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The effect of oolong tea as an adjunct to nonsurgical management of chronic periodontitis: A randomized controlled clinical trial

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ABSTRACT

Introduction: Oolong tea, a functional food, has numerous therapeutic benefits owing to the presence of bioactive polyphenols, theasinensins (TS) and catechins. The present study aimed to evaluate the influence of systemic administration of oolong tea as an adjunct to nonsurgical periodontal therapy (NSPT) in the management of chronic periodontitis (CP).
Methods: A total of 60 subjects with mild to moderate CP were randomly divided into two groups of tests (n = 30) and the controls (n = 30). They underwent NSPT with adjunctive oolong tea supplementation in the test group only. At baseline, 1, and 3 months, their gingival index (GI), plaque index (PI), probing pocket depth (PPD), clinical attachment loss (CAL), percentage of sites with bleeding on probing (BOP), and lobene stain index (LSI) were recorded. Furthermore, the levels of glutathione peroxidase (GPx), total antioxidants (TAO), and malondialdehyde (MDA) were also estimated in gingival crevicular fluid (GCF), saliva and serum. Additionally, colony-forming units (CFUs) of selective supra and subgingival plaque bacteria were estimated in the plaque samples.
Results: In both groups, at 1 month, the GI, PI, BOP, GPx, and TAO levels were improved with a reduction in the levels of MDA and CFU's and no staining of teeth ($P < 0.05$). The results were maintained in the test group at 3-month recall visit.
Conclusion: Adjunctive administration of oolong tea with NSPT reduced the local and systemic oxidative burden and rapidly resolved the inflammation in CP. This would be specifically beneficial in CP subjects with systemic conditions.

Implication for health policy/practice/research/medical education:
Oolong tea, a functional food, contains bioactive polyphenols like theasinensins and catechins that have potent antioxidant, anti-inflammatory, and antimicrobial properties. They may promote the rapid resolution of oxidative stress and inflammation in chronic periodontitis (CP) and enhance the treatment outcomes of routine nonsurgical periodontal therapy (NSPT).
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Introduction

Chronic periodontitis (CP), a multifactorial disease of complex etiology, affects about 11% of the world population (1). It is characterized by gingival bleeding, periodontal pocket formation, and alveolar bone destruction resulting in tooth loss. The disease is initiated by a consortium of dental plaque bacteria, which stimulate an abnormal host response in the body. These bacteria are engulfed by polymorphonuclear leucocytes via phagocytosis. These cells produce reactive oxygen species (ROS), like superoxides, hydrogen peroxide (H_2O_2), and hydroxyl anions (2). An overwhelming production of ROS causes oxidative stress, which oxidizes the DNA, lipids, and proteins, resulting in tissue damage. Increased levels of these metabolites have been localized in both periodontal and systemic environments and have been correlated with

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the severity of periodontal destruction. Their entry into blood circulation causes systemic oxidative stress, which is significant in the oro-systemic relationship of periodontal diseases (2). Therefore, treatment of periodontal diseases may help in reducing the local and systemic oxidative stress, which may improve the overall condition of the patients (3). The traditional nonsurgical periodontal therapy (NSPT) may be beneficial in this regard. However, adjunctive administration of dietary components like functional foods may enhance the outcomes of NSPT (4). Functional foods are healthful foods or food ingredients with potential health benefits beyond their nutrient content when consumed regularly in typical quantities as part of a varied diet (5). These include foods with active components like carotenoids, lycopene, or polyphenols.

