



Anti-Inflammatory Property of Lycopene, Vitamin E and their Combination – In Vitro Study

V. Divyadharsini¹, T.N. Uma Maheswari^{2*}, S. Rajeshkumar³

¹Post Graduate Student, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

²Professor and Head of the Admin, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

³Professor, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

***Corresponding author:** T.N. Uma Maheswari, Professor and Head of the Admin, Department of Oral Medicine and Radiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India,
Email: umamaheswaritn@saveetha.com

Submitted: 27 April 2023; Accepted: 16 May 2023; Published: 18 June 2023

ABSTRACT

Background: Lycopene is a non provitamin A carotenoid primarily found in tomatoes, papaya, pink grapefruits, and other foods. Lycopene on its own effectively reduces inflammation by inhibiting the release of Tumor Necrosis Factor - α and other inflammatory cytokines. Vitamin E is a group of eight fat soluble compounds that include four tocopherols and four tocotrienols. Vitamin E is shown to inhibit COX-2, C reactive protein, serum levels of Interleukin-6 involved in inflammatory reactions. The present study was conducted to compare the anti-inflammatory properties of Lycopene, Vitamin E and their combination.

Materials and Methods: Albumin Denaturation Assay and Egg Albumin Denaturation Assay was used to evaluate the anti-inflammatory activity of five concentrations of Lycopene, Vitamin E and their combination (10 μ l, 20 μ l, 30 μ l, 40 μ l and 50 μ l). The statistical analysis performed using the SPSS. Lycopene, Vitamin E and their combination were compared against one another by Repeated measures ANOVA and post hoc test using Tukey test for multiple independent groups.

Results: Anti-inflammatory activity of Lycopene and Vitamin E combination was as effective as individual compounds; in few instances better than individual compounds. The anti inflammatory activity had a dose dependent effect; the anti-inflammatory action of all the compounds increased while increasing the concentration. The results were statistically significant (P value <0.05).

Conclusion: From the above results, it can be concluded that the combination of herbal extracts has better anti-inflammatory properties. Lycopene and Vitamin E used alone or in combination with other drugs, can be formulated in topical forms and used for the chemoprevention of oral inflammatory lesions.

Keywords: *Anti-inflammatory, Carotenoids, Interleukins, Tocopherols, Tomato*

J Popul Ther Clin Pharmacol Vol 30(16):e288–e294; 18 June 2023.

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INTRODUCTION

Lycopene is a non-provitamin A carotenoid which is found in tomatoes, pink grapefruit, watermelon, papaya, guava and other red colored fruits and vegetables.(Imran et al., 2020) The use of lycopene as a nutraceutical has increased in the last few years, especially due to its anti-inflammatory and antitumor properties. These properties rely on inhibiting several endogenous interleukins (IL) and the activation of several antitumor mechanisms like the inhibition of the production of nitric oxide and inflammatory cytokines, such as tumor necrosis factor alpha; interleukins of the families 1, 6, and 8; and interferon gamma.(Magne et al., 2022) Clinical studies have shown that lycopene decreases the incidence of chronic inflammatory related diseases, such as coronary heart disease.(Gómez-Romero et al., 2007) Chronic inflammation is a complex biological process, involving different pathophysiological processes such as radical formation and cytokine production. In vitro and in vivo tests suggest that Lycopene are excellent antioxidants, having the ability to sequester and inactivate free radicals.(Pu and Tang, 2017)

Vitamin E is a group of potent, lipid-soluble, chain-breaking antioxidants. It can be classified into tocopherol and tocotrienol based on the chemical structure.(Nazrun et al., 2012) In human subjects and animal models, high doses of vitamin E found to exhibit anti-inflammatory effects by decreasing C-reactive protein (CRP) and inhibiting the release of proinflammatory cytokines.(Singh and Devaraj, 2007) Vitamin E was also found to inhibit cyclooxygenase-2 activities, it was thought to be able to exert anti-

inflammatory and anticarcinogenic activities, especially in the colon.(Jiang et al., 2008) Literature search revealed a systematic review assessing the effect of Vitamin E on inflammatory biomarkers and concluded that CRP and IL-6.(Asbaghi et al., 2020)

Lycopene and Vitamin E in isolation, inhibits the inflammatory cascade in cultured cells.(Feng, Ling and Duan, 2010) Evidence regarding the interplay of these bioactive compounds in inflammation has not been demonstrated. This prompted us to assess the anti-inflammatory property of Lycopene, Vitamin E and their combination.

