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SPIRULINA PLATENSIS- THE REALM OF SUPERFOOD IN THE TREATMENT OF CHRONIC PERIODONTITIS

Periodontics

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ABSTRACT

Natural phytochemicals have shown to be worthy substitutes for their synthetic and chemical-laden counterparts because of their extensive natural activity, higher safety margin and lower costsmaking them extremely beneficial, especially to the lower socioeconomic population around the world. Spirulina platensis (SP) is one such emerging modality in the treatment of periodontitis. A clinical study was conducted in 32 sites in 13 periodontitis patients in the age group of 30-60 years of both the genders. They were classified into two groups. Group 1: scaling and root planning with 1% Spirulina gel placement and Group 2: Scaling and root planning with 1 % Chlorhexidine gel application on same day, 21st day and 45th day. Clinical parameters like Plaque Index (PI), Modified Sulcus Bleeding Index (MSBI), Clinical Attachment Level (CAL) and Pocket Probing Depth (PPD); and Microbiological parameters such as Porphyromonas gingivalis (P.gingivalis), Tannerella forsythia (T.forsythia), Filifactor alocis (F.alocis) were estimated in both the groups at baseline and at 45th day using Fluoroscent In-Situ Hybridisation technique (FISH). The study revealed that 1 % Spirulina as an adjunct to scaling and root planning enhanced clinical parameters and microbiological parameters. All the parameters revealed statistically significant improvement from baseline to 45th day intragroup in both the Groups A and B. The inter group assessment revealed statistically significant improvement in PPD and CAL in group B, while the microbiological parameters showed no statistically significant results. Hence it can be concluded that 1% Spirulina can be used as an adjunct to SRP among periodontitis patients as it's effective against plaque and gingivitis, alongwith its potent anti-microbial activity.

KEYWORDS

Chronic Periodontitis, Spirulina platensis, Fluorescent In-Situ Hybridisation

INTRODUCTION

Periodontitis is a prevalent disease reported to be between 20% and 50% at a global level.[1]The removal of plaque and biofilm remain the mainstay for the periodontal disease treatment- using the mechanical plaque control methods as most of the periodontopathic bacteria are found associated the plaque mainly. To ensure eradication of all the causative microorganisms from the sub-gingival environment especially in the inaccessible areas of the oral cavity, where inadequate exposure to drug results in alteration of treatment outcomes - chemical plaque control measures in the form of local drug delivery systems have become increasingly popular adjunct therapies amongst the dentists to ensure better prognosis. Chlorhexidine stands as the gold standard due to its antiplaque action and unparalleled substantivity, however, due to the recent literature on the emergence of Chlorhexidine -resistant bacterial strains, the need for alternative naturally derived products has arisen. One such product is Spirulina, the superfood, for its potential in periodontal disease treatment.

In Periodontitis, locally delivered Spirulina gel as an adjunct to scaling and root planning has been shown to cause an improvement in the clinical parameters.[2]The sparse literature available on Spirulina has demonstrated potent anti-gingivitis[3]and anti-bacterial effect.[4]

The efficacy of a locally delivered drug cannot be successfully established solely based on the assessment of clinical parameters for the treatment outcome. Hence various advancements have been made over conventional culture methods for microbiological analysis. The advent of new molecular diagnostic methods like Fluoroscent in-situ hybridization (FISH) has enabled one to observe, in vivo, the spatial distribution of periodontal pathogens in the supragingival and subgingival biofilm[5], as well as, their ability to invade host epithelial cells.[6] As not much literature is available comparing Spirulina with chlorhexidine in periodontics, the current study was carried out to compare and evaluate the clinical and microbiological efficacy of 1% Spirulina platensis gel and 1% Chlorhexidine gel as adjuncts to scaling and root planing.

MATERIALS AND METHODS

In this study, a total of 32 sites in participants aged 30-50yrs of both the genders were examined from the Out- patient department of Periodontics, AJ Institute of Dental Sciences. An informed written consent was taken from all the subjects. All the participants were diagnosed with chronic periodontitis with probing pocket depth of 4-6 mm.

