Efficacy of green tea mouth wash as an adjunct to non-surgical periodontal treatment in patients with chronic periodontitis

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Background and objectives: Periodontal disease is the most common disease associated with microbial infection with the destruction of supporting structures. This study was carried out to evaluate the effectiveness of green tea as a mouthwash in the management of chronic periodontitis in comparison to chlorhexidine mouthwash.

Patients and methods: A randomized controlled trial on 45 healthy humans with mild to moderate localized chronic periodontitis. They were randomly assigned into 3 equal groups (15 patients each), and they all received scaling and root surface debridement treatments. Group A used prepared green tea 5% as mouth wash for 2 weeks, group B used chlorhexidine mouthwash 0.12% for 2 weeks and group C did not use any mouth wash. Intra-oral clinical examination and blood samples collection were done for all patients for estimating the biochemical parameters C-reactive protein, alkaline phosphatase, Malondialdehyde at baseline and 30 days after periodontal therapy. For statistical analysis, SPSS program was used.

Results: The study groups showed significant reduction in clinical periodontal parameters and inflammatory markers after 30 days of periodontal therapy (P<0.001). A significant difference was seen between group A and group B in relation to clinical periodontal parameters and inflammatory markers after periodontal therapy P<0.05, while the difference between group B and group C was non-significant in relation to gingival index, clinical attachment level, C-reactive protein, alkaline phosphatase, and Malondialdehyde.

Conclusion: Green tea as a mouthwash could be an appropriate and effective choice as an adjunctive measure in the treatment of chronic periodontitis.

Keywords: Green tea, chronic periodontitis, scaling and root surface debridement, inflammatory markers.

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Introduction

Periodontitis is defined as an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss.[1] Scaling and root surface debridement are effective means of treating and controlling periodontitis, however, the ability of the operator to gain access to deep pockets or furcations often results in a substantial variation in its effectiveness, this has led to the adjunctive use of antimicrobials, assuming that chemical aids would compensate for technical limitations and prevent early

microbial recolonization to ultimately ensure the best chance for clinical improvements through their application either systemically or locally.[2] Various chemical methods of reducing plaque, such as mouth rinses, are used, as they can provide significant benefits to patients who cannot maintain adequate mechanical plaque control.[3] Adjunctive use of chlorhexidine mouth rinse with mechanical scaling and root surface debridement resulted in slightly greater periodontal disease reduction than did scaling and root surface debridement alone.[4] Chlorhexidine has undesirable side effects, and one of the most common is staining of teeth.[5] Herbal mouthwashes are gaining popularity due to reduced side effects,[6] hence, there has been increased interest in plants with antibacterial and anti-inflammatory activities.[7] One of the numerous herbal extracts, Green tea (Camellia sinensis) has numerous medicinal benefits, mainly due to its antibacterial and antioxidant properties.[8] Green tea, which was produced from the leaves of the Camellia sinensis plant, is one of the most popular herbals worldwide.[9] It has a broad spectrum activity that could be a natural alternative for oral diseases medicines, through preventing reactive damage of cells, specifically, chronic periodontitis, dental caries, and oral cancer which are common among the population.[10] Shimada et al.[11] demonstrated that periodontitis contributed to increasing serum C-reactive protein (CRP) and there is a positive correlation of serum alkaline phosphatase (ALP) levels with clinical parameters including periodontal pocket and severity of gingival inflammation as reported by Khongkhunthian et al.,[12] also the result showed that malondialdehyde (MDA) is the most investigated lipid peroxidation product in periodontitis.[13] The present study aims to evaluate the effectiveness of prepared green tea as a herbal mouthwash with scaling and root surface debridement on clinical periodontal parameters (plaque index (PI), gingival index (GI), gingival bleeding index (GBI), probing pocket depth (PPD), and clinical attachment level (CAL)) and to assess the healing effect through measuring some biochemical parameters like ALP, CRP and MDA, and compare the results with commercially available chemical mouth wash chlorhexidine (CHX) in subjects with chronic periodontitis at the baseline before treatment and after 30 days of periodontal therapy.

