# Comparative Evaluation of the Chemoprevention of Oral Leukoplakia with Spirulina and Green Tea Extract

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## ABSTRACT

**Introduction:** Oral leukoplakia (OL) is a potentially malignant disorder of the oral cavity. Random regression is more common in homogeneous types than in nonhomogeneous types. OL has been treated using a variety of chemopreventive therapy techniques. The goal of the present study was to compare the clinical regression rates of oral leukoplakia chemoprevention to those of spirulina and green tea extract.

**Material and Methods:** Fifty-four participants with a clinico-histological diagnosis of OL were selected and divided into two groups, with 27 in each group, by a simple randomization method. Respective groups received spirulina and green tea extract for a period of 4 months. Parameters such as the size and number of lesions at baseline and review, as well as side effects, were used to assess efficacy.

**Results:** Z- test was applied to obtain the results. In the Spirulina group, 14/22 (64%) of subjects with homogenous leukoplakia had complete regression compared with 3/5 (60%) of subjects with non-homogenous leukoplakia. In the green tea extract group, 16/21 (76%) of subjects with homogenous leukoplakia had complete regression compared with 2/6 (40%) of subjects with non-homogenous leukoplakia. No statistically significant difference was observed between Green Tea and Spirulina with respect to the proportion of complete response ($P$>0.05), partial response ($P$>0.05) as well as no-response ($P$>0.05) in homogenous and non-homogenous leukoplakia.

**Conclusion:** In the present study of chemoprevention of OL, when efficacy was compared between the spirulina and green tea extracts, statistically insignificant results were found, which means both were equally efficient but do not yet provide evidence that such therapies are effective in preventing recurrence. It highlights the necessity of continuing OL monitoring after treatment remission.


**Keywords:** Chemoprevention, Green tea extract, Oral Leukoplakia, Spirulina

## INTRODUCTION

Chemoprevention (Sporn, 1976) is defined as the use of natural, synthetic or biological agents to reverse, suppress or prevent either the initial phases of carcinogenesis or the progression of premalignant cells to invasive disease. [1]

The extensive range of physiologically active phytochemicals found in natural substances, such as phenolics, flavonoids, carotenoids, alkaloids, and nitrogen, can inhibit both early and late stages of carcinogenesis.

The progression of oral potentially malignant disorders can be stabilised, halted, or reversed by chemoprevention techniques because epithelium carcinogenesis takes years to reach the invasive stage.

Both green tea and spirulina have been studied for their chemopreventive potential and are also readily available and low-toxic.

Oral leukoplakia (OL) is a potentially malignant oral disorder described as "a predominantly white lesion of the oral mucosa that cannot be characterised as any other definable lesion."

Two categories make up the etiopathogenesis of OL: OL related with tobacco use and OL of uncertain aetiology or idiophatic.[2]

OL on the tongue, soft palate, and floor of the mouth are regarded as high-risk lesions.[3,4] Annual malignant transformation rates for OL range from 0.1% to 17%.[5,6]

The purpose of the present study was to assess the effectiveness of chemoprevention of oral leuoplakia in comparison to spirulina and green tea extract in terms of clinical regression.

## MATERIAL AND METHODS

The study involved 60 OL patients and was carried out in the Oral Medicine and Radiology department. Sample size is determined by pilot study using formula $n = (Z_{\alpha/2}+Z_{\beta})^2 * (p_1(1-p_1)+p_2(1-p_2)) / (p_1-p_2)^2$. No. of samples with Clinical response (CR) in group (1): 0.64, No. of samples with CR in group (2): 0.23, difference in proportion: 0.41, α (probability of Type 1 error): 0.05, β (probability of Type 2 error): 0.20, Power of the test (1- β): 0.80, substituting in above formula sample size required for each group is 22. The trial began with 60 patients, 30 in each group, and was completed with the exclusion of 6 patients at the conclusion of the study due to inconsistent reporting and dropouts for personal reasons.

