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Efficacy of olive leaf extract mouthwash on clinical and inflammatory parameters of gingival inflammation in relation to chlorhexidine in acute gingivitis serum patients

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Abstract

Background: Oral and gum infections are mostly linked to poor oral hygiene. Chlorhexidine mouthwash, and teeth brushing, have been commonly recommended. There is many studies on the effectiveness of olive leaf extract (OLE) as herbal mouthwash, there is much guided on its competitive effect with chlorhexidine.

Objective: To determine the clinical and inflammatory effects of olive leaf extract (OLE) as mouthwash in the treatment of acute gingivitis and compare its effect with 0.12% chlorhexidine mouthwash (CHX).

Patients and Methods: A total of 60 subjects aged 18-35 years with acute gingivitis underwent scaling and polishing with oral hygiene instruction; 20 patients received 3% of (OLE) as mouthwash, 20 patients received of 0.12% of chlorhexidine mouthwash (CHX), and 20 patients received scaling and polishing only (SRP) without any mouthwash. Mouthwash was twice daily for 17 days. At the start of the study, clinical periodontal parameters (PI, GI and BOP) and blood samples were taken and after five weeks of treatment to estimate the levels of C-reactive protein (CRP), alkaline phosphatase (ALP) and total protein (TP).

Results: After 5 weeks of periodontal treatment, there was a significant decrease in clinical periodontal parameters ($P<0.01$) and inflammatory markers ($P<0.05$) in the OLE and CHX and S&P alone groups. From periodontal treatment, there was no significant difference between the OLE and CHX groups with clinical periodontal parameters and inflammatory markers ($P<0.05$), although there was a significant difference between the OLE, CHX groups and the S&P alone group in terms of clinical periodontal parameters and inflammatory markers ($P<0.05$).

Conclusion: Olive leaf extract (OLE) has been showed to be comparable to CHX aided in reducing gingivitis by decreasing clinical periodontal parameters and inflammatory chemical markers values such as CRP, ALP, and TP.

Keywords: olive leaf extract (OLE, mouthwash, chlorhexidine, gingivitis, CRP, ALP, TP

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Introduction

Periodontal disease is an expression used to depiction an inflammatory disease of the dental supporting tissues produced by plaque [1]. Gingivitis is one form of periodontal disease in which the gingival tissues become inflamed but not distracted [2]. According to multiple scientific findings, microbial plaque is an important factor for gingivitis, and can be avoided by decreasing microbial plaques[3].Several methods for extracting microbial plaque have been suggestion, like mechanical and chemical approaches. Mechanical plaque removal does not look to be 100 percent successful in certain people depend on manual skill, according to clues. As a result, chemical plaque management approaches have obtained a lot of coverage [4].So it is a broad-spectrum antiseptic, chlorhexidine (CHX) is deemed the mostly commonly used adjunctive antiseptic in periodontal disease treatment and the “gold standard” factor for chemical plaque control methods[5]. Depending on the concentration, chlorhexidine is either bacteriostatic or bactericidal. However, despite its effectiveness in lowering levels of microorganisms in the oral cavity, long-term use of CHX mouthwash is associated with local side effects such as impaired taste, teeth and oral prosthesis staining, improved production of supragingival calculus, and sporadic inflammation and desquamation of oral mucous membranes[6].Natural component found in medicinal plants have been seen to be an important source of biologically active components, with many of them acting as the architecture for the manufacturing of new pharmaceutical chemicals [7] . Olive leaf extract is one of the world's most distributed used and widely known medicinal plants [7]. Several phenolic compounds are among the plant's main components (sesquiterpenes and primarily terpenoids).The flavonoids apigenin, quercetin, patuletin, and luteolin are also found in olive leaf extract [8]. Quercetin and apigenin have been mostly studied for their antioxidant and anti-inflammatory properties [9,10].When used twice a day for five weeks, an olive leaf extract mouthwash minimized plaque buildup and gingival inflammation while having non-significant effect on teeth staining[11].So periodontal disease determined is almost entirely based on conventional periodontal diagnostic indeces (plaque index, gingival index, and bleeding on probing), these traditional diagnostic techniques are mostly limited in that they can only determine disease history, not mostly disease status [12]. Advance diagnostic method aimed at providing tools for quantifying and justifying periodontal risk using quantitative criteria as these biomarkers used in this study.