The patients included in the study were systemically healthy individuals with chronic periodontitis (AAP 1999), with periodontal pockets measuring 4-6 mm. Patients with a history of underlying systemic diseases and condition, history of any surgical interventions, acute illnesses, with adverse habits like smoking, pregnant and lactating women and those on contraceptives; and history of periodontal treatment 6 months prior were exempted from the study.

The clinical parameters were recorded at baseline, 21st day, and 45th day as follows:

1. Plaque index (Silness and Löe)
2. Modified Sulcus Bleeding Index (A.Mombelli et al)
3. Probing pocket depth (PPD)
4. Clinical attachment level (CAL)

1% Spirulina platensis gel preparation was done in the Srinivasa College of Pharmacy, Mangalore. The obtained gel was stored at 4°C in the refrigerator to maintain its consistency. Chlorhexidine gel used was under the brand name Hexigel containing 1% w/w chlorhexidine gluconate dispersed in flavored gel, made by ICPA health products ltd, Ankleshwar, India.

The procedure was carried out by a calibrated Periodontist. The procedure was standardized by using same periodontal probe (William's periodontal probe). Scaling and root planing was done using ultrasonic scalers (Satelec Ultrasonic scalers). The sample sites were isolated using cotton rolls and the supragingival plaque was removed to avoid contamination of the subgingival plaque deposit. Subgingival plaque samples were collected using a sterile Gracey curette with one light stroke on the root surface from the deepest periodontal pocket and transferred to the vial containing tris-EDTA. 1% Spirulina gel was then placed in the defects in Group A (test group), whereas 1% Chlorhexidine was placed in Group B (control group). The gel was applied carefully subgingivally and interproximally until excess gel was observed from the gingival margin. (Figure 1) Excess gel was removed with cotton roll.

Patients were instructed to refrain from chewing hard or sticky foods, brushing near the treated areas, or using any interdental aids for 1 week. In case of supragingival plaque formation during the recall visits, the plaque was removed using hand instruments to prevent any further contamination of the subgingival sample below. The collected subgingival plaque samples were then transported in tris- EDTA buffer to the Central Research Laboratory, Maratha Mandal Dental College, Belgaum. (Figure 2 (a) and (b))The collected samples were assessed to visualize, identify and quantitate Porphyromonas gingivalis, Tannerella forsythia and Filifactor alocis using Fluorescent In-Situ Hybridisation (FISH) technique.

Figure 1- Spirulina gel application

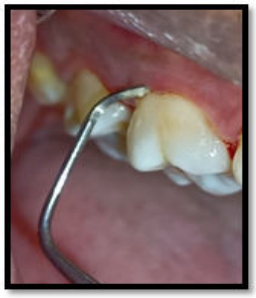


Figure 2 –(a) Subgingival plaque sample collection using Gracey curette

Figure 2-(b) Transfer of collected plaque sample into tris-EDTA for transportation

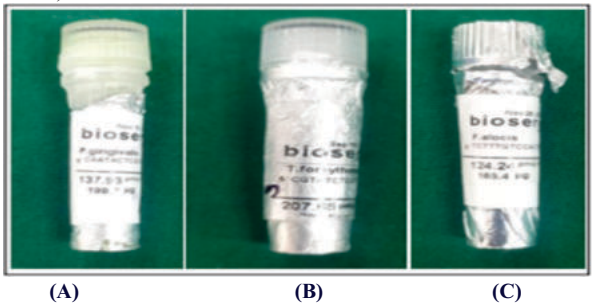
The demarcated area of the plaque material was studied using fluorescent microscope with ×20 and ×40 objectives. They were counted and graded by using a score system as- 0 for No bacteria seen, 1 for 1–10 bacilli/smear, 2 for 10-100/smear, 3 for 100–1000/smear and 4 for Large no of clumps /smear. (Figure 4)

The probe sequences used for detection were: (Figure 3)
a) Porphyromonas gingivalis 5-'
CAATACTCGTATCGCCCGTTATTC-3' (FITC labeling at 5' end)

b) Filifactor alocis 5'-
TCTTTGTCCACTATCGTTTTGA-3' (FITC labeling at 5' end)

c) Tannerella forsythia **5'-**
CGTATCTCATTTTATTCCCCTGTA-3' (FITC labeling at 5' end)

