EFFECT OF GREEN TEA CATECHIN GEL ON CREVICULAR GLUTATHIONE LEVELS IN CHRONIC PERIODONTITIS - A CLINICO-BIOCHEMICAL STUDY.

Periodontology

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ABSTRACT

The aim of the study to evaluate the effect catechin gel on glutathione levels in GCF following nonsurgical periodontal therapy (NST) in chronic periodontitis patient. Thirty patients with chronic periodontitis (113 sites) were randomly divided into two groups, one was treated with scaling and root planing(SRP) + placebo(Group A) and other with SRP+Catechin gel(Group B).All clinical parameter were assessed prior to treatment (baseline), 1month and 3months. Result: Twenty patients completed the study, group B showed significant improvements over group A. Conclusion: Catechin gel with nonsurgical periodontal therapy improved GSH levels and clinical parameters as compared to nonsurgical therapy alone.

KEYWORDS

Introduction-The periodontium consists of four principal components namely gingiva, periodontal ligament, alveolar bone and cementum. These structures help to provide support necessary to maintain teeth in function. The role of bacteria in the initiation of periodontitis is well documented and destruction of the alveolar bone and periodontal connective tissue is clearly observed (Kornman et al. 2008). The reduction and elimination of pathogenic bacteria have been accomplished in large part by the use of mechanical treatment approaches such as scaling and root planing (SRP), which removes the deposits from the tooth surface and shifts the pathogenic microbiota to one compatible with periodontal health (Tenenbaum et al. 2112, Socransky et al. 1992). However, the periodontal pocket anatomy is a significant limiting factor in mechanical access, and sufficient reduction of the bacterial load may not be provided (Rabbani et al. 1981). Systemic antibiotics require the administration of large doses in order to gain sufficient concentrations at the disease sites, and suffer from potential for the development of bacterial resistance, side-effects, drug interactions or inconsistent patient compliance (Slots et al. 2000). The inherent limitations of systemic or topical chemotherapies led to the development of local delivery systems for the administration of antimicrobials directly into the periodontal pockets (Raheja et al. 2013). This form of therapy offers little or no systemic drug uptake, reduced risk of drug resistance, reduced side-effects and high concentrations at the targeted sites (Friedman et al 1990). The effectiveness of this form of therapy is that, it reaches the base of periodontal pocket and is maintained for an adequate time for the antimicrobial effect to occur. Other advantages include higher drug concentration, minimal side effects, less reliance on patients for taking medication and avoid treating the patient systemically (Kalsi et al.2011, Divya et al. 2006). Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side-effects. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals (Palombo et al. 2011, Bairwa et al. 2012). Green Tea has received considerable attention because of its numerable scientifically proven health benefits, due to polyphenols like (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epicatechin gallate (ECG),(-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) (Cabrera et al 2006, Kushiyama et al. 2009, Palwankar et al. 2015). Green tea catechin was observed to have a bactericidal effect against black pigmented rods. The combined use of mechanical treatment and the application of green tea catechin using a slow release local delivery system were found to be effective in improving periodontal status (Hirasawa et al. 2002, Sakanaka et al. 2004).

Glutathione (GSH) is a ubiquitous tripeptide made from the combination of three amino acids, that is, cysteine, glutamate, and glycine. It is a low molecular weight thiol (up to 5–10 mM) present in the cell and existing in two forms which are oxidized glutathione

(GSSG) and reduced GSH forms (Townsend 2003). It is present predominantly in gingival crevicular fluid, is reported to be the most important redox regulator and controls inflammatory process (Chapple 1996). Periodontal therapy aims at reducing oxidative stress and restoring redox balance to healthy levels.

Hence, the aim of this study was to evaluate the potential effect of Green tea catechin gel on clinical parameters and its effect on glutathione levels in gingival crevicular fluid as an adjunct to SRP over SRP alone during nonsurgical therapy in patients with chronic periodontitis.