CRP is an acute-phase reactant plasma protein formed in response to different inflammatory stimuli. It is a plasma protein made in acute-phase reactions in response to multiple inflammatory stimuli. It was used in gingivitis and periodontitis as an important biochemical marker to evaluate and determined the activities of periodontal disease [13]. Alkaline phosphatase (ALP) is a biochemical marker that has special property of being introduced in periodontal inflammation; also, it is an important biochemical marker that is characterized by its involvements in periodontal inflammation

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[14]. Total protein (TP) used as a potential biochemical marker for determining periodontal disease occurrence in gingivitis and periodontitis. It is used as a potential diagnostic biomarker for active disease status in periodontal tissues and to determine successful treatment and prevention strategies [15].

In literature review, research on the use of 3% olive leaf extract (OLE) as a mouthwash for the treatment of acute gingivitis has been done in Diyala Medical University. This is the first research to compare the efficacy of olive leaf extract mouthwash with 0.12 % chlorhexidine mouthwash (CHX) on both clinical and biochemical parameters after 5 weeks of periodontal treatment, including plaque index (PI), gingival index (GI), bleeding on poking (BOP), and serum C-reactive protein (CRP), serum alkaline phosphatase (ALP), and serum total protein (TP).

Patients and Methods

Setting of the study

The research was carried out at College of Medicine University of Diyala. The research took place between July 2020 and January 2021.

Study design

A comparative clinical research was carried out on 60 systemically healthy patient without any signs and symptoms of any systemic disease persons only with acute gingivitis, male and female, ranging in age from 18 to 35 years. All the patients met the following inclusion criteria: had more than 20 teeth, on the other hand, probing depth > 3 mm; use of anti-inflammatory medications; tobacco smoking; use of local or systemic antibiotic therapy or antibiotic prophylaxis in the previous month; pregnancy; use of anticoagulant drugs (due to the use of coumarin in olive leaf extract); and orthodontic appliances were among the exclusion criteria. The subjects were divided into three main groups; first group (group OLE) consisted of 20 subjects underwent scaling and polishing with oral hygiene instruction with (OLE) 0.3% for three weeks, use mouthwash (10 ml, twice daily), second group (group CHX) consisted of 20 subjects underwent scaling and polishing with oral hygiene instruction, for three weeks, use 0.12 % chlorhexidine mouthwash (10 ml, twice daily)., and the third group (group S&P) consisted of 20 patients received scaling and polishing with oral hygiene instruction only. After a half-hour of tooth brushing, a one-minute mouth rinse was done, followed by at least a half-hour of eating and drinking. Before the study began, all participants signed an informed consent form. The patients had a full-mouth periodontal examination before treatment and after 5 weeks of periodontal therapy. Clinically, plaque thickness was determined using a straight sharp explorer and measuring the amount of bacterial plaque according to plaque index (PI) 1[6]on four surfaces of all the examined teeth, with a score varying from 0-3. Gingival inflammation was determined by using the gingival index (GI) [17] naked eye inspection with gentle probing by using Williams periodontal probe for four gingival surfaces on all examined teeth, and a score was given from 0-3. Bleeding on probing (BOP %) was measured by softly running a periodontal probe (William) along the inner surface wall of the gingival sulcus, and bleeding on probing was

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noted as absent (-) or present (+) after 30 seconds [18].

Olive leaf extract mouthwash Preparation

Olive leaf extract (1 kg) were washed and air-dried, then percolated with 55 % EtOH at room temperature. The dried residue (78.8 g) was suspended in water after the combined EtOH extracts were purified with liquefier and evaporated under vacuum at low temperature. As a result, 1 kilogram of concentrated liquid extract (olive leaf extract mouthwash) was produced. Concentration of 3% is obtained by dilute 3 mL of mouthwash in 100 mL of pre-boiled water [11-19].

