control group ($p < 0.001$) with more reduction for total colony count seen in test group. At 90 days, there was no change in total colony count for test group while for the control group slight increase in the total colony count was observed.

DISCUSSION

Essential goal of current periodontal therapy is successful management of the suspected bacterial pathogens to the extent that destruction of the periodontium is arrested. A number of different nonsurgical and surgical therapies have been successful in achieving this goal. Mechanical debridement with or without surgical manipulations, to disrupt the subgingival flora and to provide clean, smooth, and biological compatible roots surfaces, had been the therapy to treat periodontal diseases till the early 1970s. Mechanical therapy may however fail to eliminate the pathogenic bacteria because of

their location within gingival tissues or in other areas inaccessible to periodontal instruments.8

The scientific rationale for adding locally applied anti- infective agents to SRP is that certain broad-spectrum antimicrobial agents can theoretically reduce the number of subgingival bacteria left behind after SRP. Almost immediately after SRP, bacteria left behind begin to recolonize the subgingival environment to form a new biofilm.9

Various clinical studies have shown beneficial effects of green tea on periodontal health. Green tea being incorporated in various delivery systems like dentifrices, chewing gum, candies10, mouthwash11, 12, and strips 62, 63has shown improvement in various parameters.

In vitro studies showed that green tea catechin inhibits the growth of Porphyromonasgingivalis, Prevotellaintermedia, and Prevotellanigrescens and the adherence of P. gingivalis onto human buccal epithelial cells. In addition, green tea catechins with the steric structures of 3-galloyl radial, EGCg, (-)-epicatechingallate (ECg), and ()-gallocatechingallate, which are the major tea polyphenols, inhibit the production of toxic end metabolites of P. gingivalis.5, 13

The present study was carried out with the objective to evaluate the efficacy of green tea catechin containing local delivery system over scaling and root planning for a period of 21 days and 90days. The assessment was done at day 21 to allow for soft tissue maturation as the time required for healing of the periodontium demonstrates an initial gain of clinical attachment at 3 weeks following non surgical therapy. The clinical parameters were also assessed at baseline and 21 days as the flora is supposedly said to return to pretreatment patterns following 3 weeks of scaling and root planning.

The placement of green tea chips in our study enabled therapeutic concentration to be maintained for sufficient period of time in the pocket to influence the improvement in clinical and microbiological parameters. The results were in accordance with the study by Hirsawa (2002)14, where he reported that green tea catechin absorbed by the oral epithelial cells in the subgingival pocket, continuously released from HPC strips until the of the experimental period and may inhibit the growth of gram negative anaerobic rods for 24 hours. The concentration of green tea catechin exhibit antimicrobial activity against gram negative anaerobic rods at minimum inhibitory concentrations (MIC) of 1 mg/ml.

Statistically significant reduction in the gingival index, plaque index and sulcular bleeding index scores were seen in both the control and test groups from baseline to 21 days and 90 days. There was also highly significant reduction of probing pocket depth in the test group from baseline to 21days. Probing depth value when compared between the test and control group showed a statistically significant difference.

The reduction in the gingival index and plaque index was in accordance with the study conducted by Hirasawa et al14 which demonstrated a clinical and microbial improvement following insertion of green tea catechin using a slow release local delivery system when compared to subgingival debridement alone. A study by Kushiyamaet al.15 showed that the intake of green tea was inversely correlated with the mean probing depth, mean clinical attachment level, and bleeding on probing.

The reduction in the plaque scores in our study could be attributed to the anti-plaque activity of green tea. It has been suggested that green tea tannins forms stable complex with proline-rich proteins, which is involved in the adsorption of oral bacteria to the pellicle. The galloyl radical in green tea catechins is responsible for the inhibition of acid production in dental plaque bacteria. Improvement in plaque index scores in our study are in accordance with Hirsawa (2006)16, Kudwa et al5, Hattarki et al17 which shows that use of C sinensis tea 1-3 cups/day resulted in reduction of plaque scores in school children.

The significant reduction in the gingival inflammation in the test group could be attributed to the antioxidant activity of green tea polyphenols which scavenges the reactive oxygen species. As a result there is reduction in gingival oxidative stress which causes decrease production of inflammatory cytokines. Green tea also contains tannins and vitamin K which may result in improved bleeding index. This is in agreement with the previous studies where Takayuki et al, 18reported that green tea

catechin blocks NF-b, a transcriptional factor involved in regulating the expression of cytokines and generation of Reactive oxygen species. Nakamura et al, 19 demonstrated that in response to lipopolysaccharide administration, green tea catechins cause reduction in interleukin 1 ß positive cells.

Statistically significant reduction in the probing depth and clinical attachment level were seen in test groups when compared with the control group from baseline to 21 days and 90 days and same levels were maintained throughout the experimental period in the test group.