Material and Methods- A randomized case control study was conducted after approval by the ethical committee of B.D Sharma University of health sciences Rohtak, Patients with probing pocket depth 4-6 mm in the age group of 30-50 years were included in the study. All forms of tobacco users, history of periodontal therapy or systemic disease or use of antibiotics 6 months prior to the study were excluded from the study. All selected patients received full mouth scaling and root planing. They were then assigned to either the group A or group B by flip of coin. Group A-Scaling and Root planing(SRP) + placebo gel and Group B-Scaling and Root planing(SRP) + catechin gel. The clinical parameters Plaque Index (Turesky et al modification of Quigely and Hein, 1970), Gingival index (Löe and Silness, 1963), Probing Pocket depth (PD) were recorded. At baseline, the pockets was irrigated with normal saline and then dried with paper points. The gel was then applied directly from the syringe with blunt needle into the pocket and then, while continuing to extrude the material, the needle was slowly withdrawn till it reached the superior portion of the pocket.

Catechin gel and placebo gel syringes were encoded so that neither patient nor investigator knows about the gel incorporated in the syringes. At the end of the study, decoding was done to compare and relate the data to Group A and Group B sites by the study coordinator.

In vitro preparation of Green tea catechin gel preparation -The gel was procured from Jagadguru Sri Shivarathreeshwara Pharmacy College, Ooty, India. Green tea extract powder was provided by Anthem Cellutions Pvt Ltd, Delhi, INDIA. Gel were sterilized by gamma radiation at 2.5 Mrad by Shriman centre for industrial research, Delhi university, and sterilized were refrigerated at 2 degrees - 8 degrees Celsius.

No dietary limitations were imposed during or after treatment. Patients were instructed not to use any chemotherapeutic mouth rinses or oral irrigation devices. Patients were asked to report immediately if pain, swelling or any other problem occurred. Modified Bass brushing technique was standardized but the patients were instructed not to use floss or interdental aids for next 24hrs. Patients were recalled after one month.

North Carolina (UNC) 15 probe), Clinical attachment level (using UNC 15 probe) and levels of glutathione were estimated at the baseline (Pretreatment) and at an interval of 1 month and 3 months. The probing site and measurements were standardized in all areas by using an acrylic stent, considering the lower margin of stent as reference point and groove as reference

Sampling for biochemical analysis: After careful isolation of the area with cotton rolls to prevent contamination by saliva of the sites to be treated, 1µl of Gingival crevicular fluid (GCF) was collected from sites using calibrated micro capillary pipettes (@ Sigma Aldrich USA) at baseline and at 1, 3 months. Then the samples were carefully transferred in Eppendorf tubes. The Eppendorf tubes were then stored at ≤-20o c until further analysis. Reduced Gluthathione (GSH) were analyzed using "Beutler" spectrophotometric (Beutler et al 1963)

Statistical Analysis
The statistical analysis was performed using SPSS Version 18 software. The student t-test was carried out for the unpaired observations (inter-group comparison) and the paired t-test for the paired observations (intragroup comparison). Mean values and standard deviations were calculated for each variable and at stipulated intervals respectively.

Results
Table-1 Intergroup Comparison of Clinical Parameters of Group B and Group A

	Groups	N	Mean	Std. Deviation	t-value	p-value
PI Baseline	Group B	10	1.42	0.26	1.348	0.194
	Group A	10	1.57	0.24		
PI 1M	Group B	10	0.87	0.16	3.011	0.008
	Group A	10	1.03	0.05		
PI 3M	Group B	10	0.81	0.15	3.098	0.006
	Group A	10	0.98	0.09		
GI Baseline	Group B	10	1.32	0.28	0.624	0.54
	Group A	10	1.40	0.28		
GI 1M	Group B	10	0.85	0.11	0.757	0.459
	Group A	10	0.89	0.11		
GI 3M	Group B	10	0.82	0.12	0.873	0.394
	Group A	10	0.87	0.10		
PD Baseline	Group B	52	5.63	0.93	0.93	0.354
	Group A	61	5.48	0.89		
PD1M	Group B	52	3.63	0.91	1.693	0.093
	Group A	61	3.90	0.77		
PD3M	Group B	52	3.27	0.72	4.134	<0.001
	Group A	61	3.82	0.70		
CAL Baseline	Group B	52	6.27	1.03	0.798	0.426
	Group A	61	6.13	0.81		
CAL1M	Group B	52	3.81	0.93	1.664	0.099
	Group A	61	4.08	0.82		
CAL 3M	Group B	52	3.56	0.78	3.205	0.002
	Group A	61	4.02	0.74		

