Research Article

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ASSESSING THE EFFICIENCY OF SYSTEMICALLY ADMINISTERED LYCOPENE IN THE TREATMENT OF GINGIVITIS – A RANDOMISED CONTROLLED CLINICAL TRIAL

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Abstract:

Oxygen is an element indispensible for life, can under certain situation have deleterious effect on the human body. Harmful effect of oxygen is due to the formation of a number of compounds known as reactive oxygen species. Reactive oxygen species have a role in pathogenesis of periodontal disease. The aim of the study was to evaluate the effect of systemically administered lycopene as a monotherapy and as an adjunct to scaling in gingivitis patients. The study also attempts to evaluate the level of uric acid in saliva before and after lycopene administration .30 patients reporting to the Department of Periodontics KLE'S VK Institute of Dental Science were included in the randomized controlled clinical trial. All the subjects were given 8 mg of lyco red tablets daily in two equally divided doses for two weeks. Plaque and gingival index were recorded at baseline, 1st and 2nd week. There was a statistically significant reduction in the mean gingival and plaque index scores from Baseline to 1st and 2nd week for the test and control site. There was a statistically significant increase in mean salivary uric acid level following lycopene administration. Lycopene shows great promise as a treatment modality in gingivitis patients. The possibility of obtaining an additive effect by combining oral prophylaxis with lycopene is also a possibility which deserves further study.

Key Words: Lycopene , Antioxidants , Reactive oxygen species, Uric acid.

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Introduction:

Gingivitis may be defined as an inflammatory lesion mediated by the host parasite interaction that is confined to the gingival tissue. The major cause of gingivitis is accumulation of microbial plaque in and around the dentogingival complex

which when removed results in complete resolution of inflammatory lesion.[1] Polymorphonuclear neutrophil produce reactive oxygen species via respiratory burst as a part of host response to infection. Patients with periodontal disease display increased PMN number and activity.[2] A free radical may be defined as any species capable of independent existence that contains one or more unpaired electrons. While most reactive oxygen species have extremely short half life (10^{-9} to 10^{-6}), they can cause substantial tissue damage by initiating free radical chain reaction.[3] In recent years attention has been focused on the role of reactive oxygen species, antioxidant system and products of oxidative stress in the pathogenesis of periodontitis[4].

Antioxidants may be regarded as those substances which when present at low concentration compared to those of an oxidisable substrate will significantly delay or inhibit oxidation of that substrate[5]. Oxidative stress is a state of altered physiological equilibrium within a cell or tissue/organ. It is defined as a condition arising when there is a serious imbalance between the level of free radical in a cell and its antioxidant defence in favour of the former[6]. Oxidative stress is implicated in the pathogenesis of several chronic inflammatory disease of which periodontal disease is no exception.[5] Many chemotherapeutic agents used in periodontics in addition to their antiseptic properties are known to have an antioxidative activity against spontaneous oxidation.[7] Modulation of free radical production seems to be essential for the inhibition of tissue destruction and treatment with drugs that block the production of free reactive oxygen species or blocks its effect might be therapeutically valuable.

Lycopene is predominately present in plasma with tomatoes being the main dietary source; other sources include red grape fruit and watermelon. Lycopene exhibits the highest physical quenching rate constant with singlet oxygen. Lycopene has been found to be at least three fold more effective than beta carotene in preventing cell death by quenching free radical.The commercially available antioxidant used in the study (lyco red) manufactured by Jagsonpal Pharmaceuticals contains 100 % natural lycopene with added phytonutrients. Mixtures of carotenoids are more effective than single compounds. This synergistic effect is more pronounced when combined with Lutein. In the present study zinc and selenium were used, which are potent antioxidants[8].

Saliva is armed with various defence mechanisms such as immunological and enzymatic defence system. Salivary antioxidants provide the first line of defense[9]. Saliva contains several antioxidants like uric acid, ascorbic acid and glutathione. Uric acid is the major antioxidant in saliva constituting to about 70%[8]. Thus the aim of the present study was to evaluate over a period of two weeks observation the effect of systemically administered lycopene (lyco red) as a monotherapy and as an adjunct to scaling in gingivitis patients. The present study also attempts to evaluate the level of uric acid in saliva, before and after lycopene administration.

Materials and Methods:

Ethical clearance from KLES VK Institute of Dental Science was obtained.