This result was in accordance with study conducted, by Okamoto et al.20suggested that the green tea catechin may have the potential to reduce periodontal breakdown resulting from the potent protein as e activity of Porphyromonas gingivalis. Sakanaka in 200421 reported that Green tea catechin (EGCG) inhibits the expression of gelatinase (MMP-9 RNA), and the formation of osteoclasts, thereby reduces the alveolar bone resorption that occurs in periodontal disease40.Kudwa et al5 demonstrated improvement in clinical parameters including probing pocket depth following placement of green tea catechin as local drug delivery into periodontal pocket.

The statistically significant reduction in the colony forming units of anaerobic bacteria was observed in present study. This result shows the efficacy of green tea as an antimicrobial agent when applied as a local drug delivery. Scaling and root planning alone causes profound shift in the composition of the subgingival microbiota.

The greater reduction in the microbial load at test site as compared to control site can be explained on the basis of antibacterial activity of green tea extract used at the test site which may have decreased the pathogenicity of the anaerobic obligate bacteria residing in the bacterial environment. This is in accordance with studies by Makimura et al 19934, Sakanaka et al 199621, Hirsawa et al 200214 and Kudwa et al 20125.The antibacterial action of green tea is attributed to galloradical present in EGC, ECGg which possesses strong bacterial activity, inhibitory activity on both the toxic metabolities of P.gingivalis and collagenase of eukaryotic and prokaryotic cell.

Plaque biofilm on the tooth surface can act as source of endotoxins, serve as nidus for bacterial accumulation and may be impervious to local drug delivery. Thorough scaling and root planning enables disruption of this biofilm complex which allows enhancing the action of local drug delivery. Green tea exhibiting antioxidant and antimicrobial activity against several periopathogens may be used as therapeutic and prophylactic agent for periodontitis. Thus administration of green tea chips as local drug delivery with scaling and root planning could be viable treatment option for treating moderate pocket depth of 4-6 mm in chronic periodontitis patients.

CONCLUSION

The present three month study was designed to reduce the surgical intervention in the treatment of periodontal pocket and to use locally available material so as to reduce the financial burden on the patient & thereby making cost effective management. The continuous application of green tea catechin on a daily basis may be useful and practical method for the prevention of periodontal disease. The development of a new ointment base that can maintain long term effectiveness in periodontal tissue by one –time drug administration is expected. Additional standardized trial designs are recommended to evaluate the clinical and microbiological effects of green tea. Further research with relatively large sample size, longer follow up period, and use of advanced periodontal probes are advocated to further study the efficacy of green tea as effective local drug delivery agent.

Table 1: Inter Group Comparison of Mean Value for Test and Control for Gingival Index

		CONTROL		TEST		P-Value
		Mean	SD	Mean	SD	
Gingival Index	Baseline	2.67	.29	2.67	.29	
	21 day	0.53	.55	0.51	.55	0.334;NS
	90 day	1.37	.23	1.20	.23	0.001;Sig

Table 2: Inter Group Comparison of Mean Values for Test and Control for Plaque Index

		CONTROL		TEST		P-Value
		Mean	SD	Mean	SD	
Plaque Index	Baseline	2.75	.28	2.77	.26	0.334;NS
	21 day	1.32	.26	0.95	.05	<0.001;Sig
	90 day	1.81	.36	1.25	.08	<0.001;Sig

Table 3: Inter Group Comparison of Mean Values for Test and Control for Sulcular Bleeding Index

		CONTROL		TEST		P-Value
		Mean	SD	Mean	SD	
SBI	Baseline	3.47	.38	3.87	.34	<0.001;Sig
	21 day	2.15	.44	2.05	.46	0.090
	90 day	2.28	.51	1.29	.37	<0.001;Sig

Table 4: Inter Group Comparison of Mean Values for Test and Control for Probing Depth

		CONTROL		TEST		P-Value
		Mean	SD	Mean	SD	
PD	Baseline	5.73	.70	5.73	.70	
	21 day	3.27	.46	2.27	.46	<0.001;Sig

	90 day	3.27	.46	2.27	.46	<0.001;Sig

Table 5: Inter group Comparison of Mean Values for Test and Control for Clinical Attachment Level (CAL)

		CONTROL		TEST		P-Value
		Mean	**SD**	**Mean**	**SD**	
CFU	**Baseline**	5.60	.51	5.60	.51	
	21 day	2.80	.55	1.43	.49	<0.001;Sig
	90 day	3.27	.46	1.43	.49	<0.001;Sig

