RESEARCH ARTICLE

The Effects of *Salvia officinalis* Gel as an Adjunct to Scaling and Root Planning in Patients with Periodontitis (Clinical and Immunological Study)

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ABSTRACT

Background: Phytotherapy is the usage of herbal species with medicinal properties for the management of various diseases. Gingivitis and periodontitis are diseases that involve the role of both the bacteria and the host immune response. Over the years, various researches have shown the importance of herbal products in the management of periodontal diseases.

Aims of the study: To evaluate the efficacy of locally applied *Salvia officinalis* gel as adjunctive in the treatment of chronic periodontitis.

Subjects and methods: Fourteen patients (10 males and 4 females) with chronic periodontitis were enrolled in the present study with total number of twenty-eight periodontal pockets utilizing a split mouth design, the pockets were divided into two groups, the test group which was treated with scaling and root planning procedure and the application of the *S. officinalis* gel, and the control group that treated with scaling and root planning only. Plaque index (PI) and gingival index (GI) were recorded for each site. Gingival crevicular fluid (GCF) was collected from each site by using PerioCol paper strips. The concentration of the transforming growth factor beta-1 in the gingival crevicular fluid was quantified by a high sensitivity enzyme-linked immunosorbent assay.

Results: The test group demonstrated a significant reduction in GI at 1-week and 1-month after the treatment comparing to baseline (1.14 *vs.* 1.64, p = 0.003, and 1.21 *vs.* 1.64, p = 0.028, respectively), while no significant reduction in the PI at recall visits comparing to baseline. The control group demonstrated no significant reduction in PI and GI at recall visits, comparing to baseline. Both the test and control groups demonstrated no significant reduction in the transforming growth factor-beta 1 concentration in the gingival crevicular fluid at one week after the treatment comparing to baseline, while at one month after the treatment only the test group demonstrated a significant reduction comparing to baseline (3.91 *vs.* 9.62, p = 0.044).

Conclusion: The findings of the present study indicated that the *S. officinalis* gel has a potential anti-inflammatory role in the treatment of chronic periodontitis by monitoring both the clinical and immunological parameters.

Keywords: Carbopol, Chronic periodontitis, Herbal extraction, Maceration, Periodontal pocket, Phytotherapy, *Salvia officinalis*, Scaling and root planing, Spray dryer, Transforming growth factor-beta 1.

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INTRODUCTION

Periodontitis is a multifactorial disease that causes tooth loss. The complex pathogenesis of periodontitis implies the involvement of a susceptible host and a bacterial challenge.[1]

The aim of periodontal treatment is to arrest inflammation, prevent contamination of underlying tissue, and create favorable conditions for healing and regeneration of periodontal tissue.[2]

Antibiotics and nonsteroidal anti-inflammatory drugs have been studied as an adjunctive to periodontal therapy. Yet, these drugs are related to antimicrobial resistance, gastrointestinal intolerance, and systemic alterations.[3-5] Thus, phytochemicals can be an alternative of drugs with desirable properties and reduced side effects.[6]

Researchers have found that bacteria that lead to periodontal diseases can be suppressed by phytotherapeutic agents. Hence, there is a pronounced interest in the development of phytotherapeutic agents for periodontal therapy. Over the past decades, pharmaceutical companies

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have increased their interest in investigating plants as sources for new phytotherapeutic agents with proven efficacy, quality, and safety.[7] The plants have therapeutic benefits, such as, antioxidant, anti-inflammatory, antimicrobial, antiseptic, and anti-collagenase properties.[8]

S. officinalis Linnaeus (sage) belongs to the Lamiaceae family; it is a perennial sub-shrub species of woody stems. This aromatic plant can be used for culinary, medicinal, and commercial (fragrance) purposes. In addition, some biologic effects, including antibacterial, antifungal, antileishmanial, anti-inflammatory, antitumor, antioxidant, antinociceptive, mnemonic, and antiangiogenic activities, have been attributed to *S. officinalis* products, such as, extracts, essential oils, and bioactive molecules.[9] *S. officinalis* has many phytochemicals like carnosol, rosmarinic acid, carnosic acid, and ursolic acid, which is responsible for its antioxidant and anti-inflammatory effect.[10]

