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Original Article

EFFECTIVENESS OF SPIRULINA MOUTHWASH ON REDUCTION OF DENTAL PLAQUE AND GINGIVITIS: A CLINICAL STUDY

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ABSTRACT

Objective: The present study evaluated the effectiveness of *Spirulina* mouthwash on the reduction of dental plaque and gingivitis.

Methods: A single-blind clinical trial was conducted among thirty patient's aged 18-40 y visiting dental college and hospital in Bangalore city. Mouthwash was prepared using 0.5% *Spirulina*. Intervention protocol consisted of instructing the patients to rinse with 10 ml of mouthwash for 1 minute twice daily for 7 d. Plaque index and Gingival index were used to assess the variables at the baseline and after the intervention. The perception of the individual subjects with regard to the use of mouthwash was assessed using 10 cm long visual analog scale (VAS). Statistical analysis was carried out using Wilcoxon signed rank test for mean pre and post plaque and gingival scores respectively. Descriptive statistics was performed for VAS questionnaire

Results: The results showed a highly significant difference ($p<0.001$) between the mean plaque scores at the baseline (2.16±0.34) and at the follow up (1.27±0.46). The mean gingival scores at the baseline (1.86±0.38) and at the follow-up (1.05±0.43) also showed a highly significant difference ($p<0.001$). Regarding the Visual Analog Scale, the mean values of 5 or greater than suggested the responses to be favourable as the values were reflected

Conclusion: The study showed that *Spirulina* mouthwash resulted in significant reduction in dental plaque and gingivitis. Also, the mouthwash was convenient to use without any adverse effects. Hence, the use of herbal mouth rinses such as *Spirulina* should be supported.

Keywords: Gingivitis, Herbal mouthrinse, Phycocyanin, *Spirulina*

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INTRODUCTION

The advances in science and technology have made treatment of general and oral health diseases now available to human mankind, thus increasing the longevity of life along with retention of teeth. WHO recently published a global review of oral health which emphasized that despite great improvements in the oral health of populations in several countries, global problems still persist [1]. The incidence of gingival and periodontal diseases is rising steadily affecting all the age groups. Dental plaque is a host associated biofilm, which exists as soft deposits adhering to tooth surfaces or other hard surfaces in the oral cavity, and consists of bacteria embedded in a matrix of polymers of bacterial and salivary origin [2]. There is a causal relationship between dental plaque and gingivitis that was established decades ago [3]. Clinical control of these diseases can most readily be achieved by reducing the oral microbial load of the plaque biofilm [4]. Thus, plaque reduction remains the mainstay of preventive dentistry. For this various mechanical plaque control measures have been practised.

Despite the potential for adequate mechanical plaque control, clinical experience and population-based studies demonstrate that such methods are not being employed sufficiently by large numbers of the population as it requires time, motivation and manual dexterity [5, 6]. As an adjunct to this, chemical plaque control measures have been employed which includes the use of various anti-plaque agents and mouthwashes. Such products influence plaque accumulation by preventing bacterial attachment and removing bacterial biofilm [4]. Various mouthwashes are available containing triclosan, metronidazole, chlorhexidine and many more. The use of these agents has certain unpleasant effects such as altered taste sensation, staining of teeth which often deters its use. Hence, the use of plants and plant products can be an alternative solution to this.

Phytotherapy has been widely practiced in India since ages. *Spirulina* is a *cyanobacterium* or *blue-green algae* (BGA) that is associated with a wide range of nutritional and health benefits. *Spirulina* is known to have an antimicrobial effect against *S. aureus, E. coli, P. aeruginosa, Klebsiella sp, Proteus sp* and *Embedobacter sp* [7]. At present, there is a mass of evidence in favour of the antioxidant properties of Phycocyanin, a major pigment of *Spirulina* which has been used to explain its anti-inflammatory effects.

Gingivitis is an inflammatory disease induced by bacterial biofilms that accumulate in the gingival margin, in which a series of inflammatory responses are initiated by pathogenic bacteria and ultimately results in periodontal breakdown if not treated at the initial stage. So far in dentistry, *Spirulina* was assessed for its oxidative properties in the healing of oral submucous fibrosis and leukoplakia, but its effect on plaque reduction and gingivitis remains unexplored. Thus, this is the first study of its kind assessing the effectiveness of *Spirulina* mouthwash on the reduction of dental plaque and gingivitis.

MATERIALS AND METHODS

The present study was a single-blind clinical trial conducted to assess the effectiveness of mouthwash containing *Spirulina* on the reduction of dental plaque and gingivitis measured using Plaque index [8] and Gingival index [9].

