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RESEARCH ARTICLE

POMEGRANATE SUPPLEMENTATION (40% ELLAGIC ACID) AS AN ANTIOXIDANT IN THE TREATMENT OF SMOKERS WITH CHRONIC PERIODONTITIS

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ABSTRACT

Smoking is considered to be a classical risk factor for periodontitis. It leads to a subsequent state of circulating lipid peroxidation byproducts in blood, such as lipid hydroperoxides and superoxides resulting in oxidative stress worsening periodontal health. In the past, studies have proved that the intake of fruits and vegetables can reduce oxidative damage in patients who smoke. Ellagic acid, a primary constituent of pomegranate fruit is one of the natural polyphenols with potent antioxidant capacity. In the present study we evaluated the effect of 40% ellagic acid supplementation on total antioxidant capacity and periodontal status in heavy and light smokers with chronic periodontitis. This was a randomized controlled trial with 40 smoker patients as part of the study. The patients were divided into two major groups (20 patients each): GROUP A - heavy smokers and GROUP B - light smokers. At baseline, clinical measurements and total antioxidant capacity were evaluated. Scaling and root planing was performed for all patients. Each of the major were then groups then were randomized into two subgroups each (A1, A2, B1 and B2).Groups A1 and B1 were given 40% ellagic acid supplementation, while A2 and B2 were not. Clinical parameters and total antioxidant capacity were reevaluated after 1 month. There was a significant improvement seen in the total antioxidant capacity (TAOC) in all the groups.This improvement was significantly higher in the groups which received the adjunctive ellagic acid supplementation. Within the groups; TAOC was maximum in light smokers who received the supplementation (B1) followed by heavy smokers who received the supplementation (A1). Significant improvements were seen in the clinical parameters in all groups. The results of the study show that the supplementation of Ellagic acid as an adjunct to scaling and root planing shows a promise in reducing the periodontal destruction and the total antioxidant capacity.

INTRODUCTION

Since time immemorial, periodontal disease has been remorseless towards human dentition. It starts with the inflammatory lesions of the gingiva, which, if left untreated, may progress and eventually involve and compromise the entire periodontal attachment of the affected teeth leading to tooth loss (Page RC *et al.*, 1997) Periodontal disease is initiated by the plaque biofilm (Axelsson *et al.*, 2002), but most tissue destruction results from an abnormal inflammatory immune response in patients predisposed to the condition (Page RC *et al.*, 1997). The response is characterized by hyperinflammation, which fails to remove causative pathogens and generates prolonged release of neutrophils proteolytic enzymes, proinflammatory mediators and reactive oxygen species (ROS), which in turn destroy the periodontal attachment (Ian LC *et al.*, 2007).

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The term "reactive oxygen species", has been adopted to include molecules such as hydrogen peroxide, hypochlorous acid and singlet oxygen, which whilst radical in nature, are capable of radical formation in the extra and intra cellular environment. They cause tissue destruction by variety of mechanisms including DNA damage, lipid peroxidation, protein damage, oxidation of important enzymes (anti-proteinases) and stimulatory pro – inflammatory cytokine release (Chapple *et al.*, 1997). To combat such entities, the human body is blessed with variety of inherent antioxidant mechanisms. Antioxidants maybe regarded as "those substances which when present at low concentrations, compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate" (Halliwell *et al.*, 1990). A rather popular term that summarizes the critical equation of reactive oxygen species and antioxidants is "OXIDATIVE STRESS". It is known to arise within tissues when the normal balance between ROS generation and antioxidant defense shifts in favor of the former, a situation arising from either an excess an excess of ROS and / or a

