Research Article

The clinical and immunological efficacy of a mouth rinse containing green tea and *Salvadora Persica L.* in patients with dental biofilm-induced gingivitis (randomized clinical trial)

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Abstract: Background: Dental biofilm-induced gingivitis is an inflammatory condition resulting from the interaction between dental plaque and the host's immune-inflammatory response at the site level. Objective: examine the anti-plaque and anti-inflammatory properties of GT/SP (Green Tea/Salvadora Persica Linn) mouthwash in individuals with dental biofilm-induced gingivitis. Moreover, a study was conducted to compare the effects of GT/SP with those of CHX (Chlorhexidine) and placebo mouthwashes, and to anticipate outcomes of plaque control together with proposing cut-off points for the predictors on the profile of the salivary biomarker IL-6. Material and Methods: The clinical trial was conducted using a randomized, double-blinded, and parallel design. A total of sixty patients diagnosed with generalized biofilm-induced gingivitis were randomly divided into three groups for the administration of mouthwashes: GT/SP, CHX, and placebo. The study lasted for one month, during which participants used 15 milliliters of GT/SP mouthwash formulation, CHX (0.12%), or a placebo, twice daily. Each individual had two assessments, one at the beginning and another after one month. Before and after each participant's mouthwash usage, the Patient's plaque level and bleeding on probing were assessed. The quantification of the Salivary IL-6 in saliva was assayed using enzyme-linked immunoassay (ELISA). Results: A significant decrease in salivary interleukin-6 was observed in all three groups. However, there was no significant difference between the GT/SP and CHX groups in intergroup comparisons at second visits (P>0.05). In addition, all treatments significantly decreased the build-up of Plaque, with the mouthwash containing Green Tea (GT) and Salvadora Persica Linn (SP) having a more pronounced effect compared to the other two treatments. Conclusion: Mouthwash with GT/SP exhibited both anti-plaque and anti-inflammatory properties. The above findings suggest that GT/SP may have positive effects in treating dental biofilm-induced gingivitis, making GT/SP a suitable alternative to chlorhexidine.

Keywords: Salvadora, Green tea, Interleukin-6, Gingivitis, Dental plaque.

Introduction

Dental biofilm-induced gingivitis is an inflammatory condition resulting from the interaction between dental plaque and the host's immune-inflammatory response at the site level. The inflammation

is restricted to the gingiva and is not distributed to the underlying supporting periodontal tissues [1, 2]. In a clinical context, the gingival tissues exhibit certain characteristics such as swelling, redness, discomfort, a glossy appearance, and bleeding upon gentle probing [3]. If left untreated during its initial stages, this condition has the capacity to escalate into more severe forms of periodontal disease [4]. Cytokines are small proteins that are released in response to an antigen and act as chemical messengers to regulate the innate and adaptive immune systems. The synthesis and secretion of cytokines are induced by the activation of cytokine-producing cells [5, 6].

Interleukin-6 (IL-6), is a pleiotropic cytokine that acts as a pro-inflammatory and anti-inflammatory activity [7]. Interleukin-6 (IL-6) is a cytokine that exhibits diverse physiological effects, such as the differentiation and activation of macrophages and T cells, the growth and differentiation of B cells, the stimulation of hematopoiesis, the differentiation of osteoclasts, and the resorption of bone [8]. IL-6 has been found in elevated levels in gingival crevicular fluid (GCF) and saliva. It has been associated with disease extent/severity and has been shown to decrease with successful therapy [9]. The mouthwash containing chlorhexidine (CHX) has recently become the most powerful chemotherapeutic drug and the standard for decreasing *S. mutates* and oral biofilm [10]. Nevertheless, the utilization of this substance may result in various adverse effects, such as dry mouth, decreased taste sensation, and tongue discoloration. Long-term use may also lead to the formation of calculus and staining on the teeth. Whereas less frequent side effects include enlargement of the parotid gland, oral paranesthesia, glossodynia, hypersensitivity, burning sensation, and desquamation of the oral mucosa. However, tooth discoloration continues to be the principal adverse effect that prevents people from using chlorhexidine [11].

The development of alternative mouthwashes with comparable efficacy, but without these adverse effects is desirable. Traditional medicinal plants may possess bioactive properties that can improve oral health. The present study aimed to examine the efficacy of a mouth rinse solution comprised of green tea (GT) and Salvadora Persica Linn (SP). The anti-bacterial effects of green tea (Gt) extract from Camellia sinensis var. assamica (family: Theaceae) against bacterial species found in dental plaque have been documented [12]. Furthermore, the root extracts of *Salvadora persica L.*, a member of the Salvadoraceae family, have been extensively studied for their antibacterial characteristics in preventing dental plaque [13]. The study found that individuals who use chewing sticks have lower levels of primary plaque colonizers such as *Streptococcus mitis*, *S. sanguinis*, *S. oralis*, and *S. salivarius* in their saliva compared to those who use conventional toothbrushes [14]. The aim of the study is to evaluate the anti-plaque and anti-inflammatory efficacy of mouthwash with a combination of green tea and *Salvadora persica L.* aqueous extracts over a period of one month among individuals diagnosed with dental biofilm-induced gingivitis and to juxtapose these results with those obtained from CHX and a placebo solution.