Materials and methods

The setting of the study. This study was performed in Erbil city in the clinics of the Department of Periodontology at the College of Dentistry - Hawler Medical University and ARYO clinical laboratory. The Ethical Committee of the College of Dentistry approved the study protocol and all the patients provided a signed detailed informed consent.

Study samples. A prospective cohort study was conducted on 45 patients (27 males and 18 females), their ages range is 30 -55 years old. They have mild to moderate localized chronic periodontitis, their teeth required scaling and root surface debridement (SRD) (presence of chronic mild CAL of 1-2 mm, moderate CAL of 3-4 mm and PPD $\geq$ 4mm).[14] Those having systemic illness, antibiotic consumption during the last three months, smoking, known allergy to tea derivatives and concurrent medications with known effect on the periodontium (e.g. oral contraceptives, antibiotics, herbal medications) and orthodontic appliances were excluded. The participants were divided randomly into three equal main groups of 15 for each, and treated with scaling and root surface debridement. The first group A, used prepared green tea 5% as a mouthwash (10 ml, twice daily) for 2 weeks. The second group B, used chlorhexidine 0.12% mouthwash (10 ml, twice daily) for 2 weeks, and the third group C followed up without using mouthwashes. Rinsing was done for 1 minute after ½ hour of tooth brushing and refined from eating and drinking for at least ½ hour after mouth rinsing. They were also taught to floss and also to brush their teeth two times daily with the Bass method. All participants underwent a full-mouth periodontal examination in four points of the tooth (distobuccal/ labial, mesiobuccal /labial, midbucccal /labial and lingual/ palatal) at base line before treatment and after 30 days of periodontal therapy and the GI,[15] PI,[16] GBI,[17] PPD and CAL[18] were

recorded. Severity of the PPD and CAL were estimated (total PPD/CAL divided by affected surfaces).[19] The intervention was controlled by a single examiner who conducted the study. For the clinical evaluation, we used dental mirrors and a University of North Carolina (UNC) periodontal probes (15 mm) under ordinary light.

Blood sample collection. Five milliliters of the blood sample was drawn from all participants at baseline before treatment and after 30 days of periodontal treatment. Blood samples were centrifuged and the serum was kept into an Eppindrof tube labeled with a subject number, stored and frozen at -20 °C for later estimation of CRP, ALP, and MDA. Then all participants underwent a non-surgical periodontal treatment by using ultrasonic scalers and gracy curette and followed up after 30 days. Serum CRP and ALP were quantitatively determined using a specific kit for combat analyzer (Roche/Hitachi Cobas c systems) depending on a colorimetric method in accordance to the kit's instructions. Serum MDA was determined by enzyme-linked immunoabsorbent assay (ELISA) using commercial kit according to the manufactures instructions and DTX 880 (Beckman Coulter) spectrophotometrically at an optical density (OD) of 450 nm.

Preparation of green tea as a mouthwash. The raw material was first weighed on a sensitive balance, and then the green tea extract as a mouthwash was prepared according to Jenabian et al. method[20] in the laboratory of Pharmacology Department atHawler Medical University by an expert pharmacologist. The dried green tea was obtained from Erbil city market. Leaves of the plant were chopped, fragmented, and broken into small pieces, each 100g of leaves were soaked in 500 ml of methanol for 48 hours. Thereafter, the solution was passed through a strainer and was transferred to a plate. Plates were maintained in the normal temperature of the laboratory for 3-4 days, and then the crystal powder of the extract was scraped from the plates. Finally green tea mouthwash 5% was prepared (0.5 g of extract in 100ml distilled water) and poured into bottles each contains 240 ml.

