



Lycopene Nanoparticles Ameliorate The Antioxidants, Antimicrobial And Anticancer Potencies Of Tomato Pomace.

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Abstract

Nanoparticles are the basic essential element of nanotechnology and it exhibits characteristic features based on their properties such as size, morphology and other size dependent properties. Their applications on agriculture, food and medicine are widely used nowadays. Agro-waste materials cause a global environmental problem worldwide. Maximizing the benefits of such waste material is a very important issue. The present investigation aimed to use nano-formulation techniques by using polyethylene glycol 8000 to produce nano-lycopene extracted from tomato waste (pomace) as well as evaluation the antioxidant, antimicrobial and anticancer activity of produced lycopene-NPs. The results of antioxidant activity using ABTS and DPPH assays revealed that nano-formulation of Lycopene increases the scavenging activity compared with lycopene. Also, both lycopene and lycopene-NPs showed antibacterial activity against all tested bacteria and antifungal activity against tested fungal species. MIC for lycopene and lycopene-NPs was determined. DNA damage induced by oxidative stress protection analysis indicated that lycopene-NPs has a great potential in DNA damage prevention compared to their respective controls. The MTT assay exhibited cytotoxic activity against three cancerous cell lines, MCF-7, HCT-116 and HepG2. As well as lycopene-NPs biosafety was tested against normal cell lines (BJ-1 and MCF-12F) compared to lycopene.

Key words: Lycopene, Agro-waste, Antioxidant, DNA damage, Antimicrobial, Anticancer activity.

1. Introduction

In the last two decades, the nano-sized materials production and their pharmaceutical activity have been extensively investigated. Thus, the physical and chemical characteristics of substances are changed, including quantum size effect, mesoscopic effect, solubility, the surface effect, and pharmaceutical activities [1]. The nano-materials have a wide range of applications in the science and technology fields, such as biomedical applications, electronic IT applications, as well as environmental remediation [2]. Moreover, drugs coupled nanocarrier are the most important and successful application in drug delivery, due to their biological activity in vivo are mainly dependent on their nanoproperties [3,4]. Several strategies which used in the production of nano materials could alter different parameters of nanoproducts including,

surface modification, size and shape [5,6]. Among them, surface coating of polyethylene glycol (PEG) is the most frequent and effective method to produce nanoparticles, because of their “stealth” properties, that accelerate their clinical transformations [7]. The “stealth” properties of PEG nanoparticles have long been considered to be determined the surface PEG length and density, both of which are hard to finely control [8].

Tomato (*Solanum lycopersicum L.*) is an annual or perennial plant of the Solanaceae family. It is one of the widely consumed vegetables that is available both raw and in the form of various types of processed products, such as ketchup, juice, and sauce [9]. Consumption of tomatoes and tomato-based food reduce the risk of different types of human cancers (oral cavity, pharynx, esophagus, stomach, rectum, colon, urinary bladder, prostate and breast cancer)

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[10]. Reutilization of agroindustrial by-products have been implicated in animal feeding and reduces the environmental problems associated with their accumulation. Tomato pomace is a by-product of the tomato industry have high nutritional value. However, tomato pomace is important for its high contents of carotenoids and lycopene. Carotenoids show strong antioxidant activities and also important source for pro-vitamin A to the human body [11]. Lycopene is the natural red color pigment provided by red fruits and vegetables such as guava, watermelon, papaya, grapefruit and tomato. Lycopene is economically relevant as red color food additive, because its intense red color [12]. Furthermore, lycopene displays an antioxidant activity as it reduces risk of chronic diseases. In addition, reduction of cholesterol level in blood and therefore, protects against cardiovascular diseases [13]. The structure function relationship of lycopene indicated that it contains an acyclic isomer of β -carotene, which is one of the members of carotenoid family Figure 1. It is a lipid soluble antioxidant produced naturally by various plants, microorganisms and certain algae and fungi but not synthesized by animals and humans [14]. In plants, lycopene acts as an accessory light-gathering pigment that protects the plants against the toxic effects of oxygen and light. Chemically, lycopene has a unique highly unsaturated structure with 11 conjugated and 2 unconjugated double bonds. As a polyene, it undergoes cis-trans isomerization induced by light, thermal energy and chemical reactions.

