

EFFECT OF LOCALLY DELIVERED SPIRULINA GEL ON THE TREATMENT OF STAGE II, GRADE B PERIODONTITIS: A RANDOMIZE CONTROL CLINICAL STUDY

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ABSTRACT

Background and Aim: a potent antioxidant and anti-inflammatory can offer improved results in periodontitis cases. The current study set out to evaluate the clinical and immunological effects of spirulina gel as a scaling and root planing adjuvant for stage II, grade B periodontitis.

Methods: Two groups of forty patients with stage II, grade B periodontitis were created. Group I (control group) (n = 20) received SRP with placebo gel in the pocket. Group II (spirulina group) (n = 20) received treatment with both spirulina gel and scaling and root planing (SRP). Plaque index (PI), gingival index (GI), clinical attachment level (CAL), and probing pocket depth (PPD) were the clinical parameters that were measured at baseline, one, three, and six months following therapy. Using an enzyme-linked immunological test, the levels of interleukin 6 (IL-6) in gingival crevicular fluid were measured at baseline, 1, 3, and 6 months following treatment.

Results: Both groups' clinical and biochemical markers significantly improved. In comparison to the control group, the spirulina group had statistically significant improvements in clinical PPD, CAL, and IL-6 at 1, 3, and 6 months following therapy.

Conclusions: Local drug delivery of spirulina gel adjunctive to SRP has potent anti-inflammatory in treatment of stage II, grade B periodontitis. Spirulina is promising treatment of periodontitis.

KEYWORDS: Stage II, B periodontitis, Spirulina, Interleukin 6

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INTRODUCTION

A chronic inflammatory disease that affects the tissues surrounding and supporting teeth is called periodontitis. Periodontal diseases, which lead to the loss of periodontal attachment, are caused by microorganisms interacting with a host. Bacterial elements such as lipopolysaccharides (LPS) stimulate different host cells including leukocytes, fibroblasts, and epithelial cells by Toll-like receptors (TLRs). Signaling through these receptors induces proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6). These inflammatory mediators advocate a number of events that lead to destruction of periodontal tissues. Interleukin-6 (IL-6) is a pleomorphic cytokine involved in a number of physiologic and pathologic processes including response to infection and development and progression of inflammation and malignancy. ⁽¹⁾

Treatment methods for periodontal disease include both surgical and non-surgical procedures. Scaling and root planing (SRP) is a mechanical, non-surgical therapy that is thought to be the fundamental, gold standard treatment for periodontal diseases. SRP can reduce the associated periodontal infections. ⁽²⁾

It has been observed that SRP has transient effects. The pocket depth, the intricate architecture of the root surface, the shape of the lesion, the practitioner's expertise, and the patient's cooperation all affect how the patient response to SRP. After a single session of periodontal debridement, a pathogenic subgingival microbial pathogen may reestablish in 42–60 days with good oral hygiene. Therefore, systemic, and local administration of was implemented as a supplement to SRP. ⁽³⁾ Drug toxicity, emergence of resistant strains, and drug interactions are all side effects of systemic antibiotic therapy. As a result, several local adjuvant therapies have begun to be employed to manage periodontal diseases. ⁽⁴⁾

Because of its high concentration of natural nutrients, the filamentous cyanobacterium spirulina

has a variety of biological activities and nutritional significance. It also has immunomodulatory and bio-modulatory properties. Spirulina contains a high quantity of super molecule, carbohydrates, fats, dietary fibers, sugar, vitamins, vit C, vitamin E, B complex. Arthrospira, as "spirulina," has several benefits that have been established. ⁽⁵⁾ Spirulina has been shown to have antioxidant, antihyperlipidemic, and anti-inflammatory properties. ⁽⁶⁻⁸⁾ Recent studies on animal models have shown that spirulina can lower pro-inflammatory cytokines such necrosis factor alpha (TNF- α), interleukin 1 (IL-1), and interleukin 6 (IL-6). ⁽⁹⁾

The aim of the study was to evaluate the clinical and immunological (interleukin 6) of spirulina in the treatment of stage II, grade B periodontitis.

