



RESEARCH ARTICLE

EFFECT OF ARTHROSPIRA PLATENSIS (SPIRULINA) AS AN ADJUNCT TO SCALING AND ROOT PLANING ON SALIVARY ANTIOXIDANT LEVELS IN CHRONIC PERIODONTITIS SUBJECTS - A RANDOMIZED, DOUBLE-BLIND, CLINICO-BIOCHEMICAL TRIAL

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ABSTRACT

Purpose: Oxidative stress plays a causative role in pathogenesis of periodontal disease.

It has been reported that *Spirulina platensis* or its active ingredient C phycocyanin (CPC) exerts anti-inflammatory, anti-oxidative and inhibitory effects on prostaglandin and leukotriene biosynthesis. So aim of this study was to evaluate the effect of dietary spirulina as an adjunct to SRP on salivary antioxidants level in CP subjects.

Materials and Methods: The study was a randomized double-blind clinical and biochemical trial in which 70 subjects with CP in the age group of 30-55 years, reporting to Department of Periodontology, TKDC and RC, New Pargaon, were equally and randomly divided into Test Group (TG) and Control Group (CG). TG was given 2 gm Spirulina capsules daily, after meals for 1 month and CG was not given any supplementary diet. SRP was done at baseline in TG and CG. Whole saliva sample was collected. Clinical and biochemical parameters (SOD & MDA) will be recorded at baseline, 1 month & 3 months.

Result: Reductions in clinical & biochemical parameters were statistically highly significant in TG compared to CG after 1month ($P < 0.05^{**}$). Reduction in clinical & biochemical parameters were clinically significant but statistically non significant in TG group Compared to CG after 3month.

Conclusion: Spirulina appears to be promising. It exerts strong anti-inflammatory effects which are closely connected with its antioxidative activity. This study can have a significant impact on the treatment procedures of periodontitis, with the use of blue green algae in the future.

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INTRODUCTION

Chronic periodontitis (CP) is a plaque biofilm induced inflammatory process, leading to loss of periodontal attachment and supporting alveolar bone (Trivedi, 2014). The majority of periodontal destruction occurs due to an unsuitable host response to these pathogens (Lamster, 1992). Neutrophils and macrophages negate the periodontal pathogens with inherent oxidative and non oxidative mechanisms. Both neutrophils and macrophages experience a 'respiratory burst', the main feature of which is, an increase in oxygen consumption, activation of the hexose-monophosphate (HMP) shunt and production of free radicals (FR), reactive species and their metabolic products (Firatli, 1994). The sites of

chronic inflammation are characterized by considerable increase in the production of FR and reactive oxygen species (ROS). Whenever there is insufficiency of antioxidant defense and an overproduction of FR or a fall in level of antioxidants it will lead to an imbalance which causes deleterious effects known as oxidative stress (OS) (Brock, 2004). *Spirulina* is a free-floating filamentous microalgae with spiral characteristics of its filaments. It is formally called *Arthrospira*. They belong to the class of cyanobacteria which has characteristic photosynthetic capability (Sapp, 2005, Kom'arek, 2009). Spirulina contains many important nutrients, which include protein, complex carbohydrates, iron, and vitamins A, K, B complexes chlorophyll, fatty and nucleic acids, and lipids. It has a high supply of carotenoids such as beta carotene and yellow xanthophylls which have antioxidant properties. It also contains other nutrients such as iron, manganese, zinc, copper, selenium, and chromium. These nutrients help in combating free radicals, cell-damaging molecules absorbed by the body

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through injury, pollution, poor diet, or stress (Robert Henrikson 1998). One of the major pigment constituents of Spirulina is Phycocyanin (Pc), whose nutritional and therapeutic values have been very well known (Bhat, 2000; Kay, 1991). It was found that *Spirulina* dose-dependently inactivated FRs which was generated during an oxidative burst (Dartsch PC 2008). *Spirulina* also alleviates the metabolic activity of functional neutrophils. Treatment of CP includes primarily scaling and root planing (SRP). Any other therapy should be adjunctive and never in lieu of it. Therefore the goal of the present study was that clinical oral administration of a natural antioxidant in the form of Spirulina capsules can be beneficial in reducing OS as an adjuvant in treatment of CP along with SRP.

MATERIALS AND METHODS

A double blind randomized clinical study was carried out on eligible patients who reported to the Department of Peridontology, Tatyasaheb Kore Dental College & Research Centre, New Pargaon. Prior approval for the study was obtained from the Local Ethical Committee. Clinical trial is registered at www.clinicaltrials.gov. (NCT02886676)

Sample size

Considering SD 15% with confidence level 95% and power of study 80%, the minimum required sample size was calculated as 35 for each group.

