RESEARCH PAPER

Comparative Assessment of In Vitro Antioxidant Potential of Lycopene-Loaded Nanostructured Lipid Carriers and Conventional Suspension

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ABSTRACT

Naturally occurring lycopene is widely recognized as a potential antioxidant. It also has therapeutic potential to prevent oxidative stress-induced disorders. The clinical applications of lycopene have several limitations due to poor solubility and low bioavailability. Encapsulation of lycopene in nanostructured lipid carriers enhances solubility and bioavailability. The present study was aimed to evaluate the in-vitro antioxidant potential of lycopene-loaded Nanostructured Lipid Carriers (NLCs) and conventional suspension. The lycopene-encapsulated NLCs were formulated by melt emulsification followed by ultrasonication method using solid lipid (glyceryl monostearate) and flax seed oil which acts as a liquid lipid. The particle size, PDI, zeta potential, and percentage entrapment efficiency of NLCs were found to be 559.7 nm, 0.73, -27.24 mV, and 84.62 ± 1.93. The antioxidant activity of both LYC-loaded NLCs and suspension was analyzed by DPPH radical scavenging assay and hydrogen peroxide assay with standard (ascorbic acid). From antioxidant activity, it was concluded that the LYC-loaded NLCs showed significantly higher radical scavenging property than the conventional suspension at the same concentration, and both showed increased antioxidant property with an increase in concentration. The IC_{50} value was found to be 15.88 µg/ml and 22.15 µg/ml for LYC NLCs and LYC suspension, respectively, for the DPPH assay, and 14.78 µg/ml and 21.65 µg/ml for the hydrogen peroxide assay. The IC_{50} value of the NLC formulation was lower than that of the suspension, indicating enhanced antioxidant efficacy. The particle size was found in the nanometric range, and due to this, the entrapment efficiency was increased. Hence, these findings suggest that NLCs represent an auspicious delivery system for improving the antioxidant performance of lycopene.

Keywords: Nano-lipid Carriers (NLCs), Lycopene, Antioxidant Activity, DPPH Radical Scavenging Assay.

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Introduction

Oxidative stress is responsible for the development of various chronic diseases such as cardiovascular disease, neurodegenerative disorders, diabetes and cancer many more[1, 2]. In normal metabolism process the reactive oxygen species generated and destroys the DNA, proteins and lipids. Antioxidants help to reduce this oxidation process by scavenging the free radicals and prevents the changes in biomolecules structure[3]. A red piment carotenoid known as lycopene (LYC) is found in various fruits and vegetables basically from tomatoes and tomato based dietry products which having some antioxidant activity[4]. It can reduce the damage of proteins and lipids due the quenching of single oxygen species. It also shows the increased endogenous antioxidant enzyme activity which mitigates various chronic disease and metabolic disorders [5]. Although the various biological benefits the application of lycopene is restricted as clinically due to its strong lipophilic nature which retards the aqueous solubility of lycopene[6, 7]. This behaviour lowers the GI absorption of active moiety. Lycopene is prone to degrade in light and paraded instability which can diminish the bioavailability in the form of conventional preparations [8].

The nanotechnology an attractive approach to overcome the problem associated with poor solubility and bioavailability of lycopene. The NLCs are second

generation of solid lipid nanoparticles having affinity to enhance the drug loading, entrapment efficiency stability and bioavailability due to the presence of solid and liquid lipid which provide a rough surface for the binding of active drug[9]. The encapsulation of active material may alter the surface area which leads a greater solubility and protect to drug from degradation resulting in enhance bioavailability and pharmacological properties [10]. From the various studies it was proven that the antioxidant activity of various products may can be amplified when delivered by nanocarriers[11, 12]. On the bases of these finding the present study having the goal to develop the nano lipid carriers of lycopene and assessment of their in vitro antioxidant potential against the conventional suspension by using DPPH and Hydrogen peroxide radical scavenging assay[13].

2 Materials and Methods

2.1 Materials

Standard lycopene was obtained from Panacea biotech ltd. as a gift sample. Glyceryl monostearate and stearic acid were purchased from Merck (Mumbai, India). Polyethylene glycol 4000, Tween 80, Methanol, Hydrogen peroxide, ascorbic acid and carboxy methyl cellulose were purchased from CDH (P) Ltd. New Delhi. DPPH and Flaxseed oil were purchased from SRL Mumbai and Dev Herbes.