MATERIALS AND METHODS

A prospective randomized trial was carried out in the Department of Oral Medicine and Periodontology, Faculty of Dentistry, Kafrelsheikh University from July 2022 to March 2023. A total of 40 sites were selected, 20 sites for each group. All patients from stage II, grade B periodontitis with attachment loss 3-4 mm and probing pocket depth \leq 5 The research was conducted in compliance with the 2004 revision of the Declaration of Helsinki, and it was approved by the Kafrelsheikh University Faculty of Oral and Dental Medicine Research Ethical Committee (KD/33/22). After approval from the institutional ethics review committee, the clinical trial was registered at Clinical Trials.gov (NCT06016933). All participants received full written and verbal information about the study and signed the informed consent form.

The sample size for this study was calculated according to Arkin, 1984 ⁽¹⁰⁾ using the following equation:

$$N = \frac{(Z_{\alpha})^2 * (SD)^2}{(d)^2}$$

N= Total sample size

Z_{α} = normal variate and 4.52

SD= Standard deviation of variable

d= Absolute error or precision

<i>Z</i>	<i>SD</i>	<i>d</i>
4.52	1.96	2

The criteria used for sample size calculation were as follows:

-95% confidence limit

-84% power of the study

sample size based on the results of Mahendra et al.,⁽¹¹⁾.

$$\text{Total sample size } n = \frac{(4.52)^2 * (1.96)^2}{(d)^2}$$

= 19.62 ≈ 20 cases in each group.

Inclusion criteria:

1. Patients age ranged from 20 to 50 of both genders.
2. Patients are free from any systemic diseases affecting periodontium.
3. Ability to maintain good oral hygiene as evidenced in recall visits.

Exclusion Criteria for this Study Include:

- Pregnant, postmenopausal women.
- Smokers
- Patient with gingival recession.
- People who take anti-inflammatory drugs, antibiotics, or vitamins within the previous 3.
- People who use mouth washes regularly
- Participation in other clinical trials.

Materials:

1. In the Kafrelsheikh University Faculty of Pharmacy, pure extracted spirulina algae powder was obtained (2019©, Egypt, TEL:16246), and an aqueous based hydrogel was developed. To obtain the gel, a homogenizer was used to

combine 4 grammes of powdered spirulina, 12 grammes of gelatin, 0.2 milliliters of glycerin (wetting agent), 0.1 milliliters of peppermint oil (agent), 0.1 milliliters of sodium saccharine (sweetening agent), and 100 milliliters of filtered water. Every component of the gel acted as an inert carrier for the medication.

2. Placebo gel (methyl cellulose): To manufacture methylcellulose gel, seven grammes of methyl cellulose powder were dissolved in 100 milliliters of boiling water. The gel was then autoclaved for 20 minutes to ensure sterilization.

Patient Groups:

Patients who met the inclusion/exclusion criteria were randomly divided by a computer-generated process to receive one of the following treatments:

Group I (control group): Oral hygiene guidelines, a full mouth SRP of all teeth, and a placebo gel injection in the pocket.

Group II (test group): Oral hygiene guidelines, a full mouth SRP of all teeth, and a spirulina gel injection in the pocket after drying the area around it.

Full mouth SRP was performed in a single visit by only one periodontologist (AE) using ultrasonic scalers and hand. The time spent in each case ranged from 30 to 45 min. The oral hygiene instructions involved the use of round-ended, soft-to-medium nylon bristle brushes arranged in three or four tuft rows. The Fones Circular Brushing Method for use of the selected brush was demonstrated to the patients, and no mouthwash was allowed. Oral hygiene was practiced twice daily for 2 mins. For ten days following implantation of the gel, patients were advised not to use dental floss near the locations of spirulina gel inserts due to the possibility of the gel coming loose. Oral hygiene can carry on as usual. There was no need for dietary restrictions.

The prepared gels (methyl cellulose gel and spirulina gel) were applied into deepest part of the

pockets using a standardized plastic syringe with a 24 gauge and 0.55 diameter bend at the tip. On 7,14,21 day after initial treatment. The spirulina and placebo gel were repeated insertion for all sites. A cotton roll was used to remove any extra gel.

Clinical assessment:

The following measurements were used to assess the individuals' periodontal status:

- Plaque Index (PI)⁽¹²⁾
- Gingival index (GI)⁽¹³⁾
- Determining the deepest interproximal pocket by probing pocket depth (PPD).⁽¹⁴⁾
- Determining the deepest interproximal clinical attachment level (CAL).⁽¹⁴⁾

At the beginning of treatment and one, three, and six months later, all clinical parameters were noted.