Intergroup Comparison (Table 1) - There is a statistically significant improvement in plaque index at 1 month and 3 months in group B than group A, for the same period. There is no significant difference in Gingival Index at 1 month and 3 months in group B and group A. Pocket depth reduction showed a statistically significant difference at 3 months in group B with no significant difference is at 3 months in group A. CAL showed a significant improvement at 1 month in both the groups. At 3 months the CAL showed improvement in group B but same was not observed in group A.

Table-2 Intergroup comparison of mean values of reduced Gluthathione levels.

Group		N	Mean	Std. Deviation	t-value	p-value	
GLU Baseline	Group B	10	266.23	17.64	1.653	0.116	
	Group A	10	256.43	6.35			
GLU 1M	Group B	10	339.26	25.07	6.44	<0.001	
	Group A	10	281.95	12.78			
GLU 3M	Group B	10	406.10	32.26	7.582	<0.001	
	Group A	10	316.66	18.73			

GLU- reduced glutathione (GSH)

A significant increase of reduced glutathione levels at 1 month in group B and group A was observed. At 3 months the improvement in reduced glutathione levels was seen in group B whereas in group A no difference was observed (Table 2)

Table 3– Intragroup Clinical Parameters of Group B and Group A

Periodontal Parameters	Group	Time interval	Mean ± SD	Mean Difference from baseline	p value	Mean Difference from 1 month	p value
Plaque Index	B	Baseline	1.42±0.26	-	-	-	-
		month	0.87±0.16	0.55	0.001	-	-
		3 months	0.81±0.15	0.61	0.001	0.06	0.024
	A	Baseline	1.57±0.24	-	-	-	-
		1 month	1.03±0.05	0.54	<0.001*	-	-
		3 months	0.98+0.09	0.59	<0.001*	0.05	0.063
Gingival Index	B	Baseline	1.32±0.28	-	-	-	-
		1 month	0.85±0.10	0.47	<0.001	-	-
		3 months	0.82±0.30	0.50	<0.001	0.03	0.178
	A	Baseline	1.40±0.28	-	-	-	-
		1 month	0.89±0.11	0.51	<0.001	-	-
		3 months	0.87±0.10	0.53	<0.001	0.02	0.104
Periodontal Parameters	Group	Time interval	Mean± SD	Mean Difference from baseline	p value	Mean Difference from 1 month	p value
Probing depth	B	Baseline	5.63±0.93	-	-	-	-
		1 month	3.63±0.91	2.00	<0.001	-	-
		3 months	3.27±0.72	2.36	<0.001	0.36	<0.001
	A	Baseline	5.48±0.89	-	-	-	-
		1 month	3.90±0.77	1.58	<0.001	-	-
		3 months	3.82±0.70	1.66	<0.001	0.08	0.228
Clinical Attachment Level	B	Baseline	6.31±1.00	-	-	-	-
		1 month	4.40±1.05	1.91	0.001	-	-
		3 months	4.04±1.04	2.27	0.014	0.36	0.008
	A	Baseline	6.13±0.80	-	-	-	-
		1 month	4.57±0.84	1.56	0.05	-	-
		3 months	4.49±0.78	1.64	0.231	0.08	0.199