Material and Methods

Study design

This study was a randomized, parallel double-blinded three-arm control clinical trial. The trial was conducted from January 2024 to June 2024. The participants were recruited at the clinics of the periodontic Department, College of Dentistry, University of Baghdad. The study protocol was approved by the ethical committee of the College of Dentistry at the University of Baghdad. (Ref. 857623; Dec. 3, 2023). The study followed the Consolidation Standards of Reporting Trials (CONSORT) and was registered at (https://clinicaltrials.gov). "US National Institute of Health Registry" in 2024 under Identifier number (NCT06211309).

Study Subjects

The participants were recruited from the clinics of the periodontic Department at the College of Dentistry, University of Baghdad. Eligible participants were invited to take part in this study based on meeting the inclusion and exclusion criteria. The study's inclusion criteria encompassed individuals who were in good systemic health, did not smoke, possessed over 20 natural teeth, and had been diagnosed with dental biofilm-induced generalized gingivitis. This diagnosis was characterized by the presence of over 30% bleeding sites with no PPD greater than 3 mm, an intact periodontium, and no loss of periodontal attachment[3]. The exclusion criteria consisted of individuals who had (1) active cavity caries and/or periodontal disease, (2) ongoing orthodontic treatment, (3) a history of antibiotics within the past 3 months, (4) a need for prophylactic antibiotic coverage, (5) a requirement for systemic and/or topical non-steroidal anti-inflammatory drugs for the past 4 months, (6) pregnancy or intention to become pregnant, (7) lactating mothers, (8) known intolerance or allergy to mouthwashes, and (9) any systemic disease. Written informed consents were obtained from all eligible participants prior to the start of the trial.

Sample size

The sample size was determined based on a pilot study involving five patients from each group. The data obtained from the use of different mouthwashes as an adjunct was analyzed to determine the required number of patients for each group. The primary outcome of the present study was the reduction in plaque index to establish good gingival health. Using G*Power software, a sample size of 15 patients in each group was estimated to reject the study's null hypothesis at a probability of 0.05 and a power of 0.80. Furthermore, to avoid a possible dropout of patients during follow-up, which was further increased to include 20 patients. A total of 60 subjects who were free from any systemic health conditions were divided into three groups. Group I: The patient uses combination mouthwash (GT/SP) with manual tooth brushing. Group II: The patient uses 0.12% CHX mouthwash with manual toothbrushing. Group III: The patient uses a Placebo mouthwash with manual tooth brushing.

Calibration

The validity and repeatability of the clinical periodontal measure, specifically the plaque index, were evaluated through intra- and inter-examiner assessments. The examiner measured periodontal parameters on two occasions, with a 2-hour gap, for a total of five subjects. The threshold for agreement on all clinical parameters was set at a minimum value of 0.75. The Kappa value for the parameter in this research was 0.91.

Study Interventions

The three-arm control clinical trial involves the use of different mouth rinses. These include a 0.12% CHX mouthwash (KIN Gingival, KIN, Barcelona, Spain) as a positive control, distilled water with food additives as a placebo (negative control), and a combination mouthwash containing green tea and Salvadora Persica as the test intervention [15,16]. The participants were randomly divided into three groups according to intervention intake. The mouthwashes are fully described in Table 1.

Table 1: Description of interventions(15).

Interventions	Positive control	Test formulation	Negative control
Ingredients & Concentration	0.12%Chlorhexidin gluconate (w/v) (Active ingredient)	Combination of Camellia sinensis var.assamica (0.25mg) +Salvadora persica L. (7.82mg) extracts/1ml distilled water.	Distilled water with food additives
Dosage/ Regimen	15ml twice daily /rinse for 1min / refrain from eating or drinking for 30min	15ml twice daily /rinse for 1min / refrain from eating or drinking for 30min	15ml twice daily /rinse for1min / refrain from eating or drinking for 30min
Duration	1 month	1 month	1month
Color	Pink	Light yellow	Blue
Preparation	Used commercial oral rinse (KIN ™ Gingival)	Prepared in chemical engineering Research Laboratory, University of Technology, Baghdad, Iraq.	Commercially available distilled water (deionized water)

Plant extracts

The complete details regarding the leafs of Camellia sinensis var. assamica (family: Theaceae) and the roots of *Salvadora persica L.* (family: Salvadoraceae), including their source, preparation of aqueous extracts, and formulation for combination, are discussed in [16].

Randomization & blindness

The examiner performed block randomization to enroll patients into three groups. Each group was assigned a letter (A, B, or C) corresponding to the intervention used. A random number generator was used to randomly assign the order of the groups and participants. They were subsequently assigned to specific groups using a 1:1:1 allocation method, with an equal distribution of subjects. The allocation procedure was expedited using a random table in Microsoft Excel. Therefore, each participant had a fair chance to be assigned to the sequence of interventions. In order to ensure blindness in the study, the mouthwash was placed in identical bottles that were not transparent. These bottles will be assigned random letter codes (A, B, and C) by a third party who is not involved in the clinical study. This ensured that both the patient and the examiner were double-blind throughout the clinical experiment. The codes were revealed at the end of the study, during data analysis.