Eligibility criteria

Inclusion criteria included subjects with CP (moderate and severe) as per CDC criteria 2007 (Page RC 2007) without any known systemic diseases. Patients who have taken anti-inflammatory/antibiotic/antioxidant within 3 months of beginning of study, subjects who have undergone periodontal treatment within six months of inception of study, patient having tobacco habits in any form and alcoholics, pregnant and lactating women were excluded from the study. Participants were recruited from May 2015 to May 2016 & attended clinic visits at baseline, 1month & 3months.

Randomization

The treatment group (TG) & control group (CG) of 35 participants each were created through randomization in accordance with 2010 CONSORT (Moher D 2012) guidelines. Single study coordinator randomized patients into two groups by using Quick Cals, Graphpad software and was stratified with a 1:1 allocation. Allocation was done using lottery method of randomization. The study was explained, including the benefits, risks, and alternative treatments, the patients signed an informed consent form indicating their agreement to participate in the study, and each patient was assigned a patient number in ascending order to maintain the masking of evaluators. For e.g. first patient was given a number D1, Second D2, and so on.

Intervention

Patients in TG will be provided with Spirulina capsules 2gm daily, after meals for 1 month. Patients in CG will not be provided any supplementary diet. The study co-ordinator

prepared packets of spirulina capsules and served them to patients according to randomization done. SRP was performed by one trained Periodontist under local anaesthesia (if required) using Piezoelectric scaler (Satelec ACTEON P5™), Hand scalers, Universal curette and Gracey Curette. Clinical and biochemical parameters were recorded by another trained examiner. Instructions regarding oral hygiene maintenance were reinforced in both the groups. Patients from both groups were recalled after 1 month & after 3 months for saliva collection, biochemical investigation and recording clinical parameters. All study personnel, including examiner, biostatistician, study co-ordinator and participants were kept blinded. Assessment of patients was done based on various clinical parameters with laboratory values of Superoxide dismutase (SOD) & Malondialdehyde (MDA) in saliva. Clinical parameters and laboratory values of SOD and MDA were measured at baseline, after 1month and 3 months. Clinical parameters assessed for both CG and TG were, Plaque Index (PI)-Loe & Silness (1964), Gingival Index (GI)-Silness & Loe (1963), Sulcus Bleeding Index (SBI) – Muhleman's (1971), Pocket Depth (PD), Clinical Attachment Level. (CAL). CAL measurements were recorded at six sites around each tooth (i.e. mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) from the cemento-enamel junction to the base of the pocket. All clinical measurements were recorded by a single trained investigator using University of Carolina (UNC-15 probe). The investigator was blinded to the study subjects of both the groups. Salivary SOD was measured by Kazari Das method. Salivary MDA was measured by Kei Satoh method.

Statistical analysis

Descriptive statistics were expressed as mean \pm standard deviation (SD) for each group. The change in mean scores of various parameters over a period of 3 months in each group was analyzed using repeated measures ANOVA test. Intergroup comparison was done using unpaired t test. In the above tests, p value less than or equal to 0.05 ($p \leq 0.05$) was taken to be statistically significant. All analyses were performed using SPSS software version 17.

RESULTS

Seventy patients comprising of males and females with an age range of 30 to 55 years (mean age: 40.7 years) were included in this study. Subjects were classified into two groups (35 patients in each group). None of the patients reported of any change in their general health or medical status during the period of the study. One patient from the TG and two patients from CG did not maintain follow-up after first visit, so were considered as drop-outs. (Figure 1)

The demographic distribution within the TG & CG revealed no statistical differences between groups with respect to age & sex ($p > 0.05$) (Table 1). As previously stated, all clinical and biochemical parameters were assessed in both groups at the baseline and again in 1month, & 3months after treatment. At baseline, no significant differences between the groups were found among any of the clinical parameters and salivary SOD levels (Table 1). After thorough SRP followed by regular maintenance, the level of plaque control in all the patients was satisfactory. Throughout the study, plaque accumulation was minimal with no significant differences between the two groups.