Source of data:

30 patients both male and female who showed clinical signs of gingivitis with the gingival index score of 2 and above (Loe and Sillness 1963), reporting to the out patient Department of Periodontics, KLE'S V.K. Institute of Dental Sciences, Belgaum, who met the inclusion criteria were included in this double blind randomized controlled clinical study.

Method of collection of data:

Inclusion criteria: All patients between the age group 20 – 40 years. Patients showing clinical signs of gingivitis with the gingival score of 2 and above in all the four quadrants

Exclusion criteria: Smokers**,** Patients with systemic diseases like Diabetes, Cardiovascular diseases. Patients who are on medications like antibiotics, or antiseptics for the past 6 week or over the counter antioxidants such as Vitamin C, Vitamin E or beta carotene for the past 3 months **,**Pregnant females or females on any hormonal therapy. Gout, Renal disease and patients with any other systemic disease that causes alteration in uric acid level .Patient with high protein diet.

Procedure:

An informed consent was taken from the participants before starting the study. All the subjects included in the study were given 8 mgs of lycopene daily in 2 equally divided doses for 2 weeks.

A split mouth design was followed for all the subjects included in the study. The quadrant allocation for the subjects (1^{st} and 3^{rd} quadrant) and (2^{nd} and 4^{th} quadrant) was assigned by randomized method of sampling technique. Thus after allocation of quadrants we obtained.

- Test site (quadrant 1 and 3) – oral prophylaxis
- Control site (quadrant 2 and 4) – no oral prophylaxis.

No dietary limitations were imposed during the study time. Normal oral hygiene procedures were permitted except for the use of chemotherapeutic mouth rinse. Horizontal method of brushing technique was advised for all the patients included in the study.

The following indices were recorded at baseline, 1^{st} week and 2^{nd} week.

1. Gingival index (Loe and Silness 1963)
2. Plaque index (Silness and Loe 1964)

Saliva collection and Preparation:

The antioxidant systems of saliva are highly complex and rich in several antioxidants such as uric acid, glutathione and ascorbic acid. Uric acid is a major anti oxidant present in saliva whose level decreases in gingivitis & periodontal disease.

Unstimulated saliva for uric acid estimation was collected in a sterile test tube and transferred to the department of biochemistry for analysis. The samples were stored in tightly capped test tubes at 2-8ºC and analysis was performed on the same day. Saliva samples were centrifuged at 4000 g for 10 min at 4^{o} C, the upper parts were drawn in small aliquots at 40^{o} C .

Results:

Statistical analysis

Results were analyzed as follows

The two sites were compared with respect to 1st week and 2nd week, mean plaque scores and gingival index score by taking baseline scores as co variants by analysis of co variance

The reduction from baseline to 1st week,and 2nd week follow up in plaque scores and gingival index scores were analyzed using unpaired t test .

Salivary uric acid analysis was done using paired t – test.

Table 1: Plaque Index Scores at baseline 1st week and 2nd week

Site	Baseline	1st week	2nd week	%reduction from baseline to 1st week	%reduction from baseline to 2nd week
Control	1.7±0.10	1.50 ±0 .19	1.1±0.17	14.7±8.76	34.1±9.89
Test site	1.8±0.06	0.8±0.14	0.6±0.16	53.8±7.49	68.3±9.09
t value	1.529	15.336	13.484	18.597	13.949
P value	0.132	<.000	<.000	<.000	<.000

A statistically significant reduction in mean plaque scores was observed for test and control site at 1st and 2nd week

(t value 15.336 , p value<0.001)

(t value13.484, p value<0.001)

The % reduction in plaque scores at 1st week for the control site was 14.7±8.76 and for the test site was 53.8±7.49

(t value 18.597, p<0.001).

The % reduction in plaque sores at 2nd week for the control site was 34.1±9.89 and test site was 68.3±9.09

(t value13.949 ,p value <0.001)

Thus a statistically significant % reduction in plaque score for the test and control site was observed from baseline to 1st week and from baseline to 2nd week (t value 18.597 p value<.001) (t value13.949 p value>0.001)

Table 2: Gingival Index Scores at baseline 1st week and 2nd week

Sites	Baseline	1st week	2nd week	% reduction from baseline to 1st week	% reduction from baseline to 2nd week
Control site	1.7±o.08	1.3±0.18	1.0±0.17	21.8±8.01	40.6±8.32
Test site	1.7±0.08	0.8±0.10	0.5±0.23	50.9±4.87	70.6±12.84
t value	1.086	13.254	9.664	17.030	10.740
p value	0.282	<0.001	<0.001	<0.001	<0.001

A statically significant decrease in mean gingival scores was observed at 1st and 2nd week

(t value 13.25, p value<.001)

(t value 9.664 , p value<0 .001)

The % reduction in gingival scores at 1st week for test site was 50.9±4.87 and for the control site was 21.8±8.01.