Tea (*Camellia sinensis*) is a functional food owing to the presence of about 4000 chemicals with health-promoting properties (6-8). It is the second most popular drink globally after water (6). Based on the degree of fermentation during processing, it can be divided into three major types: unfermented (e.g., green tea), partially fermented (e.g., oolong tea), and fully fermented black tea (6). Among the three types, oolong tea is chiefly consumed in Taiwan and China (6). Its health benefits are mainly related to the polyphenols that comprise about 30% of the total dry weight of the fresh tea leaves. The polyphenols in oolong tea are monomeric catechins like epigallocatechin-3-gallate (EGCG), epigallocatechin, epicatechin-3-gallate, epicatechin, and catechin. Additionally, oxidized polymeric forms of catechins known as theasinensins (TS), theaflavins, thearubigins, and natural amino acids are present. These components together produce potent anti-oxidative, anti-inflammatory, antibacterial, anticancer, and anti-obesity effects (6). Oolong tea has potent anti-inflammatory effects when compared to green and black teas, owing to the presence of TS and EGCG (6). Its daily consumption may reduce the risks of head and neck cancer, as well as cardiovascular and hepatic diseases (9). The latter effect was seen in mice with alcohol liver injury where oolong tea extract significantly reduced the serum levels of aspartate aminotransferase, alanine aminotransferase, and malondialdehyde (MDA) (9). It restored the activities of liver superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Even though oolong tea has numerous health benefits, its oral health effects have rarely been investigated. Recently, its effectiveness against oral bacteria like *Streptococcus mutans* and *Porphyromonas gingivalis* was reported (10, 11). Owing to these beneficial properties, its role in CP is worth exploring. Therefore, the present study aimed to evaluate the influence of systemic administration of oolong tea as an adjunct to NSPT on GPx, total antioxidants (TOA), and MDA levels in gingival crevicular fluid (GCF), saliva, and serum, as well as its effect on clinical periodontal parameters and selective dental plaque bacteria in patients with CP.

Materials and Methods

Study design

This randomized, controlled, single-centre, parallel-arm clinical trial was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki and was approved by the institutional ethics committee (IEC 34/2017). The scope of the study with possible benefits and harms were explained to all the participants, and written informed consents were obtained from them. Further, the trial was registered with the clinical trial registry of India (CTRI/2017/02/007938). The study was done for a period of fifteen months, from March 2017 to June 2018.

Participants

About 169 subjects, who came to the department of periodontology for treatment, were screened for the presence of CP. Only systemically healthy subjects (aged 20 to 60 years) with generalized mild to moderate CP were recruited (Figure 1). The inclusion criteria were CP subjects with a minimum of 20 teeth with probing pocket depth (PPD) (≥4 mm) and clinical attachment loss (CAL) of ≤4 mm on at least two interproximal sites. The subjects on antibiotics, anti-inflammatory medications, mouthwashes, high polyphenol diet (e.g., green tea), herbal or similar products in the past six months or with orthodontic or prosthodontic appliances or having oral abusive habits such as smoking, alcohol intake, betel nut chewing, or any other form of tobacco intake or pregnant, lactating mothers or any form of periodontal treatment in the last six months were not included in the study.

Determination of the sample size and randomization

The sample size was calculated based on the results of a previous study assuming a statistical power of 80% and the confidence interval of 95% (12). The standard deviation (SD) was set at 0.128 and the mean difference at 0.1. On the basis of these factors, the estimated sample size was 26 in each group (total 52). However, to prevent any potential fallouts, a total of 60 subjects (30 in each group) were included. They were randomly assigned to one of the treatment groups using a coin toss method with the heads included in the test (n=30) and the tails in the control group (n=30).

Intervention

The oolong tea used in this study was commercially available Himalayan oolong tea (Udyan tea, Siliguri, West Bengal, India; FSSL 12813006001028; Grade-SFTGFOP1; Family- Theaceae and species- *C sinensis*). About 2 g of tea leaves (sealed in one-time use pre-weighed sachets) were steeped in 300 mL of hot water (90 to 95°C) for 5 minutes (13). The subjects were asked to bring the water to a boil and then cool it down for about 2 minutes so that the ideal temperature was reached. They were advised to refrain from additives such as milk, sugar, and honey and not to consume any other type of tea, coffee, or beverages during