MATERIALS AND METHODS

The study was approved by the Scientific Review Board and Institutional Human Ethical committee of Saveetha Dental College and Hospital, SIMATS University.

Preparation of Lycopene

Lycopene extract powder was obtained from Herbadiet. 2 gm of lycopene was weighed in a digital weighing machine (Figure 1-A) and then dissolved in 100 ml distilled water (Figure 1-B). The mixture was mixed well and was subjected to boiling at 90 degree celsius (Figure 1-C) until the aqueous mixture was well concentrated (Figure 1-D). The concentrated mixture was then subject to filtration (Figure 1-E). The obtained filtrate was subjected to more heating (Figure 1-F), till the volume of the filtrate decreased upto 2 ml (Figure 1-G).



FIGURE 1: Preparation of Lycopene



Figure 1: illustrating the steps involved in the preparation of lycopene

Preparation of Vitamin E

Vitamin E or α -tocopherol was procured from sigma-aldrich. Vitamin E was directly used for the in vitro studies.

Preparation of Lycopene and Vitamin E Combination

2 ml of lycopene concentrate was mixed with 2g of vitamin E. It was mixed by hand for 5 minutes and then placed in a vortex for 10 minutes. Then it was placed in a digital sonicator for 30 minutes. Once both the compounds were dissolved it was used for further studies (Figure 2).



FIGURE 2: Preparation of Lycopene and Vitamin E Combination

Anti-Inflammatory Property of Lycopene, Vitamin E and their Combination Albumin Denaturation Assay

The anti-inflammatory activity for lycopene, vitamin E and their combination was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations. 0.05 mL of lycopene, vitamin E and their combination of various fixation (10 μ L, 20 μ L, 30 μ L, 40 μ L and 50 μ L) was added to 0.45

mL bovine serum albumin (1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control.

Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

Egg Albumin Denaturation Assay

A 5ml solution was made which consisted of 2.8ml of freshly prepared phosphate buffered

saline of pH - 6.3, 0.2 ml of egg albumin extracted from hens egg. Specific concentrations were prepared separately for lycopene, vitamin E and their combination as (10 μ L, 20 μ L, 30 μ L, 40 μ L and 50 μ L). Diclofenac sodium was used as the positive control. Then the mixtures were heated in a water bath at 37°C for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm (Figure 3).

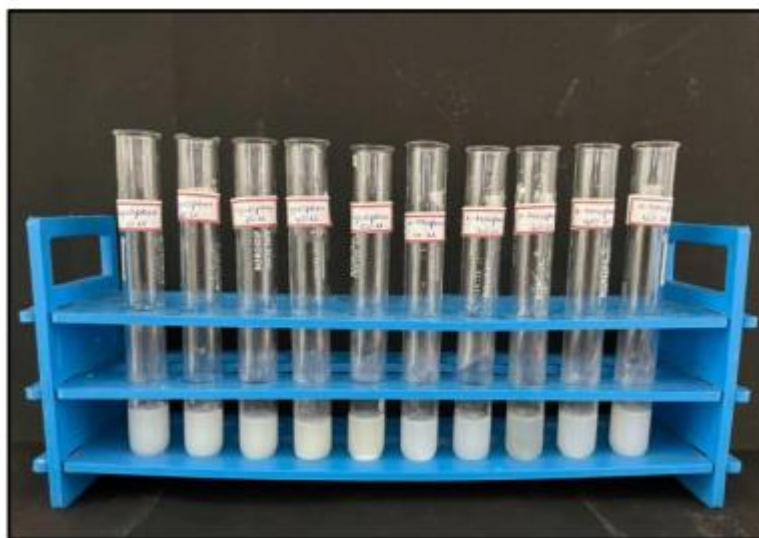


FIGURE 3: Egg Albumin Denaturation assay to test the anti-inflammatory activity of Lycopene, Vitamin E and their combination

Statistical Analysis

The statistical analysis performed using the SPSS (Version 9.05, Chicago, IL, U.S.A). Lycopene, Vitamin E and their combination were compared against one another by Repeated measures ANOVA and post hoc test using Tukey test for multiple independent groups. Significantly variances among sample resources was evaluated by Duncan's multiple comparison test within $p < 0.05$.