GCF is predominantly formed by serum transudate and/or inflammatory exudate derived from the periodontal tissues. Therefore, the analysis of biomarkers in GCF is a widely used non-invasive method to study the status of inflammation of periodontal tissues and the host response to different periodontal therapies.[11]

TGF-β1 is a cytokine with pleiotropic properties, it may be classified as pro-inflammatory because it is chemotactic for neutrophils, monocytes, mast cells, and lymphocytes, but it also has potent immunosuppressive activities that suppress cell-mediated, as well as, humoral immunity.[12] Chronic periodontitis patients had significantly elevated GCF TGF-β1 total amount compared to healthy patients, TGF-β1 is contributed to the pathogenesis of chronic periodontitis, TGF-β1 may, thus be one of the components modulating exaggerated host response together with other major mediators of inflammation.[13]

MATERIALS AND METHOD

Plant Material

The *S. officinalis* subshrubs were obtained from Jordan, the leaves were separated from the stem, the leaves were dried by air-drying by exposing the plant leaves to air at ambient temperature (20–25°C), and the temperature was monitored by thermometer. This drying method does not force dried plant materials using high temperature; hence, heat-labile compounds are preserved.[14]

Herbal Extraction

The procedure was carried out at the Ministry of Industry and Research, Commission of Research and Industrial Development, Ibn Al-Bitar Research Centre. The dried leaves were grinded by using FW177 herbal medicine grinding machine, the dried and pulverized leaves were submitted to maceration extraction by 70% ethanol, the ratio of plant material and extracting solvent was 1:10 w/v,[15] that means 100 grams of the leaves were macerated with 1,000 mL of 70% ethanol (700 mL of ethanol absolute and 300 mL of distilled water). After that the maceration procedure completed using Lab Companion® SL-600R benchtop shaker, the maceration time for about 5 hours, and an extraction temperature of 45°C was used.[16] After maceration, the herbal extract were filtered by two steps, the first step by using gauze pads fitted in conical flask, the second step is vacuum filtration, by using filter paper fitted in Buchner funnel in a Buchner flask with the use of vacuum pump. The extract then dried by using spray dryer (BUCHI® mini spray dryer B-290), the inlet temperature was in the range of 48 to 50°C, and the range of outlet temperature was 42 to 44°C, but never exceeding 50°C.[16]

Test for Flavonoids

One mL of herbal extract was taken in a test tube, and then 1 mL of potassium hydroxide (5%) was added; a dark yellow color precipitate indicates the presence of flavonoid compound.[17-19]

Experimental Study

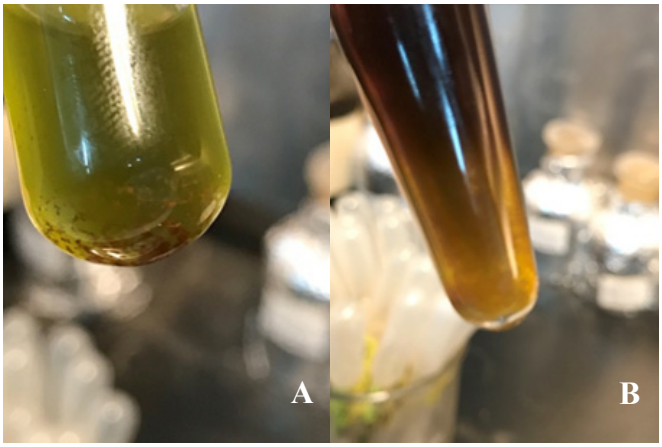
To test the extraction conditions, after the completion of maceration in the shaker, we did not start filtration of the herbal extract immediately; instead, we covered the flask with parafilm tape and stored it in the refrigerator overnight, then we started the filtration process in the morning and after completion we did the test for flavonoids by potassium hydroxide, no or merely seen yellow precipitate was seen, unlike the previous test (Figures 1A and B).

Preparation of the *S. officinalis* Gel

One gram of carbopol 940 was dissolved in 25 mL of distilled water to obtain 4% gel, 0.4 grams of *S. officinalis* alcoholic extract was dissolved in 10 mL of 5 mL of ethanol absolute, 3 mL of propylene glycol, and 2 mL of distilled water, to obtain 4% of active ingredients, after that 10 mL of the gel was added to 10 mL of active ingredients, triethanolamine was added to adjust the pH above 7. The gel was prepared with the final concentration of 2% for both of the gelling agent and the *S. officinalis* extract, and the gel set aside for 24 hours before loading in the syringes.