Saliva samples collection and preparation

The participants were asked to refrain from eating, drinking, or performing oral hygiene procedures for at least two hours prior to saliva collection. Then, they were asked to rinse their mouths with water for 30 seconds approximately 10 min prior to saliva collection. Each participant was positioned in an upright and comfortable posture. Then, by tilting their head forward, they let the saliva accumulate on the bottom of their mouth. This enabled the collection of the entire combination of unstimulated mixed saliva. After that dropping, his/her saliva was placed into the graduated sterile plastic collection tube without any stimulation or spitting for 5 minutes. The volume of saliva collected per subject was approximately 2ml,

and a standardized passive saliva drooling method was used to collect the whole saliva. The saliva was aspirated using a micropipette and transferred into a plastic test tube. The subject's number was written on the tube's label, corresponding to the number previously written on the case sheet. Then, the samples were stored in a compact cooling box. The salivary supernatants were separated from the cellular debris by centrifuging the samples at 4000 rpm for 15 minutes. The salivary fluid was aspirated and transferred into a clean and labeled Eppendorf tube after centrifugation. The sample was subsequently frozen at -20 degrees Celsius until the day of analysis [17,18].

Clinical measurements

Plaque Index (PLI)

The amount of accumulated dental plaque on teeth was measured using the modified Quigley Hein plaque index (PLI) [19]. The plaque accumulation detected by the disclosing Solution (Bioclear™ Dual Colour Disclosing Solution) are able to stain bacterial biofilm on teeth, gingiva, and tongue, enabling researchers to identify the production of plaque. The examiner prepared the participants' teeth by drying them and applying a disclosing solution using a brush applicator. The plaque biofilm was then stained and scored using the Modified Quigley-Hein Plaque Index (MQHI) on a scale of 0 to 5, based on the amount of dental plaque present. After that the participants were instructed to rinse their mouth with water to remove any disclosing remnants. The distance from the gingival margin to the edge of the disclosed area was measured using a calibrated periodontal probe, with measurements recorded to the nearest 0.5 mm on a PLI record form for each participant. The participants' PLI mean was calculated by dividing the total scores by the number of surfaces examined.

Bleeding on Probing (BOP)

This index was used to quantify bleeding on probing, which was done by carefully inserting the periodontal probe into the gingival sulcus/pocket until minimum resistance was detected [20]. An evaluation was conducted at six distinct surfaces (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) on each tooth. If bleeding occurred within 15-30 seconds of probing at a site, it was assigned a score of (1). Conversely, a site with no bleeding received a score of (0).

Probing depth (PPD)

The measurement of PPD was conducted solely at the initial visit to establish a diagnosis. The measurement was taken from the gingival border to the sulcus base or pocket.

Study visits

Baseline visit:

At baseline, demographic data was collected for the selected subjects that included the subject’s name, age, and sex. In addition, the complete medical history of the subject and the history of the previous periodontal treatment were recorded. Then, an unstimulated saliva sample was collected for IL-6 quantification. After that, recording of clinical periodontal parameters (PLI, BOP, and PPD). Furthermore, all the participants received oral hygiene motivation and instructions (OHI), moreover underwent scaling and polishing, or merely polishing using an ultrasonic scaler, rubber cup, and pumice. The examiner performed all procedures while being blinded to the intervention administered to each patient.

After that, one of three interventions was given to the participants. The participants were instructed to rinse with 15 ml of the assigned mouth rinse solution twice a day for 1 minute each time, 30 minutes after brushing their teeth. They were additionally instructed not to eat or drink anything for 30 minutes after rinsing. For standardization, all participants used the same toothbrush with medium-hardness bristles (COLGATE®) and fluoridated toothpaste (COLGATE®, Colgate-Palmolive, NY, USA) provided by the examiner.

Furthermore, after each week, a third party summoned all groups to return the empty bottle for evaluation of subject compliance with mouthwash usage. Subsequently, a new 225ml bottle of mouthwash (GT/SP, CHX, Placebo) was provided to each group. In addition, all participants received oral hygiene instructions and a repetition of the mouthwash usage method. Additionally, an intra-oral examination was conducted to assess any potential side effects of the mouthwashes.

Second visit (one month following the baseline visit)

A salivary sample was collected from each participant. Then, the clinical periodontal parameters (PLI and BOP) were recorded again as in the baseline visit. The mouthwash bottles were collected, and the remaining amount was measured to assess adherence to mouth rinse. At the end of the trial, all participants received a questionnaire using a visual analog scale (VAS) designed to assess their opinions about the product they had used.

Statistical analysis

Both inferential and descriptive statistical procedures were conducted using Statistical Package for Social Science (SPSS version 26) (Chicago, USA, Illinois), and GraphPad Prism (version 9) software. A p-value less than 0.05 was set as the threshold for considering significant differences in the results. Continuous data was described using mean and standard deviation, while categorical data was given frequency and percentage. At first, the Shapiro–Wilk test verified data distribution. Parametric data was analyzed using One Way Analysis of Variance (ANOVA), a statistical test for the difference between independent groups using Tukey HSD posthoc test while non-parametric data was analyzed using Kruskal Wallis and Dunn-Bonferroni post hoc tests. The effect size was determined using G Power. The effect sizes were categorized into three ranges: small (0-0.499), medium (0.5-0.799), and large (≥ 0.8).

Categorical variables were analyzed using the Chi-square test (a statistical test used to compare observed results with expected results). Cohen's kappa test is used to measure inter and intra reliability for qualitative variables. Receiver Operating Characteristic (ROC) curve analysis and area under curve(AUC) calculation were conducted to assess the model's accuracy in predicting plaque control results and to identify optimal cut-off points for the predictors.