RESULTS

Lycopene, Vitamin E and their combination exhibited significant dose-dependent anti-inflammatory activity, the result was mentioned in Table 1. Lycopene and Vitamin E combination showed a very good anti-inflammatory effect when compared to standard and individual

compounds. The anti-inflammatory action of all the compounds increased while increasing the concentration.

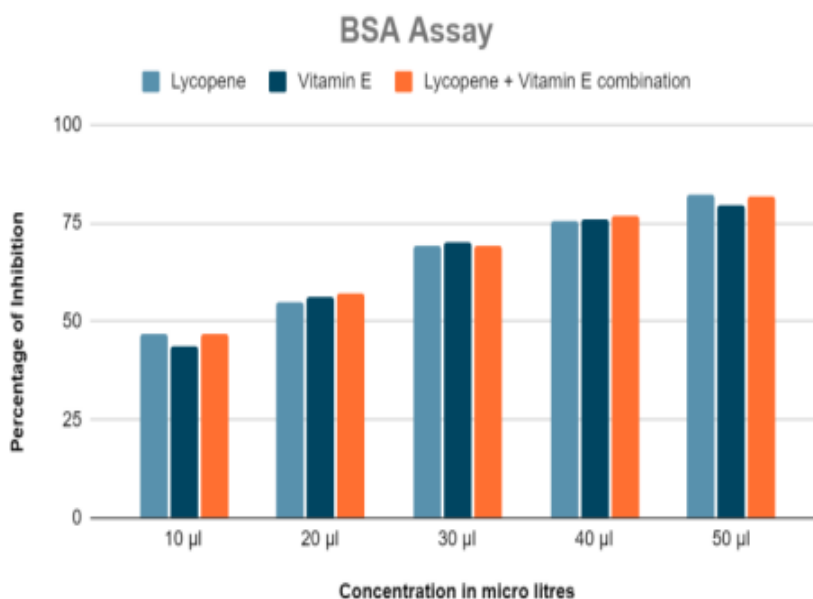
There is a significant increase in the percentage inhibition of Lycopene and Vitamin E combination compared to individual compounds and standard medication by Albumin Denaturation assay ($p=0.027$). Graph 1 shows the anti-inflammatory property of lycopene, Vitamin E and their combination by BSA assay.

Similarly, Lycopene and Vitamin E combination exhibited increased percentage inhibition compared to other medications and standard medication at all concentrations by EA assay ($p=0.030$). Graph 6 shows the anti-inflammatory property of lycopene, Vitamin E and their combination by BSA assay.

TABLE 1: Anti-inflammatory Property at different concentrations

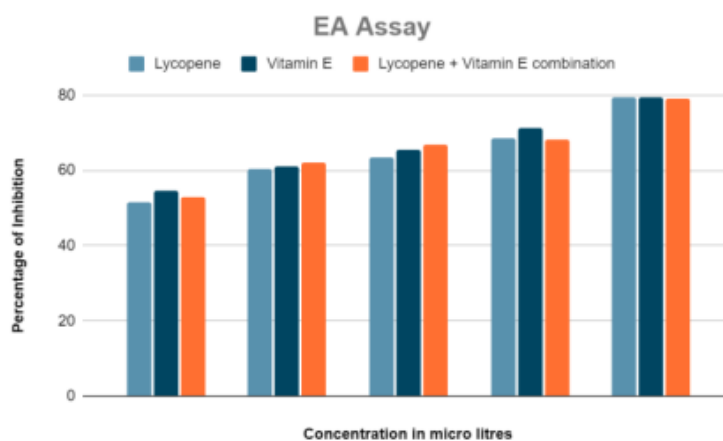
Anti-inflammatory Assay	Concentration	Mean ± SD			P Value
		Lycopene	Vitamin E	Combination	
BSA Assay	10 µl	46.67 ± 1.155	43.67 ± 1.527	46.67 ± 1.527	0.027
	20 µl	55.00 ± 1.135	56.33 ± 0.577	56.89 ± 1.00	
	30 µl	69.00 ± 1.084	70.00 ± 1.004	69.33 ± 1.155	
	40 µl	75.33 ± 0.577	76.00 ± 2.491	76.67 ± 0.577	
	50 µl	82.00 ± 1.000	79.33 ± 0.57	81.67 ± 1.155	
EA Assay	10 µl	51.67 ± 0.577	54.67 ± 1.155	52.78 ± 1.582	0.030
	20 µl	60.33 ± 1.527	61.00 ± 1.502	62.04 ± 1.285	
	30 µl	63.33 ± 0.577	65.50 ± 1.293	66.93 ± 1.119	
	40 µl	68.67 ± 1.155	71.33 ± 1.155	68.33 ± 1.155	
	50 µl	79.33 ± 1.527	79.33 ± 0.577	79.11 ± 1.616	