Study Sample

Fourteen patients with chronic periodontitis (10 males and 4 females) in the age group of 25 to 47 years (mean age 37.1 ± 7.46) from the patients attending the Department of



Figures 1A and B: Test for flavonoids for overnight maceration (**A**); and 5 hours maceration (**B**)

Periodontology, Teaching Hospital, College of Dentistry, University of Baghdad, were included in this study. This study was approved by the ethical committee of the College of Dentistry, University of Baghdad, and all patients were given detailed information relating to the study aims and an informed consent representing the patient's approval to participate in this study.

Inclusion Criteria

- Patients with chronic periodontitis with probing pocket depth ≥ 5 mm
- Patients with moderately or severely inflamed gingiva that bleeds on probing
- Patients able to follow the required instructions

Exclusion Criteria

- Subjects wearing removable partial dentures or undergoing orthodontic treatment
- Teeth showing endo-perio lesions
- Patients taking anti-inflammatory drugs, antibiotics, immunosuppressant, or oral contraceptives for last 3 months
- Patients with systemic diseases that influence the condition
- Pregnant or lactating women
- Patients with smoking habit

The Clinical Intervention

The research utilized a split-mouth technique in the application of the gel, two sites treated in each patient, the included sites pockets must be ≥5. All patients were agreed to participate in the research. Written informed consent was obtained from all participants in this research study. During the first visit, the patient received oral hygiene instructions, recording the plaque and gingival indices and full mouth scaling with ultrasonic instruments, in the next visit after one week, the first site treated conventionally with root planing (controlled site), the second site was treated with root planing and the application of the *S. officinalis* gel, the site isolated with cotton rolls to prevent contamination from saliva, a 2 mL disposable syringe equipped with a blunted 25 gauge needle bent along its shank at an approximately 130 degree was used, the syringe was inserted up to the base of the pocket, measurement of the plaque and gingival indices for the two sites were taken previous to the treatment commencement to establish the baseline record for further follow up and clinical improvement monitoring, the first recall for the patient was after one week for measuring the clinical parameters (PI and GI), and second application of the gel, the gel was applied at the entrance of the pocket, the syringe was not inserted up to the base of the pocket, so as not to disturb healing, the second recall visit was after one month from baseline for measuring the plaque and gingival indices. The GCF sample was collected at baseline, first recall, and second recall by intracrevicular method utilizing PerioCol paper strips (PerioCol, Orafollow, USA). To ensure excellent collection of the GCF we must make sure that the sites are dried and not contaminated with saliva. Patients were scheduled for sample collection during the morning hours, that is, from 9 to 11 AM, to prevent any diurnal variations affecting the crevicular fluid volume.[20] GCF collection was the first step done during the appointment before recording the clinical parameters to prevent contamination from bleeding during the recording or stimulation of saliva. Cotton rolls were inserted all around the intended sites before the insertion of the PerioCol paper strip; gentle force was used to avoid bleeding; the PerioCol paper strip was placed for 30 seconds deep inside the pocket. The sample then transferred into Eppendorf tubes containing 300 µL of PBS, and after extraction of the GCF (elution and centrifugal procedure from the paper strip), the sample stored at –80°C by ultra-low temperature freezer until the completion of sample collection.

Statistical Analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean and standard deviation. Independent t test (two-tailed) was used to compare the continuous variables accordingly. Paired t test was used to compare the continuous variables at baseline with 1st and 2nd visits. p value less than 0.05 was considered statistically significant.

RESULTS

Clinical Periodontal Parameters (from Baseline to 2nd Recall)

Baseline

The comparison between study groups by clinical periodontal parameters at baseline is shown in Table 1. In this study, there were no statistical significant differences ($p \geq 0.05$) between study groups in the plaque and gingival indices at baseline.

1st and 2nd Recall Visits

PI

Table 2 shows the comparison in the PI at the 1st and 2nd recall visits compared to baseline level in each study group. No statistical significant change ($p \geq 0.05$) in the PI at the 1st and 2nd recall visits compared to baseline readings in all study groups.