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At the end of the trial, 54 patients were present, 27 of whom were in the spirulina group and 27 in the green tea extract group. Males to females in green tea extract group is 18:9 and 25:2 in spirulina group. The entire process complied with the Helsinki Declaration's ethical principles.[7] For a period of 4 months, the Spirulina group was given oral spirulina capsules containing 500 mg twice daily, and the Green Tea Extract group was given oral green tea extract capsules containing 500 mg twice daily. Clinically and histopathologically confirmed cases of OL met the inclusion criteria, and subjects with hypertension, diabetes mellitus, stomatitis, radiation fibrosis, scleroderma, immunosuppressive diseases, peptic ulcer bleeding disorders, cardiac disorders, allergy, and hypersensitivity to study medications met the exclusion criteria.

The individual was made to feel comfortable in the dental chair, and a thorough history was taken that covered the subject's chewing and smoking habits as well as their type, form, brand, frequency, and duration. The results of a comprehensive clinical examination were documented. At baseline and at each review, the number, kind, and size of the lesions were noted. Both during recruiting and throughout evaluations, the subjects received advice to break their habits. Tests of kidney and liver function were performed to look for any abnormalities. During visits, the individuals were examined every 15 days. Patients were instructed to report any adverse effects right away. A biopsy was performed on suspicious lesions in order to rule out malignancy. The size and number of lesions at baseline and review were used to gauge response. Individual pre- and post-treatment comparisons were conducted for each group as well as between the groups.

A complete response (CR) was defined as the total elimination of the lesion(s) as assessed by visual inspection; a partial response was defined as a reduction of at least 50% in the size of a single lesion or the sum of the sizes of several lesions; stable and progressive lesions were rated as no response.

## STATISTICAL ANALYSIS

**Null Hypothesis*:*** There is no significant difference in the proportion of samples in Green Tea Extract and Spirulina with respect to the response i.e. $\rho_1=\rho_2$

**Alternate Hypothesis*:*** There is a significant difference in the proportion of samples in Green Tea Extract and Spirulina with respect to the response i.e. $\rho_1\neq\rho_2$

**Level of Significance*:*** $\alpha=0.05$

**Statistical test used*:*** Z- test for proportions

**Decision Criterion:** We compare the P-Value with the level of significance. If $P<0.05$, we reject the null hypothesis and accept the alternate hypothesis. If $P\geq0.05$, we accept the null hypothesis.

**Computations*:*** The tables 1&2 give us the various computations and the P-Value.

## RESULTS

No statistically significant difference was observed between Green Tea and Spirulina in homogenous lesions with respect to the proportions of complete response ($P > 0.05$), partial response ($P > 0.05$), and no response ($P > 0.05$). (Table 1).

In nonhomogeneous lesions, there was no statistically significant difference between Green Tea and Spirulina in terms of the proportion of complete responses ($P > 0.05$) or no responses ($P > 0.05$). (Table 2).

In the Spirulina group, 14/22 (64%) of subjects with homogenous leukoplakia had complete regression

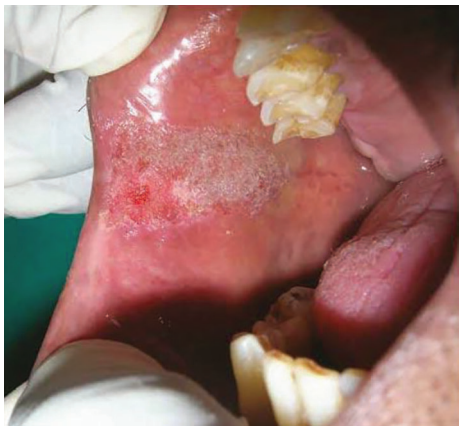

**Figure-1:** Pre-treatment clinical photograph of a patient in the spirulina group

| Response | Green Tea (N=21) | | Spirulina (N=22) | | Difference in Proportions | Z | P-Value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| Complete | 16 | 76% | 14 | 64% | 0.126 | 0.900 | 0.370 |
| Partial | 2 | 10% | 3 | 14% | -0.041 | -0.420 | 0.674 |
| No-Response | 3 | 14% | 5 | 23% | -0.084 | -0.710 | 0.477 |