FIGURE 1: PARTICIPANT FLOW CHART

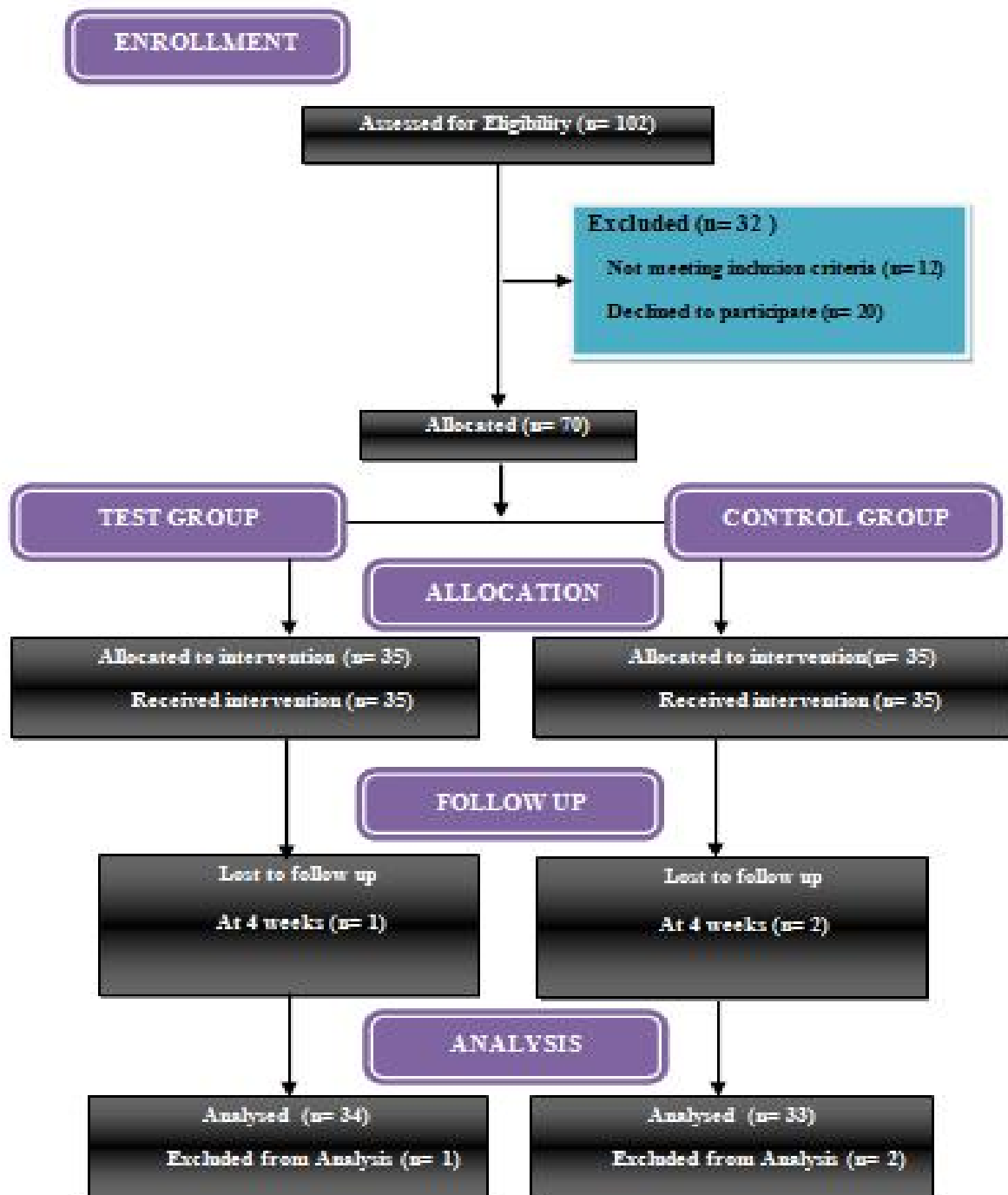


Table 1. Demographics of subject population and baseline periodontal parameters

Baseline characteristics	Test Group(n=35)	Control Group (n=35)
Age(year)	41.40±5.93	42.70±4.82
Plaque index	2.79±0.07	2.75±0.14
Gingival index	2.70±0.23	2.80±0.10
Sulcus bleeding index	4.08±0.26	4.08±0.27
Pocket depth	5.21±0.47	5.22±0.48
Clinical attachment level	5.70±0.18	5.73±0.22
Superoxide dismutase	2.49±0.42	2.47±0.40
Malondialdehyde	7.30±0.40	7.28±0.38

Values are presented as mean± standard deviation.