Fig 1: Green Tea Strips and Placebo Strips

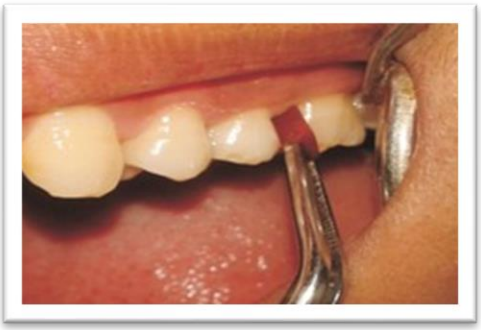

Fig 2: Placement of Green Tea Strips at Site

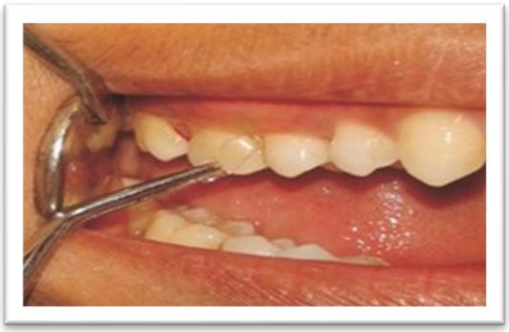

Fig 3: Placement of Placebo Strips at Control Site

REFERENCES

1. Deshpande N, Deshpande A, Mafoud S. Evaluation of intake of green tea on gingival and periodontal status: An experimental study. Journal o Interdisciplinary Dentistry 2012; 2(2).
2. Chava, Vedula: Thermo-Reversible Green Tea Catechin Gel for Local Application in Chronic Periodontitis. A 4-Week Clinical Trial. J Periodontol. 2013 Sep; 84(9): 1290-6.
3. Hanes PJ, Purvis JP. Local anti-infective therapy:Pharmacological agents. A Systematic Review. Ann Periodontal 2003;8:79-98.

4. Makimura M, Hirasawa M, Kobayashi K, et al. Inhibitory effect of tea catechins on collagenase activity. J Periodontol 1993;64:630-636.
5. Kudva P, SyedaTabasum T, Shekhawat N.Effect of green tea catechin, a local drug delivery system as an adjunct to scaling and root planning in chronic periodontitis patients: A clinico-microbiological study JIndianSocPeriodontol 2011; 15(1): 39-45.
6. Wen WC, Po-Jan Kuo, Cheng-Yang Chiang, Yu-Tang Chin, Martin M. J. Fu, and EarlFu Epigallocatech in-3-Gallate Attenuates Porphyromonasgingivalis Lipopolysaccharide - Enhanced Matrix Metalloproteinase-1 Production Through Inhibition of Interleukin-6 in Gingival Fibroblasts J Periodontol 2014;85:868-875.
7. S. Coimbra et al. The effect of green tea in oxidative stress. Clinical Nutrition 2006; 25: 790–796.
8. Thomas E.R, Slots J et al. Local delivery of antimicrobial agents in the periodontal pocket. Periodontol 2000 1996; 10: 139-159.
9. Hanes PJ, Purvis JP. Local anti-infective therapy: Pharmacological agents. A Systematic Review. Ann Periodontal 2003;8:79-98.
10. Sakanaka S (1997). Green tea polyphenols for prevention of dental caries. In "Chemical Applications of Green Tea" (T. Yamamoto, L. R.Juneja, D.-C. Chu and M. Ki, CRC Press, Boca Raton, FL, pp. 87-101.
11. Moghbel AH, Farajzadeh A, Aghel N, Raisi N. Formulation and Evaluation of Green Tea Antibacterial Mouthwash Effect on the Aerobic Mouth Bacterial Load. Sci. Med. J 2010; 9: 317-330.
12. Orafai H, Forouzanfar A, Maroofian A. Formulation of green tea mouthwash as an Effervescent tablet from dried green tea leaf of north Iran. 2013; 1-11.
13. Bruce LP, William FA. American academy of periodontology: Treatment of gingivitis and periodontitis (Position paper). J Periodontol 1997;68:1246-53.
14. Hirasawa M, Takada K, Makimura M, Otake S. Improvement of periodontal status by green tea catechin using a local delivery system: A clinical pilot study. J Periodontal Res 2002;37:433-438.
15. Kushiyama M, Shimazaki Y. Relationship between intake of green tea and periodontal disease. J Periodontol 2009;80(3): 372-377.
16. Hirsawa M, Takada K, Otake S. Inhibition of acid productionin dental plaque bacteria by green tea catechins. Caries Res 2006;40(3):265-70.
17. Hattarki S, Pushpa SP, Bhatt K.Evaluation of the efficacy of green tea catechins as an adjunct to scaling and root planning in the management of chronic periodontitis using PCR analysis: a clinical and microbiological study. JIndianSocPeriodontol2013; 17(2): 204-9.
18. Maruyama T et al. Supplementation of green tea catechins in dentifrices suppresses gingival oxidative stress and periodontal inflammation. Archives of oral biology 2011; 56: 48–53.
19. Nakamura H, Ukai T, Yoshimura A, Kozuka Y, Yoshioka H, Yoshinaga Y, et al. In vivoGreen tea catechin inhibits lipopolysaccharide-induced bone resorption. J Periodontal Res 2009;45:23-30.
20. Okamoto M., Sugimoto A., Leung K.P., Nakayama K., Kamaguchi A., Maeda N., Inhibitory effect of green tea catechins on cysteine proteinases in Porphyromonasgingivalis. Oral Microbiol. Immunol 2004; 19:118–120.
21. Sakanaka S, Aizawa M, Kim M, Yamamoto T. Inhibitory effects of green tea polyphenols on growth and cellular adherence of an oral bacterium, Porphyromonas gingivalis. Biosci Biotechnol Biochem 1996;60:745-9.