2.2 Preparation of Lycopene -Loaded Nanostructured Lipid Carriers

Lycopene-loaded nanostructured lipid carriers (NLCs) were prepared using a melt emulsification-followed-by-sonication approach. Briefly, a precisely weighed mixture of GMS as solid lipid and Flax seed oil as liquid lipid was melted at a temperature 69±10 °C above the melting point of the solid lipid under continuous stirring at 1000 rpm to obtain a clear, homogeneous lipid phase. Lycopene was dissolved in the molten lipid mixture to ensure uniform distribution, while an aqueous surfactant (Tween 80) solution was separately heated to the same temperature to avoid rapid solidification during emulsification.

The hot aqueous phase was then added dropwise to the hot lipid phase with continuous stirring to form a coarse pre-emulsion[14]. This pre-emulsion was subjected to probe ultrasonication for 10 minutes to reduce particle size and achieve a narrow size distribution of lycopene-loaded NLCs. The resulting formulation was then characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, and antioxidant activity[15].

2.3 Preparation of Conventional Lycopene Suspension

The conventional Lycopene suspension was prepared by dispersing the lycopene in aqueous phase with 0.25% CMC as a suspending agent. The mixture was stirred with continuous stirring to obtained a uniform suspension and served as a control formulation.

2.4 Characterization of Nanostructured Lipid Carriers

2.4.1 Particle size and Polydispersity Index and Zeta- Potential

The particle size and zeta potential of lycopene loaded NLCs was analysed by using Malvern Zeta-sizer Nano ZS instrument based on dynamic light scattering. 1 ml sample was diluted with distilled water and volume make up to the 20 ml for removal of multiple scattering. The PDI characterized the homogeneity and varies from 0 to 1. The zeta potential indicates the particle surface charge which measures the electrophoretic mobility of particles and converted it to zeta potential[16].

2.4.2 Entrapment Efficiency

EE efficiency of lycopene loaded nano lipid carriers was determined by calculating the free amount of lycopene for this 1 ml of NLCs was mixed with 9 ml of solvent (chloroform: methanol, 1:2). The free drug was solubilized in particular solvent. The prepared solution was centrifuge for 3 min at 2000 rpm. Supernatant was collected and analysed by spectrophotometer in 472 nm for the determination of free lycopene [17]. The % EE was calculated by as follows-

$$EE\% = \frac{(LYC\ total - LYC\ free)}{LYC\ total} \times 100$$

2.4.3 Morphological study

The morphology of LYC loaded NLCs was analysed by transmission electron microscope (TEM). Sample was diluted and a drop of that sample as stained negatively by 2 % solution of phosphotungstic acid. The sample was dried after application of carbon coated grid on the slide. After this the slide was subjected in TEM for the morphological evaluation of NLCs [18].

2.5 In vitro Antioxidant Activity

2.5.1 DPPH Radical Scavenging Assay

DPPH radical scavenging assay was performed by mixing the both lycopene loaded NLCs and conventional suspension of lycopene in 0.025% methanolic solution of DPPH in a fixed volume. For the optimization of IC50 known as the concentration used for reduction of 50% of the absorbance of free radicals of different concentration of NLCS and suspension 5-25 µg/mL were prepared and DPPH solution was added. All the samples were incubated in the dark for 30 minutes and absorbance was measured

at 517 nm [19]. The % RSA was calculated by using formula-

%RSA= [(A control−A sample/ A control] ×100

Where

A control= absorbance of control (Ascorbic acid)

A sample= absorbance of sample (Nlcs and suspension)

2.5.2 Hydrogen Peroxide Scavenging Assay

The ability of lycopene loaded NLCs and conventional suspension to scavenge the H_2O_2 was determined by preparing the 4mM solution of H_2O_2 in phosphate buffer saline pH 7.4 at 20° C. The different concentration 5-25 µg/mL of NLCs formulation and suspension were prepared and equal amount of H_2O_2 solution were added in each sample. After that the concentration of H_2O_2 was determined by spectrophotometrically at 230 nm [20].