Intragroup Comparison – The mean difference of plaque index for Group B at 1 month and 3 months from baseline was 0.55 and 0.61 respectively, which was significant (p<0.05).The mean difference of plaque index for Group A at 1 month and 3 months from baseline was 0.54 and 0.59 respectively, which was significant (p<0.05). The mean difference of gingival index for Group B at 1 month and 3 months from baseline was 0.47 and 0.50 respectively, which was significant (p<0.05) (Table-5).The mean difference of gingival index for Group A at 1 month and 3 months from baseline was 0.51 and 0.53 respectively, which was significant (p<0.05) . The mean pocket depth reduction for the Group B when compared from baseline to 1 month and 3 months was 2 and 2.36 respectively, which was significant. (p<0.05) (Table-7)The mean pocket depth reduction for the Group A when compared from baseline to 1 month and 3 months was 1.58 and 1.66 respectively, which was significant. (p<0.05) . The mean attachment gain for the group B when compared from baseline to 1 month and 3 months was 1.91 and 2.27 respectively, which was significant (p<0.05) . The mean attachment gain for the Group B when compared from 1 month to 3 months was 0.36, which was significant (p<0.05). The mean gain in reduced glutathione levels for the Group B when compared from baseline to 1 month and 3 months was 73.03 and 139.87 respectively, which was significant (p<0.05) (Table-3).

Table-4 Intragroup Comparison of Mean Values of Rseduced Glutathione Level

Group	Time interval	Mean± SD	Mean Difference from baseline	p value	Mean Difference from 1 month	p value
B	Baseline	266.23±17.64	-	-	-	-
	1 month	339.26±25.07	73.03	<0.001	-	-
	3 months	406.10±32.26	139.87	<0.001	66.84	<0.001
A	Baseline	2563.4±6.35	-	-	-	-
	1 month	281.95±12.78	25.52	<0.001	-	-
	3 months	316.66±18.73	60.23	<0.001	34.71	<0.001

*Statistically significant at p <0.05   **SD - Standard deviation

The mean gain in reduced glutathione levels for the Group B when compared from 1 month to 3 months was 66.84 , which was significant (p<0.001) (Table-4). The mean gain in reduced glutathione levels for the Group A when compared from baseline to 1 month and 3 months was 25.52 and 60.23 respectively, which was significant (p<0.05).The mean gain in reduced glutathione levels for the Group A when compared from 1 month to 3 months was 34.71, which was significant (p<0.001).

Discussion
All the patients showed statistically and clinically significant improvements in plaque indices at follow-up visits when compared with the baseline levels in Group B. The mean reduction in plaque index score in Group B from baseline to 1 and 3 months were 0.55 and 0.61 respectively and for Group A were 0.54 and 0.59 respectively. This is supported by the molecular scenario following treatment as the crevicular epithelium is not subjected to oxidative stress, hence bringing the homeostasis back to redox balance (Palwankar p et al 2016).The mean reduction in gingival index score for Group B from baseline to 1 and 3 months were 0.47 and 0.50 respectively and for Group A were 0.51 and 0.53 respectively. This could be attributed to elimination of local etiology by scaling because this harbors numerous bacterial species with potent virulent factors that are known to damage the host periodontal tissues. This is in accordance with Hinrichs et. al 1985 and Cugini et. al 2000.

The reduction in plaque and gingival scores from baseline could be due to the recording of these parameters before scaling and root planing at the baseline visit. The antibacterial role of green tea catechin gel may have leaked out of periodontal pockets which may have contributed to the clinical outcome15. Similar effects of improvement in gingival and plaque index scores was observed by using aloe vera gel as a dentifrice (Pradeep et. al 2012) and used mucoadhesive gel containing neem leaf extract (Pai et. al 2013)

The Clinical Attachment Level (CAL) showed mean difference from baseline to 1 month and 3 months at in Groups B 1.91 and 2.27 and mean difference from baseline to 1 and 3 months in control group as 1.56 and 1.64 respectively. This could be attributed to elimination of local etiology by scaling because this harbors numerous bacterial species with potent virulent factors that are known to damage the host periodontal tissues. This is in accordance with Hinrichs et al and Cugini et al. similar observations were made by Hirasawa et. al 2002, and Chava et. al 2013.