(t value 17.030) (p value<0.001)

The % reduction in gingival score at 2nd week for test site was 70.6±12.84 and for the control site 40.6±8.32

(t value 10.740) (p value<0.001)

Table 3: Uric Acid analysis

Before lycopene administration	After lycopene administration	Mean difference
2.6±1.16	3.6±0.82	0.97±0.57

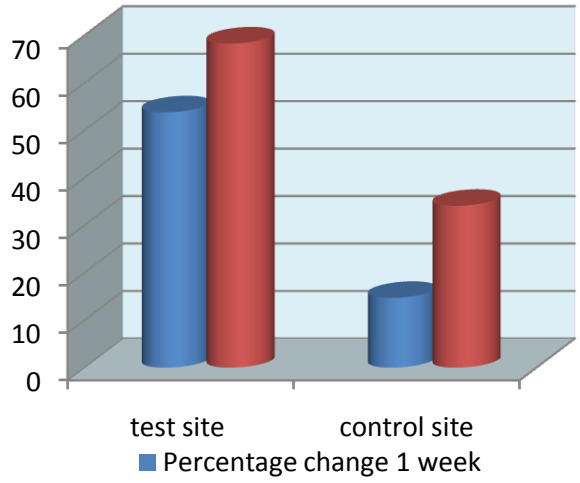
t value =9.301 p value <0.001

A statistically significant increase in mean salivary uric acid was observed after lycopene administration.

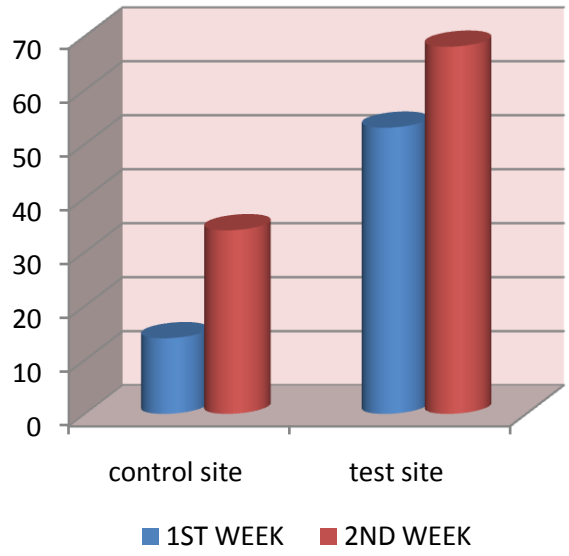
(mean difference =3.6±0.82)

(p value<0.001)

Graph 1: Percentage Change in plaque values from baseline to 1st and 2nd week



Graph 2: Percentage change in gingival index values from baseline to 1st and 2nd week



Discussion:

Reactive oxygen species are free radicals which have one or more free floating electrons rather than having paired electrons. They are highly reactive and unstable.[10] Reactive oxygen species include: hydrogen peroxide, superoxide, hydroxyl radicals and nitric oxide. There is increasing evidence available to implicate reactive oxygen species (ROS) in the pathogenesis of a variety of inflammatory disorder of which periodontal disease is no exception[8]. Antioxidant may be regarded as those substance which when present at low concentration compared to those of an oxidisable substrate will significantly delay or inhibit oxidation of that substance[3]. Mechanical plaque control is an indispensible phase of periodontal therapy but there are factors such as accessibility or the presence of plaque retentive areas that can limit clinical and microbiological response[11]. This has led researchers to look to antioxidant therapy as a possible strategy for the treatment of gingival disease. With the above details in mind this study was done to evaluate over a two week observation period the effect of systemically administered lycopene as a monotherapy and as an adjunct to scaling in gingivitis patients.