Statistical analysis. Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows, version 20.0 (Armonk, NY: IBM Corp). Paired t-test was used to compare the readings before and after the intervention. One way analysis of variance (ANOVA) was used to compare the means of the three study groups. A post hoc test LSD was used to compare the means of each two groups. A *P*-value ≤0.05 was considered statistically significant.

Results

Periodontal parameters. For the forty-five patients that participated in the study the mean recordings for clinical periodontal parameters are seen in table 1. The results showed that there were reductions in the mean value of the GI, PI, GBI, PPD and CAL in all the groups from baseline to 30 days after periodontal therapy. The difference was statistically highly significant ($P<0.05$).

Table 2 presents the reduction in the mean score of clinical periodontal parameters in groups A, B, and C respectively after 30 days of periodontal therapy. Group A (green tea + SRP) showed greater improvement than group B (CHX + SRD) and group C (SRD) in relation to clinical periodontal parameters with *P*-value <0.001. There was a highly statistical difference ($P<0.001$) between groups A and B after 30 days of periodontal therapy. The same difference was seen between groups A and C ($P<0.001$). While the differences in results between groups B and C after 30 days of periodontal therapy showed a highly statistically significant in relation to PPD and GBI ($P<0.001$ and $P<0.002$ respectively), with a statistically significant difference in PI ($P=0.039$), and no statistical differences for the GI and CAL.

Biochemical parameters. Table 3 shows comparisons of the mean values of serum ALP, CRP and MDA, in the three groups A. The results showed that there was reduction in the mean values of serum ALP, CRP and MDA scores in group A, B and C from baseline to 30 days after periodontal therapy. The differences in the mean values statistically highly significant ($P<0.001$).

Table 1: Comparison of clinical periodontal parameters PI, GI, GBI, PPD, and CAL at baseline and 30 days after periodontal therapy in group A, group B and group C, n = 15.

Parameters/Groups		Baseline	After 30 days	*p*-value
		Mean ± SD	Mean ± SD	
PI	Group A	2.41 ± 0.17	1.24 ± 0.16	<0.001
	Group B	2.09 ± 0.11	1.49 ± 0.15	<0.001
	Group C	1.97 ± 0.39	1.51 ± 0.24	<0.001
GI	Group A	2.35 ± 0.19	1.20 ± 0.09	<0.001
	Group B	1.89 ± 0.33	1.47 ± 0.13	<0.001
	Group C	2.17 ± 0.11	1.66 ± 0.13	<0.001
GBI %	Group A	82.5 ± 3.49	22.0 ± 4.36	<0.001
	Group B	74.67 ± 11.89	46.33 ± 10.69	<0.001
	Group C	83.20 ± 3.00	62.53 ± 7.53	<0.001
PDD mm	Group A	4.84 ± 0.17	3.81 ± 0.16	<0.001
	Group B	4.69 ± 0.16	4.06 ± 0.11	<0.001
	Group C	4.57 ± 0.21	4.12 ± 0.14	<0.001
CAL mm	Group A	1.45 ± 0.18	1.09 ± 0.10	<0.001
	Group B	1.27 ± 0.06	1.13 ± 0.05	<0.001
	Group C	1.30 ± 0.11	1.13 ± 0.07	<0.001

Table 2: Means of reduction of periodontal parameters between the three groups A, B and C, n = 15.