There were no significant difference between two groups ($p>0.05$)

Table 2. Full mouth values for periodontal measurement before & after treatment

Parameters	Test Group (n=35)	Control Group (n=35)	(p-value)
Plaque index			
Baseline	2.79±0.07	2.75±0.14	0.912
1month	2.62±0.14	2.64±0.13	0.620
3 month	2.60±0.07	2.55±0.14	0.780
Gingival index			
Baseline	2.70±0.23	2.80±0.10	0.912
1month	1.54±0.20	1.78±0.12	0.001x
3 month	1.35±0.20	1.42±0.12	0.986
Sulcus bleeding index			
Baseline	4.08±0.26	4.08±0.27	1.00
1month	1.37±0.18	1.68±0.25	0.001x
3 month	1.20±0.18	1.30±0.25	0.130
Pocket depth			
Baseline	5.21±0.47	5.22±0.48	0.992
1month	3.62±0.31	3.77±0.25	0.032x
3 month	3.58±0.31	3.63±0.25	0.566
Clinical attachment level			
Baseline	5.70±0.18	5.73±0.22	0.891
1month	4.21±0.22	4.52±0.33	0.001x
3 month	4.00±0.42	4.11±0.33	0.817
Superoxide dismutase			
Baseline	2.49±0.42	2.47±0.40	0.932
1month	3.71±0.29	3.48±0.29	0.041x
3 month	3.82±0.30	3.62±0.29	0.142
Malondialdehyde			
Baseline	7.30±0.40	7.28±0.38	0.910
1month	5.22±0.41	5.46±0.24	0.036x
3 month	5.12±0.41	5.16±0.24	0.745

x- p<0.05

No statistically significant difference was found for PI between the TG and CG after 1 and 3 months. The GI, SBI, PD, CAL, & MDA significantly decreased in the TG compared to the CG after 1 month ($P<0.001$). These parameters were not statistically significant between groups (intergroup) at 3 months of the study. SOD value significantly increased after 1 month & 3 month in both groups. But statistically significant difference found in both groups at 1 month ($p<0.04$) (Table 2).

DISCUSSION

Periodontitis, a common impending source of low-grade inflammation, is associated with a systemic OS state and reduced global anti-oxidant capacity. A direct, although weak, linear correlation among clinical measures of periodontitis, systemic inflammation, and systemic OS may exist (D'Aiuto, 2010). OS has been associated with both start of periodontal tissue destruction and systemic inflammation (Volchegorskii, 2010). OS is worse during periodontitis. Evidence has claimed that the imbalances in the levels of FR and antioxidants in saliva may play an important role in the onset of PD; therefore measurement of OS in saliva may provide an accurate account of oral environment (Giustanini, 2009). Saliva may constitute a first line defense against FR-mediated OS, since the process of mastication promotes a variety of such reactions including lipid peroxidation (LPO). Human saliva plays an important role in maintaining the physiological conditions of oral tissues. During gingival inflammation GCF flow increases, adding to saliva with products from the inflammatory response. That is why the antioxidant capacity of saliva is of increasing interest (Sculley, 2002). SOD is a significant antioxidant enzyme within mammalian tissues, which catalyzes the dismutation of O_2^- to H_2O_2 and O_2 . SOD has also been localized within the human PDL and may represent an important defense mechanism within gingival cells against O_2 release. LPO has been implicated in the pathogenesis of several pathological disorders including PD. ROS can attack polyunsaturated fatty

acids (PFA) and induce formation of LPO products such as MDA. It is a stable end product of LPO by ROS. Treatment of PD reduces OS by a simultaneous reduction in inflammatory load by enhancing antioxidant levels, irrespective of the medium, GCF or saliva. There was an increase of the antioxidants concentration post-treatment showing a definite association between even minor levels of inflammation and sensitivity of the antioxidant system. Even minimal levels of OS are perceived and the protective antioxidant mechanism is set into action, which is essential for the maintenance of the structural integrity of proteins (Kohen, 2002, Terao, 1991, Bartold, 1986). The significance of the host inflammatory response in the pathogenesis of PD presents an opportunity for exploiting new treatment strategies via host response modulation. Studies have indicated altered clinical parameters, an imbalance of antioxidant levels and OS situation in CP as compared to healthy controls (Chapple, 2007). In the present study, on intragroup comparison for PI, GI and SBI; both TG and CG showed statistically significant difference from baseline to 1 month & 3 months. On intergroup comparison, PI did not show statistically significant difference whereas statistically significant reduction was observed for GI and SBI in TG when compared with the CG.