The specificity of the probe was confirmed by testing against known strains of ***Porphyromonas gingivalis*** (ATCC No. 33277), ***Filifactor alocis*** (CCUG No. 47790) and ***Tannerella forsythia*** (ATCC No. 43037)



(A) (B) (C)

Figure 3- Oligonucleotide probes- (A) P.gingivalis, (B)T.forsythia AND (C) F.alocis

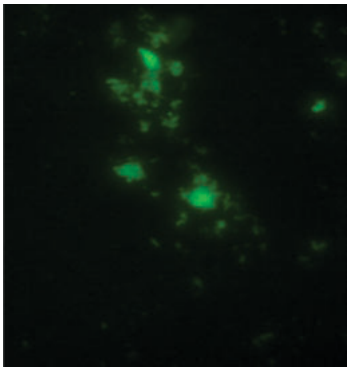


Figure 4- Microorganisms visualized under the Fluoroscent microscope

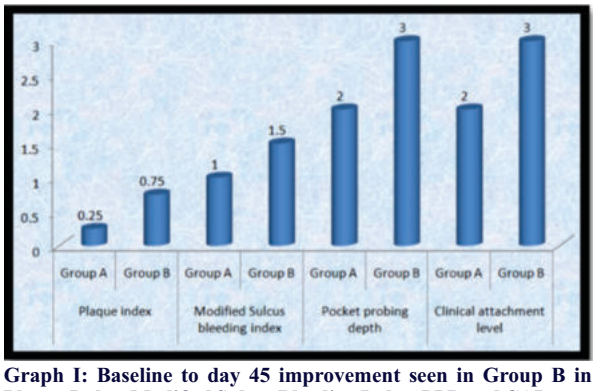
Statistical Analysis
Statistical analysis of the data was performed using SPSS 20.0. The Categorical variables were presented as frequency and percentage. The continuous variables were presented as median and Interquartile range. Comparison of categorical variables was performed using chi square test. Comparison of Plaque Index, Modified Sulcus Bleeding Index, Pocket Probing Depth and Clinical Attachment Level within groups A and B for baseline, day 21st and day 45th was done using Kruskal Wallis ANOVA. Comparison of Plaque Index, Modified Sulcus Bleeding Index, Pocket Probing Depth and Clinical Attachment Level between group A and B was done using Mann Whitney U test. Pre and Post comparison was done using Wilcoxon signed rank test. $p < 0.05$ value was considered statistically significant.

RESULTS
32 sites in 13 patients were evaluated, out of which all of them reported for the follow-ups . All subjects showed uneventful healing, and there was no allergic reaction to Spirulina.

Table I- Showing comparison of Plaque Index, Modified Sulcus Bleeding Index, Pocket Probing Depth and Clinical Attachment Level between group A and B

Clinical parameters	Group	Average Difference	IQR
Plaque index	Group A	0.25	(0.3-0.8)
	Group B	0.75	(0.2-1.0)
Sulcus bleeding index	Group A	1.0	(1.0-1.0)
	Group B	1.5	(1.0-2.0)
Pocket probing depth	Group A	2.0	(1.5-3.0)
	Group B	3.0	(3.0-4.0)
Clinical attachment level	Group A	2.0	(1.5-3.0)
	Group B	3.0	(3.0-4.0)

The above table depicts PPD and CAL improvement from baseline to day 45, which significantly differs between Groups A and B. The improvement is more statistically significant in group B (Chlorhexidine). PPD improvement was seen in group B (Chlorhexidine)= 3.0 when compared to group A(Spirulina) = 2.0. Also CAL improvement was seen in Group B=3.0 when compared to Group A(Spirulina)=2.0 .

