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Efficacy of oral lycopene in the treatment of oral leukoplakia

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KEYWORDS

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Summary This study evaluates the efficacy of lycopene in the treatment of oral leukoplakia and compares two different doses with a placebo. Fifty-eight clinically and histologically diagnosed patients of oral leukoplakia were selected for the study. They were randomly divided into three groups. Group A: ($n = 20$; 8 mg lycopene/day), Group B: ($n = 20$; 4 mg lycopene/day) and Group C: ($n = 18$; placebo). The duration of the therapy was three months. Outcome was assessed clinically as well as histologically. Post-treatment patients were on follow-up for two months. Student's '*t*' test was used for statistical evaluation. Clinically the patients in Groups A, B, C had a mean response of 80%, 66.25% and 12.5% respectively. Histological evaluation too had similar results. Patients receiving lycopene in both regimes show highly significant difference in response as compared to placebo (Group C). The observed effect of lycopene suggests that it can be effectively and safely used for the management of oral leukoplakia.

Introduction

Leukoplakia is the most common pre-cancerous lesion in the oral cavity. Malignant potential of leukoplakia was hinted by Sugar and Banoczy way back in 1957.[1] Association between tobacco chewing and smoking with oral leukoplakia is established beyond doubt.[2,3] Tobacco smoke contains NOO^- radicals, which are carcinogenic. Free radical scavengers should be the necessary part of the treatment regimen in tobacco chewers or smokers to prevent the formation, induce the remission or inhibit the progression of pre-cancerous lesions into malignancies. Lycopene, the carotenoid that gives the ripe tomato its bright red color, is a very effective natural antioxidant and quencher of free radicals.[4] It is also found in various fruits such as watermelons, guava and pink grapefruit.[5] Reactive oxygen species (ROS) is generated in tissues and can damage DNA, proteins, carbohydrates and lipids.

Lycopene exhibits the highest physical quenching rate constant with singlet oxygen.[6] Lycopene

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has been found to be at least 3-fold more effective than β-carotene in preventing cell death by quenching of NOO^- radicals.[7] It also protects DNA damage induced by 1-methyl-3-nitro-1-nitrosoguanidine and H_2O_2.[8]

Lycopene also increases the expression of a gene encoding connexin-43, a gap junction protein, effect being independent of pro vitamin-A or antioxidant properties.[9] Lycopene and β-carotene are two major carotenoids found in human buccal mucosal cells. Protective effect of tomato consumption has been observed in oral leukoplakia in a population based case control study.[10,11] Tomato, tomato products and lycopene consumption is associated with reduction in upper aero-digestive tract cancers like oral cavities, pharynx, larynx and oesophagus.[12] Administration of lycopene suppresses DMBA-induced oral carcinogenesis.[13] The first report of efficacy of lycopene against human oral cancer cell was recently published describing the significant therapeutic effect.[14] The following study reports the efficacy of lycopene as a treatment modality in oral leukoplakia and its probable chemopreventive effect.

Materials and methods

Fifty-eight oral leukoplakia patients were confirmed by history, clinical and histological examination. These patients were randomly categorized in three groups:

Group A: $(n = 20)$ 8 mg lycopene daily in two equally divided doses.
Group B: $(n = 18)$ 4 mg lycopene per day in two equally divided doses.
Group C: $(n = 18)$ controls to whom placebo capsules were given.

Lycopene used in the study was LycoRed™ 2 mg softgels, manufactured by Jagsonpal Pharmaceuticals Ltd., New Delhi, India, under license from LycoRed Natural Products Industries Ltd., Beer-Sheva, Israel, makers of natural lycopene, LYC-O-MATO™.

The therapy was instituted for three months and subjects were followed up for another two months. The nature and purpose of the study were fully discussed with each patient. Each participating institute's review board for research approved the study protocol and consent was obtained from the patient.

Patients were evaluated every 7–10 days during the treatment period and then every 15 days during the follow up period. Routine evaluation included recording the history of tobacco and ethanol usage, physical examination.

Outcome was assessed both clinically and histologically. The clinical objective response was evaluated by bi-dimensional measurement of the lesions and color photography.[15] These assessments were made at entry, at the first evidence of response or progression and at the third month of the study.

Histological examination and grading of dysplasia were performed in a blind fashion before treatment and subsequently upon its completion.