Blood sample collection

A total of five milliliters of blood is collected from each subject at the start of the study and again after five weeks of periodontal therapy. The samples were directly sent to the laboratory for estimated of C- reactive protein (CRP), alkaline phosphatase (ALP), and total protein in plain tubes without anticoagulant (TP). Based on the colorimetric process, serum ALP was quantified using a special package for a combat analyzer (Roche/Hitachi Cobas c systems). A specific package for Cobas analyzer (Roche/Hitachi Cobas systems) was used to quantify serum TP. Serum CRP was measured quantitatively using a combat analyzer kit (Roche/Hitachi Cobas c systems) and a colorimetric approach according to the kit's manufacture instructions.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) for Windows, version 20.0, was used to analyze and estimate the results (Armonk, NY: IBM Corp). To evaluate the readings before and after the treatment, a paired t-test was used. The three sample groups' means were compared using one-way measurement of variance (ANOVA). To equate the means of the two classes, a post hoc test (LSD) was used. Statistical significance was defined as a P-value of less than 0.05.

Results

This study included 60 adults, 30 females and 30 males between the ages of 18 and 35 years. Table (1) indicates a significant reduction in the mean value of the PI, GI, and BOP from baseline (before treatment) to 5 weeks after periodontal therapy in groups (OLE), (CHX), and (S&P). The difference was statistically significant ($P<0.01$). After 5 weeks of periodontal treatment, Table (3) shows the differences in mean scores of clinical periodontal parameters in groups (OLE), (CHX), and (S&P). For PI, the greater improvement in mean difference was shown in CHX group (0.95±0.48), followed by (OLE) and (S&P) as 0.79±0.26 and 0.58±0.5 respectively. Significant differences ($P<0.05$) were detected between the three main groups regarding the mean differences of PI. Comparison in mean differences showed no statistically significant differences was shown between OLE and CHX ($P=0.651$), OLE and S&P ($P=0.333$); while significant differences was shown between CHX and S&P ($P <0.05$). For GI, the reduction in mean differences of GI were 0.63±0.48 in OLE group, 0.78±0.5 in CHX group, and 0.35±0.43in S&P group ($P<0.05$). For comparison between the three main groups regarding the mean difference of GI, no significant differences had been existed between OLE and CHX ($P=0.575$), and highly significant differences had existed between OLE and S&P and CHX and S&P groups ($P <0.05$). Finally, for BOP, the result

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showed the mean differences in group OLE, CHX and S&P were 37.08±0.85, 37.29±1.25 and 25.01±1.99 respectively(P<0.001). For comparison between the three main groups regarding the mean difference of BOP, no significant differences had been existed between OLE and CHX (P=0.239), and highly significant differences had existed between OLE and S&P and CHX and S&P groups (P <0.001).

Table (1): Comparison of clinical periodontal parameters PI, GI, BOP before therapy and 4 weeks after periodontal therapy in OLE, CHX and S&P groups, n = 20

Group	Parameters	Before therapy	After therapy	*P*-value
		Mean± SD	Mean± SD	
Olive extract 0.3% (OLE)	PI	1.81±0.23	1.00±0.71	< 0.001
	GI	1.70±0.26	1.10±0.84	< 0.001
	BOP%	70.17±9.15	34.11±8.33	< 0.001
Chlorhexidine 0.12% (CHX)	PI	1.89±1.21	0.90±0.72	<0.001
	GI	1.85±0.76	1.07±0.16	< 0.001
	BOP%	68.53±7.66	30.22±6.43	< 0.001
Scaling &Polishing (S&P)	PI	1.71±0.45	1.12±0.15	< 0.001
	GI	1.75±0.12	1.43±0.53	< 0.001
	BOP%	71.20±8.22	48.17±6.23	< 0.001

Table (2): Comparison of biochemical parameters serum ALP, CRP and TP before therapy and 5 weeks after periodontal therapy in group OLE, group CHX and group S&P, n = 20

Group	Parameters	Before therapy	After therapy	*P*-value
		Mean± SD	Mean± SD	
Olive extract 0.1% (OLE)	ALP (U/L)	81.45±9.72	76.21±11.43	0.001
	CRP (mg/L)	3.51±1.17	2.77±1.23	0.02
	TP (g/dl)	7.02±0.75	6.71±0.46	0.02
Chlorhexidine 0.12%(CHX)	ALP (U/L)	77.65±11.98	68.43±10.74	0.005
	CRP (mg/L)	3.56±2.66	2.75±1.38	0.01
	TP (g/dl)	7.02±0.48	6.41±0.11	0.01
Scaling& Polishing (S&P)	ALP (U/L)	79.47±11.45	76.16±9.25	0.01
	CRP (mg/L)	3.71±1.43	3.31±1.59	0.05
	TP (g/dl)	7.17±0.39	7.13±0.62	0.04