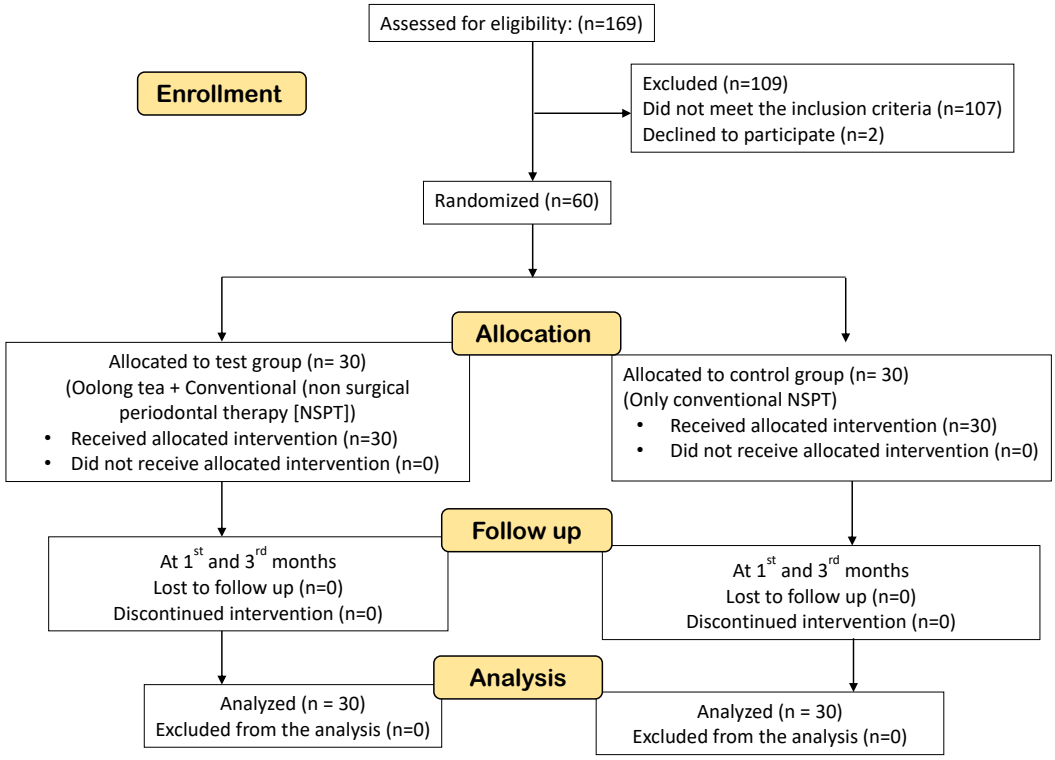

Figure 1. Consort flowchart of the study. NSPT: Nonsurgical periodontal therapy.

the duration of the study.

The test group (n = 30) underwent NSPT and drank oolong tea, while the controls (n = 30) underwent NSPT only. The NSPT included supragingival scaling followed by subgingival scaling and root planning in the areas with CAL. The test subjects drank two cups of oolong tea per day (once in the morning and once in at the night, 1 hour after the meals) i.e., 4 g tea per day for 30 days. They were asked to swish the tea for 1 minute in the mouth before swallowing. They were reminded for the intake of tea every day through messaging and phone calls.

At baseline, clinical periodontal parameters including gingival index (GI), plaque index (PI), PPD, CAL, percentage sites with bleeding on probing (BOP), and lobene stain index (LSI) were recorded (14). The biochemical parameters (GPx, TAO, and MDA) were recorded in serum, saliva, and GCF. The microbiological parameters were recorded in supragingival dental plaque, which was collected using a Gracey curette and transferred to thioglycolate transport medium immediately. Later, they were subjected to serial dilutions and incubated at 37°C for 48 hours. The blood samples were collected in a vacutainer and centrifuged to separate the serum, which was stored at -70°C until further estimation.

All the participants underwent supragingival scaling prior to GCF collection to reduce the plaque scores to 0 or 1. The site (PPD of ≥4 mm) was isolated with cotton rolls, and GCF was collected with Whatman- No.1 filter paper strips. The samples were transferred to vials containing 0.5 mL of phosphate buffer saline and refrigerated at -70°C until further assay. Whole unstimulated saliva samples were collected between 9 AM to 12 PM to avoid diurnal variations. The subjects were advised to refrain from eating or drinking 2 hours prior to sample collection. The samples were centrifuged for biochemical analysis.

Estimation of GPx, TOA, and MDA

The GPx was assessed by the continuous spectrophotometric rate determination, utilizing EDTA, sodium azide, and glutathione reductase (15). The optical density of the solution was measured at 340 nm for 1 minute, at an interval of 5 minutes. The TOA was measured by ferric reducing antioxidant power (FRAP assay), a redox linked colorimetric method wherein the samples were mixed with 3 mL of working FRAP reagent and absorbance measured at 593 nm (16). The levels of MDA were estimated by thiobarbituric acid (TBA) reaction where the samples were centrifuged at 13 000 rpm for 30 minutes resulting in a pink coloured complex. It was measured at 532 nm using a spectrophotometer (17).