depletion of antioxidants resulting in a state of altered physiological equilibrium within a cell or tissue/ organ. (Halliwell *et al.,* 1989). Along with periodontitis, oxidative stress is also implicated in other chronic inflammatory diseases like type -2 diabetes (Evans *et al.,* 2002), vascular disease (Faraci *et al.,* 2005) and chronic inflammatory lung disease (Rahman *et al.,* 1996). Environmental factors are one of the many risk factors for periodontitis. Recent studies have shown tobacco use may be one of the most significant risk factors in the development and progression of periodontal disease because it promotes a high degree of ROS release, culminating in heightened oxidative damage to gingival tissue, periodontal ligament, and alveolar bone. Many studies have shown the effects of continued smoking on persistent gingival bleeding, vertical bone loss, and poor treatment outcomes (Ian *et al.,* 2007).Nicotine, the major component of tobacco smoke, has the potential to inhibit neutrophil ROS release in low doses. In contrast when present in high doses it actually stimulates ROS release that causes oxidative stress mediated tissue damage in gingivoperiodontal tissues (Mathews *et al.,* 2011). Antioxidant micronutrients are known to combat proinflammatory cascades through modulation of oxidative stress by directly scavenging reactive oxidative species and also by down regulation of some redox – sensitive pro inflammatory gene transcription factors and upregulating anti – inflammatory gene transcription factors (Ian *et al.,* 2012).

These natural antioxidant products have been tried in the past research aiming in reducing the oxidative burden to the human body and in the oral cavity, thereby improving the periodontal status. Antioxidant preparations have been used as part of oral care as mouthrinses, dentifrices, gels as well as direct systemic supplementation as encapsualated fruits, vegetable and berry juice concentrate, Amla, Noni, Lycopene and many other ingredients have been used effectively. Adjunctive juice powder concentrates has been used in patients with chronic periodontitis and have shown initial pocket depth reductions, and subsequent additional improvements in bleeding on probing and plaque scores (Ian *et al.,* 2012). In of the recent studies amla juice was given as regular supplementation in smokers and was beneficial for the improvements of antioxidant status which in turn reduces the risk of smoking related health hazard (Goswami *et al.,* 2013). Lycopene gel formulation has also been found to be effective in increasing clinical attachment and reducing gingival inflammation, probing depth, and oxidative injury compared with the placebo in smoking and nonsmoking subjects (Rampalli *et al.,* 2012). Pomegranate, Punica granatum, has been employed in the treatment and prevention of cancer, cardiovascular disease, erectile dysfunction and protection from ultraviolet radiation. Current research seems to indicate that of the most therapeutically beneficial pomegranate constituents ellagic acid (EA), including punicalagins, plays a major role (Julie Jurenka *et al.,* 2008). In vivo studies with EA have shown improvement in generalized antioxidant status of superoxide dismutase (SOD), and gluthione levels (Dong Hoon Han *et al.,* 2006). Although; this sacred fruit has been used in the treatment of periodontal disease in the past, its true potential has not been tapped. Previously, it has been most commonly used as a juice incorporated in a mouthrinse. The effect of this form on plaque microorganisms was tested in a clinical trial as an antiplaque mouthrinse effectively reducing Pg, Aa and Pi counts. Local drug delivery in the form of biodergradable chips have been made P.granatum and used to treat pockets of 5-8mm with significant improvement (Smruti *et al.,* 2011). We hypothesize that the supplementation of ellagic acid as an adjunct to scaling and root planing in smokers with periodontitis in reducing the oxidative stress caused due to smoking as well as periodontitis. Hence, with the given background, the present study was conducted to evaluate efficacy of ellagic supplementation (obtained from pomegranate seeds) on total antioxidant capacity, and the treatment of periodontitis in smokers with chronic periodontitis.

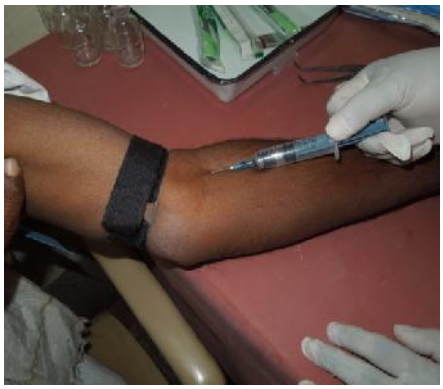
Objective

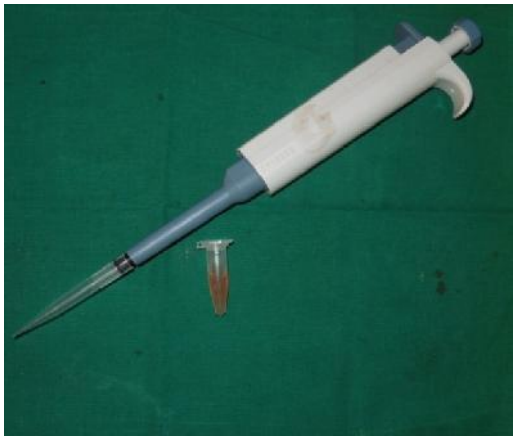
To evaluate the effect of 40% ellagic acid on periodontal status and total antioxidant capacity in heavy and light smokers with chronic periodontitis