TABLE (1) Mean± SD of age and sex, among the study groups.

	Group 1	Group 2	P- value
Age	43.45 ± 4.38	41.65 ± 4.41	0.204 ^{ns}
Sex	9 Male / 11Female	8 Male / 12Female	1.000 ^{ns}

P- value determined using independent samples t-test, using the Chi-squared test.

Immunological Analysis:

Enzyme assay for the quantitative measurement of interleukin 6 concentration in gingival fluid (GCF) at baseline and at 1, 3 and 6 months post-treatment. First, the area was isolated with cotton rolls and gently air dried, then GCF samples were taken from the sites. The inclusion criteria using sterile paper were inserted at the entrance of the test pocket for 30 s and blood contaminated samples discarded. The samples were collected in sterile Eppendorf with 1 ml phosphate saline. The paper points were taken out after 15 minutes and the samples were frozen for examination. IL-6 was determined for each

sample by electrochemiluminescence immunoassay (Elecsys® model E411; Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis:

Using computer software [Statistical Package for Social Science (SPSS)] version 20, all the data were tabulated and statistically analyzed. At a level of considered significant independent samples tests were conducted between the studied groups and within the studied groups using paired tests.

RESULTS

Forty patients with stage II, grade B periodontitis who met the inclusion criteria were included in the current study and completed followed up for three study periods (17 males and 23 females). The patients responded favorably to every therapy modality and experienced no side effects. The mean age of the patients in Group I was 43.45 ± 4.38 years old, whereas in Group II it was 41.65 ± 4.41 years old. However, there was no statistically significant difference between the two groups' ages (P>0.05). A statistical analysis showed that there was no difference between the two participant groups.

Clinical Results (Table 2):

The mean baseline values of the clinical attachment level (CAL), probing pocket depth (PPD), gingival index (GI), and plaque index (PI) did not differ between the two groups (P>0.05).

In the current study, the mean PI and GI scores were significantly lower with both treatment methods than with the mean baseline values at 6 months evaluation period (P<0.001). Furthermore, there was never a difference in the mean PI or GI between the two groups; nevertheless, at the one-month follow-up, the mean GI showed a statistically significant decrease in the spirulina group as compared to the control group (P<0.05, Table 2).

Additionally, group I and group II data showed that the mean PPD and CAL measures at 1, 3 and 6 months after therapy were significantly lower than the mean baseline values. The mean PPD and CAL readings the experimental group were significantly reduced during follow-up periods the control group, according to statistical analysis ($P < 0.01$, $P < 0.001$; Table 2).

Immunological (Table 2):

In reference to IL-6, the results of group I and group II demonstrated a statistically significant decrease in mean IL-6 in both groups at 1, 3, and 6 months relative to baseline ($P < 0.001$). Additionally, the test group's IL-6 level was lower than the control group during both assessment periods, according to the independent samples test.

TABLE (2) Mean values of plaque index (PI), gingival index (GI) and probing pocket depth (PPD) clinical attachment loss (CAL) and interleukin 6 (IL 6) among the study groups at baseline, 1 month, 3 month and 6 month after treatment.

Variable	Time	Groups		P- value
		Group 1 (n=20)	Group 2 (n=20)	
PI	Baseline (1)	1.412 ± 0.374	1.512 ± 0.308	0.362 ^{ns}
	1 month (2)	0.500 ± 0.344	0.487 ± 0.249	0.896 ^{ns}
	3 months (3)	0.575 ± 0.304	0.587 ± 0.247	0.887 ^{ns}
	6 months (4)	0.687 ± 0.241	0.625 ± 0.250	0.087 ^{ns}
		1 vs 2	1 vs 2	
		P = 0.000***	P = 0.000***	
		1 vs 3	1 vs 3	
		P = 0.000***	P = 0.000***	
		2 vs 3	2 vs 3	
		P = 0.163 ^{ns}	P = 0.057 ^{ns}	
GI	Baseline (1)	1.687 ± 0.352	1.612 ± 0.357	0.508 ^{ns}
	1 month (2)	0.437 ± 0.159	0.312 ± 0.196	0.034*
	3 months (3)	0.500 ± 0.162	0.387 ± 0.236	0.087 ^{ns}
	6 months (4)	0.562 ± 0.212	0.462 ± 0.295	0.087 ^{ns}
		1 vs 2	1 vs 2	
		P = 0.000***	P = 0.000***	
		1 vs 3	1 vs 3	
		P = 0.000***	P = 0.000***	
		2 vs 3	2 vs 3	
		P = 0.135 ^{ns}	P = 0.163 ^{ns}	
PPD	Baseline (1)	4.900 ± 0.261	4.950 ± 0.034	0.466 ^{ns}
	1 month (2)	3.900 ± 0.307	3.350 ± 0.461	0.000***
	3 months (3)	3.950 ± 0.276	3.125 ± 0.222	0.000***
	6 months (4)	4.00 ± 0.229	3.15 ± 0.243	0.000***
		1 vs 2	1 vs 2	
		P = 0.000***	P = 0.000***	
		1 vs 3	1 vs 3	
		P = 0.000***	P = 0.000***	
		2 vs 3	2 vs 3	
		P = 0.163	P = 0.046*	
	3 vs 4	3 vs 4		
	P = 0.163 ^{ns}	P = 0.619 ^{ns}		