Estimation of colony forming units (CFUs) of dental plaque bacteria

The CFUs of supragingival plaque bacteria like *Staphylococcus aureus*, *S. mutans,* and selective subgingival

plaque bacteria like *P. gingivalis*, *Prevotella intermedia, Fusobacterium nucleatum, Streptococcus salivarius,* and *Streptococcus sanguis* were evaluated. The total number of CFUs were then converted to CFU/mL using the formula:

CFU/mL = (no. of colonies × dilution factor)/volume of culture plate (18).

Follow-up evaluation

The levels of antioxidants GPx, TAO, and MDA, CF's, and the clinical periodontal parameters were reassessed at 1 and 3 months.

Statistical analysis

Descriptive and analytical statistics were done, and the normality of the data was analyzed by the Shapiro-Wilks test. As the data did not follow normal distribution, non-parametric tests like Mann-Whitney U and Wilcoxon Signed Rank tests were used to evaluate the differences in the means. The SPSS (Statistical Package for Social Sciences) version 20.1 (IBM Corporation, Chicago, USA) was used for analysis. The results were considered statistically significant if $P \leq 0.05$.

Results

Among the 60 subjects recruited for the study, 28 were male and 32 were female subjects with a mean age of 41.50±10.58 years and 41.60±7.80 years in the test and control groups, respectively ($P > 0.05$). The oolong tea was acceptable to all the test group participants, and they did not report any discomfort with its daily intake.

At baseline

Intergroup comparisons using Mann-Whitney U test revealed that the mean levels of clinical periodontal, biochemical, and microbiological parameters were not significantly different ($P > 0.05$) (Table 1). The exceptions to this were slightly higher serum GPx levels and slightly lower MDA levels in the test group ($P < 0.05$). Besides, this group had lower CFUs ($P < 0.05$).

At one month

Intragroup comparisons using Wilcoxon Signed rank test showed a significant increase in the mean levels of GPx and TOA and reduction in the MDA levels in GCF, saliva, and serum, and CFUs in both groups (Table 2). The mean levels of all the clinical periodontal parameters were reduced significantly ($P < 0.001$) (Table 3). However, intergroup comparisons using the Mann-Whitney U test showed that in the test group, there was a significant increase in the levels of GPx in the GCF only while the TOA increased in all three fluids. Likewise, the MDA levels and CFUs were significantly reduced in this group ($P < 0.001$) (Table 4). The mean GI levels were significantly reduced in the tests ($P = 0.049$) (Table 4).

At three months

Intra group comparisons using Wilcoxon Signed rank test showed a significant increase in the mean levels of GPx and TOA and a decrease in the MDA levels in both the groups (Table 2). However, when compared to baseline and one month levels, the changes were more significant in the tests ($P < 0.001$). Furthermore, the reduction in

Table 1. Intergroup comparisons of clinical periodontal, biochemical, and microbiological parameters at baseline

Parameter		Test (n=30) Mean ± SD	Control (n=30) Mean ± SD	*P* value[a]
GI		2.07±0.33	2.06±0.26	0.976
PI		1.91±0.45	1.88±0.44	0.739
BOP (% sites)		83.41±21.08	89.58±18.79	0.170
PPD (mm)		6.13±0.73	6.13±0.89	0.900
CAL (mm)		3.76±0.56	3.76±0.67	0.589
LSI		1.00±0.00	1.00±0.00	1.000
TAO (µM)	GCF	2.06±0.90	2.13±0.98	0.877
	Saliva	2.40±0.93	2.60±0.93	0.496
	Serum	9.14±1.50	9.54±1.66	0.264
GPx (units/mL)	GCF	0.99±0.44	0.88±0.33	0.564
	Saliva	1.27±0.41	1.13±0.31	0.277
	Serum	1.30±0.35	1.08±0.29	0.032*
MDA (nmol/mL)	GCF	42.73±11.08	46.36±10.49	0.084
	Saliva	43.50±10.43	46.30±8.72	0.078
	Serum	43.10±9.77	47.70±7.29	0.004*
CFUs		2.32±0.25	2.49±0.28	0.024*

Abbreviations: GI, Gingival index; PI, Plaque index; BOP, Bleeding on probing; PPD, Probing pocket depth; CAL, Clinical attachment loss; LSI, Lobene stain index; TAO, Total antioxidants; GCF, Gingival crevicular fluid; GPx, Glutathione peroxidase; MDA, Malondialdehyde; CFUs, Colony forming units
[a]Mann-Whitney U test; *Significant at $P < 0.05$.