Results

A double-blind, randomized parallel trial was conducted to assess the effects of different interventions on plaque accumulation and gingival inflammation. The study measured the mean plaque index (PLI), Bleeding on probing (BOP) and the change in interleukin-6 (IL-6) levels after one month of rinsing. Seventy participants were examined for their eligibility and sixty participants were included in the current trial. Five participants were lost to follow-up from each group for different reasons; fifteen participants in each group have completed the study.

Demographic characteristics

The statistical test for analyzing the age parameter was detailed in Table 2. The CHX group had the highest mean value, followed by the placebo group, and the GT/SP group had the lowest mean value, as shown in the table below. These findings revealed no statistically significant difference among groups. The Sex distribution was also illustrated in Table 2 as follows: In the GT/SP group, (53.3%) were males and (46.7%) were females. In the CHX group, (46.7%) were males, and (53.3%) were females. While, in the placebo group, (40%) were males, and (60%) were females. These findings revealed no statistically significant difference among groups.

Table 2: Demographic data of participants.

Characteristic	GT\SP Group (N=15)	0.12%CHX Group (N=15)	Placebo Group (N=15)	Overall (N=45)	P value
Age (years)					
Mean (standard deviation)	24.80 (3.44)	26.93 (3.15)	25.00 (4.03)	25.58 (3.61)	0.385 ns
Sex					
Female: N. (%)	7 (46.7)	8 (53.3)	9 (60)	24 (53.3)	0.778 ns
Male: N. (%)	8 (53.3)	7 (46.7)	6 (40)	21 (46.7)	

Comparison of age was done by ANOVA; Comparison of gender was done by chi-square; GT/SP: green tea/Salvadora persica, CHX: chlorhexidine, level of significance ≤ 0.05**;** ns: non-significant; N: number; %: percentage.

Plaque index

The mean values of PLI of the study groups were presented in Table 3. Concerning the baseline data, the comparison of the PLI mean values among the three groups did not exhibit any statistically significant difference ($p > 0.27$). On the 30th day of the study, there were a significant drop in the mean PLI values in all groups. The mean PLI values were 0.81, 0.74, and 1.22 for GT/SP, CHX, and Placebo respectively. The difference among the groups were statistically significant. Additionally, the same table showed the mean change of PLI among study groups, The higher mean change of PLI in the GT/SP mouthwash group then the CHX group and lastly Placebo group with a significant difference. While the highest effect size was in the CHX group (2.433), followed by the GT/SP group (2.116), and the placebo group (0.710) had the lowest effect size.

As demonstrated below in Table 4, multiple pair-wise comparisons between groups for PLI mean values with achieved effect size and power at the second visit. A significant difference was observed between the Placebo and CHX groups. Also, a significant difference was found between Placebo and GT/SP groups. While a non-significant difference was observed between the CHX and GT/SP groups. The mean PLI of the GT/SP group was significantly lower than that of the placebo group ($p<0.005$, effect size = 1.117, power = 0.909, $\alpha = 0.05$). The mean PLI of the CHX group was significantly lower than that of the placebo group ($p<0.001$), with an effect size of 1.272 and achieved power of 0.960. There was no statistically significant difference in the mean plaque index (PLI) between the chlorhexidine (CHX) and the (GT/SP) groups.

Bleeding on probing

Mean values of the BOP of the studied groups were presented in Table 3. Concerning the baseline data the comparison of the mean percentage of BOP among the three groups exhibits a statistically significant difference ($p < 0.029$). On the 30th day of the study, there were significant drop in the mean BOP% values in all study groups. The mean percentage of BOP values were 22.06, 17.80, and 36.80 for GT/SP, CHX, and Placebo groups respectively. The difference among the groups were statistically significant. The intra-group comparison showed a significance difference in each group. In addition, the same table revealed the mean change of BOP at study groups, The highest mean change of BOP in the GT/SP group then in the CHX, and lastly in the Placebo group with no significant difference. Also, the GT/SP group had the highest effect size (2.990), followed by the CHX group (2.329), and the placebo group (1.189) had the lowest effect size.

As demonstrated below in Table 4, multiple pair-wise comparisons between groups for the mean percentage of BOP values at the second visit regarding the mean BOP of the GT/SP group was significantly lower than the mean BOP of placebo ($p<0.001$) with an achieved effect size of 1.168 and power of 0.929. Interestingly, the mean BOP of the CHX was significantly lower than that of the placebo group ($p<0.001$) with an achieved effect size of 1.674 and power of 0. 997. Furthermore, there was no significant difference between the mean BOP of the CHX and GT/SP groups.

Interleukins -6

The level of IL-6 in saliva after using the study mouthwashes (GT\SP, CHX, and Placebo) was shown in Table (3).In the current study at the baseline visit, the comparison of the IL-6 concentration mean values among the three groups exhibited statistically significant differences. On the 30th day of the study, there was a significant drop in the mean values of IL-6 concentration in all study groups, the mean values of IL-6 concentration (pg/mL) were 1.41,1.79 and 4.57 for GT/SP, CHX, and Placebo respectively. These differences among the groups were statistically significant. Similarly, in the groups comparison, the mean change of IL-6 level shows a statistically significant difference associated with a higher reduction in the GT\SP group with the highest effect size (4.245) than CHX and placebo groups, which had the lowest effect size. Furthermore, the salivary biomarker has shown excellent ability to predict the outcome of plaque control using different mouthwashes. As well as between periodontal health and disease as presented by the Roc curve in Figure 1 with a cutoff value of 3.629 ng/ml.