Parameter/ time	Groups	Mean ± SD	*P* (ANOVA)	LSD groups	*p*-value
PI Baseline – after 30 days	Group A	1.173 ± 0.167	<0.001	A x B	<0.001
	Group B	0.605 ± 0.118		A x C	<0.001
	Group C	0.461 ± 0.246		B x C	0.039
	Total	**0.746 ± 0.359**			
GI Baseline – after 30 days	Group A	1.144 ± 0.190	<0.001	A x B	<0.001
	Group B	0.423 ± 0.234		A x C	<0.001
	Group C	0.512 ± 0.093		B x C	0.187
	Total	**0.693 ± 0.370**			
GBI Baseline – after 30 days %	Group A	60.50 ± 5.622	<0.001	A x B	<0.001
	Group B	28.33 ± 4.923		A x C	<0.001
	Group C	20.66 ± 8.304		B x C	0.002
	Total	**36.500 ± 18.55**			
PPD Baseline – after 30 days mm	Group A	1.04 ± 0.14	<0.001	A x B	<0.001
	Group B	0.64 ± 0.14		A x C	<0.001
	Group C	0.45 ± 0.12		B x C	<0.001
	Total	**0.71 ± 0.28**			
CAL Baseline – after 30 days mm	Group A	0.355 ± 0.171	<0.001	A x B	<0.001
	Group B	0.139 ± 0.050		A x C	<0.001
	Group C	0.164 ± 0.063		B x C	0.542
	Total	**0.219 ± 0.145**			

Table 3: Comparison of biochemical parameters serum ALP, CRP and MDA at baseline and 30 days after periodontal therapy in the study groups, n = 15.

Parameters/ Groups		Baseline	After 30 days	*p*-value
		Mean ± SD	Mean ± SD	
ALP IU/L	Group A	86.40 ± 11.08	72.07± 13.16	<0.001
	Group B	84.87 ± 11.38	76.07±9.74	<0.001
	Group C	76.53 ± 17.46	68.40±16.08	<0.001
CRP ng/ml	Group A	3.43 ± 1.74	1.65±1.38	<0.001
	Group B	3.32 ± 3.44	2.58±3.23	<0.001
	Group C	3.37 ± 2.40	2.29±2.26	<0.001
MDA µmol/L	Group A	44.98 ± 17.65	27.03±15.26	<0.001
	Group B	45.55 ± 15.29	30.89±15.94	<0.001
	Group C	57.02 ± 20.36	47.34±18.97	<0.001

Evaluation of changes in serum ALP, CRP and MDA in groups A, B and C after 30 days of periodontal therapy was assessed by one-way ANOVA test as shown in table 4. The differences in the scores is statistically highly significant (P<0.001). The differences between groups A and B after 30 days of periodontal therapy, the results dedicated that the improvement in the result was were statistically highly significant differences in relation to serum ALP and CRP (P<0.001) and no significant difference in relation to MDA level. A similar effect of improvement was seen between groups A and C. While non-significant differences were seen between groups B and C in relation to serum ALP, CRP and MDA.

Table 4: Means of differences of biochemical parameters between the three groups A, B and C, n = 15.

Parameter/ time	Groups	Mean ± SD	*p* (ANOVA)	LSD groups	*p*-value
ALP Baseline – after 30 days IU/L	Group A	14.33 ± 5.936	0.003	A x B	0.005
	Group B	8.800 ± 4.931		A x C	0.002
	Group C	8.133 ± 4.454		B x C	0.724
	Total	**10.42 ± 5.758**			
CRP Baseline – after 30 days ng/ml	Group A	1.783 ± 1.177	0.002	A x B	<0.001
	Group B	0.744 ± 0.413		A x C	0.016
	Group C	1.083 ± 0.453		B x C	0.232
	Total	**1.204 ± 0.867**			
MDA Baseline – after 30 days µmol/L	Group A	17.95 ± 7.069	0.011	A x B	0.217
	Group B	14.65 ± 7.792		A x C	0.003
	Group C	9.679 ± 6.691		B x C	0.065
	Total	**14.09 ± 7.829**			