Table 2. Intragroup comparisons of biochemical and microbiological parameters at baseline, 1 and 3 months follow up

Parameter	Sample of estimation	Baseline Mean± SD	Baseline v/s 1 month	Baseline v/s 1 month	*P* value[a]		
					Baseline v/s 1 month	Baseline v/s 3 months	1 month v/s 3 months
Group = Test							
TAO (μM)	GCF	2.06± 0.90	17.76± 2.34	21.61± 1.97	<0.001*	<0.001*	<0.001*
	Saliva	2.40±0.93	19.23±2.15	24.59±1.86			
	Serum	9.14±1.50	20.82±1.50	27.53±1.20			
GPx (units/mL)	GCF	0.99±0.44	2.22±0.71	3.29±0.68	<0.001*	<0.001*	<0.001*
	Saliva	1.27±0.41	2.69±0.71	4.07±0.54			
	Serum	1.30±0.35	3.01±0.62	4.21±0.53			
MDA (nmol/mL)	GCF	42.73±11.0	38.46±10.4	34.66±10.1	<0.001*	<0.001*	<0.001*
	Saliva	43.50±10.4	39.73±10.4	36.66±10.5			
	Serum	43.10±9.77	39.46±9.84	36.96±9.9			
CFUs	Plaque	2.32±0.25	0.91±0.13	1.04±0.11	<0.001†	<0.001†	<0.001†
Group = Controls							
TAO (μM)	GCF	2.13±0.98	14.53±1.73	15.35±2.21	<0.001*	<0.001*	<0.001*
	Saliva	2.60±0.93	16.23±1.66	17.58±2.41			
	Serum	9.54±1.66	16.79±3.33	18.84±2.44			
GPx (units/mL)	GCF	0.88±0.33	1.51±0.53	2.64±0.33	<0.001*	<0.001*	<0.001*
	Saliva	1.13±0.31	2.57±0.30	2.76±0.28			
	Serum	1.08±0.29	2.79±0.26	3.00±0.23			
MDA (nmol/mL)	GCF	46.36±10.5	42.76±11.4	40.23±11.3	<0.001*	<0.001*	<0.001*
	Saliva	46.30±8.72	43.50±8.72	41.56±8.75			
	Serum	47.70±7.3	45.23±7.3	43.60±7.4			
CFUs	Plaque	2.49±0.28	1.36±0.32	1.53±0.33	<0.001*	<0.001*	<0.001*

Abbreviations: TAO, Total antioxidants; GCF, Gingival crevicular fluid; GPx, Glutathione peroxidase; MDA, Malondialdehyde; CFUs, Colony forming units.
[a]Wilcoxon signed rank test; *Significant at $P < 0.05$.

Table 3. Intragroup comparisons of clinical periodontal parameters at baseline, 1 month and 3 months follow up

Parameter	Group = Test			*P* value[a]		
	Baseline Mean ± SD	1 month Mean ± SD	3 months Mean ± SD	Baseline v/s 1 month	Baseline v/s 3 months	1 month v/s 3 months
GI	2.07±0.33	1.40±0.19	1.24±0.20	<0.001*	<0.001*	<0.001*
PI	1.91±0.45	1.06±0.22	1.04±0.14			0.049*
BOP (% sites)	83.41±21.08	38.13±12.25	32.86±11.12			<0.001*
PPD (mm)	6.13±0.73	4.73±0.90	4.36±0.85			
CAL (mm)	3.76±0.56	2.43±0.67	2.06±0.73			
LSI	1.00±0.00	0.00±0.00	0.00±0.00			
Group = Controls						
GI	2.06±0.26	1.47±0.20	1.61±0.23	<0.001*	<0.001*	<0.001*
PI	1.88±0.44	1.08 ± 0.29	1.19±0.27			<0.002*
BOP (% sites)	89.58±18.79	41.71±13.02	45.97±13.61			<0.001*
PPD (mm)	6.13±0.89	4.63±1.09	4.63±1.29			<0.001*
CAL (mm)	3.76±0.67	2.26±1.11	2.30±1.11			0.763
LSI	1.00±0.00	0.00±0.00	0.00±0.00			<0.001*