Parameters in biochemistry. The mean values of serum ALP, CRP, and TP in the three groups are compared in Table (2). The result detect that from baseline (before treatment) to 5 weeks after periodontal therapy, the mean values of serum ALP, CRP, and TP scores in the OLE, CHX, and S&P groups decreased. The differences in mean values are statistically significant (P=0.001). Table (3) shows the improvements in serum ALP, CRP, and TP in the OLE, CHX, and S&P groups after 5 weeks of periodontal therapy.

For ALP enzyme, the mean differences of ALP enzyme in OLE group were 6.25±1.71, and CHX was 7.21±1.24 and 3.27±2.2 in S&P group (P<0.001). For comparison between the three main groups, regarding the mean difference of ALP, no significant differences had been existed between OLE and CHX (P=0.197), and highly significant

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differences had existed between OLE and S&P and CHX and S&P groups (P <0.001). Regarding CRP, the mean differences were 0.75±0.05 in OLE group, 0.85±1.4 in CHX group, and 0.43±0.17 in S&P group. For comparison in the mean differences of CRP between the three main groups, no significant differences had been existed between OLE and CHX (P=0.253), and highly significant differences had existed between OLE and S&P and CHX and S&P groups (P <0.001). For TP, the mean difference of TP was 0.33±0.27 in OLE group, 0.58±0 in CHX group and 0.09±0.25 in S&P group (P <0.001). For comparison in the mean differences of CRP between the three main groups, no significant differences had been existed between OLE and CHX (P= 0.053), and highly significant differences had existed between OLE and S&P and CHX and S&P groups (P <0.001).

Table (3): Means of reduction of clinical periodontal parameters and biochemical parameters between OLE group, CHX group and S&P group, n = 20

Parameter/ time	Groups	Mean ± SD	*P* (ANOVA)	LSD groups	*P*- value
PI Before therapy- after 5 weeks	(OLE)	0.79±0.26	0.0ᵒ	OLE x CHX	0.651
	(CHX)	0.95±0.48		OLE x S&P	0.335
	(S&P)	0.58±0.5		CHX x S&P	0.02
	Total	0.77±0.35			
GI Before therapy- after 5 weeks	(OLE)	0.63±0.48	0.01	OLE x CHX	0.575
	(CHX)	0.78±0.5		OLE x S&P	0.01
	(S&P)	0.35±0.43		CHX x S&P	0.001
	Total	0.58±0.49			
BOP% Before therapy - after 5 weeks	(OLE)	37.08±0.85	0.001	OLE x CHX	0.239
	(CHX)	37.29±1.25		OLE x S&P	< 0.001
	(S&P)	25.01±1.99		CHX x S&P	<0.001
	Total	32.79±1.35			
ALP(U/L) Before therapy - after 5 weeks	(OLE)	6.25±1.71	0.01	OLE x CHX	0.197
	(CHX)	7.21±1.24		OLE x S&P	0.02
	(S&P)	3.27±2.2		CHX x S&P	0.005
	Total	6.24±1.71			
CRP (mg/L) Before therapy - after 5 weeks	(OLE)	0.75±0.05	0.02	OLE x CHX	0.253
	(CHX)	0.85±1.4		OLE x S&P	0.01
	(S&P)	0.43±0.17		CHX x S&P	0.001
	Total	0.66±0.50			
TP(g/dl) Before therapy - after 5 weeks	(OLE)	0.33±0.27	< 0.001	OLE x CHX	0.053
	(CHX)	0.57±0.5		OLE x S&P	<0.001
	(S&P)	0.09±0.25		CHX x S&P	<0.001
	Total	0.32±0.27			

Discussion

Olive leaf extract is known as it contained a variety of active flavonoids, also terpenoids such as α -bisabolol, azulene, matricin, and chamazulene in its volatile oil. These contents give Olive leaf extract its anti-inflammatory, antispasmodic, and antibacterial characters [20], its pharmacological activity has been determined, mostly for its antioxidant