In the present study, the observations showed statistically significant reduction in probing depth from baseline to 1 month and 3 months in Group B compared to Group A. The mean pocket depth reduction from baseline to 1 month and 3 months was found to be 2 and 2.36 respectively for Group B and 1.58 and 1.66 respectively for Group A. The results were consistent with the findings of Chava et. al 2013. This may be due to the inhibitory effect of green tea catechins on cysteine proteinases (Arg-gingipain and Lys-gingipain) of P. gingivalis and protein tyrosine phosphatase of P. intermedia which are considered as potent virulence factors in the development of periodontitis. (Okamoto et. al 2003, Holt et. al 1999, Haffajje et. al 1994). This may also be because of absorption of green tea into the epithelial cells in the subgingival pocket, thus inhibiting the growth of black pigmented rods responsible for periodontal disease.

At termination of the study, test group B in intergroup comparison showed an additional pocket depth reduction of 0.42 mm from baseline to 1 month and 0.28 mm from 1 month to 3 months. With regard to CAL gain, the difference for test and control group was reported to be 0.35mm and 0.28 mm from baseline to 1 month and from 1 month to 3 months respectively.

In the present study, a significant increase in reduced GSH levels were observed following non-surgical periodontal therapy, which were consistent with the observations made by Palwankar et al. The mean reduced glutathione levels of group B from baseline, 1month and 3 months are 73.03 and 139.87 respectively and group A was found to be 25.52 and 60.23.There was statistical significant improvement in reduced Gluthathione levels in Group B as compared to Group A. This suggested that there is improvement in antioxidant status and reduction in oxidative stress both locally and systemically in periodontitis patients with Catechin   gel. Also, the comparison of group B and group A from baseline to 1 month was reported to be 47.51 and from 1 month to 3 months were reported to be 32.13. The change in glutathione level in the present study is due to reduction in amount of GSSG (Oxidised Glutathione) and maintenance of GSH (Gluthathione) levels within GCF after treatment.

Limitations- Catechin gel therapy can be reliable treatment modality in treating chronic periodontitis. This study opens up newer avenues in the field of research with catechin gel which is constituent of Green tea in treating periodontal disease and increase the level of GSH, the potent antioxidant. Further studies on GSH as a diagnostic marker in periodontitis and newer methods of its evaluation have to be undertaken.

Conclusion
On the basis of this study, it can be said that local drug therapy using herbal formulations when used as an adjunct markedly improves the benefits of scaling and root planing in chronic periodontitis patients. Further long term studies can be undertaken to evaluate the efficacy of catechin gel for improving the buffering capacity within periodontal tissues by elevating the GSH levels.

The present study opens up scope for various future studies dealing with role of glutathione as a biological disease marker, antioxidant, detoxifier and immune modulator

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33. Introduction-The periodontium consists of four principal components namely gingiva, periodontal ligament, alveolar bone and cementum. These structures help to provide support necessary to maintain teeth in function. The role of bacteria in the initiation of periodontitis is well documented and destruction of the alveolar bone and periodontal connective tissue is clearly observed (Kornman et al. 2008). The reduction and elimination of pathogenic bacteria have been accomplished in large part by the use of mechanical treatment approaches such as scaling and root planing (SRP), which removes the deposits from the tooth surface and shifts the pathogenic microbiota to one compatible with periodontal health (Tenenbaum et al. 2112, Socransky et al. 1992). However, the periodontal pocket anatomy is a significant limiting factor in mechanical access, and sufficient reduction of the bacterial load may not be provided (Rabbani et al. 1981). Systemic antibiotics require the administration of large doses in order to gain sufficient concentrations at the disease sites, and suffer from potential for the development of bacterial resistance, side-effects, drug interactions or inconsistent patient compliance (Slots et al. 2000). The inherent limitations of systemic or topical chemotherapies led to the development of local delivery systems for the administration of antimicrobials directly into the periodontal pockets (Raheja et al. 2013). This form of therapy offers little or no systemic drug uptake, reduced risk of drug resistance, reduced side-effects and high concentrations at the targeted sites (Friedman et al 1990). The effectiveness of this form of therapy is that, it reaches the base of periodontal pocket and is maintained for an adequate time for the antimicrobial effect to occur. Other advantages include higher drug concentration, minimal side effects, less reliance on patients for taking medication and avoid treating the patient systemically (Kalsi et al.2011, Divya et al. 2006). Despite several agents being commercially available, these chemicals can alter oral microbiota and have undesirable side-effects. Hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as good alternatives to synthetic chemicals (Palombo et al. 2011, Bairwa et al. 2012). Green Tea has received considerable attention because of its numerable scientifically proven health benefits, due to polyphenols like (+)-catechin (C), (-)-epicatechin (EC), (+)-gallocatechin (GC), (-)-epicatechin gallate (ECG),(-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG) (Cabrera et al 2006, Kushiyama et al. 2009, Palwankar et al. 2015). Green tea catechin was observed to have a bactericidal effect against black pigmented rods. The combined use of mechanical treatment and the application of green tea catechin using a slow release local delivery system have found to be effective in improving periodontal status (Hirasawa et al. 2002, Sakanaka et al. 2004).