**Table-1:** Comparison of Homogeneous Samples:

| Response | Green Tea (N=6) | | Spirulina (N=5) | | Difference in Proportions | Z | P-Value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| Complete | 2 | 40% | 3 | 60% | -0.200 | -0.880 | 0.376 |
| Partial | 0 | 0% | 0 | 0% | 0.000 | - | - |
| No-Response | 4 | 80% | 2 | 40% | 0.400 | 0.880 | 0.376 |

**Table** 2- Comparison in Non-Homogeneous Samples:

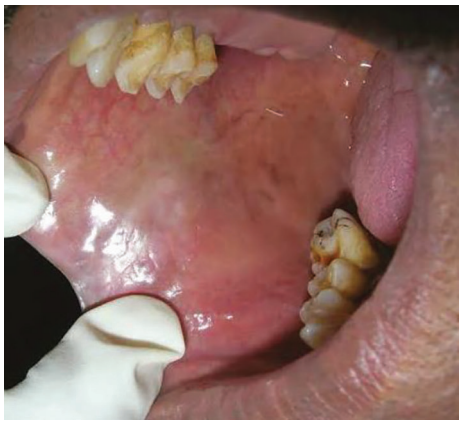

**Figure-2:** Post-treatment clinical photograph of the same patient in the spirulina group

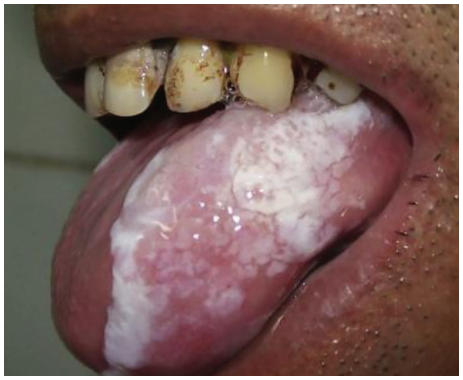

**Figure-3:** Pre-treatment clinical photograph of a patient in the Green tea extract group

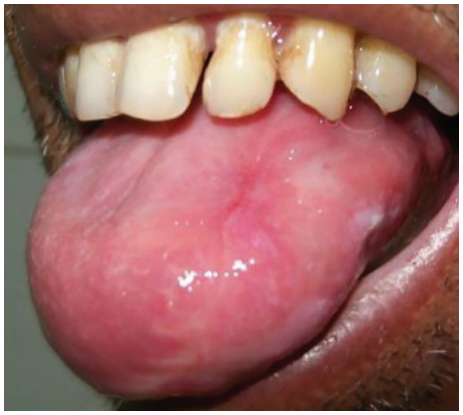

**Figure-4:** Post-treatment clinical photograph of the same patient in the Green tea extract group

compared with 3/5 (60%) of subjects with non-homogenous leukoplakia. In the green tea extract group, 16/21 (76%) of subjects with homogenous leukoplakia had complete regression compared with 2/6 (40%) of subjects with non-homogenous leukoplakia. (Table 1&2).

## DISCUSSION

OL is an Oral Potentially malignant disorder and is considered as major oral health problem with high degree of malignant potential. For treatment of OL, the degree of epithelial dysplasia may be assessed. In the presence of moderate to severe epithelial dysplasia, surgical treatment either though conventional surgery, electro cauterization, laser ablation, or cryosurgery is recommended.[8,9] After surgical intervention, OL has been documented to recur in 10% to 35% of instances.[10,11,12]

Nonsurgical treatment may also be considered this modality offers minimal adverse effects to patients, especially for patients with widespread OL that involves a large area of oral mucosa or patients with medical problems and, consequently, high surgical risks with potential advantages of easy application that does not require treatment at a medical centre and relative low cost.[13,14,15]