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properties [21]. The aims of this research was to determine the mean values of gingival inflammatory indices and biochemical parameters before and after the use of 3% of Olive leaf extract as mouthwash (OLE) and to compare this effect with 0.12 % of chlorhexidine mouthwash in the treatment of acute gingivitis. In this study, using 3% Olive leaf extract mouthwash resulted in statistically significant decreases in clinical periodontal parameters. Reduced inflammation due to OLE's antimicrobial properties, which are attributed to its terpenic components chamazulene and β -bisabolol [22]. Bisabolol, an important oil included in OLE extract, has been shown to decrease edema, leukocyte infiltration, protein extravasation, tumor necrosis factor-alpha and other factors release, finally neutrophil degranulation [23]. Chamazulene, another OLE constituent, has been shown to contentiously protect against lipid peroxidation [24]. These sings and notes are consistent with other researchers' findings, on the effects of matrica mouthwash on bacterial plaque and gingival inflammation, which found that this mouthwash has a major effect on plaque collection, bleeding on probing, and gingival inflammation 11;25. In the treatment of acute gingivitis, a related process could be found in the effect of 0.12 % CHX mouthwash after scaling and polishing, result in the reduction of clinical periodontal parameters such as PI, GI, and BOP compared to scaling and polishing alone [26]. The decline result may be due to CHX mouthwash's positive effect on plaque formation by its antibacterial activity, which greatly minimized and give controls for gingival inflammation in patients with poor oral hygiene [27]. The results showed no significant difference in the mean values of gingival indices between the chlorhexidine and OLE as mouthwashes [19]. Nevertheless, the result showed that the plaque inhibitory effects of 0.12 % CHX were slightly greater than that of 3% of OLE, this is because chlorhexidine has a widely spectrum of action, on both types of bacteria that are Gram positive and Gram negative. Has a bactericidal proberty at high concentrations, result in cell wall lysis [28], in addition, Varoni *et al*., [29] concluded that as compared to other topical antimicrobial agents used as prophylactically and therapeutically in gingivitis, chlorhexidine has shown the best and ideal clinical effects in biofilm control. The findings and results of the present study matched those of Hallmon & Rees [30], who found a significant improvement in gingival inflammatory indices in the S&P group alone, which they attributed to increase in patients' attention to oral hygiene instruction involving plaque removal and prophylaxis.

Noack *et al*.,[31] found a strong relationship between periodontal disease and increase CRP levels in patient blood. The serum level of CRP in the present study was significantly reduced in the OLE group, CHX group, and S&P group alone from baseline to 5 weeks after scaling and polishing with the reduction in the level of periodontal clinical parameters. This result is consistent with that of Zhou *et al*.[32]. Ide *et al*. [33] did not see a reduction in serum CRP following phase 1 therapy, which is in contrast to the present results. This result is due to the fact that periodontal debridement alone is ineffective in prevention of periodontal disease in all

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cases of periodontal disease patients. The present study reported that after 5 weeks of therapy, serum ALP and TP levels in patients with chronic gingivitis decreased significantly in both MTC and CHX groups, with considerable variation relative to the S&P alone group. Since bisabolol and chamazulene have potent antioxidant effects, this results provided evidence that OLE has antioxidant properties [34]. In addition, by different chemical processes, OLE plant was able to inhibit reactive chemical species formation and block lipid peroxidation. Furthermore, Fe2+/ascorbate-induced lipid peroxidation and dimethyl sulfoxide (DMSO) autoxidation is blocked by OLE [35,36]. The results of the present study found that OLE mouthwash, like chlorhexidine, had significant effects on CRP, ALP, and TP levels; evidence improved that OLE has immunomodulatory proberties [37]. In addition, the flavonoid apigenin, which is present in OLE extract, has anti-inflammatory and antioxidant properties in addition to other properties like it prevents the synthesis of nitric oxide (NO) in addition to the actions of hyaluronidase, collagenase, and cyclooxygenases enzymes that introduced in the inflammatory process [9]. The anti-inflammatory and antimicrobial action of CHX, which prevents the decolonization of putative pathogenic bacteria by formation of a bacteriostatic mileu [38], was observed in the CHX group as well. More research is needed to determine the effectiveness of OLE as an adjunctive aids to standard acute gingivitis therapy by evaluating their bioactive components.