34. Glutathione (GSH) is a ubiquitous tripeptide made from the combination of three amino acids, that is, cysteine, glutamate, and glycine. It is a low molecular weight thiol (up to 5–10 mM) present in the cell and existing in two forms which are oxidized glutathione (GSSG) and reduced GSH forms (Townsend 2003). It is present predominantly in gingival crevicular fluid, is reported to be the most important redox regulator and controls inflammatory process (Chapple 1996). Periodontal therapy aims at reducing oxidative stress and restoring redox balance to healthy levels.

35. Hence, the aim of this study was to evaluate the potential effect of Green tea catechin gel on clinical parameters and its effect on glutathione levels in gingival crevicular fluid as an adjunct to SRP over SRP alone during nonsurgical therapy in patients with chronic periodontitis.

36. Material and Methods- A randomized case control study was conducted after approval by the ethical committee of B.D Sharma University of health sciences Rohtak, Patients with probing pocket depth 4-6 mm in the age group of 30-50 years were included in the study. All forms of tobacco users, history of periodontal therapy or systemic disease or use of antibiotics 6 months prior to the study were excluded from the study. All selected patients received full mouth scaling and root planing. They were then assigned to either the group A or group B by flip of coin. Group A-Scaling and Root planing(SRP) + placebo gel and Group B-Scaling and Root planing(SRP) + catechin gel. The clinical parameters Plaque Index (Turesky et al modification of Quigely and Hein, 1970), Gingival index (Löe and Silness, 1963), Probing Pocket depth (PD) were recorded. At baseline, the pockets was irrigated with normal saline and then dried with paper points. The gel was then applied directly from the syringe with blunt needle into the pocket and then, while continuing to extrude the material, the needle was slowly withdrawn till it reached the superior portion of the pocket.

37. Catechin gel and placebo gel syringes were encoded so that neither patient nor investigator knows about the gel incorporated in the syringes. At the end of the study, decoding was done to compare and relate the data to Group A and Group B sites by the study coordinator.

38. In vitro preparation of Green tea catechin gel preparation -The gel was procured from Jagadguru Sri Shivarathreeshwara Pharmacy College, Ooty, India. Green tea extract powder was provided by Anthem Cellutions Pvt Ltd, Delhi, INDIA. Gel were sterilized by gamma radiation at 2.5 Mrad by Shriman centre for industrial research, Delhi university, and sterilized were refrigerated at 2 degrees - 8 degrees Celsius.

39. No dietary limitations were imposed during or after treatment. Patients were instructed not to use any chemotherapeutic mouth rinses or oral irrigation devices. Patients were asked to report immediately if pain, swelling or any other problem occurred. Modified Bass brushing technique was standardized but the patients were instructed not to use floss or interdental aids for next 24hrs. Patients were recalled after one month.