Abbreviations: GI, Gingival index; PI, Plaque index; BOP, Bleeding on probing; PPD, Probing pocket depth; CAL, Clinical attachment loss; LSI, Lobene stain index;
[a]Wilcoxon signed rank test; *Significant at $P < 0.05$.

the levels of all the clinical periodontal parameters was maintained within the test group while they deteriorated in the controls ($P < 0.001$) (Table 3). The mean LSI levels were maintained at 0 in both groups ($P < 0.001$). The intergroup comparisons using the Mann-Whitney U test showed a significant increase in the levels of GPX and TAO and a reduction in the levels of MDA, GI, PI, BOP, and CFUs in the tests ($P < 0.05$) (Table 4).

Table 4. Intergroup comparisons of clinical periodontal, biochemical and microbiological parameters at 1 and 3 months follow up

Parameter		Intergroup comparison at 1 month			Intergroup comparison at 3 months		
		Test (n = 30) Mean ± SD	Control (n = 30) Mean ± SD	*P* value[a]	Test (n = 30) Mean ± SD	Control (n = 30) Mean ± SD	*P* value[a]
GI		1.40±0.19	1.47±0.20	0.049*	1.24 ± 0.20	1.61±0.23	<0.001*
PI		1.06±0.22	1.08±0.29	0.866	1.04 ± 0.14	1.19 ±0.27	0.045*
BOP (% sites)		38.13±12.25	41.71±13.02	0.204	32.86 ± 11.12	45.97 ±13.61	<0.001*
PPD (mm)		4.73±0.90	4.63±1.09	0.769	4.36±0.85	4.63 ±1.29	0.220
CAL (mm)		2.43±0.67	2.26±1.11	0.239	2.06±0.73	2.30 ±1.11	0.548
LSI		0.00±0.00	0.00±0.00	1.000	0.00±0.00	0.00±0.00	1.000
TAO (µM)	GCF	17.76±2.34	14.53±1.73	<0.001*	21.61 ± 1.97	15.35 ± 2.21	<0.001*
	Saliva	19.23±2.15	16.23±1.66	<0.001*	24.59 ± 1.86	17.58 ± 2.41	<0.001*
	Serum	20.82±1.50	16.79±3.33	<0.001*	27.53 ± 1.20	18.84 ± 2.44	<0.001*
GPx (units/mL)	GCF	2.22±0.71	1.51±0.53	<0.001*	3.29 ± 0.68	2.64 ± 0.33	<0.001*
	Saliva	2.69±0.71	2.57±0.30	0.252	4.07 ± 0.54	2.76 ± 0.28	<0.001*
	Serum	3.01±0.62	2.79±0.26	0.074	4.21 ± 0.53	3.00 ± 0.23	<0.001*
MDA (nmol/mL)	GCF	38.46±10.40	42.76±11.43	0.065	34.66 ± 10.12	40.23 ± 11.30	<0.019*
	Saliva	39.73±10.37	43.50±8.72	0.030*	36.66 ± 10.50	41.56 ± 8.75	0.013*
	Serum	39.46±9.84	45.23±7.32	<0.001*	36.96 ± 9.90	43.60 ± 7.38	<0.001*
CFU		0.91±0.13	1.36±0.32	<0.001*	1.04 ± 0.11	1.53 ± 0.33	<0.001

Abbreviations: GI, Gingival index; PI, Plaque index; BOP, Bleeding on probing; PPD, Probing pocket depth; CAL, Clinical attachment loss; LSI, Lobene stain index; TAO, Total antioxidants; GCF, Gingival crevicular fluid; GPx, Glutathione peroxidase; MDA, Malondialdehyde; CFUs, Colony forming units
[a]Mann-Whitney U test; *Significant at $P < 0.05$.