MATERIALS AND METHODS

The present study was conducted in the Department of Periodontology, JSS Dental College and Hospital, constituent college of JSS University between June 2012 and February 2013. Ethical clearance was obtained from the Ethical review board, JSS University. Written informed consent was taken from the subjects who participated in the study. The study subjects included systemically healthy smokers, within the age of 18 to 45 years. Patients had atleast 20 teeth with more than 2 teeth per quadrant having probing pocket depth >4 mm. The overall bleeding score of all the patients was more than10 %. All patients were consulted about their dietary habits and were asked to maintain a diet diary. Patients were questioned regarding their smoking status and systemic disease, such as diabetes, rheumatoid arthritis and cardiovascular problems. The presence of any systemic disease was an exclusion criteria. Other patients who had been on a course of non-steroidal anti-inflammatory drugs / antimicrobial drugs / vitamin supplements within a 3-month period before the start of the study, pregnant or lactating mothers, regular mouthwash users and patients with special dietary requirements were also excluded from the current study. 40 patients who fulfilled the inclusion criteria were recruited in the study. The patients were divided into two major groups: GROUP A - heavy smokers (> 10 cigarettes / day) and GROUP B - light smokers (<10 cigarettes / day). Each group consisted of 20 patients each. At baseline, clinical measurements were recorded for all patients. Clinical examination included the recording of all teeth except for third molars. Plaque index (Sillness and Loe), Gingival index (Loe and Sillness) and Bleeding index (Ainamo and Bay) were measured. Probing depth and clinical attachment level were also recorded with UNC 15 probe. All recording were made by a single trained examiner. Baseline venous blood samples were also collected, after overnight fasting to estimate the total antioxidant capacity (Fig 1-3). The total antioxidant capacity of whole blood was estimated using the reduction of nitroblue tetrazolium reduction test (NBT) with modifications (Demehin *et al.,* 2001).Two ml of peripheral venous blood was taken in the morning before meals. Blood clotting was controlled with heparin (15 units/mL). The test

tubes with blood were placed in a thermostat at the temperature of 37°C and kept for 5 min. The obtained plasma was sent in ice boxes to Maratha Mandal Dental College and Hospital, Belgaum, Karnataka where the biochemical analysis was done. To the samples, nitroblue tetrazolium, the final concentration of which ranged to 1×10^{-4}, was added to the blood in the test tubes, which were kept for 20 min at the temperature of 37°C. On completion of incubation, the tubes were centrifuged for 5 min at 2000 rpm to sediment any cells. The supernatant was decanted into fresh test tubes, and the absorbance of NBT was measured in the samples using a spectrophotometer at wave length of 580 nm at 37ºC against a blank. Scaling and root planing was performed over a period of two weeks for patients in both groups A and B. After 2 weeks patients were recalled for oral hygiene maintenance and the major groups were randomized into the respective groups by a simple randomized method:





Group A1: Heavy smokers receiving 40% Ellagic acid supplementation
Group A2: Heavy smokers with no supplementation
Group B1: Light smokers receiving 40% Ellagic acid supplementation
Group B2: Light smokers with no supplementation

Formulation of drug (Fig.4) The drug was formulated at the JSS college of Pharmacy, Mysore under strict sterile conditions. Per oral tablet contained active principle 112 mg of pomegranate extract (containing 40% Ellagic acid equal to 100 mg Ellagic acid). Other ingredients included lactose, starch paste (10% w/w), Mannitol, Magnesium stearate and Talc. All excipients were certified as generally regarded as safe (GRAS by US- FDA). The formulated drug was given twice daily over a period of one month by a single examiner. The patients were informed to report any side effects whatsoever following drug intake. Patients were recalled one month post therapy. Clinical parameters and total antioxidant capacity was reevaluated. The patients were informed to return the medication bottles to evaluate the patient compliance.