- An objective response was classified as complete when gross inspection had revealed no evidence of a lesion for at least four weeks (approx. 100%).
- A partial response was defined as a decrease of more than 50% of the lesion size (approx. 75%).
- A response was classified as stable when the decrease in the lesion size was less than 50% of the base line measurements (no response).
- Disease progression was defined as an unequivocal increase in the size of any lesion during the treatment or as the appearance of a new lesion (−25%).

All the variables computed from the study, for example the measurement of the size of the lesion before the treatment and after the treatment were recorded. The response was assessed from the range of 100%, 75% to −25% (for progression).

The histological response was assessed according to the following categories:[15]

(1) Atypical hyperplasia
(2) Mild dysplasia
(3) Moderate dysplasia
(4) Severe dysplasia or CA in situ

For histological evaluation five stages were taken as normal, atypical hyperplasia, mild dysplasia, moderate dysplasia and severe dysplasia. They were ranked as 0, 1, 2, 3, and 4 respectively so that change could be quantified in terms of these ranks. For example a case from stage moderate dysplasia comes to stage mild dysplasia post-treatment; the improvement was considered as $3 - 2 = 1$ unit.

Both the response scores were analyzed statistically for mean values, standard deviation, standard error and range. Unpaired student's 't' test was used to assess the statistical significance between the mean values for the respective variables.

Results

In this study majority were males 44 (76%) as compared to 14 females (24%) (Table 1). Most of the patients were in their middle age. 13 (65%) patients in Group A, 16 (80%) patients in Group B, and 13 (72.22%) patients in Group C and were belonging to the age group ranging from 31 to 60 years (Table 2, panels a–c).

Almost 80% of patients in all the three groups had the lesions on the buccal mucosa, which represented the commonest site followed by gingiva, tongue, palate and lip (Table 3, panels a–c).
Among the clinical forms studied patients with homogenous variety were maximum 85% followed by speckled variety and verrucous type (Table 4, panels a–c).

Table 1 Sex distribution of patients

Groups	Male	Female	Total
Group A	15 (75%)	5 (25%)	20
Group B	14 (70%)	6 (30%)	20
Group C	15 (83.33%)	3 (16.66%)	18

Table 2 Age-wise distribution of patients

Age groups (years)	Male	Female	Total (%)
Panel a: Group A ($n = 20$)			
10–20	2	–	2 (10%)
21–30	3	1	4 (20%)
31–40	5	2	7 (35%)
41–50	3	1	4 (20%)
51–60	1	1	2 (10%)
61–70	1	–	1 (5%)
71–80	–		
Panel b: Group B ($n = 20$)			
10–20	1	–	1 (5%)
21–30	2	–	2 (10%)
31–40	4	1	5 (25%)
41–50	3	3	6 (30%)
51–60	3	2	5 (25%)
61–70	1	–	1 (5%)
71–80	–	–	–
Panel c: Group C ($n = 20$)			
10–20	1	–	1 (5.56%)
21–30	2	–	2 (11.12%)
31–40	4	1	5 (27.77%)
41–50	3	1	4 (22.22%)
51–60	3	1	4 (22.22%)
61–70	2	–	2 (11.12%)
71–80	–	–	–

Table 3 Site distribution of the lesions

Location of lesion	Total no. of patients
Panel a: Group A ($n = 20$)	
Buccal mucosa	16
Tongue	4
Gingiva	2
Palate	6
Lip	2
Floor of the mouth	1
Panel b: Group B ($n = 20$)	
Buccal mucosa	17
Tongue	2
Gingiva	4
Palate	4
Lip	4
Floor of the mouth	–
Panel c: Group C ($n = 18$)	
Buccal mucosa	16
Tongue	–
Gingiva	4
Palate	1
Lip	5
Floor of the mouth	–

Statistical analysis for histological response

The statistical analysis of histological response showed that the response of Group A compared to Group B was significant ($p < 0.05$). On comparison with Group C it was found to be highly significant ($p < 0.001$). Group B patients' response when compared with Group C patients' response also proved to be significant ($p < 0.05$) (Table 5 and Fig. 1a and b).

Statistical analysis for clinical response

It was noted that patients receiving 8 mg lycopene per day, that is Group A, 11 patients completely responded, seven showed partial response, two were in stable condition.

In Group B (4 mg lycopene per day), complete response was seen with five patients, partial response with eight patients, seven were in stable condition.

In Group C (control group) no patient showed complete response, three patients showed partial response, 15 were in stable condition and there was no progression of the disease (Table 6, panels a–c).