3 Result and Discussion

3.1 Particle size and Polydispersity Index and Zeta-Potential

The particle size of Lycopene loaded NLCs was measured by Zeta sizer. The mean particle size and PDI (Polydispersity index) was found to be 559.7 nm and 0.73. The value of PDI was less than 1 which shows the mono dispersion of particles. The zeta potential was found to be -27-24 mv which indicates that the NLC formulation was stable.

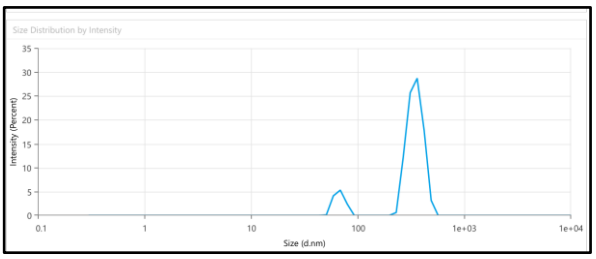


Figure 1 Particle Size of Lycopene Loaded NLCs

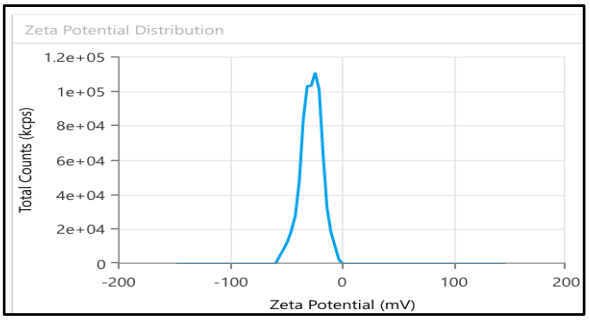


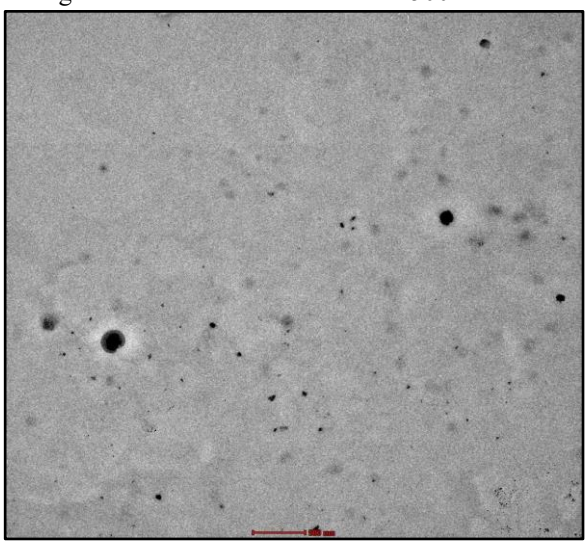
Figure 2 Zeta Potential of Lycopene Loaded NLCs

3.2 Entrapment Efficiency

The entrapment efficiency of LYC loaded NLCs was found to be 84.62±1.93. Which indicates that the higher amount of Lycopene was entrapped in lipid layer and the NLCs have greater capability to hold the active material [21].

3.4 Morphological Study

The morphology of NLCs was analysed by transmission electron microscope (TEM) and the result was shown in figure 3 which indicates that the NLCs having round surface and nano in size 500 nm.



4.1 In vitro Antioxidant Activity

4.1.1 DPPH Radical Scavenging Assay

The antioxidant activity of lycopene loaded NLCs and lycopene conventional suspension was measured by the scavenging capacity of DPPH. In this study the DPPH scavenging ability of lycopene NLCs and lycopene suspension were measured by using ascorbic acid as standard and shown in Table 1. From the study it was found that the with increase in the concentration of NLCs and suspension the antioxidant activity was increased. The NLCs have the significantly higher scavenging ability than the lycopene suspension at same concentration but lower than the ascorbic acid. The IC_{50} value was found to be 15.88 µg/ml and 22.15 µg/ml for LYC NLCs and LYC suspension respectively [22].