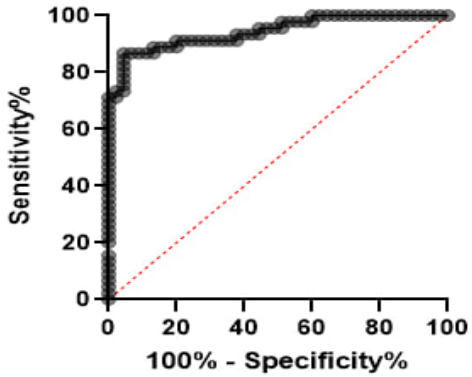

Figure 1: ROC curve predicting the outcome of plaque control and differentiating generalized gingivitis from clinically healthy gingiva at p-value <0.0001, the area under the curve 0.9432, and 95% confidence interval 0.8972 to 0.9893.

Table 3: Means and Means change of PLI, BOP, and IL-6 before and after using the study interventions.

	Study Group	Baseline N=15 Mean ±SD	1 month N=15 Mean±SD	P-value	Mean change Baseline - after 1 month	Effective size	P -value*
PLI	GT\SP group	1.84 ± 0.56	0.81 ± 0.24	0.000	1.03±0.37	2.116	
	CHX group	1.60 ± 0.40	0.74 ± 0.27	0.000	0.86±0.26	2.433	
	Placebo group	1.57 ± 0.52	1.22 ± 0.46	0.000	0.35±0.11	0.710	0.001
	p-value**	0.27	0.001				
BOP	GT\SP group	49.15±11.45	22.06±8.75	0.001	27.06±9.05	2.990	
	CHX group	44.66±10.56	17.80±3.98	0.001	26.86±11.53	2.329	
	Placebo group	59.40±20.64	36.80±15.55	0.001	22.60±19.01	1.189	0.612
	p-value**	0.029	0.001				
IL-6	GT\SP group	4.66±0.75	1.41±0.78	0.001	3.25±0.72	4.245	
	CHX group	4.42±1.43	1.79±0.86	0.001	2.62±1.66	2.109	
	Placebo group	5.61±1.41	4.57±1.14	0.001	1.03±0.95	0.802	0.001
	p-value**	0.030	0.001				

*Paired T-test; ** one-way ANOVA test; significant at p<0.05, not significant at p>0.05, SD: standard deviation

Table 4: Multiple pairwise comparisons of mean PLI, BOP%, and IL-6 between intervention groups with achieved effect size and power at the second visit.

	Intervention group comparisons	P value*	Achieved effect size	Achieved power at α error probability 0.05
PLI	Placebo VS. GT\SP	0.005	1.117	0.909
	Placebo VS. CHX	0.001	1.272	0.960
	GT\SP VS. CHX	0.868	0.274	0.180
BOP	Placebo VS. GT\SP	0.001	1.168	0.929
	Placebo VS. CHX	0.001	1.674	0.997
	GT\SP VS. CHX	0.515	0.626	0.511
IL-6	Placebo VS. GT\SP	0.001	3.585	1.000
	Placebo VS. CHX	0.001	1.976	0.999
	GT\SP VS. CHX	0.517	0.924	0.777

* Tukey HSD test, significant at p<0.05, not significant at p>0.05.

Discussion

The present study was conducted as a randomized controlled trial, which offers the most reliable and conclusive evidence regarding the effectiveness of medical interventions [21]. In addition, a properly designed and executed randomized controlled trial may successfully manage confounding factors and reduce bias. Therefore, it is capable of generating the most accurate estimate of the effects of the intervention [22]. Furthermore, the blindness procedure enhances the validity of the results and the study's strength. In addition, a block randomization method was developed to allocate subjects into groups, ensuring equal sample sizes and controlling for variability, resulting in enhanced accuracy. Moreover, a parallel trial study design was chosen for this research. The key strength of this design is that each participant received only one treatment throughout the study duration, which reduced the carryover effect and period effects [23].

The study found that using a mouthwash containing a combination of 0.25 mg/ml Gt and 7.82 mg/ml Sp aqueous extracts (referred to as the "test" mouthwash) resulted in a substantial reduction in plaque accumulation compared to both the CHX mouthwash and the placebo mouthwash after a period of 1 month. The formulated mouthwash was found to effectively reduce salivary concentrations of IL-6 in individuals with dental biofilm-induced gingivitis. In a previous in vitro study, it was found that this specific combination showed similar antibacterial effects as 12% CHX against primary plaque colonizers, namely *S. mitis, S. sanguinis, and A. viscosus*. The bacteria's adherence to saliva-covered glass beads, which mimic tooth surfaces, was significantly reduced. When comparing the anti-adherence impact of the combination with CHX, there was a decrease in the number of primary colonizers that adhered, although this decrease was not statistically significant [16]. The initial attachment of primary colonizers to oral surfaces plays a critical role in creating new binding sites for the subsequent attachment of secondary bacterial colonizers. This process promotes the development of dental plaque [24].