On statistical evaluation it was found that there was insignificant comparison between Group A and

Table 4 Histologic response

Stage	No. of patients pre-treatment	No. of patients post-treatment
Panel a: Group A $(n = 20)$		
Atypical hyperplasia	7	6—N 1—AH
Mild dysplasia	6	3—N 3—AH
Moderate dysplasia	4	1—N 1—AH 2—MD
Severe dysplasia	3	1—N 2—AH
Panel b: Group B $(n = 20)$		
Atypical hyperplasia	9	N—4 AH—5
Mild dysplasia	6	N—1 AH—4 MD—1
Moderate dysplasia	3	AH—1 MD—2
Severe dysplasia	2	MD—1 S.D.—1
Panel c: Group C $(n = 18)$		
Atypical hyperplasia	8	N—1 AH—7
Mild dysplasia	6	AH—1 MD—5
Moderate dysplasia	4	MD—1 MOD—3
Severe dysplasia	—	—

Key: N—Normal, AH—atypical hyperplasia, MD—mild dysplasia, MOD—moderate dysplasia.

Group B patients. But response was highly significant when Group A and Group B were compared with placebo $(p < 0.001)$.

Table 5 Frequency table for the three treatments for different ranks

Unit of improvement	Group A	Group B	Placebo
−1	0	0	0
0	1	7	15
1	11	10	3
2	4	3	0
3	3	0	0
4	1	0	0
Total	20	20	18
Mean	1.50	0.70	0.17
S.D.	1.1180	0.7810	0.3727
	A—B	B—C	A—C
'*t*' value	2—56 *	2—57 *	4—70 *
	$P < 0.05$ Significant	$P < 0.05$ Significant	$P < 0.001$ Highly significant

On statistical evaluation it was found the patients in Group A (8 mg regimen) had a mean response of 80% with a standard deviation of 29.1548. Student's '*t*' test proved the response to be highly significant when compared to the Group C (placebo) patients with significance value $p < 0.001$. On comparison with Group B (4 mg regimen) patients the response was not significant.

Mean response rate with Group B patients was 66.25%, standard deviation was 27.6981. When this response was compared with Group C patients it was found to be highly significant $(p < 0.001)$.

Mean response rate with Group C patients (placebo) was 12.5% with standard deviation of 27.9508. It is apparent that lycopene is more efficacious as compared to a placebo. We see the response was better in the patients administered 8 mg lycopene in comparison to those administered 4 mg lycopene (Table 7).

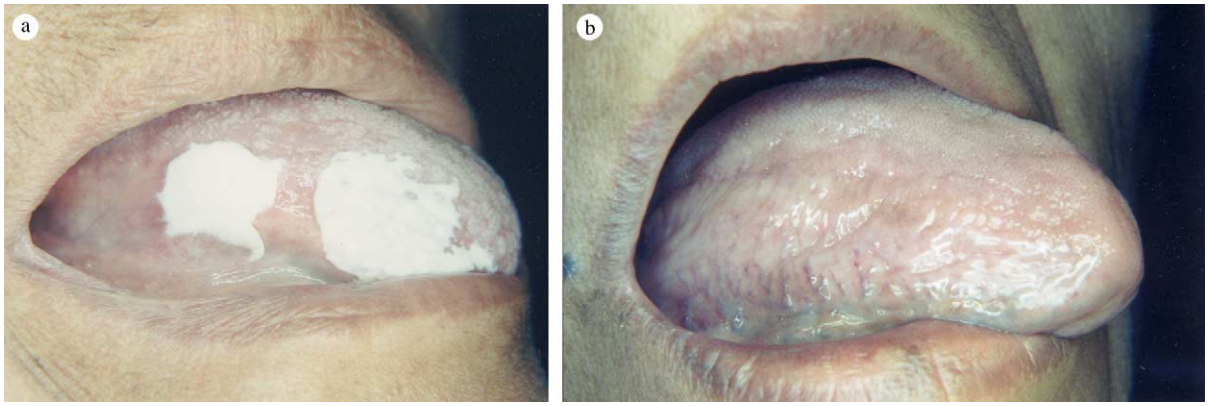

Figure 1 (a) Before starting 8 mg lycopene therapy. (b) Complete response seen after 8 mg lycopene therapy.