The groundbreaking retinoid study by Sporn *et al.* demonstrated chemoprevention in the field of conventional cancer research.[16] The vitamin A derivative 13-cis-retinoic acid (13cRA) was found in landmark trials to reverse dysplasia by shrinking precancerous lesions in 67% of patients when compared to 10% of the placebo group.[17] It has been demonstrated that high dose 13cRA, α-interferon, and vitamin E work well together.[18] Assessment of the topical fenretinide use in OL/lichen planus patients (100 mg BID for 2 months) revealed premalignant leukoplakia reduction with no negative side effects and negligible drug levels in the serum.

The objective of this present study was to compare the clinical regression efficiency of chemoprevention of OL to that of spirulina and green tea extract. The study's participants' ages ranged from 35 to 65. Epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epgallocatechin-3-gallate (EGCG), are the four polyphenols as well as glycosides, leucoanthocyanins, and phenolic acid, are found in green tea extract, which is obtained from the plant Camellia sinensis. Since these polyphenols activate p53 and its targets p21 and Bax, they can cause cell cycle arrest or apoptosis.[19,20,21] Tsao *et al.* phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions received randomly 3 doses of GTE (500,750, 1000 mg/m$^2$) vs placebo thrice a day for 12-weeks and evaluated the biomarkers in the baseline and 12- week biopsied tissues. This study showed a greater clinical response with the 750 and 1000 mg/m$^2$ GTE (58.5%) and 500 mg/m$^2$ (36.4%) vs the placebo arm (18.2%) suggesting a good dose-response effect.[22]

Spirulina is blue green algae with rich natural source of proteins, carotenoids and other micronutrients. Experimental studies in animal models have demonstrated an inhibitory effect of spirulina algae on oral carcinogenesis. Studies among preschool children in India have demonstrated spirulina fusiformis (SF) to be effective source of dietary vitamin A. Mathew *et al.* evaluated the chemo preventive activity of spirulina (1g/day for 12 mos) in reversing OL in pan tobacco chewers in Kerala, India. Complete regression of lesions was observed in 20 of 44 (45%) evaluate subjects supplemented with SF, as opposed to 3 of 43 (7%) in the placebo arm ($p<0.0001$). [23]

In the present study, when efficacy was compared between the spirulina and green tea extract, statistically insignificant results were found which means both were equally efficient. (Table 1&2). Both showed no side effects and efficacy which was similar to the study by Mathew *et al.* and Tsao *et al.* [22, 23].

However, present research was limited by the inconsistent patient reporting, the small sample size, and the lack of long-term follow-up. However, multicentre research should be conducted for increased consistency and improved results.

## CONCLUSION:

The use of supplements in the treatment of OL has been examined in a number of clinical trials. Except for lycopene, the use of antioxidizing medicines requires strict management due to their negative effects and contraindications. Randomized controlled trials for nonsurgical therapy of OL currently offer no proof that such treatments are successful in avoiding malignant change and recurrence. It underlines the need for ongoing OL monitoring following therapeutic resolution.