GI

Table 3 shows the comparison in the GI at the 1st and 2nd recall visits compared to baseline level in each study group. Means of GI were significantly decreased in the 1st and 2nd recall visits compared to baseline in the test group (1.14 *vs.* 1.64, $p = 0.003$, and 1.21 *vs.* 1.64, $p = 0.028$, respectively).

Table 1: Comparison between study groups by clinical periodontal parameters at baseline

	Study group		
Clinical parameters	*Test mean ± SD*	*Control mean ± SD*	*p value*
Plaque index	1 ± 0	1 ± 0	-
Gingival index	1.64 ± 0.5	1.64 ± 0.5	1

Table 2: Comparison in the PI at the 1st and 2nd recall visits compared to baseline level in each study group

	Plaque index				
Study group	*Baseline (mean ± SD)*	*1st recall visit (mean ± SD)*	*p value*	*2nd recall visit (mean ± SD)*	*p value*
Test	1 ± 0	1 ± 0.55	1	1.35 ± 0.63	0.055
Control	1 ± 0	0.92 ± 0.47	0.583	1.07 ± 0.61	0.671

Table 3: Comparison in the GI at the 1st and 2nd recall visits compared to baseline level in each study group

	Gingival index				
Study group	*Baseline (mean ± SD)*	*1st recall visit (mean ± SD)*	*p value*	*2nd recall visit (mean ± SD)*	*p value*
Test	1.64 ± 0.5	1.14 ± 0.36	0.003	1.21 ± 0.42	0.028
Control	1.64 ± 0.5	1.35 ± 0.5	0.104	1.35 ± 0.5	0.165

Table 4: Comparison between study groups by TGF-β1 concentration at baseline

	Study group		
Inflammatory markers	*Test (mean ± SD)*	*Control (mean ± SD)*	*p value*
TGF-β1 (pg/μL)	9.62 ± 8.4	6.74 ± 4	0.27

Table 5: Comparisons in TGF-β1 concentration between baseline and 1st recall visit in each study group

	TGF-β1 (pg/μL)		
Study group	*Baseline (mean ± SD)*	*1st recall visit (mean ± SD)*	*p value*
Test	9.62 ± 8.4	5.78 ± 4.9	0.148
Control	6.74 ± 4	4.75 ± 4.8	0.285

Table 6: Comparisons in TGF-β1 concentration between baseline and the 2nd recall visit in each study group

	TGF-β1 (pg/μL)		
Study group	*Baseline (mean ± SD)*	*2nd recall visit (mean ± SD)*	*p value*
Test	9.62 ± 8.4	3.91 ± 5.36	0.044
Control	6.74 ± 4	10.95 ± 23.9	0.543

No statistical significant change ($p \geq 0.05$) in the GI at the 1st and 2nd recall visits compared to baseline readings in the control group.

TGF-β1

Baseline

The comparison between study groups by TGF-β1 concentration at baseline is shown in Table 4. In this study, there were no statistical significant differences ($p \geq 0.05$) between study groups in the TGF-β1 concentration at baseline.

1st Recall Visit after Treatment

The comparisons in TGF-β1 concentration between baseline and the 1st recall visit in each study group are shown in Table 5. No statistical significant change ($p \geq 0.05$) in TGF-β1 concentration at the 1st recall visit compared to baseline readings in both groups.

2nd Recall Visit

The comparisons in TGF-β1 concentration between baseline and the 2nd recall visit in each study group are shown in Table 6. The mean of TGF-β1 was significantly decreased at the 2nd recall visit compared to baseline reading in the test group (3.91 *vs.* 9.62, $p = 0.044$). No statistical significant change ($p \geq 0.05$) in TGF-β1 concentration at the 2nd recall visit compared to baseline reading in the control group.

DISCUSSION

We should mention that this is the first study regarding *S. officinalis* gel in periodontal treatment as, according to our knowledge, we are the first who synthesize a gel from the herbal extract of the *S. officinalis*.

There are few studies regarding the treatment of periodontal diseases by herbal remedies prepared from *S. officinalis*, in spite of the recommendation by the European Pharmacopeia for the use of aqueous *S. officinalis* leaf extract as an oral rinse to relieve inflammation and pain in diseased gingival tissues.[21]

In our current study, there were no statistical significant differences ($p \geq 0.05$) between the study groups in the plaque and gingival indices at baseline that means the sites respond to scaling with the same outcome, and the patient keeps the same oral hygiene for both sites.