Conclusions

In conclusion, OLE minimized biofilm accumulation and gingival bleeding in acute gingivitis patients, due to its antimicrobial and anti-inflammatory properties, as compared to CHX. To prove that this 3% herbal product is effective in the treatment of periodontal diseases, large-scale trials are required.

Recommendations

Further studies are suggested to identify to the efficacy and side effects of different types of mouth rinses with and without alcohol. Since the exact mechanism for antimicrobial effects of OLE mouthwashes is still unknown in acute gingivitis, microbiological, histological and genetic studies are recommended to strengthen the interrelation.

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Conflict of interest: Nill

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فعالية غسول الزيتون وما يتعلق بالكلورهيكسيدين على عوامل التهاب اللثة والعوامل السريرية في مصل الدم لمرضى التهاب اللثه الحاد

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الملخص

خلفية الدراسة: ترتبط العدوى الفموية والجهازية ارتباطًا وثيقًا بسوء نظافة الفم. يوصى عادة بغسول الفم الكلورهيكسيدين ، بالإضافة إلى تفريش الأسنان. هناك الكثير من الدراسات حول فعالية الزيتون كغسول عشبي للفم ، ولكن لا توجد أدلة كثيرة على تأثيره التنافسي مع الكلورهيكسيدين.

اهداف الدراسة: لتحديد التأثيرات السريرية والالتهابية لنسبة ٣٪ من الزيتون(OLE) كغسول للفم في علاج التهاب اللثة المزمن ومقارنة تأثيره مع ٠,١٢٪ كلورهيكسيدين .(CHX).

المرضى والطرائق: تم جمع ما مجموعه ٦٠ شخصا مصابين بالتهاب اللثه الحاد تتراوح أعمارهم بين ١٨-٣٥ سنة وخضعوا لتعليمات النظافة الفموية، التنظيف والتلميع. قسموا الى ٢٠ مريضا تلقوا ٣٪ من (OLE) كغسول للفم ، ٢٠ مريضا تلقوا ٠,١٢٪ من غسول الفم الكلورهيكسيدين ، و ٢٠ مريضا تلقوا التنظيف والتلميع فقط (SRP). . كان غسول الفم يستخدم مرتين يوميًا لمدة ١٧ يومًا. تم جمع مؤشرات التهاب اللثه السريرية بما في ذلك مؤشر الصفيحة الجرثومية (PI)، مؤشر التهاب اللثه (GI)و مؤشر نزيف اللثة (BOP)، وعينات الدم في بداية الدراسة وبعد خمسة أسابيع من العلاج لتقدير مستويات البروتين التفاعلي (C (CRP والفوسفاتيز القلوي (ALP) والبروتين الكلي (TP).

النتائج: بعد ٥ أسابيع من علاج اللثة ، النتائج اظهرت انه كان هناك انخفاض كبير في مؤشرات التهاب اللثه السريرية (P <0.01) وعلامات الالتهاب (P <0.05) في كل من مجموعات OLE و CHX و S&P وحدها. من علاج اللثة ،يتبين لنا انه لم يكن هناك فرق بين مجموعتي OLE و CHX من خلال مؤشرات التهاب اللثه السريرية وعلامات الالتهاب (P <0.05) ، على الرغم من وجود فرق كبير بين تلك المجموعتين OLE و CHX مع مجموعة S&P وحدها من حيث مؤشرات التهاب اللثه السريرية وعلامات الالتهاب (P <0.05).

الاستنتاجات: لقد ثبت أن الزيتون يمكن مقارنته بـ CHX كعامل مساعد لعلاج اللثه و الحد من التهاب اللثة عن طريق تقليل مؤشرات التهاب اللثه السريرية وعلامات وقيم الالتهابية الكيميائية مثل CRP و ALP و TP.

الكلمات المفتاحية: مستخلص أوراق الزيتون (OLE) ؛ غسول الفم الكلورهيكسيدين. التهاب اللثة ، CRP، ALP، TP.

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