Discussion

The results from the present study suggest that systemic administration of oolong tea as an adjunct to NSPT in subjects with CP is superior to NSPT alone. It produced potent antioxidant, anti-inflammatory, and antimicrobial effects (Figure 2). There was an increase in the levels of GPx and TOA and a reduction in the MDA levels in the localized periodontal and systemic environments. The CFUs of selected periodontopathogens were also reduced. These subsequently improved the clinical periodontal parameters, which were more evident in the test group.

As already stated, neutrophils, the first line of defence against bacterial infection in CP, accumulate in the periodontal tissues and gingival sulcus. They produce ROS via the metabolic pathway of *"respiratory burst"* (2). This process is catalysed by the nicotinamide adenine dinucleotide phosphate oxidase during phagocytosis resulting in the production of H_2O_2, hypochlorous acid, hydroxyl radical, and singlet oxygen in the phagosomal and extracellular environment (2). It has been reported that CP subjects have phenotypically hyperactive peripheral blood neutrophils, which produce more ROS, compared to healthy individuals (19,20). Under normal physiological conditions, there is a balance between ROS and antioxidants (2). However, excessive production of ROS or inadequate levels of antioxidants may cause oxidative stress. The antioxidants may be enzymes like GPx, SOD, catalase, and glutathione reductase or chain-breaking molecules like polyphenols (flavonoids) (2).

In the present study, low levels of GPx and TOA and increased levels of MDA were seen in both groups at baseline. This was in accordance with other studies that correlated low enzymatic antioxidants and increased MDA levels in human gingival tissues with increased PPD (3). Furthermore, studies have reported that NSPT enhances the levels of salivary GPx and TOA in CP (3). This was seen in the present study in both groups after NSPT. Studies have shown that although NSPT reduces FcγR-stimulated ROS, it has marginal or no effect on unstimulated extracellular ROS production (21). This hyperactivity of neutrophils is higher in CP and has been correlated to both reactive and constitutional mechanisms, including genetics (21, 22). Furthermore, enhanced production of ROS from pathogen stimulated monocytes, gingival fibroblasts and periodontal ligament cells adds to the ROS burden (2). These ROS are neutralized by the antioxidants like GPx and TOA which may improve periodontal health (4). However, NSPT alone may be insufficient in this regard (21). This was evident in the present study whereby the levels of antioxidants, clinical periodontal, and microbiological parameters improved at one month in both groups after NSPT, but were not sustained in the controls at three months. Therefore, adjunctive application of antioxidants and their enhancers like functional foods may be advocated to improve periodontal health (4).

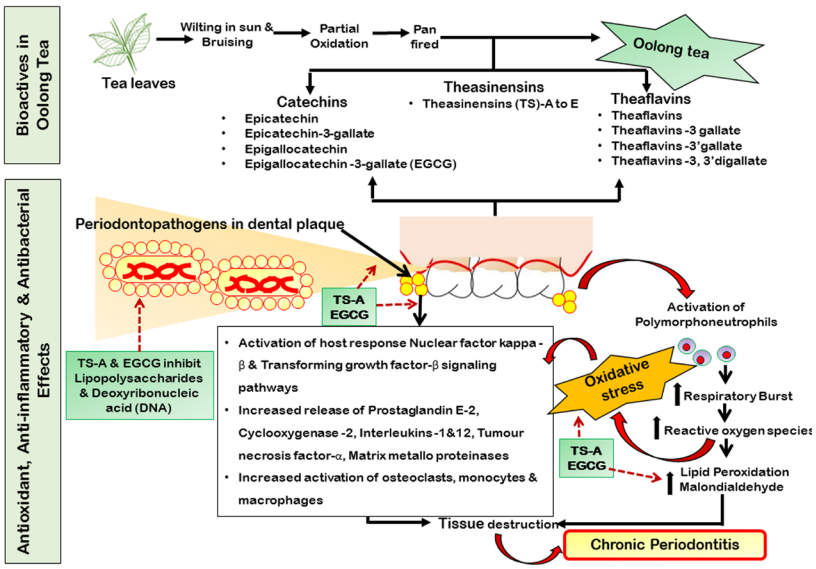

Figure 2. Bioactives in oolong tea and their roles in periodontal health.