Table 1: % RSA, IC50 value of ascorbic acid, lycopene NLCs and Lycopene suspension By DPPH Assay

Sample	Concentration (µg/ml)	% RSA	IC_{50} Value (µg/ml)
Standard (Ascorbic acid)	5	36.37	11.61 µg/ml
	10	44.58	
	15	61.43	
	20	70.42	
	25	82.55	
Lycopene NLCs	5	1.17	15.88 µg/ml
	10	35.25	

	15	47.55	
	20	61.53	
	25	74.07	
Lycopene suspension	5	6.17 %	**22.15 µg/ml**
	10	14.25 %	
	15	30.55 %	
	20	41.53 %	
	25	61.17 %	

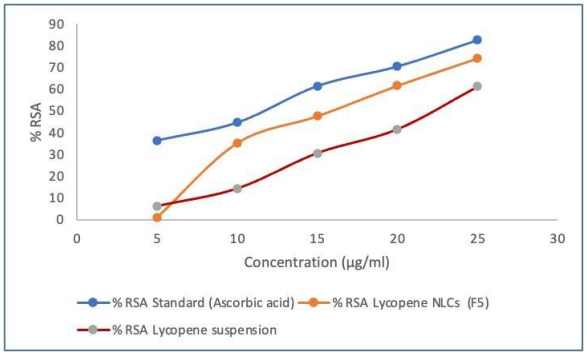


Figure 4: % RSA, IC50 value of ascorbic acid, lycopene NLCs and Lycopene suspension By DPPH Assay

4.1.2 Hydrogen Peroxide Scavenging Assay

The antioxidant activity of lycopene loaded NLCs and lycopene conventional suspension was measured by the scavenging capacity of hydrogen peroxide. In this study the DPPH scavenging ability of lycopene NLCs and lycopene suspension were measured by using ascorbic acid as standard and shown in Table 2. The NLCs have the significantly higher scavenging ability 9.17 at 5 µg/ml – 77.07 at 25 µg/ml than the lycopene suspension at same concentration 5.15 at 5 µg/ml – 63.17 at 25 µg/ml but lower than the ascorbic acid. The IC_{50} value was found to be 14.78 µg/ml and 21.65 µg/ml for LYC NLCs and LYC suspension respectively.

Table 2 % RSA, IC50 value of ascorbic acid, lycopene NLCs and Lycopene suspension By Hydrogen Peroxide Assay

Sample	Concentration (µg/ml)	% RSA	IC_{50} Value (µg/ml)
	5	37.37	
Standard (Ascorbic acid)	10	45.58	**11.31 µg/ml**
	15	62.43	
	20	71.42	
	25	83.55	
Lycopene NLCs	5	9.17	**14.78 µg/ml**
	10	38.25	
	15	50.55	
	20	64.53	
	25	77.07	
Lycopene suspension	5	5.17	**21.65 µg/ml**
	10	16.25	
	15	32.55	
	20	43.53	
	25	63.17	

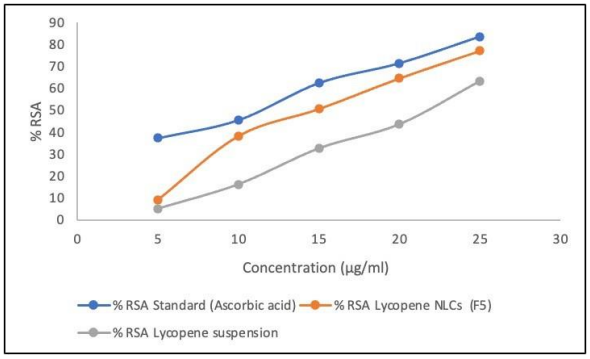


Figure 5 % RSA, IC50 value of ascorbic acid, lycopene NLCs and Lycopene suspension By Hydrogen Peroxide Assay

5 Conclusion

The lycopene loaded NLCs and suspension was successfully prepared and comparatively evaluated for antioxidant activity with ascorbic acid. The LYC-NLCs showed favourable physicochemical characteristics and also having greater free radical scavenging property. The reason behind the improved antioxidant study may be the enhancement in dispersion, greater surface area and protection of lycopene in lipid matrix. So, from this we can conclude that the nanostructured lipid carrier is a promising system to improve the antioxidant property of lycopene.

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