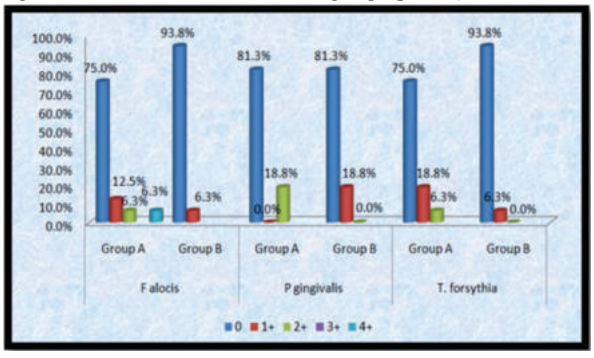


Graph I: Baseline to day 45 improvement seen in Group B in Plaque Index, Modified Sulcus Bleeding Index, PPD and CAL

Table 2: Showing comparison of specific microorganisms between groups A and B on Day 45

No. Of	Filifactor alocis		Porphyromonas gingivalis		Tannerella forsythia	
bacilli	Group A	Group B	Group A	Group B	Group A	Group B
0	75.00%	93.80%	81.30%	81.30%	75.00%	93.80%
1+	12.50%	6.30%	0.00%	18.80%	18.80%	6.30%
2+	6.30%	0	18.80%	0.00%	6.30%	0.00%
4+	6.30%	0	NIL	NIL	NIL	NIL

On microbiological analysis using FISH method, it was noted that there was comparatively better reduction in Filifactor alocis, Tannerella forsythia and Porphyromonas gingivalis in Group B (chlorhexidine) when compared to that of Group A (Spirulina) on the 45th day. However the improvement on day 45 showed no statistically significant difference between both the groups (p>0.05).



Graph 2: Showing Filifactor alocis, Porphyromonas gingivalis and Tannerella forsythia in group A and group B on day 45

DISCUSSION

Spirulina platensis (SP) is an emerging herbal remedy for chronic diseases and conditions that has been considered as safe by the United States Food and Drug Administration (FDA).[7] Spirulina's unique constitution contributes to anti inflammatory, immunomodulatory, anti-bacterial and antioxidant properties. Hence Spirulina has been recurrently cited in literature for its potential in the management of chronic periodontitis.

To the best of our knowledge, no study has been reported in literature comparing the efficacy of Spirulina gel with that of chlorhexidine gel in the management of chronic periodontitis. Hence the current study evaluated the effectiveness of 1% Spirulina gel subgingival application as an adjunctive therapy to scaling and root planing in chronic periodontitis patients in comparison with 1% chlorhexidine. The effect of each treatment modality on the periodontal pathogens and the clinical parameters were also evaluated. As very sparse literature is available on the utility of Spirulina platensis in the gel form in the management of chronic periodontitis, in this study, we have utilized Spirulina gel formulation method as suggested by Mahendra et al [2] by homogenization of Spirulina powder with gelatin to obtain suitable gel consistency at 1% concentration.

It was observed that both the groups assessed in this study showed reduction in all the assessed clinical parameters like Plaque Index, Modified Sulcus Bleeding Index, PPD and CAL at different time intervals when compared to baseline in both the groups.

Mahendra et al [2] reported similar results of reduction of PPD and CAL, which have been attributed to the anti-inflammatory characteristics of phycocyanin, a constituent pigment of spirulina, resulting in its ability to scavenge reactive oxygen species, as well as from its ability to suppress cyclooxygenase-2 activity and mast cell release of histamine. Phycocyanin exerts inhibitory effects on the release of tumour necrosis factor alpha, other cytokines and leukotrienes, especially Leukotriene B4, which has potent chemotactic properties on neutrophils. [8]

In the current study, on intergroup comparison of the clinical parameters, statistically significant reduction was seen with PPD and CAL in group B using 1% CHX gel when compared to group A using 1% SP gel, citing that CHX is the prevailing gold standard.

The present study also showed improvement in the plaque index at the end of 45th day. This is in accordance with the study conducted by Maniyar et al [3], which showed a significant reduction in the plaque index and gingival index scores using Spirulina mouthwash twice daily at the end of 7th day. The anti-gingivitis and anti-inflammatory effect of spirulina mouthwash can also be attributed to its component gamma-linolenic acid (GLA), where GLA can be metabolized to produce anti-inflammatory eicosanoids.[9,10] However the plaque index score in the present study showed no improvement from 21st day to 45th day in group A.