CAL	Baseline (1)	3.700 ± 0.470	3.800 ± 0.140	0.478 ^{ns}
	1 month (2)	2.500 ± 0.487	2.100 ± 0.308	0.004**
	3 months (3)	2.600 ± 0.475	1.900 ± 0.416	0.000***
	6 months (4)	2.725 ± 0.443	2.000 ± 0.280	0.000***
		1 vs 2 P = 0.000***	1 vs 2 P = 0.000***	
	1 vs 3 P = 0.000***	1 vs 3 P = 0.000***		
	2 vs 3 P = 0.104 ^{ns}	2 vs 3 P = 0.028*		
	3 vs 4 P = 0.096 ^{ns}	3 vs 4 P = 0.163 ^{ns}		
IL-6	Baseline (1)	24.65 ± 3.881	25.21 ± 3.151	0.617 ^{ns}
	1 month (2)	13.98 ± 3.814	10.31 ± 2.763	0.001**
	3 months (3)	15.56 ± 2.713	11.29 ± 2.658	0.000***
	6 months (4)	16.61 ± 2.307	11.66 ± 2.412	0.000***
		1 vs 2 P = 0.000***	1 vs 2 P = 0.000***	
	1 vs 3 P = 0.000***	1 vs 3 P = 0.000***		
	2 vs 3 P = 0.110 ^{ns}	2 vs 3 P = 0.264 ^{ns}		
	3 vs 4 P = 0.112 ^{ns}	3 vs 4 P = 0.1637 ^{ns}		
Paired test	samples			

Significance: *P<0.05, **P<0.01, ***P<0.001 ns = not significant

Group I = Patients treated with oral hygiene instructions and SRP of all teeth + placebo gel

Group II = Patients treated with oral hygiene instructions and SRP of all teeth + spirulina gel

DISCUSSION

The sixth most common disease affecting humans is periodontitis, which affects 50% of people worldwide varying degrees. It is characterized by the gradual loss of the tooth's supporting structures, which leads to tooth loss. (15) Periodontitis is a bacterial infectious disease, and several inflammatory cytokines control its pathogenesis through crosstalk between tissue cells and immune cells. An essential cytokine in the control of the host's reaction to bacterial infection is interleukin (IL)-6. (16)

treatment of periodontal diseases includes professional removal of both supragingival and subgingival dental plaque and calculus. In addition, SRP is mechanical therapy that can be used to suppress suspected periodontal bacteria. (17) Achieving sufficient home care is necessary

for periodontal disease prevention, periodontal therapy success, and long-term dentition retention. Patients should be informed by their doctors about the value of eliminating dental biofilm at home, especially before beginning active periodontal therapy. Throughout the first and subsequent phases of periodontal therapy, the importance of adequate home care should be reinforced frequently. In addition, scaling and root (SRP) is mechanical therapy that can be used to suppress suspected periodontal bacteria. (18)

All patients in the current study were free from any relevant systemic diseases to avoid the possible impact of systemic disorders on the periodontal condition and their possible effect on the clinical parameters, microbial environment, and any modulation of inflammatory mediators as IL-6. (19) All patients received full mouth SRP in one visit to prevent cross contamination or what is called

intraoral translocation of periodontal pathogens from the untreated sites to the treated sites.⁽²⁰⁾

Traditional mechanical SRP for treating periodontitis, does not ensure complete remission of the disease. Antimicrobial therapy administered directly into periodontal pockets is known as local delivery of antimicrobial drugs in periodontitis. The benefit of this method of treatment is that it greatly exceeds the minimum inhibitory concentration (MIC) and lasts for up to several weeks after application. As a result, numerous locally applied device systems using a wide range of antibiotics or antiseptics have been created.⁽²¹⁾ The aim of the present study was to assess the clinical and immunological of spirulina in treatment of stage II, grade B periodontitis.