Statistical Analysis

The increase in the total antioxidant capacity was the primary outcome measure in the present study. Secondary outcome measures were the improvements in clinical parameters. The values obtained from the clinical and biochemical data was subjected to statistical analysis by SPSS 16.0.Descriptive statistics, Paired samples “t” test and independent sample “t” test were used. The measurements were recorded at baseline and 1 month. To ensure reproducibility, intra examiner kappa analysis was done (0.82).

RESULTS

The present study was conducted to evaluate the effect of ellagic acid supplementation on the clinical parameters and total antioxidant capacity in heavy and light smokers with chronic periodontitis. All male patients were taken as part of the study within the 18 to 45 years (average = 34.525 years) (Table 1). The total antioxidant capacity (TAOC) was significantly increased for all the patients ($p<0.05$). The improvement in TAOC was maximum in light smokers who received the supplementation (B1). This was followed by heavy smokers who received the supplementation (A1). Intragroup analysis suggested that the TAOC in smokers who

received the drug (A1, B1) remained statistically significant compared to their counterparts not receiving medication ($p<0.05$). (Table 2, Figure 1). No major side effects were reported by the patients. Probing depth decreased significantly for all groups ($p <0.05$). When the mean difference in reduction was compared, it remained non- significant with no group showing better reduction ($p>0.05$). Clinical attachment level also demonstrated significant gain for all patients. While the gain was almost similar for all the groups, it was seen more in patients receiving the supplementation. The patients receiving the supplementation showed significantly more reduction compared to their counterparts. There was a significant improvement in the plaque scores in all patients ($p<0.05$). Intra group comparison between the test and control for both groups A and B remained insignificant. Similar result was observed for gingival index scores (Table 3).

Table 1. Age distribution across the groups

GROUPS	N	Mean	Std. Deviation	Std. Error
A1	10	36.4000	4.16867	1.31825
A2	10	34.6000	3.97772	1.25786
B1	10	35.5000	4.64878	1.47007
B2	10	35.1000	4.45845	1.40989

Table 2. Table showing TAOC (Total antioxidant capacity)

Groups	Mean TAOC Baseline	Mean TAOC 1 Month	Mean difference of TAOC	P value
A1	27.240 +/- 0.69	37.9+/-1.36	-10.6900	0.000
A2	27.90 +/- 1.20	33.38+/- 1.21	-5.4800	0.000
B1	31.85+/-1.311	44.39+/-0.945	-12.5400	0.000
B2	32.00+/-1.388	38.74+/-0.800	-6.7400	0.000

Table 3. Table showing the change in clinical parameters over the study period on one month

Groups	Mean pocket depth reduction (mm)	Mean increase in clinical attachment level gain(mm)	Mean difference in plaque scores	Mean difference in gingival scores
A1	1.03	0.18	0.95	0.43
A2	0.90	0.22	0.89	0.31
B1	1.06	0.24	0.89	0.34
B2	0.94	0.20	0.89	0.33

DISCUSSION

Current evidence indicates that the periodontal disease occurs in predisposed individuals that have an aberrant inflammatory or immune response to the microbial plaque adjacent to the gingival margin (Frediksson *et al.,* 1998). The excessive or prolonged release of neutrophil enzymes and reactive oxygen species is responsible for the majority of host tissue destruction in periodontitis (Gustaffson *et al.,* 1997). To combat reactive oxygen species (ROS) production, the body possesses a variety of antioxidant defense mechanism which act in concert. Their role is to protect vital cells and tissue structures and their biomolecules from host derived ROS as well as those of parasitic origin (Chapple *et al.,* 1996), by removing them as they form and repairing the damage they cause. A delicate balance exists between antioxidant systems and pro-oxidant mechanisms of tissue destruction and if balance is tipped in favor of ROS activities significant tissue damage ensues (Chapple *et al.,* 2002). Recent research into antioxidant defense in patients with periodontal disease has demonstrated reduced peripheral (plasma) and local (gingival crevicular fluid) total antioxidant capacity (Brock *et al.,* 2004). Previously, in a study the relationship between cigarette smoking and periodontal damage was observed in smoking and non smoking individuals with periodontitis. The results showed that there was a significant increase in serum lipid peroxidation and nitric oxide in both groups of periodonitits patients and suggested that smoking increases the level of free radicals in periodontal tissues (Dhotre *et al.,* 2012). The presence of nicotine and other harmful constituents of cigarette smoking further deplete the antioxidants severing the periodontal tissue damage. Authors suggest that tobacco smoke exposure significantly decreases both GCF and serum antioxidant capacity in patients suffering from chronic periodontitis. (Anand Mohan *et al.,* 2011).