On intragroup comparison, mean PD in TG and CG significantly reduced from baseline to 1 month & 3 months. On intergroup comparison between TG and CG, TG showed reduction in PD as compared to CG but it was not statistically significant at 3 months. Also within the TG and CG mean CAL showed significant gain from baseline to 1 month. On intergroup comparison however, the gain in CAL was not statistically significant in TG when compared with the CG at 3 months. Similar findings were observed in a study by Novaković *et al.* (Novaković, 2013). The NSPT was effective in improving clinical parameters and reducing ROS. This was in accordance with studies by Tamaki *et al.* (Tamaki 2011) Karim *et al.* (Karim, 2012) Present study showed

decreased SOD activity in CP patients. The salivary SOD levels significantly increased in both TG and CG from baseline to 1month & 3months. On intergroup comparison, statistically significant increase in SOD levels was seen in TG after 1 month when compared to the CG. In contrast to our findings, various studies conducted by Panjamurthy *et al.* (Panjamurthy, 2005), Wei *et al.* (Wei, 2010) have observed higher SOD activity in chronic periodontitis group than in controls. In a study done by Wei *et al.* (Wei, 2010) Had shown that MDA and SOD both increased during OS. LPO was higher in the periodontal disease sites while SOD increased locally and peripherally. However, consistent with our finding, Ellis *et al.* (Ellis, 1998) found a significant and progressive reduction in SOD activity with the chronic periodontitis patients. Similar results with Akalin *et al.* (Akalin, 2007) also found that SOD activity was lower in periodontitis groups than the matched CG and type 2 diabetes mellitus patients with healthy PDL had the highest, but the CP group had the lowest SOD levels. Present study showed that SOD levels increased after NSPT and results in TG were higher as compared to CG. This is in accordance with study by Karim *et al.* (Karim, 2012) where in the SOD levels significantly improved post NSPT after 15 days. On intragroup comparison the MDA levels significantly reduced from baseline to 1month & 3month in both TG and CG. When TG was compared to CG, the MDA levels significantly reduced in TG as compared to the CG after 1month. Khalili *et al.* (Khalili, 2008) and Tonque MO *et al.* (Tonguç, 2011) have also observed higher MDA levels in CP.

In the present study post treatment MDA levels were in accordance with Wei *et al.* (Wei, 2010) and Tsai *et al.* (Tsai, 2005) who has reported that LPO was significantly correlated with clinical parameters of PD, and periodontitis patients showed significantly lower LPO concentration after treatment than before therapy. Diab Ladki *et al.* (Diab-Ladki, 2003) Salivary antioxidant levels were observed to be lower in periodontitis patients. Markers of oxidative damage such as MDA were found to be higher in saliva of patients with periodontitis which decreased following initial treatment approaching mean control values. In our study, a significant decrease in PPD was seen in both the groups; similarly, gain in CAL was seen in both the groups at the end of the study where TG showed significant improvement compared to CG. In our study there was improvement in clinical parameters in TG compared to CG. This was similar to the study done by J. Mahendra *et al.* (Mahendra, 2013) in which they confirmed that Group A (SRP along with spirulina) showed statistically significant decrease in mean PPD and gain in the CAL after 120 days as compared to Group B SRP alone. Pc in spirulina is the main anti-inflammatory and anti-arthritis agent in microalgae, which exerts strong anti-inflammatory effects (Romay, 2003) In addition, Panigrahi *et al.* (Panigrahi, 2011) showed a significant improvement in the wound healing activity with use of spirulina in an vitro study. One of the advantages of using spirulina capsules is that unlike other NSAIDs, spirulina seems to have low toxicity and lack of adverse effects. The favorable characteristics of Pc are mainly as follows:

- 1) A distinct cyto and tissue-protective potential, in particular against oxidative stress.
- 2) An anti-inflammatory potential which is caused by a set of multi-site actions such as, scavenging of various ROS, anti-lipoperoxidative and inhibitory effects on both pathways of AA metabolism (COX-2 inhibitor),

inhibition of Hi release from mast cells and inhibitor of TNF-alpha), taking into account that Pc is a major constituent of microalgae Spirulina. So it exerts the therapeutic effects when it is administered alone or included in the microalgae used as dietary supplement (Romay C 2003).

Conclusion

From the conducted study, it can be concluded that Spirulina capsules shows an inverse relationship with periodontitis. SRP plus spirulina capsules have favorable results in chronic periodontitis. It is also important to lay additional emphasis in modulation of host response while treating periodontitis. Spirulina appears to be promising. It exerts strong anti-inflammatory effects which are closely connected with its antioxidative activity, low toxicity and lack of adverse effects. In support RCTs with larger sample size and longer duration should be carried out. If spirulina found effective, this therapeutic modality may prove to be a cheaper and safer adjunctive for the prevention and treatment of periodontitis.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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