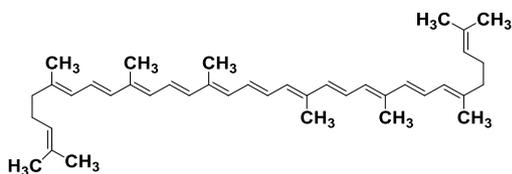


Figure 1. Lycopene chemical structure

Lycopene obtained from plant exists predominantly in an all-trans configuration, which is the most stable form, thermodynamically [15]. Lycopene is present as an isomeric mixture, with 50% as cis isomers in human plasma [16]. It is one of the most potent antioxidants, which has a singlet-oxygen-quenching ability twice as high as that of β -carotene and it has 10 times higher ability than that of α -tocopherol [17]. The aim of this work is to produce nano-formulated lycopene extracted from tomato waste, then examine the impact of lycopene-NPs as antioxidant, antimicrobial and anticancer in comparison to lycopene.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma (USA) and Fluka (Switzerland) at analytical grade. The tomato's pomace was obtained from Heinz industrial, 6 October-City, Egypt. Then the air dried plant materials were grinded to obtain a homogenous sample.

Crude lycopene extraction.

About 200 g of dried tomato's pomace were extracted by 180 ml ethyl acetate into separating funnel and shaken vigorously and set at room temperature for 24 h. Then the upper layer was then separated and the solvent was evaporated by rotary evaporator at 40°C. The extracted lycopene was covered with aluminum foil and stored in the freezer until further analysis. The final extract consists of tomato oil in which lycopene together with a number of other constituents that occur naturally in tomato, are dissolved and dispersed. [18].

Lycopene nanoparticles preparation:

Lycopene loaded to nanoparticles were prepared according to method described by (Werdirn González *et al.*, 2014 [19]) with slightly modifications. In brief, 50 g of Poly Ethylene Glycol (PEG) 8,000 were melted at 65 °C on a hotplate stirrer in a clean flask. While stirring a 16.5 g of lycopene previously dissolved into 3 ml of Tween 80 were added in a dropwise to the melted PEG, then the mixture was sonicated using a ultrasonic processor (DAIGGER ULTRASONIC Model GEX 750, USA) for 15 min. The mixture was then cooled at 4 °C for 2h and completely ground in a refrigerated mortar. Finally, the product was sieved using a stainless steel sieve (230 mesh), stored at 25 ± 0.5 °C in an airtight container and used for the subsequent bioassay Figure 2.

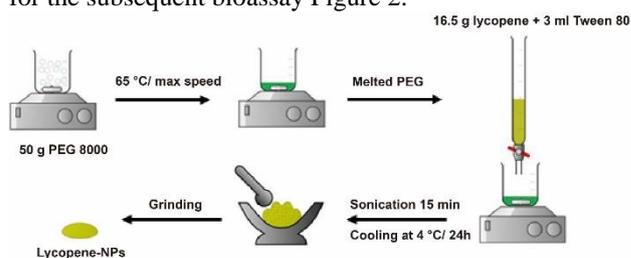


Figure 2. Scheme of Lycopene nano-preparation.

Nano-lycopene characterizations:

A Dynamic Light Scattering (DLS) particle size analyzer (Nano- ZS, Malvern instruments Ltd.,UK) was used to evaluate the NPs surface charge at 25 °C, indicated by the zeta potential values, and the NP dimension, expressed in terms of Z-average size (d), and polydispersity index (PDI). After 24 h of Lycopene-NP preparation, aliquots of each Lycopene-NP were suspended in 10 mL of distilled water and

then the suspension was filtered using Whatman n° 1 filter paper. The morphology of the Lycopene-NPs was visualized using transmission electron microscope TEM (JEM 2100 HRT, HIGH RESOLUTION MADE IN JAPAN). The association level between materials during nanoparticles production was evaluated using Fourier transform infrared spectroscopy FTIR (VERTEX 80v, BRUKER, Germany) at 4 cm⁻¹ resolution and measurement scale range of 4000-400 cm⁻¹ [20].

Biological characterization of Lycopene-NPs.