A total of 30 patients both male and females who showed clinical signs of gingivitis with the gingival score of 2 and above were included in the randomized controlled clinical trial. Gingival index and plaque index were recorded at baseline,1st week and 2nd week respectively .The plaque index score at baseline for the control and test site was (1.7±0.10) and (1.8±0.06) respectively. This suggest that the major cause of gingivitis is accumulation of microbial plaque in and around the dentogingival complex, which when removed, results in complete resolution of the inflammatory lesion[12]. The Mean plaque score for the control site at 1st week was (1.50±0.19) and for the test site it was (0.8±0.14) whereas the mean plaque score at the 2nd week for the control site was (1.1±0.17) and for the test site was (0.6±0.16) respectively. It was also observed that the % reduction in the mean plaque score for the test site was almost twice as compared to the control site, which was statistically significant (table 1). The severity of gingivitis can be assessed effectively by gingival index given by (Loe and Silness 1963)[13] .The mean Baseline scores for the gingival index for control site and test site was (1.7±0.08). Bleeding on probing is widely used by clinicians and epidemiologist to measure disease prevalence and progression as it is easily detected clinically. It is an objective sign of inflammation.

Environmental condition such as bleeding might establish a local environment that facilitates colonization with or shift in to pathogenic flora that is responsible for active phase of periodontal disease[14]. It has been demonstrated that fusobacterium species, a frequent isolate from chronic gingivitis can induce increased production of oxygen radicals, cytokines and elastase by leucocytes activated under condition which might be a possible pathogenic factor in gingival disease, thus stating that reactive oxygen species is common to both bacterial and host mediated pathway of tissue damage[8].

Many chemotherapeutic agents used in periodontics in addition to their antiseptic or anti inflammatory properties are known to have antioxidative activity against spontaneously oxidation. Treatment with drugs that block the production of free radical (ROS) or blocks its effect might be therapeutically valuable. Lycopene the carotenoid that gives ripe tomato its bright red colour is an effective natural antioxidant and quencher of free radical. Lycopene exhibits the highest physical quenching rate constant with singlet oxygen and atleast three fold more effective than beta carotene in preventing cell death by quenching nitric oxide radicals.[8]

In the present study (Lyco red) was tested over a period of 2 weeks since the effect of antioxidants can be best observed within 15 days of administration. There was a statistically significant reduction in the mean gingival scores for the test and control sites. The Gingival index score for the test site at 1st week was (1.3±0.18) ,for the control site was (0.8±0.10) where as a the mean gingival scores at the 2nd week for the test site was (1.0±0.17) and for the control site was (0.5±0.23) respectively (table 2). The beneficial effect of lycopene may be a result of lowering gingival tissue free radical concentration thus arresting disease progression. In the present study the mean reduction in gingival inflammation for the test site was almost twice as compared to the control site thus stating that lycopene showed more promising results when combined with oral prophylaxis[15] But whether lycopene can be utilized as a stop gap monotherapy for control of gingivitis particularly during period when oral prophylaxis needs to be deferred to a later date requires investigation[8]. The result of the study are in agreement with the work done by Muoz et al 2001 ,Hirasawa et al 2002, ,Dipaola et al 2004 , Rampalli Viswa Chandra et al 2007, who found significant improvement in gingivitis patients when they were supplemented with antioxidants compared with placebo group.

Saliva is a heterogeneous fluid comprising of proteins, glycoproteins, electrolytes, small organic molecules and compounds transported from blood which constantly bathes the teeth and oral mucosa.[16] Whole saliva offers numerous possibility for marking disease activity for the development of techniques suitable for salivary antioxidant evaluation and evaluating treatment outcome[17].Saliva is a combination of GCF which has a composition similar to Serum and Fluids released from salivary glands[12], thus in the present study unstimulated saliva was collected. Saliva contains various antioxidant and hence constitute first line of defence against free radical mediated oxidative stress[9]. It is rich in several antioxidants such as Uric acid, Glutathione and Ascorbic acid. Uric acid is a major antioxidant in saliva

constituting to about 70%. In the present study salivary uric acid estimation was done by centrifugation method where saliva samples were centrifuged at 4000g for 10 min at 4^0c^8. Uric acid is a relatively powerful scavenging antioxidant of water soluble radical such as hypocholorous acid and singlet oxygen[18]. The antioxidant activity of uric acid includes scavenger of singlet oxygen, scavenger of hydroxyl radical, scavenger of hypocholorous acid, protection of α1 antitrypsin when combined with ascorbate, binding of divalent metal ions preventing Fenton chemistry[19]. The results of this study showed that there was a significant increase in salivary uric acid levels after lycopene administration which was also followed by resolution of gingival inflammation. The mean uric acid level in saliva before lycopene administration was (2.6± 1.16) and after lycopene administration was (3.6±0.82). The results of the study are in agreement with previous studies which reported that the uric acid level in saliva increased following antioxidant therapy[8]

Thus in general whether antioxidant therapy has a corrective action on the natural antioxidants need to be further investigated. As per the result present in the current study we can suggest that lycopene shows great promise as a treatment modality in gingivitis. The possibility of obtaining an additive effect by combining routine oral prophylaxis with lycopene is also a possibility which deserves further study.