The current study also showed improvement in the Modified Sulcus Bleeding Index at the end of 45th day which could be attributed to its prominent anti inflammatory property due to its constituent phycocyanin. A study conducted by Butera et al[11] showed that the improvement of bleeding on probing was more evident in the biorepair parodontgel plus toothpaste group which contained Spirulina platensis extract along with lactoferrin, hamamelis virginiana leaf extract, calendula officinalis flower extract, and tocopheryl acetate; which is in accordance with the results of the present study.

The present study also showed reduction in the 3 periodontal pathogens assessed at the end of 45th day. In group A, except for Tannerella forsythia, all the other organisms showed statistically significant reduction; while group B showed statistically significant reduction with respect to all the assessed microorganisms. However on intergroup comparison, no statistically significant difference was noted.

Spirulina (arthrospira) platensis is able to inhibit the growth of a range of gram-negative and gram-positive bacteria as it shows high concentration of antimicrobial active lipids and active fatty acids. It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. [12]

Kaushik et al [4] evaluated Spirulina platensis for its antibacterial efficacy in an in-vitro investigation against gram-positive Staphylococcus aureus and four gram-negative bacteria, including Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, and Klebsiella pneumonia. When compared to other organic spirulina extracts, the Minimum Inhibitory Concentration of spirulina platensis' methanolic extract displayed strong antibacterial activity. In a pioneering in-vitro study conducted by Dharma et al [13], minimum inhibitory concentration and disc diffusion tests showed that Spirulina has antibacterial properties against Porphyromonas gingivalis and Tannerella forsythia; nevertheless it was shown that chlorhexidine still comparatively has superior antibacterial property, which was also concurrent in the present study.

The advent of FISH, which utilizes fluorescently labelled whole cell hybridization using 16 subtype ribosomal Ribonucleic acid oligonucleotide probes, has enabled detection and quantification of various fastidious novel pathogens which are directly involved in the pathogenesis of periodontal disease.[14] Of these, Filifactor alocis is directly implicated in the progression of periodontitis as it tends to co-exist with Porphyromonas gingivalis. The high frequency of Filifactor alocis in periodontitis may be explained by its distinct virulence traits, including tolerance to oxidative stress, generation of proinflammatory cytokines, and by secreting a host of proteases that participate in periodontal biofilm formation.[14] Hence in this study, FISH method was used to enable detection of Filifactor alocis, Porphyromonas gingivalis and Tannerella forsythia.

In a study conducted by Bhat et al [15], the rapid detection and quantification of Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola was enabled from the subgingival plaque samples collected from healthy individuals as well as those diagnosed with chronic periodontitis.

An overall effective clinical and microbiologic reduction noted in this study suggests the utilization of 1% SP gel as a potential herbal adjunctive agent in the non surgical management of chronic periodontitis.

Future Direction

In order to increase the overall substantivity of Spirulina at the periodontal pocket site, various carriers are now being devised in the form of micro particles and nanoparticles. The green synthesis of silver

nanoparticles described by Govindaraju et al. [16] includes the choice of a solvent medium, a degrading agent, and a non-toxic stabilising molecule or substance, also known as a capping agent, which prevents the nanoparticles from aggregating. Due to their low toxicity, these nanoparticles can be employed to contain therapeutic molecules.

Mahdieh et al. [17] prepared silver nanoparticles from Spirulina. The silver nanoparticles were formulated by taking 5g of completely washed Spirulina platensis biomass from a rapid change growth phase in a 250 ml, which can be seen as an important addition to the future of nanotechnology in periodontics.

CONCLUSION

From the present study it can be concluded that 1% Spirulina gel is effective against plaque and gingivitis. Its supplemental anti-microbial resulted in amelioration of the clinical and microbiological parameters in patients diagnosed with chronic periodontitis. Usage of appropriate culture-independent diagnostic techniques like Fluorescent In-Situ Hybridisation (FISH) method can help us in customisation of treatment approach in periodontal therapy. However the outcome of the study depends on the extent of patient motivation to follow the oral hygiene instructions post operatively, contributing to confounding bias. Also as the study is first of its kind in vivo, additional research will be required in the future with larger sample size.

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