## REFERENCES


1. Steward WP, Brown K. Cancer chemoprevention: a rapidly evolving field. British journal of cancer. 2013 Jul;109(1):1-7.
2. Axéll T, Pindborg JJ, Smith CJ, Van der Waal I, an International Collaborative Group on Oral White Lesions. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18–21 1994. Journal of oral pathology & medicine. 1996 Feb;25(2):49-54.
3. Zhang L, Cheung Jr KJ, Lam WL, Cheng X, Poh C, Priddy R, Epstein J, Le ND, Rosin MP. Increased genetic damage in oral leukoplakia from high risk sites: potential impact on staging and clinical management. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2001 Jun 1;91(11):2148-55.
4. Neville BW, Day TA. Oral cancer and precancerous lesions. CA: a cancer journal for clinicians. 2002 Jul;52(4):195-215.
5. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Critical Reviews in Oral Biology & Medicine. 2003 Jan;14(1):47-62.
6. Lodi G, Porter S. Management of potentially malignant disorders: evidence and critique. Journal of oral pathology & medicine. 2008 Feb;37(2):63-9.
7. General Assembly of the World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. The Journal of the American College of Dentists. 2014;81(3):14-8.
8. AL-DROUBY HL. Oral leukoplakia and cryotherapy. British dental journal. 1983;155(4):124-5.
9. Ishii J, Fujita K, Munemoto S, Komori T. Management of oral leukoplakia by laser surgery: relation between recurrence and malignant transformation and clinicopathological features. Journal of clinical laser medicine & surgery. 2004 Feb 1;22(1):27-33.
10. Pindborg JJ, Jølst O, Renstrup G, Roed-Petersen B. Studies in oral leukoplakia: a preliminary report on the period pervalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. The Journal of the American Dental Association. 1968 Apr 1;76(4):767-71.
11. Jr SS, Gorsky M, Ms FL. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984 Feb 1;53(3):563-8.
12. Vedtofte P, Holmstrup P, Hjørting-Hansen E, Pindborg JJ. Surgical treatment of premalignant lesions of the oral mucosa. International journal of oral and maxillofacial surgery. 1987 Dec 1;16(6):656-64.
13. Fernandes G. Beta-carotene supplementation: friend or foe?. The Journal of laboratory and clinical medicine. 1997 Mar 1;129(3):285-7.
14. Girod SC, Pfahl M. Retinoid actions and implications for prevention and therapy of oral cancer. International journal of oral and maxillofacial surgery. 1996 Feb 1;25(1):69-73.
15. Hammersley N, Ferguson MM, Rennie JS. Topical bleomycin in the treatment of oral leukoplakia: a pilot study. British Journal of Oral and Maxillofacial Surgery. 1985 Aug 1;23(4):251-8.
16. Sporn MB, Dunlop NM, Newton DL, Smith JM. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). InFederation proceedings 1976 May 1 (Vol. 35, No. 6, pp. 1332-1338).
17. Hong WK, Endicott J, Itri LM, Doos W, Batsakis JG, Bell R, Fofonoff S, Byers R, Atkinson EN, Vaughan C, Toth BB. 13-cis-retinoic acid in the treatment of oral leukoplakia. New England Journal of Medicine. 1986 Dec 11;315(24):1501-5.
18. Shin DM, Khuri FR, Murphy B, Garden AS, Clayman G, Francisco M, Liu D, Glisson BS, Ginsberg L, Papadimitrakopoulou V, Myers J. Combined interferon-alfa, 13-cis-retinoic acid, and alpha-tocopherol in locally advanced head and neck squamous cell carcinoma: novel bioadjuvant phase II trial. Journal of Clinical Oncology. 2001 Jun 15;19(12):3010-7.
19. Graham HN. Green tea composition, consumption, and polyphenol chemistry. Preventive medicine. 1992 May 1;21(3):334-50.
20. Mukhtar H, Ahmad N. Tea polyphenols: prevention of cancer and optimizing health. The American journal of clinical nutrition. 2000 Jun 1;71(6):1698S-702S.
21. Hastak K, Gupta S, Ahmad N, Agarwal MK, Agarwal ML, Mukhtar H. Role of p53 and NF-κB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells. Oncogene. 2003 Jul;22(31):4851-9.
22. Tsao AS, Liu D, Martin J, Tang XM, Lee JJ, El-Naggar AK, Wistuba I, Culotta KS, Mao L, Gillenwater A, Sagesaka YM. Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. Cancer prevention research. 2009 Nov;2(11):931-41.
23. Mathew B, Sankaranarayanan R, Nair PP, Varghese C, Somanathan T, Amma BP, Amma NS, Nair MK. Evaluation of chemoprevention of oral cancer with Spirulina fusiformis.


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