The human body is blessed with concrete systems of antioxidants. In the present study we estimated the total antioxidant status of the patients. The assessment of total antioxidant capacity have the advantage of analyzing the combined effectiveness of contributing antioxidant species, which may be greater than the sum of the effects of the individual antioxidants that are as yet undiscovered or difficult to assay (Ali E. Sulaiman *et al.,* 2010). On the same basis, the present study estimation of whole blood total antioxidant capacity in heavy and light smokers post ellagic acid supplementation. There was a significant improvement seen in both the groups of heavy and light smokers who received the supplementation compared to their counterparts. This obtained results highlighted the supreme antioxidant capacity of ellagic acid. Previously, superoxide dismutase enzyme levels have been studied with a progressive reduction seen from healthy non smokers to light smokers to healthy smokers (Rupali Agnihotri *et al.,* 2009). Antioxidants may be regarded as "those substances which when present at low concentrations, compared to those of an oxidisable substrate, will significantly delay or inhibit oxidation of substrate (Halliwell *et al.,* 1989). They are classified according to their mode of action, location, solubility, structural dependence and source of origin. They have been used to combat free radicals in periodontal disease periodontits in local and systemic form. Amla, vitamin C, noni juice, berry juice concentrates are few of the many tried antioxidants to treat periodontal disease. Pomegranate is another wonder fruit containing polyphenols, tannins, ellagic acid and anthocyanins. These compounds are powerful antioxidants (Dr. Somya kote *et al.,* 2011). In the present study we took 40% ellagic acid extract of the pomegranate seeds. The extract was formulated in a tablet form given to smokers suffering from periodontitis and a subsequent compromised antioxidant status. The results obtained were highly encouraging with a significant change seen in both light and heavy smokers who received the supplementation of 40% ellagic acid compared to their counterparts. This concentration of ellagic acid was well within the safety dose of the drug. In a previous study, pomegranate juice was found effective against dental plaque microorganisms displaying it antimicrobial property (Dr. Somya Kote *et al.,* 2011). The same property has been studied by a few other authors with a positive result (Prashant *et al.,* 2001; Machado *et al.,* 2003). Similarly

pomegranate mouthrinse has been found to have an antiplaque effect (Smruti *et al.,* 2011). Biodegradable chips impregnated with Centella asiatica and Punica granatum pericarp have also been formulate with a significant decrease in pocket depth (Julie Jurenka *et al.,* 2008.). In a follow up of the same study, anti inflammatory markers like IL – 1b and IL – 6 were also evaluated and significant decrease was seen in the same at 3 and 6 months. As mentioned afore, the antiplaque and anti-inflammatory activity of pomegranate has been effectively tested. In contrast the antioxidant capacity of the same fruit has not been explored in the treatment of periodontal disease. To the best of our knowledge, our study is one of the primary studies where the antioxidant capacity was estimated in smoker patients in effect to ellagic acid supplementation. Although in the present study patients were classified on the basis of the number of cigarette smoked per day, the pack years were not calculated. This can be considered as one of the limitations of the study. Also, the patients were not divided on the basis of severity of periodontal disease which may have altered the antioxidant status. The formal release pattern of the formulated drug was not studied and the study duration was only one month. These can be the other drawbacks of the study. Further long term clinical trials are required to substantiate the obtained result.

Conclusion

Since a long time, scaling and root planing is considered to be the gold standard for the treatment of periodontitis. The present study was conducted to evaluate the adjunctive effect of 40% ellagic acid supplementation to scaling and root planing. There appeared to be definitive improvement in the clinical parameters, but this change was insignificant within the groups. On the other hand, whole blood total antioxidant capacity was significantly higher in heavy and light smokers who received the medication in comparison to their counterparts. Ellagic acid, a prime constituent of pomegranate thus demonstrated remarkable antioxidant property. The present study reinforces this form of therapy can be especially beneficial for smokers and other patients who are at risk to suffer from periodontal disease and have severe reduced antioxidant capacity. Further long term studies need to be performed to strongly substantiate the obtained results and deliver the benefits of the "*NATURE'S POWER FRUIT*" to mankind.

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