Thus, the antiplaque activity of the combination mouthwash might be explained by the reduction in the primary plaque colonizers, therefore reducing the binding sites available for the secondary plaque colonizers and resulting in retardation of the plaque formation. A recent study found that individuals who used *Salvadora persica L.* chewing sticks had lower salivary levels of primary plaque colonizers compared to those who used toothbrushes. This finding has been attributed to the chemical constituents of the *Salvadora persica L.* Several anionic components have been identified in *Salvadora persica L.* and thought to have potent effects on salivary peroxidase thiocyanate and hydrogen peroxide antimicrobial system [14]. Furthermore, studies have shown that tannins can hinder the activity of salivary α-amylase and interact with salivary histatin and proline-rich proteins [25].

Both of these actives have been researched separately in vitro in addition to in vivo models, and their features are well recognized. It was also found that green tea polyphenols have antimicrobial activity against a number of oral microorganisms including *S.sanguis, S. sobrinus, S. mitis, and S. salivarius* [12]. A separate study discovered that a concentration that ranged from 1 to 4 mg/ml of tea polyphenols effectively suppressed the initial attachment of *S. mutans, A. viscosus*, and *Lactobacillus* to an experimental pellicle (C-HA) [26]. The observed results can be explained by the polyphenols' capacity to bind to the acquired pellicle, alter its composition, and consequently disrupt the bacteria's ability to attach to the tooth surface [27]. Green tea exhibits antioxidant effects on the reactive oxygen species component, which plays an essential role in periodontal destruction [28].

Salvadora persica L. and green tea were reported to have some constituents that exhibited beneficial intraoral effects. The compound EGCG contained in green tea was discovered to hinder the production of insoluble glucan in the mouth by inhibiting the activity of glucosyltransferase enzyme in *S. mutans* bacteria [29]. It was

postulated that EGCG had the ability to inhibit GTF genes in *S. mutans* at the transcriptional level, hence disrupting the early adherence of *S. mutans* and impeding the formation of mature plaque [30]. Green tea catechins have been found to hinder the binding of *S. mutans* to saliva-coated hydroxyapatite discs by altering the structure of bacterial cells [26].

The presence of thiocyanate in the extract of *Salvadora persica L.* was found to enhance the antibacterial effectiveness of the salivary peroxidase-thiocyanate and hydrogen peroxidase system. This process takes place through the oxidation of thiocyanate, resulting in the production of hypothiocyanate. It is facilitated by salivary peroxidase in the presence of hydrogen peroxide, which is produced by oral bacteria and leukocytes. The oxidation of sulfhydryl groups in bacterial cytoplasmic membranes by the hypothiocyanate results in their breakdown [31].

Salvadora persica L. has been found to contain fluoride. Therefore, a mouthwash containing extracts of *Salvadora persica L.* can deliver fluoride topically to the oral cavity. Fluoride has been found to exhibit antibacterial effects through the inhibition of bacterial enzymes. Additionally, it can prevent the demineralization of tooth surfaces and promote their re-mineralization [32]. Previous clinical studies have examined the effectiveness of Gt and Sp extracts in reducing plaque formation separately. A study found that rinsing with 10 ml of an extract from *Salvadora persica L.* at a concentration of 100 mg/ml, three times a day, led to a notable decrease in dental plaque. Nevertheless, the reduction was smaller compared to the 0.2% CHX [33]. In previous research, it was found that using green tea extracts at concentrations of 50 and 250mg/ml resulted in a notable decrease in dental plaque. The reduction observed with both concentrations was similar to that achieved with a 0.12% CHX solution [34, 35]. A recent study examined the antiplaque efficacy of a combination of *Salvadora persica L.* and green tea. The findings of this study showed a substantial decrease in plaque buildup after 24 hours [15]. An additional study demonstrated that the act of gargling with a mixture of 0.25 mg/ml green tea and 7.82 mg/ml *Salvadora persica L.* aqueous extracts, in a quantity of 15 ml, twice a day, can effectively diminish the accumulation of dental plaque during a span of 4 days [36].

Regarding, the findings of CHX mouthwash, it exhibited a statistically significant reduction in the mean PLI, which is in accordance with previous studies that have investigated the impact of CHX mouthwash on reducing the mean PLI among individuals with dental biofilm-induced gingivitis [37, 38]. The study's significant result in PLI is due to the cationic properties of CHX molecules enabling them to adhere easily to oral surfaces, such as mucous membranes, teeth, and salivary glycoproteins, due to the negative charge of these surfaces. This interference with bacterial adhesion allows the CHX to remain effective for 12 hours [39].

The findings of our study indicated a statistically significant variance in the plaque index among all mouthwash treatments (GT/ SP, CHX, and Placebo) before and following one month of use. Nevertheless, the intergroup comparisons regarding the plaque index (PLI) during the second visit did not exhibit any significant disparity between the GT/SP and CHX groups ($P>0.05$). The results of our study indicated a significant reduction in PLI scores when using the GT+SP mouthwash in comparison to both the baseline data and the negative control.