In the present study, the results showed no significant differences regarding the PI at baseline and at all evaluation periods between the tested groups. Showed that there was a reduction in the mean PI scores during the study periods in both groups compared to baseline values. At all evaluation periods, the reduction PI in both groups may be attributed to adequate maintenance of oral hygiene, which was to each patient.

The current study's findings revealed no discernible variations in the PI across the examined groups at baseline or during any of the evaluation intervals. indicated that, in comparison to baseline values, the mean PI scores in both groups decreased during the research periods. The decrease in patient-reported illness (PI) in both groups during all assessment periods can be ascribed to each patient maintaining proper dental hygiene.

Compared to the control group, the spirulina group had a reduced mean GI at the one-month follow-up. The anti-inflammatory qualities of spirulina, which include its ability to scavenge reactive oxygen species (ROS) and its ability to decrease the activity of cyclooxygenase 2 (COX-2) and the release of histamine from mast cells, may

be responsible for the test group's notable reduction in GI.⁽²²⁾

In both groups, there was a significant reduction in PPD and CAL at both evaluation periods compared to baseline. However, the use of spirulina was found to reduce PPD and CAL more than SRP during our evaluation periods. Following the removal of the contaminated cementum layer during scaling and curettage treatments, the tissues' capacity to generate thin junctional epithelium leads to healing and an improvement in pocket depth.⁽²³⁾ The significant improvement in our tested group agrees with the result of Mahendra et al.,⁽¹¹⁾ who evaluated the spirulina gel's therapeutic effects when used in conjunction with SRP to treat patients with chronic periodontitis. In contrast to SRP alone, SRP plus spirulina demonstrated a statistically significant decrease in mean CAL and PPD after 120 days in this trial.

The improvement of CAL and PPD in spirulina group was demonstrated by Kaipa et al.,⁽¹⁸⁾ who examined the effect of spirulina when delivered sub gingivally in patients with chronic periodontitis. They found that after 90 days in clinical parameters were statistically significant in SRP with spirulina compared to SRP group. They concluded that local drug delivery of Spirulina adjunctive to SRP improved the clinical parameters.

Oral bacteria, complex-structured compounds, leukocytes, and periodontium structure cells are all present in gingival crevicular fluid. Many cytokines, including IL-1, IL-6, and TNF α , are generated when a person has periodontitis, which causes periodontal damage.⁽²²⁾ Thus, the current study set out to evaluate spirulina's impact on IL-6. After one, three, and six months, Groups I and II showed a statistically significant decrease in the mean IL-6. What's more intriguing about the data is that, at every follow-up period, the mean IL-6 in the spirulina group was significantly lower than in the control group.

Spirulina inhibits the release of TNF- α , other cytokines, and leukotrienes, including LTB₄, which has potent neutrophil chemotactic properties. ⁽²³⁾ The mechanism and effects of spirulina maxima on periodontitis caused by *P. gingivalis* were studied by Kang et al. ⁽²⁴⁾ They discovered that spirulina administration decreased gingival tissue levels of pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6, and inflammatory transcription factor NF- κ B. The ratio of osteoprotegerin (OPG) to receptor activator of NF- κ B ligand (RANKL) expression indicates the balance between osteoclasts and osteoblasts, and it was higher in the groups that received spirulina maxima treatment. In the spirulina maxima-treated groups, osteoblast count increased while osteoclast cell counts, and alveolar bone loss decreased.

The main limitation of this study is the small number of patients and larger scale studies are recommended to prove our results. In addition, more studies are needed to compare the results of local instillation of spirulina to of local instillation of different local delivery drugs.

CONCLUSIONS

The current study's findings suggest that applying local spirulina gel may have therapeutic advantages in enhancing the effects of scaling and root planing. Moreover, emphasises how spirulina's anti-inflammatory qualities can benefit periodontal tissues by lowering persistent inflammation. Spirulina is therefore a potentially beneficial adjunct to supportive periodontal care.

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