40. North Carolina (UNC) 15 probe), Clinical attachment level (using UNC 15 probe) and

levels of glutathione were estimated at the baseline (Pretreatment) and at an interval of 1 month and

41. 3 months. The probing site and measurements were standardized in all areas by using an

42. acrylic stent, considering the lower margin of stent as reference point and groove as reference

43. Sampling for biochemical analysis: After careful isolation of the area with cotton rolls to prevent contamination by saliva of the sites to be treated, 1µl of Gingival crevicular fluid (GCF) was collected from sites using calibrated micro capillary pipettes (@ Sigma Aldrich USA) at baseline and at 1, 3 months. Then the samples were carefully transferred in Eppendorf tubes. The Eppendorf tubes were then stored at ≤-20o c until further analysis. Reduced Gluthathione (GSH) were analyzed using "Beutler" spectrophotometric (Beutler et al 1963)

44. Statistical Analysis

45. The statistical analysis was performed using SPSS Version 18 software. The student t-test was carried out for the unpaired observations (inter-group comparison) and the paired t-test for the paired observations (intragroup comparison). Mean values and standard deviations were calculated for each variable and at stipulated intervals respectively.

46. Results

47. Intergroup Comparison (Table 1) - There is a statistically significant improvement in plaque index at 1 month and 3 months in group B than group A, for the same period. There is no significant difference in Gingival Index at 1 month and 3 months in group B and group A. Pocket depth reduction showed a statistically significant difference at 3 months in group B with no significant difference is at 3 months in group A. CAL showed a significant improvement at 1 month in both the groups. At 3 months the CAL showed improvement in group B but same was not observed in group A. A significant increase of reduced glutathione levels at 1 month in group B and group A was observed. At 3 months the improvement in reduced glutathione levels was seen in group B whereas in group A no difference was observed (Table 2)

48. Intragroup Comparison – The mean difference of plaque index for Group B at 1 month and 3 months from baseline was 0.55 and 0.61 respectively, which was significant (p<0.05).The mean difference of plaque index for Group A at 1 month and 3 months from baseline was 0.54 and 0.59 respectively, which was significant (p<0.05). The mean difference of gingival index for Group B at 1 month and 3 months from baseline was 0.47 and 0.50 respectively, which was significant (p<0.05) (Table-5).The mean difference of gingival index for Group A at 1 month and 3 months from baseline was 0.51 and 0.53 respectively, which was significant (p<0.05) . The mean pocket depth reduction for the Group B when compared from baseline to 1 month and 3 months was 2 and 2.36 respectively, which was significant. (p<0.05) (Table-7)The mean pocket depth reduction for the Group A when compared from baseline to 1 month and 3 months was 1.58 and 1.66 respectively, which was significant. (p<0.05) . The mean attachment gain for the group B when compared from baseline to 1 month and 3 months was 1.91 and 2.27 respectively, which was significant (p<0.05) . The mean attachment gain for the Group B when compared from 1 month to 3 months was 0.36, which was significant (p<0.05). The mean gain in reduced glutathione levels for the Group B when compared from baseline to 1 month and 3 months was 73.03 and 139.87 respectively, which was significant (p<0.05) (Table-3). The mean gain in reduced glutathione levels for the Group B when compared from 1 month to 3 months was 66.84 , which was significant (p<0.001) (Table-4). The mean gain in reduced glutathione levels for the Group A when compared from baseline to 1 month and 3 months was 25.52 and 60.23 respectively, which was significant (p<0.05).The mean gain in reduced glutathione levels for the Group A when compared from 1 month to 3 months was 34.71, which was significant (p<0.001).

49. Discussion

50. All the patients showed statistically and clinically significant improvements in plaque indices at follow-up visits when compared with the baseline levels in Group B. The mean reduction in plaque index score in Group B from baseline to 1 and 3 months were 0.55 and 0.61 respectively and for Group A were 0.54 and 0.59 respectively. This is supported by the molecular scenario following treatment as the crevicular epithelium is not subjected to oxidative stress, hence bringing the homeostasis back to redox balance (Palwankar p et al 2016).The mean reduction in gingival index score for Group B from baseline to 1 and 3 months were 0.47 and 0.50 respectively and for Group A were 0.51 and 0.53 respectively. This could be attributed to elimination of local etiology by scaling because this harbors numerous bacterial species with potent virulent factors that are known to damage the host periodontal tissues. This is in accordance with Hinrichs et. al 1985 and Cugini et. al 2000.