The study was conducted among the patients visiting a dental hospital in Bangalore.

Ethical clearance (EC-393) was obtained from the Institutional Ethics Committee of the dental college and hospital. Informed consent was obtained from the participants after explaining the methodology, benefits and adverse effects of the study. The required sample size was estimated based on the difference in the pre and post plaque and gingival scores among the study group. It was calculated based on the minimum difference of 0.5 expected between the pre and post scores among the study group which was 25. Considering the loss to follow-up, the sample size was rounded off to 30. The inclusion and exclusion criteria are as follows:

Inclusion criteria

Individuals aged 18-40 y with a minimum of 20 teeth present, with bleeding on probing present clinically, with at least fair plaque and mild gingival scores respectively, and who had not received any periodontal therapy for the past 6 mo.

Exclusion criteria

Individuals with probing depths>4 mm, undergoing orthodontic treatment, wearing a removable prosthesis, with a history of systemic disease, or had taken any systemic/topical antibiotics during the past 3 mo and who were currently using any mouthwash or have used mouthwash in past 15 d were excluded. Pregnant women, lactating mothers, smokers, alcoholics and subjects with a history of allergy to any chemical or herbal product were also excluded from the study.

The investigator was trained and calibrated before the start of the study in order to limit the intra-examiner variability. The intra-examiner variability was calculated using kappa statistics. The Cohen's kappa value was 0.82 for plaque index and 0.85 for gingival index.

The plaque index and gingival index scores were recorded at the beginning of the intervention to obtain the baseline data, then the subjects were instructed to use the mouthwash for one week. The plaque scores and gingival scores were reassessed at the end of one week to obtain the final scores. The type III clinical examination was followed throughout the study.

The participating subjects were asked to fill a questionnaire using a visual analog scale (VAS) designed to evaluate their perception with regard to the mouthwash used. Subjects were asked to mark a point on a 10-cm-long uncalibrated line with the negative extreme response (0) at the left end and the positive extreme response (10) at the right end [10].

Intervention

The mouthwash was prepared using 0.5% Spirulina with the help of pharmaceutical agency in Bangalore, India. Blinding was carried out by giving the mouthwash in an amber coloured bottle without any labelling to the participating subjects. They were asked to swish the oral cavity using 10 ml of the mouthwash for 1 minute, twice a day, after breakfast and dinner. The subjects were instructed not to eat or drink anything for at least one hour after rinsing with mouthwash. The subjects were reminded from time to time for a period of one week. The contact number of the investigator was provided to the subjects to report any inconvenience or adverse effect observed if any. No oral prophylaxis was done prior to commencement of intervention. They were allowed to follow their individual oral hygiene procedures.

Statistical analysis

The statistical analysis was performed using SPSS version 22.0. The mean plaque and gingival scores between pre and post intervention were compared using Wilcoxon signed rank test respectively. Descriptive statistics was performed for VAS questionnaire.

RESULTS

All participants (n=30) completed the trial and there were no missing values. There was an equitable distribution regarding the gender among the participants (M = 15; F = 15). The age of the participants ranged between 18-40 y with mean age being 28.5±7.3.

The mean plaque scores at the baseline were 2.16±0.34 and after seven days, at follow-up were 1.27±0.46. (fig. 1) Statistical analysis using Wilcoxon signed rank test showed that there was a highly significant difference ($p \leq 0.001$) between the scores at baseline and follow-up indicating the effectiveness of *Spirulina* on the reduction of dental plaque.

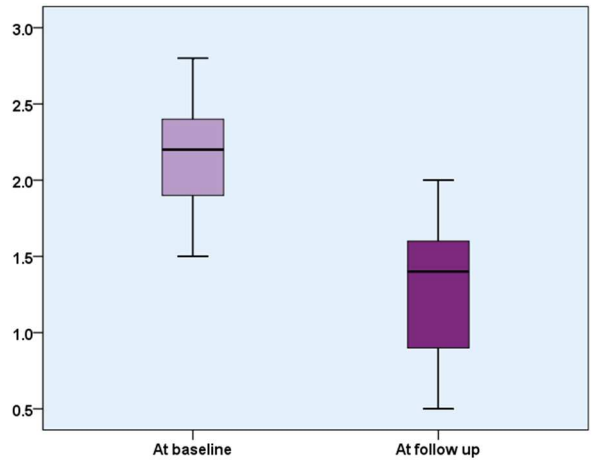

Fig. 1: Comparison of mean plaque scores before and after the intervention

The mean gingival scores at baseline were 1.86±0.38 and at follow-up were 1.05±0.43. The difference between the scores was seen to be highly significant ($p \leq 0.001$) using Wilcoxon signed rank test.(fig. 2)