Conclusion

In the light of the observation from the current study it can be concluded that modest administration of lycopene, which is an effective natural antioxidant could be an effective approach in reducing gingival inflammation. Lycopene when used as a monotherapy did not show as promising results when compared to lycopene combined with routine oral prophylaxis. Lycopene is an effective natural antioxidant obtained from diet or from supplementation can be used as a valuable adjunct along with routine oral prophylaxis in the treatment of mild form of gingivitis. Uric acid levels in saliva were increased following lycopene administration, thus reducing gingival inflammation Currently few studies are available to extrapolate the therapeutic effects of antioxidants in dental practice. Although there has been promising results, over all benefit : risk ratio should be considered . Large scale randomized controlled clinical trials and unbiased studies addressing the safety and standardization issue of antioxidants in dentistry are also required.

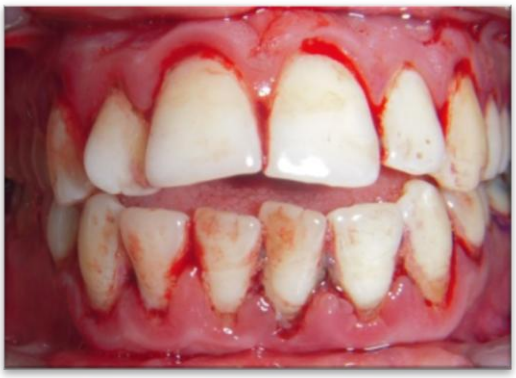


Figure 1 Generalized bleeding on probing

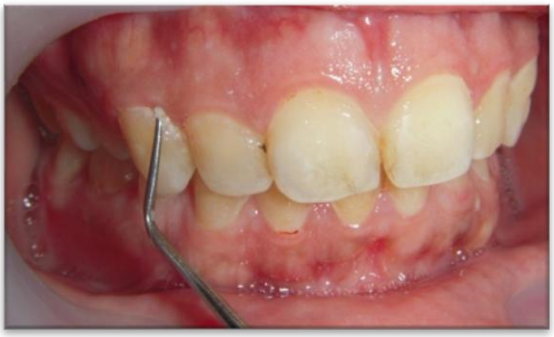


Figure 2 Baseline Plaque Index

Figure 3 Lycored tablets

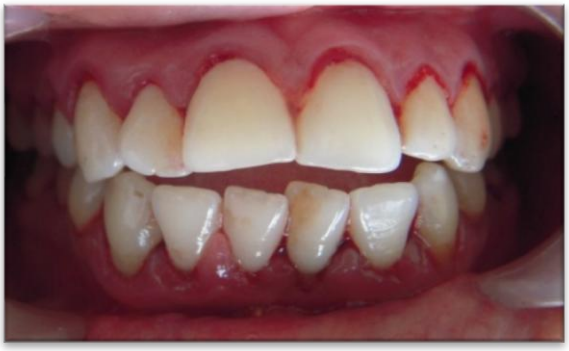


Figure 4 Bleeding on probing at 2nd week

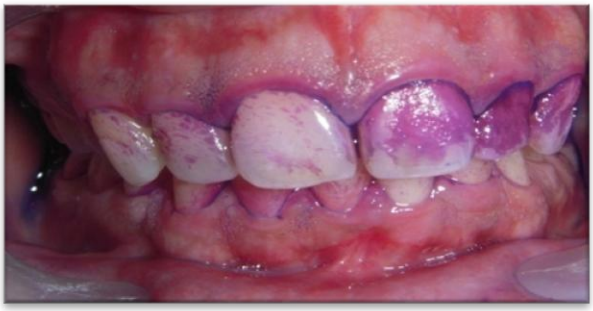


Figure 5 Plaque Index at 2nd week

Figure 6 Digital Spectrophotometer for salivary Uric Acid estimation

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