Table 6 Clinical response

Response	No. of patients
Panel a: Group A $(n = 20)$	
Complete response 100%	11
Partial response 75%	7
Stable response 50%	2
Progression 25%	0
Panel b: Group B $(n = 20)$	
Complete response 100%	5
Partial response 75%	8
Stable response 50%	7
Progression 25%	0
Panel c: Group C $(n = 20)$	
Complete response 100%	0
Partial response 75%	3
Stable response 50%	15
Progression 25%	0

Table 7 Frequency table for three treatment regimen responses

Response	Group A	Group B	Group C
Complete 100% response	11	5	0
Partial response 75%	7	8	3
Stable response 50%	2	7	15
Progression 25%	0	0	0
Mean	80%	66.25%	12.5%
S.D.	29.1548	27.6981	27.9508
't' value	1.49	5.79	7.07
	NS	***	***
	—	$P < 0.001$	$P < 0.001$

Discussion

Leukoplakia is defined as a white patch or plaque on the oral mucosa that cannot be scraped off and cannot be attributed to any other diagnosable disease, but habit of tobacco is always present.

More than 70% of patients in the present study were belonging to the age range of 31–70 years. Thoma[16] reported similar incidence, out of 321 patients within this age range, 70% of them had leukoplakia. Leukoplakia is now being reported in patients under 20 years of age.[17]

Out of 58 cases studied, 44 (76%) were males and 14 (24%) were women. Dolby[18] reported the sex ratio to be 95:5; male:female in 1940. But recently the trend has changed and according to Burket[17] the ratio reported is 3:2, due to change in smoking habits of females.

In the present study it was noted that the clinical response of patients receiving 8 mg lycopene (Group A) was more as compared to 4 mg lycopene (Group B). However the difference was not statistically significant. Lycopene supplementation was highly significant $(p < 0.001)$, when compared to placebo (Group C).

Histological response showed that the response of 8 mg lycopene treatment (Group A) as compared to 4 mg lycopene treatment (Group B) was significant $(p < 0.05)$. Response of 8 mg lycopene (Group A) as compared to placebo (Group C) was highly significant $(p < 0.001)$ whereas histological response of 4 mg lycopene (Group B) when compared to placebo (Group C) was also significant with $p < 0.05$.

The difference seen in the clinical and histological response is due to the different quantifications taken to assess the responses. Lycopene supplementation (both 8 and 4 mg) reversed hyperkeratosis with similar efficacy; clinical assessment was made with measuring the lesion size that is apparent due to hyperkeratosis.

Very recent study by Bertha Shwartz[14] proposed that lycopene could kill oral cancer cells by reestablishing the communication between them. In the present study we find that the histological picture had significantly improved in both the regimens. 8 mg regimen group patients showed very significant improvement as compared to placebo cases. The improvement was dose dependent and improved with increase in the dose.

Bhuvaneswari[13] in their study checked the chemopreventive efficacy of lycopene on 7,12-dimethyl benz[*a*]anthracene (DMBA)-induced hamster buccal pouch (HBP) carcinogenesis. The results suggested that lycopene was very efficacious in preventing neoplasia.

In our study also, we found significant reversal of the dysplastic changes. The Group A patient benefited more as compared to the Group B patients on histological evaluation. On clinical evaluation both Group A and Group B were very significantly responsive as compared to the placebo patients (Group C). This can be noted with patient in Group A, showing complete response after the therapy (Fig. 1a (before treatment) and 1b (after treatment)). Conclusions drawn from our study are that the patients receiving lycopene supplementation in both 8 and 4 mg regimens show highly significant difference in the response as compared to the placebo group. Although the statistical significance between both Group A and Group B are not present. This could be because of the reversal of hyperkeratosis, which is the initial change in any leukoplakic lesions and secondly because of

different quantifications taken in assessing clinical and histological responses.

Histologically, Group A——8 mg regimen patients were responding significantly better than Group B patients (4 mg regimen patients). There was a very significant difference between lycopene supplemented patients and patients given placebo.

This signifies that 8 mg lycopene supplementation per day was more efficacious than 4 mg lycopene supplementation per day. Significant reversal of dysplastic changes was noted. There was very low mean response as with the placebo patients.

Most of the patients were responding in the experimental groups——Group A and Group B. Probably if the duration of the treatment were longer still better results would have been seen. No side effects, toxicity of any sort were encountered in the complete duration of the therapy. Lycopene surely appears to be an efficacious drug in the treatment of oral leukoplakia patients. The efficacy of lycopene increases with dose. 8 mg lycopene per day was found to be more efficacious than 4 mg lycopene per day. 4 mg lycopene per day also showed significant clinical efficacy and it could be used for treating oral leukoplakia but probably a longer duration of therapy may be required. It also appears to be a safe drug.

Thus lycopene appears to be a very promising antioxidant as a treatment modality in oral leukoplakia. Results indicate that lycopene can protect cells against cell damage and play a protective role against progression of dysplasia.

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