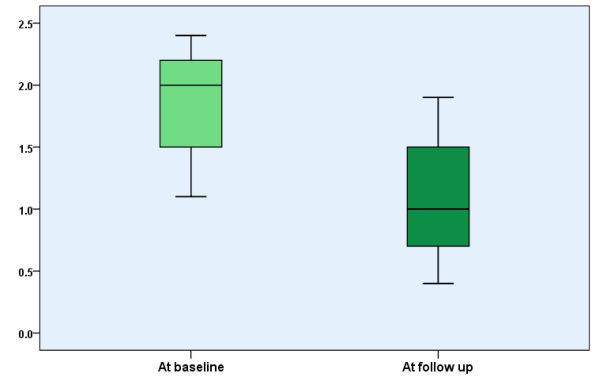

Fig. 2: Comparison of mean gingival scores before and after intervention

The subjects filled the VAS questionnaire at the end of the experimental period of 7 d. It comprised of four questions and the subjects were asked to mark a point on 10 cm long scale with the negative response at the extreme left and a positive response at the extreme right end of the scale (table 1).

Table 1: Questionnaire responses (mean±SD) determined by visual analogue scale (VAS)

Question	Response	MEAN±SD
1) How was the taste of the mouth wash?	Unacceptable……. Acceptable	5.0±0.5
2) For what duration did the taste remain in mouth after rinsing?	Long ……. Short	6.3±0.7
3) Was the taste of food and drinks affected?	Altered ……Unaltered	7.6±1.4
4) Was the use of mouthrinse convenient?	Inconvenient ……. Convenient	6.3±0.8

The mean values of 5 or greater than 5 suggested the responses be favourable, as the mean values were reflected towards the right end of the Visual Analog scale. With regard to the question on the taste of the mouthwash, the mean values were 5±0.5. When asked regarding the duration the taste remained in mouth after rinsing, the mean values were 6.3±0.7, suggesting the duration to be more towards short response. With regard to the question on whether the taste of the food was affected, the mean values were 7.6±1.4, suggesting that the taste remained unaltered. Lastly, regarding the convenience of the use of mouthwash, the mean values were 6.3±0.8, suggesting that the mouthwash was convenient to use.

DISCUSSION

The traditional methods of phytotherapy are making a comeback and an era of herbal renaissance is being revolutionised once again. The World Health Organization estimates that 65-80% of the world's population use traditional medicine as the primary form of health care [11]. Similarly, use of herbal products and dietary supplements is an emerging trend in dentistry.

Studies have shown that mechanical plaque control measures are inadequate, thus paving a pathway for the use of herbal products which are free from the adverse effects as seen by the use of synthetic chemicals. *Blue-green algae*, among the earliest life forms on earth and have been a source of food or medicine for humans since centuries. Certain BGA species include *Aphanizomenon flos-aquae* (AFA), *Spirulina platensis* (SP), *Spirulina maxima* (SM), and *Spirulina fusiformis* (SF) [12].

Spirulina is a microscopic *blue-green alga* in the shape of a spiral coil, living both in the sea and fresh water. *Spirulina* is the common name for human and animal food supplements produced primarily from two species of cyanobacteria: *Arthrospira platensis*, and *Arthrospira maxima*. It is an edible, filamentous, alkalophilic, photoautotrophic cyanobacterium belonging to the class *Cyanophyta.* It is a rich source of many important nutrients like proteins, complex carbohydrates, iron, vitamins A, K, B complexes, minerals, lipids and essential fatty acids [13-15].

Spirulina is considered as "nature's superfood". This study showed that the *Spirulina* mouthwash was effective in reducing dental plaque scores significantly when compared to the baseline scores. This could be attributed to the antimicrobial compounds found in cyanobacterial exudates which include polyphenols, fatty acids, glycolipids, terpenoids, alkaloids, carotenoids and a variety of bacteriocins. Harder was the first to observe antimicrobial substance secreted by alga. It has also been reported that they produce substances that can inhibit microbial growth. Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects. Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance [13, 16, 17].

The results of this study are in line with the *in vitro* study done by Sujatha *et al.* who reported the antibacterial activity of green seaweeds on oral bacteria. The biofilm inhibitory effect of *Spirulina* against a broad spectrum of gram positive and gram negative bacteria like *S. aureus*, *S. epidermidis*, *S. viridians*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *Vibrio* spp was also well documented by F Lewis Oscar *et al.* [18].