Regarding the PI after the treatment commencement, we noticed that no statistical significant change ($p \geq 0.05$) in the PI at the 1st and 2nd recall visits compared to baseline readings in all study groups, and this finding was in disagreement with Pistorius *et al*., who showed subgingival irrigation with a herbal-based mouth rinse containing *S. officinalis* led to a significant reduction in PI for all study groups throughout the follow-up period, with no statistically significant differences,[22] also in disagreement with George *et al*., who showed that the toothpaste containing sage cause significant reduction in plaque index,[23] and in disagreement with Smullen *et al*., who showed plant extract from *S. officinalis* inhibit plaque formation *in vitro*.[24] This finding could be explained by that in our study we use a local delivery system which is a gel and not toothpaste-like in the study that conducted by George *et al*.,[23] which has a general effect on plaque index, unlike the gel and regarding the studies that conducted by both Pistorius *et al*.,[22] and Smullen *et al*.,[24] it could be explained that in our study, the results influenced by patient's factor, like oral hygiene, especially, the Iraqi people tend to have less oral hygiene commitment.

Regarding the GI after the treatment commencement, we noticed that means of GI were significantly decreased at the 1st and 2nd recall visits compared to baseline in the test group, no statistical significant change ($p \geq 0.05$) in the GI at the 1st and 2nd recall visits compared to baseline readings in the control group. This finding was in agreement with Pistorius *et al*. who showed subgingival irrigation with a herbal-based mouth rinse containing *S. officinalis* led to a significant reduction in both SBI and GI,[22] and in agreement with George *et al*., who showed the efficacy of an herbal-based toothpaste containing sage in the control of plaque and gingivitis, which at the end of the study, there were statistically significant reductions in the GI scores within the test group.[23] This finding could be explained due to the anti-inflammatory effect of *S. officinalis*.

Regarding the TGF-β1, we noticed that there were no statistical significant differences ($p \geq 0.05$) between the study groups in TGF-β1 concentration at baseline that means the study samples suffering from comparable level of inflammation at baseline.

Previous studies had shown that transforming growth factor beta-1 (TGF-β1), a growth factor largely involved in tissue regeneration and remodeling, is upregulated in chronic periodontitis.[25] Destruction by periodontitis could be protected by suppressing the activity of pro-inflammatory cytokines by anti-inflammatory cytokines, such as, TGF-β1.[26]

In our current study, the comparisons in TGF-β1 concentration between baseline and the 1st recall visit in each study group showed that there is no statistical significant change ($p \geq 0.05$) in TGF-β1 concentration in both groups, although there is no significant change in both groups, both group decline in the mean of TGF-β1 concentration. This could be explained as both sites respond to treatment as Skaleric *et al*. stated that the levels of TGF-β1 varied with the degree of inflammation, gingival tissues taken from the sites with mild or no clinically visible inflammation generally had lower concentrations of TGF-β1 than extracts of gingival samples taken from sites with moderate clinically observable gingival inflammation,[27] sand we noticed that in our study the mean of GI in test and control groups in the 1st recall visit has declined comparing to baseline and this finding was in agreement with Skaleric *et al*.[27] While the comparisons in TGF-β1 concentration between baseline and the 2nd recall visit in each study group showed that mean of TGF-β1 was significantly decreased at the 2nd recall visit compared to baseline reading in the test group. No statistical significant change in TGF-β1 concentration at the 2nd recall visit compared to baseline reading in the control group. We noticed that the concentration of TGF-β1 was increased in the 2nd recall visit compare to baseline in the control group and this finding could be explained according to Skaleric *et al*.[27] that the control sites suffer from more inflammation and in our study, we find that the mean of GI for the control sites at the 2nd recall visit was greater than the test group, and this finding could be explained also due to the anti-inflammatory effect of *S. officinalis*.

CONCLUSION

Based on the findings of the present study, the following conclusions can be made:

S. officinalis gel has a potential anti-inflammatory role in the treatment of chronic periodontitis by monitoring both the clinical and immunological parameters.

The effect of the gel is accentuated with the second application, as we noticed a significant decrease in TGF-β1 concentration at the 2nd recall visit comparing to baseline.

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