The mean percentages of BOP significantly decreased in association with the GT/SP mouthwash compared with baseline data. The significant result of the study in terms of BOP% is explained by the synergistic effect of two active components (GT and SP), the green tea extract (GTE) possesses anti-inflammatory properties. The research has shown that extracts from Green tea have the ability to reduce the levels of pro-inflammatory cytokines and increase the levels of anti-inflammatory markers. This suggests that Green tea may have a beneficial effect on inflammation [40]. In addition, *Salvadora persica L.* demonstrates notable anti-

inflammatory and antioxidant properties. Two prominent tocopherols (-tocopherol and -tocopherol) were present in the SP seed. Both compounds demonstrated antioxidant properties equivalent to those of vitamin E, highlighting their significance for human health [41]. It also suppresses pro-inflammatory cytokines such as TNF-α, IFN, IL-1β, IL-6, and IL-8, and modifies the isoforms of nitric oxide synthase (NOS) [42], with a simultaneous release of α-Amylase enzyme promoting anti-inflammatory and antioxidative actions at the exact location of inflammation [43]. Consequently, the trigger effect of microorganisms on gingival tissue was diminished as a result of the additive effect of two active compounds in reducing the amount of dental biofilm that accumulated on teeth. This helps to restore the symbiotic microbiome and reduce associated inflammation. The second objective of our investigation was to assess a new composition including GT and SP in mitigating the salivary concentrations of IL-6 among individuals afflicted with dental biofilm-induced gingivitis caused by plaque accumulation. Numerous studies have indicated that tea polyphenols can potentially aid in the reduction of inflammation by scavenging free radicals and regulating cytokine cascades [44].

Furthermore, another study found that the secretion of IL-6 by macrophages stimulated with LPS was significantly inhibited at a concentration of 0.5 mg/mL, in comparison to the control [45]. On the other hand, a previously published study conducted by Ibrahim A.Y. et al. [46] showed that the Salvadora persica extracts have anti-inflammatory properties. The potential efficacy of *S. persica* extracts to treat inflammation is based in part on the hypothesis that it will suppress the proinflammatory cytokines.

The study's results indicated a statistically significant decrease in salivary IL-6 levels in the group that used GT/ SP mouthwash before and after use. The observed decrease in IL-6 concentrations may be attributed to the synergistic action of GT and SP, which are potent anti-inflammatory agents known to scavenge reactive free radicals and exhibit efficacy against both Gram-positive and Gram-negative bacterial strains. These factors are directly associated with the upregulation of cytokine levels [41, 47].

As this is a new substance, being the first to investigate the effectiveness of the combination of GT and SP on salivary cytokine levels, it is not feasible to make a direct comparison with previous studies. Nonetheless, there are further studies that have examined the impact of GT on IL-6 levels in a different kind of inflammation. Green tea has been shown to have a significant impact on Interleukin 6 (IL-6) levels in various studies. Research has demonstrated that green tea supplementation can lead to a decrease in IL-6 levels in obese women, indicating its potential to reduce inflammation [48].Additionally, a study on overweight middle-aged males found that green tea extract (GTE) supplementation in combination with endurance training resulted in increased irisin levels and decreased IL-6 concentrations, showcasing the anti-inflammatory effects of green tea [49]. However, a meta-analysis suggested that green tea might not significantly decrease serum IL-6 levels, indicating a need for further research in populations with higher inflammation levels to assess its effectiveness on inflammatory markers [44].

Overall, the evidence suggests that green tea can modulate IL-6 levels, highlighting its potential as a natural anti-inflammatory agent. Regarding SP as the other ingredient in the solution, the efficacy of SP on cytokines in the human body has been previously studied. *Salvadora persica L.*, commonly known for its medicinal properties, has been shown to influence the production of interleukin-6 (IL-6), a key cytokine involved in inflammation. Research indicates that extracts from *S. persica* can significantly reduce IL-6 levels in various experimental models. For instance, one study demonstrated that *S. persica* extract led to a marked decrease in IL-6 expression, suggesting its potential anti-inflammatory effects [50]. Another investigation corroborated these findings, revealing that the extract modulated inflammatory responses by downregulating IL-6, thereby highlighting its therapeutic potential in inflammatory diseases [51]. However, while the majority of studies support the anti-inflammatory role of *S. persica*, some limitations exist, such as variations in extraction methods and dosages, which may affect the consistency of IL-6 modulation [52].

Overall, *S. persica* appears to be a promising candidate for further research into its role in managing IL-6-related inflammatory conditions [53].

On the other hand, the present study's results indicated a statistically significant decrease in salivary IL-6 levels among participants who used CHX mouthwash before and after administration. The significant decrease of cytokines after using CHX mouthwash could be attributed to substantivity for 12h that prevents the growth and adhesion of oral microorganisms and subside inflammatory cytokines. Consequently, the elimination of the biofilm during periodontal therapy resulted in a decrease in the challenge and a reduction in the production of these cytokines. By inhibiting the interaction between different types of microorganisms in the subgingival biofilms and the surrounding periodontal tissues, it reduces gingival inflammation and the release of biologically active substances, such as cytokines and chemokines, into the gingival crevicular fluid and saliva [54].

This finding is consistent with prior studies on the impact of CHX on the levels of interleukin-6 (IL-6) in patients with gingivitis. Yoshida et al. discovered that periodontal therapy, which included dental hygiene instructions, conventional mechanical treatment, and 0.12%chlorhexidine used as an adjunct for 15 days, reduced salivary IL-6 in gingivitis patients [55].

Research indicates that the application of CHX leads to a reduction in IL-6 levels, which is a pro-inflammatory cytokine associated with periodontal disease. For instance, one study demonstrated that patients treated with CHX exhibited a marked decrease in IL-6 concentrations compared to baseline measurements, suggesting its efficacy in managing inflammation associated with dental biofilm-induced gingivitis [56]. Additionally, another study corroborated these findings, noting that CHX not only reduced gingival inflammation but also lowered IL-6 levels, thereby potentially improving overall periodontal health [57].Overall, the evidence supports the conclusion that CHX is effective in lowering IL-6 levels in dental biofilm-induced gingivitis patients, contributing to its role in managing periodontal inflammation [58].