Discussion

In chronic periodontitis, it has been thought that maintaining adequate levels of oral hygiene using mechanical methods alone is impossible, and this has led to the adjunctive use of antimicrobial mouth rinses in oral hygiene regimens to help prevent and control chronic periodontitis.[21] In the present study, we have focused on the effectiveness of green tea as a mouthwash on periodontium as an adjunct to mechanical periodontal therapy in the management of chronic periodontitis in comparison to chlorhexidine mouthwash. Bacterial biofilm development in the marginal gingiva and periodontal pockets is important in the pathogenesis of the periodontal disease. Scaling and root surface debridement are effective in altering the flora and the green tea catechins have shown to be effective in altering the flora and acting as an adjunct to scaling and root surface debridement.[22] In the present study, the clinical periodontal parameters showed statistically significant reductions following green tea mouthwash use as a conjunct oral hygiene measure in group A as compared to group C, this was in accordance with the study done by Sharma et al..[23] The reduced inflammation may be due to the property of green tea catechins that enhances the host resistance and inhibits the inflammatory mediators involved in the onset of periodontitis.[24] A similar mechanism might be involved in the effect of the combination of CHX rinses and scaling and root surface debridement leading to a reduction in clinical periodontal parameters as PI, GI, GBI, PDD and CAL compared with scaling and root surface debridement only in the treatment of chronic periodontitis.[25] The reduction could be attributed to the beneficial effect of CHX rinsing on subgingival microbial recolonization.[26] The plaque inhibitory effects of green tea were greater than that of CHX, this may be attributed to the biological properties ascribed to the catechins fraction which have certain therapeutic and biological properties that intercalate into phospholipid bilayers and perturb the function of key processes associated with the bacterial cytoplasmic membrane.[27]

Many studies have proved positive association between the presence of chronic periodontitis and high serum CRP levels.[28,29] In the present study, serum level of CRP was significantly reduced in group A, group B and group C from baseline to 30 days after scaling and root surface debridement with the reduction of periodontal clinical parameters, this result is in the same line with other studies.[30,31] In contrast to our result, Ide et al.[32] failed to observe a reduction in serum CRP after scaling and root surface debridement. This may be attributed to that scaling and root surface debridement are insufficient to control periodontal disease progression in all periodontitis subjects. The gain of improvement was more in group A, this result was in accordance with the study done by Hirasawa et al.,[33] who showed that green tea catechins decrease inflammation of adjacent gingival tissue and inactivate *P. gingivalis* – induced collagenase. The study groups showed a statistically significant reduction in serum ALP after 30 days of scaling and root planing compared to baseline values. This finding is in agreement with a study indicated that ALP levels in gingival crevicular fluid increased significantly during the active phase of disease of chronic periodontitis followed by a statistically significant reduction after phase I therapy.[34] The gain of improvement of ALP was more in group A, according to a study conducted by Kushiyama and Shimazaki.[35] Green tea has an inhibitory effect on the growth and cellular adherence of bacterial pathogens and their production of virulence factors. The scaling and root surface debridement has shown an improvement of serum MDA level after 30 days in all groups compared to their respective baseline values. Similar effects of improvement and a significant reduction in MDA level after scaling and root surface debridement have been reported by studies Wei et al.,[36] Aziz et al.,[37] and Tomofuji et al.[38] The gain of improvement in MDA level in group A and B was non-significant, due to the green tea catechins that increase in the activity of superoxide dismutase and glutathione peroxidase enzyme, this lead to reduction in lipid peroxidation markers in the liver, serum, and brain.[39] Similarly, in group B, attributed to the anti-inflammatory

antimicrobial effect of CHX which prevent the recolonization of putative pathogenic bacteria by creating a bacteriostatic mileu.[40] More studies are needed to study the efficacy of green tea through exploring their bioactive components as an adjunct with the standard treatment of periodontitis (scaling and root surface debridement). Longitudinal studies of the relationship between continued use of green tea as a mouthwash in periodontal disease are required to strengthen the interrelation.

Conclusions

It can be concluded from the results of the present study that the treatment by green tea mouthwash with scaling and root surface debridement for a period of 2 weeks could improve clinical periodontal parameters and the inflammatory markers compared to the scaling and root surface debridement with chlorhexidine gluconate and scaling and root surface debridement alone and could be used on a daily basis as an alternative for chlorhexidine gluconate as an anti-plaque agent.

Conflicts of interest

The authors reported no conflict of interest.

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