Functional foods like oolong tea restore the levels of antioxidants in both local and systemic environments. This tea has a high concentration of EGCG and TS, specifically TS-C, which increases the levels of GPx and decreases the MDA level. It inhibits lipid peroxidation, matrix-metalloproteinases (MMP-9), osteoclast synthesis, and interleukin-1β production, as were revealed in both animal and human studies (6,9,12). In the present study also adjunctive administration of oolong tea significantly improved the GPx and TOA and reduced the MDA levels in both local (GCF and saliva) and systemic environment (serum). However, the increase was more in the serum as compared to GCF and saliva, which may be due to its systemic administration. Furthermore, the mean levels of all clinical parameters improved significantly in test group and were stable over a three-month period. Similar results were reported in a Japanese study where the PPD and CAL were inversely correlated with the intake of green tea. An increment in intake by one cup/day, improved the parameters significantly (23). However, there is no consensus on the dosage of green or oolong teas for periodontal health. The recommendations vary from less than 1 cup to 3-4 cups/day (12,23,24). Furthermore, excessive consumption of teas may elevate bone loss due to the effects of caffeine. In the present study, the test subjects ingested 2 g of oolong tea in 300 mL of hot water in one serving per day. Studies have shown that this may result in total caffeine and polyphenol concentrations of 23.5 mg and 99.32 mg, respectively, per 100 mL of tea consumed, which was slightly more than green tea (13,25,26). As there are no direct studies relating oolong tea consumption with periodontal health, twice daily administration was advised to ensure minimum caffeine exposure and patient compliance.

Oolong tea has strong local and systemic anti-inflammatory effects; the former is attributable to the tannins, the latter is related to the galloyl moiety of TS-A. The TS-A suppresses lipopolysaccharide induced cyclooxygenase-2 and prostaglandin-2 production in a dose-dependent manner. It down-regulates the transforming growth factor-β activated kinase and the nuclear factor kappa-β signalling pathways (27). Furthermore, it attenuates the gene expression of inflammatory mediators induced by lipopolysaccharides (28). In the present study, at three months, significant reduction in the levels of both GI and BOP was seen. This could be attributed to the above-mentioned anti-inflammatory effects. Moreover, as the tea was swished in the oral cavity for one minute and swallowed, the astringent effect of tannins could have reduced the bleeding (29).

The PI reduced significantly in the tests when compared to the controls, at 1 and 3 months. It was correlated with the reduced CFUs of the bacteria and could be related to the antibacterial action of EGCG in oolong tea. The EGCG causes leakage of bacterial intracellular components through the generation of H_2O_2 in the bilayer. Additionally, it reduces the adherence of periodontopathogen like *P. gingivalis* to the oral epithelial cells. It even inhibits the collagenase activity at a concentration of 10 mg/mL. Further, its monomeric polyphenols bind to the bacterial cell surface proteins and decrease their adherence to the tooth surface (11,30). It inhibits glucan synthesis by cell-associated glucosyltransferase and decreases the adherence of bacteria like *S mutans* at a concentration of 0.5 mg/mL (31).

Tooth staining is regarded as a major drawback of tea consumption. It occurs due to the deposition of tannins on the tooth surface. The salivary proteins increase the binding of tannins to the hydroxyapatite resulting in tooth

staining (32,33). As oolong tea contains tannins, which are intermediate between green and black teas, it may produce extrinsic staining. However, it was not visible in our study sample. This may increase its acceptability if used for therapeutic purposes.

Conclusion

The present study reaffirms that oolong tea is a functional food with potent antioxidant, anti-inflammatory, and antimicrobial effects. These properties significantly reduced the local and systemic oxidative stress, along with inflammation, in the CP subjects. These findings are vital in the management of CP subjects with systemic conditions, where treatment of local conditions reduces the systemic oxidative stress and inflammatory burden.

Authors' contributions

SN: Collection and/or assembly of data, data analysis, interpretation, and manuscript writing. RA: Conception and experimental design, data analysis, interpretation, and manuscript writing and final approval of the manuscript. SUK: Data analysis and interpretation. PAS: Data analysis and interpretation. NAK: Data analysis and interpretation. DDN: Data analysis and interpretation. All read the final version and confirmed its publication.

Conflict of interests

Authors declare no conflict of interests.

Ethical considerations

The study was approved by the institutional ethics committee (Ethical code: IEC 34/2017). The trial was also registered with the Clinical trial registry of India (CTRI/2017/02/007938).

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