In a comparison between GT/SP with CHX mouthwashes, the present findings revealed that GT/SP mouthwash had the highest effect in lowering IL-6 levels when compared to CHX mouthwash. Moreover, these results revealed that there was no statistically significant difference between GT/SP and CHX mouthwash in reducing IL-6 levels at one month.

In the placebo group, the present study's findings indicated a significant reduction in the clinical periodontal parameter (PLI) as well as biomarker (IL-6) after using the placebo mouthwash compared to the baseline data. The study's findings in the placebo group can be attributed to the patients' strong motivation for practicing proper oral hygiene. This phenomenon, known as the Hawthorne effect, is a type of human behavior reactivity in which individuals modify an aspect of their behavior in response to their awareness of being observed may have contributed to the improved results in this group; due to a higher awareness of oral health care [59]. In addition, the effect of mechanical plaque control (brushing), leads to the removal of plaque from the tooth surface and reduces signs of gingival inflammation [60].

Conclusion

The findings of this study indicate that using a combination of aqueous extracts of Gt and Sp, with a concentration of 0.25 mg/ml and 7.82 mg/ml respectively, can effectively decrease plaque accumulation when rinsed twice daily for a duration of one month. The mouthwash containing GT/SP showed an anti-inflammatory effect, indicating its potential for immunomodulation. These results suggest that GT/SP

mouthwash could be an acceptable replacement for chlorhexidine (CHX) for treating dental biofilm-induced gingivitis.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

MAA; study conception and design. AS; data collection. MAA and HAA.; Methodology. MAA and AS; statistical analysis and interpretation of results. AS; original draft manuscript preparation. AS; Writing & editing. Supervision; MAA and HAA. All authors reviewed the results and approved the final version of the manuscript to be published.

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الفعالية السريرية والمناعية لمضمضة فم تحتوي على الشاي الأخضر ونبات السواك لدى مرضى التهاب اللثة الناتج عن اللويحة السنية :(تجربة سريرية عشوائية).

علياء سعيد, مها عبد العزيز, حيدر الوائلي.

المستخلص:

يعد التهاب اللثة الناتج عن اللويحة السنية حالة التهابية تنتج عن التفاعل بين البلاك البكتيري واستجابة الجهاز المناعي الالتهابية في موقع اللثة .الهدف :دراسة الخصائص المضادة للالتهاب لغسول الفم الذي يحتوي على الشاي الأخضر ونبات السواك لدى الافراد المصابين بالتهاب اللثة الناتج عن اللويحة السنية بالاضافة الى ذلك ,أجريت هذه الدراسة لمقارنة تأثيرات هذا الغسول مع غسول الكلورهكسدين وغسول الفم الوهمي .كما هدفت هذه الدراسة الى التنبؤ بنتائج التحكم في اللويحة واقتراح نقاط فاصلة للمؤشرات التنبؤية استنادا الى نمط وجود الواسم الحيوي اللعابي الانترليوكين-6 .الطريقة :تم اجراء التجربة السريرية باستخدام تصميم عشوائي مزدوج التعمية ومتوازي. تم تقسيم 60 مريضاً تم تشخيصهم بالتهاب اللثة العام الناتج عن اللويحة السنية بشكل عشوائي الى ثلاث مجموعات لتلقي غسولات فم :الشاي الأخضر/السواك،الكلورهكسدين وغسول فم وهمي .استمرت هذه الدراسة لمدة شهر واحد ،استخدم خلالها المشاركون 15 مليلتراً من تركيبة غسول الفم الشاي الأخضر/ السواك او الكلورهكسدين بتركيز 0.12 او الغسول الوهمي بمعدل مرتين يوميا خضع كل مشارك لتقييمين ،احدهما في بداية الدراسة والأخر بعد شهر .تم تقييم مستوى اللويحة السنية ونزيف اللثة عند الفحص قبل وبعد استخدام غسول الفم لكل مشارك .كما تم قياس تركيز الوسيط الحيوي اللعابي الانترليوكين-6 في اللعاب باسخدام تقنية المقايسة المناعية المرتبطة بالانزيم .النتائج:لوحظ انخفاض كبير في مستوى الانترليوكين -6 في جميع المجموعات الثلاث .ومع ذلك ،لم يكن هناك فرق ذو دلالة إحصائية بين مجموعتي الشاي الأخضر/السواك والكلوهكسيدين في المقارنة بين المجموعات خلال الزيارة الثانية .بالاضافة الى ذلك أدى جميع أنواع الغسول الى تقليل تراكم اللويحة السنية بشكل ملحوظ ،حيث اضهر

غسول الفم الذي يحتوي على الشاي الأخضر/السواك تاثيراً اكثر وضوحا مقارنةً بالعلاجين الاخرين .الاستنتاج :أظهر غسول الفم المحتوي على الشاي الأخضر /السواك خصائص مضادة للويحة السنية ومضادة للالتهاب .وتشير هذه النتائج الى انه هذا الغسول قد يكون له تأثيرات إيجابية في علاج التهاب اللثة الناتج عن التهاب اللويحة السنية مما يجعله بديلاً مناسباً للكلورهكسيدين.