51. The reduction in plaque and gingival scores from baseline could be due to the recording of these parameters before scaling and root planing at the baseline visit. The antibacterial role of green tea catechin gel may have leaked out of periodontal pockets which may have contributed to the clinical outcome15. Similar effects of improvement in gingival and plaque index scores was observed by using aloe vera gel as a dentifrice (Pradeep et. al 2012) and used mucoadhesive gel containing neem leaf extract (Pai et. al 2013)

52. The Clinical Attachment Level (CAL) showed mean difference from baseline to 1 month and 3 months at in Groups B 1.91 and 2.27 and mean difference from baseline to 1 and 3 months in control group was 1.56 and 1.64 respectively. This could be attributed to elimination of local etiology by scaling because this harbors numerous bacterial species with potent virulent factors that are known to damage the host periodontal tissues. This is in accordance with Hinrichs et al and Cugini et al. similar observations were made by Hirasawa et. al 2002, and Chava et. al 2013.

53. In the present study, the observations showed statistically significant reduction in probing depth from baseline to 1 month and 3 months in Group B compared to Group A. The mean pocket depth reduction from baseline to 1 month and 3 months was found to be 2 and 2.36 respectively for Group B and 1.58 and 1.66 respectively for Group A. The results were consistent with the findings of Chava et. al 2013. This may be due to the inhibitory effect of green tea catechins on cysteine proteinases (Arg-gingipain and Lys-gingipain) of P. gingivalis and protein tyrosine phosphatase of P. intermedia which are considered as potent virulence factors in the development of periodontitis. (Okamoto et. al 2003, Holt et. al 1999, Haffajje et. al 1994) . This may also be because of absorption of green tea into the epithelial cells in the subgingival pocket, thus inhibiting the growth of black pigmented rods responsible for periodontal disease.

54. At termination of the study, test group B in intergroup comparison showed an additional pocket depth reduction of 0.42 mm from baseline to 1 month and 0.28 mm from 1 month to 3 months. With regard to CAL gain, the difference for test and control group was reported to be 0.35mm and 0.28 mm from baseline to 1 month and from 1 month to 3 months respectively.

55. In the present study, a significant increase in reduced GSH levels were observed following non-surgical periodontal therapy, which were consistent with the observations made by Palwankar et al. The mean reduced glutathione levels of group B from baseline, 1month and 3 months are 73.03 and 139.87 respectively and group A was found to be 25.52 and 60.23.There was statistical significant improvement in reduced Gluthathione levels in Group B as compared to Group A. This suggested that there is improvement in antioxidant status and reduction in oxidative stress both locally and systemically in periodontitis patients with Catechin gel. Also, the comparison of group B and group A from baseline to 1 month was reported to be 47.51 and from 1 month to 3 months reported to be 32.13. The change in glutathione level in the present study is due to reduction in amount of GSSG (Oxidised Glutathione) and maintenance of GSH

(Gluthathione) levels within GCF after treatment.

56. Limitations- Effect of catechin gel on bone, duration of study and method of evaluation of GSH. Catechin gel therapy can be reliable treatment modality in treating chronic periodontitis. This study opens up newer avenues in the field of research with catechin gel which is constituent of Green tea in treating periodontal disease and increase the level of GSH, the potent antioxidant. Further studies on GSH as a diagnostic marker in periodontitis and newer methods of its evaluation have to be undertaken.

57. Conclusion

58. On the basis of this study, it can be said that local drug therapy using herbal formulations when used as an adjunct markedly improves the benefits of scaling and root planing in chronic periodontitis patients. Further long term studies can be undertaken to evaluate the efficacy of catechin gel for improving the buffering capacity within periodontal tissues by elevating the GSH levels.

59. The present study opens up scope for various future studies dealing with role of glutathione

60. as a biological disease marker, antioxidant, detoxifier and immune modulator

61. References

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