Comparison of Anti-inflammatory Property of Lycopene, Vitamin E and their combination in different concentrations represented as mean ± SD for triplicates



GRAPH 1: Anti-inflammatory property by BSA assay in terms of inhibition percentage at different concentration

Anti-inflammatory activity (%) on BSA and diclofenac sodium used as a standard in two different concentrations (0.4 and 0.8 mg/ml). All value is conveyed as M ± SD (n = 3)



GRAPH 2: Anti-inflammatory property by EA assay in terms of inhibition percentage at different concentration

Anti-inflammatory activity (%) on EA diclofenac sodium used as a standard in two different concentrations (0.4 and 0.8 mg/ml). All value is conveyed as $M \pm SD$ ($n = 3$)

DISCUSSION

The inflammatory response is a multifaceted biological process involving for instance the formation of reactive oxygen species, production of pro and anti inflammatory cytokines and up regulation of transcription factors and adhesion molecules. Chronic inflammatory diseases and chronic infections are putative contributors to the development of epithelial malignancies. Because of this, a large array of new compounds targeting inflammation have been developed to impede cancer progression. (Hofseth and Wargovich, 2007) Dietary supplements, such as natural antioxidants, are emerging as an important class of anti-cancer drugs by targeting inflammation. (Dueregger et al., 2014) Lycopene is a natural compound derived from plants and microorganisms. Vitamin E is a fat-soluble antioxidant that stops the production of ROS formed when fat undergoes oxidation.

Solubility of the drug was determined in different solvents such as water, methanol, ethanol, chloroform, and buffers such as phosphate buffer of pH 7.2. Lycopene is completely soluble in chloroform and phosphate buffer which is in accordance with the findings from the previous studies (Nikolova and Prokopov, 2013). Vitamin E is completely soluble in methanol and

chloroform which was in agreement with previous studies (Duhem, Danhier and Pr eat, 2014).

The anti-inflammatory activity of Lycopene, Vitamin E and their combination was estimated by comparing the percentage inhibition of BSA assay and EA assay with that of standard diclofenac sodium. In the present study, lycopene and vitamin E combination had potent anti-inflammatory properties when compared to lycopene and vitamin E used alone. The anti-inflammatory property of lycopene and vitamin E is already well proven (Jiang, 2014; van Steenwijk, Bast and de Boer, 2020). Literature search did not reveal any study assessing the synergistic anti-inflammatory effect of lycopene and vitamin E combination. In our investigation the maximum inhibition achieved was 79% at 50 $\mu\text{g/ml}$ of lycopene and vitamin E combination which is comparatively equal to that of standard.

CONCLUSION

From the above results, it can be concluded that the combination of herbal extracts has better anti-inflammatory properties. Lycopene and Vitamin E used alone or in combination with other drugs, can be formulated in topical forms and used for the chemoprevention of oral inflammatory lesions.

ACKNOWLEDGEMENT

Nil

Financial Support And Sponsorship

Study funded by Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University and Rukmani chemicals, Chennai, Tamil Nadu.

CONFLICT OF INTEREST

There are no conflicts of interest.

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