Antimicrobial active lipids and active fatty acids are present in a high concentration in this alga. It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane as well as bacteria, fungi and yeasts 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 [13].

This study showed a significant anti-gingivitis and anti-inflammatory effect of *Spirulina* mouthwash, which can be attributed to the important constituents, phycocyanin (PC) and gamma-linolenic acid (GLA). *Spirulina* contains 1.3% GLA and C-PC is a natural blue pigment accounting for 14% of *Spirulina*'s dry weight [12].

PC is thought to suppress inflammation by inhibiting the production of pro-inflammatory cytokines and by inhibiting the expressions of inducible nitric oxide synthase and cyclo-oxygeanase. GLA can be metabolized to dihomo-GLA that undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids [12, 19].

Bhat and Madyastha reported that phycocyanin inhibited about 95% of peroxyl radical-induced lipid peroxidation. The *in vitro* evidence also supports that C-PC has strong antioxidant properties by scavenging radicals and inhibiting lipid peroxidation in cell membranes [12, 20].

Lipid peroxidation mediated by Reactive Oxygen Species is believed to be an important cause of destruction and damage to cell membranes because a simple initiating event can result in the conversion of hundreds of fatty acids side chain into lipid peroxides, which alters the structural integrity and biochemical functions of membranes. It has been revealed that lipid peroxidation levels are increased during gingivitis and periodontitis. Phycocyanin also attenuated PGH2-induced Thromboxane B2 formation and platelet aggregation, implying that phycocyanin may also be a thromboxane synthase inhibitor. It also has been shown to increase the expression of essential enzymes and biochemical such as cytochrome p-450, superoxide dismutase, catalase, alanine transaminase, aspartate transaminase which further leads to the detoxification [21].

Lipopolysaccharide (LPS), an endotoxin produced by gram-negative bacteria, stimulates the metabolism of arachidonic acid. This, in turn, activates lipooxygenase and cyclo-oxygenase inflammatory pathways. LPS can also affect macrophages, monocytes, fibroblasts and, as a consequence, leads to the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β. These are amongst the most important pro-inflammatory cytokines and play a critical role in the destruction of periodontal tissue, alveolar bone, and eventually tooth loss. Also, IL-1β and TNF-α can induce the destruction of connective tissue [22]. Animal studies done with rodents showed that phycocyanin has anti-inflammatory activity due to prostaglandin E-2 inhibition and that it reduces allergic inflammatory response and histamine release from cells [23, 24].

A study done by Mahendra *et al.* showed that subgingivally delivered *Spirulina* gel resulted in a decrease in pocket probing depths as well as gain in clinical attachment levels in chronic periodontitis patients [25].

According to Miranda *et al.*, the main phenolic compounds found in *Spirulina* were salicylic, trans-cinnamic, chlorogenic, quinic and caffeic acids. These compounds are used to produce flavonoids, which possess antioxidant activity [26].

The human clinical study showed that a hot water extract of *Spirulina* rich in phycocyanin increased interferon production and NK cytotoxicity (cancer killing cells) when taken orally [27].

The results of this study could not be compared with other studies as an exploration of the available literature revealed that no studies have been carried out till date to assess the same effect *in vivo*.

CONCLUSION

The *Spirulina* mouthwash was effective in reducing dental plaque and gingivitis. *Spirulina* appears to be a promising agent with a wide array of antibacterial, antioxidant, anti-inflammatory and anti-fungal properties with low toxicity and minimal side effects. Thus, the use of herbal mouthrinse such as *Spirulina* should be supported.

As this was the first attempt to evaluate the effectiveness of *Spirulina* mouthwash on plaque and gingivitis, clinical trials of longer duration with a larger sample size should be conducted.

Also *in vitro* studies should be carried to understand the exact antimicrobial mechanism of *Spirulina* on pathogens playing an important role in gingivitis and periodontitis.

The effects of this mouthwash should also be compared with the benchmark control i.e. chlorhexidine. Further longitudinal studies and clinical trials of longer duration should be carried out to evaluate its safety.

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AUTHOR'S CONTRIBUTION

First author: Dr. Radhika Maniyar: conception and design, acquisition of data, or analysis and interpretation of data and drafting the article

Second author: Dr. Umashankar GK: supervision, proofreading and final approval

CONFLICTS OF INTERESTS

Declared none

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