Antioxidants activity

DPPH radical scavenging activity

The antioxidants activity of lycopene and nano-Lycopene compared to PEG-NPs were measured using a modified method by (Shin, 2012 [21] and Brand-Williams, *et al.* 1995 [22]). Briefly, 50 µL containing different concentration (0.05, 0.01, 0.15, 0.2, 0.5 and 1 mg/ml) of each sample were mixed with 2950 µL of DPPH (0.004% dissolved in methanol). Then, the mixture was shaken and sat to react at 37°C in a dark place for 30 min. The absorbance was measured at 517 nm, compared to vitamin C as standard and the scavenging activity % was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ scavenging activity (Inhibition \%)} = [(A_c - A_s)/A_c] \times 100$$

(A_c: absorbance of the DPPH solution and A_s: absorbance of the sample).

ABTS radical scavenging activity

The potential of ABTS radical scavenging of the above mentioned samples were measured using a modified method described by (Floegel *et al.*, 2011 [23]). Briefly, 980 µL of ABTS solution were added to a mixture of 20 µL containing different concentration (0.05, 0.01, 0.15, 0.2, 0.5 and 1 mg/ml) of each sample. The mixture was then set to react at 37 °C for 10 min in dark. The absorbance was measured at 734 nm and the ABTS radical scavenging percent is calculated using the equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

(A₀ is the ABTS^{•+} absorbance of the control reaction and A₁: is the ABTS absorbance of the sample).

DNA damage protection.

To assess whether lycopene and lycopene-NPs have a potential DNA damage prevention, Fenton's reagent was used based on a previous study reported by (Leba *et al.*, 2014 [24]). A various concentration of *Nano-lycopene and lycopene compared to Nano-control* (0.5, 0.75 and 1.0 mg/ml) were added to a mixture containing 3µl of Ribonuclease Inhibitor (RNH1) plasmid DNA (60µg/µl), 5mM of H₂O₂, 0.35mM of FeSO₄, 0.60mM of EDTA and the final volume was

completed to 25µl with phosphate buffer 8.3mM, pH 7.4. The mixture was incubated for 20 min at 37°C. Also, 3µl of RNH1 plasmid DNA (25µg/µl) were used as DNA protection control. After 20 min samples were loaded into agarose gel 1.5% and separated bands were analyzed.

Evaluation of antimicrobial activity.

The antimicrobial activity of lycopene and Nano-lycopene were tested against different strains of bacteria, including gram positive (*Bacillus cereu* EMCC 1080 and *Staphylococcus ureuse* ATCC 13565) and gram negative (*Escherichia coli* O157-H7 ATCC 51659, *Salmonella typhi* ATCC 15566 and *Pseudomonas aeruginosa* NRRL B-272). Also antifungal activity was tested against six fungal species (*Aspergillus flavus* NRRL 3357, *A.parasiticus* SSWT 2999, *A.niger* ITEM 10027, *A.ochraceus* ITAL 14, *F.proliferitum* MPVP 328 and *Penicillium verrucosum* BFE 500). Antibacterial activity was conducted using well diffusion assay on nutrient agar medium. The tested bacterial plates were incubated at 37 °C for 24 h. after incubation the inhibition zones were measured by ruler in mm. Potato dextrose agar (PDA) was used for antifungal activity using well diffusion technique. The tested plates were incubated at 25 °C for 48 h. (EVCAST, 2015 [25]).

In vitro cytotoxicity

All cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Three human, cancer cell lines were used in this study, colorectal adenocarcinoma (HCT-116, ATCC[®] CCL-247[™]), mammary adenocarcinoma (MCF-7, ATCC[®] HTB-22[™]) and hepatocellular carcinoma (HepG2, ATCC[®] HB-8065[™]), as well as two normal cell lines, skin fibroblast BJ-1 (ATCC[®] CRL-2522[™]) and epithelial breast MCF-12 (ATCC[®] CRL-10782[™]) were used. Cell lines were cultured in DMEM/high glucose media supplemented with 2 mM L-glutamine, 10% FBS and 1% penicillin/streptomycin kept in Corning[®] 75cm² U-Shaped canted neck cell culture flask with vent cap (Corning, New York, USA). Then, sub-confluent cultures (70–80%) were trypsinized (Trypsin 0.05%/0.53 mM EDTA) and split depending on the seeding ratio [26, 27].

MTT assay

Cells (1 × 10⁵/well) were plated into 100 µl of medium/well in 96-well plates (Hi media). After 48 hours incubation the cell reaches the confluence. Then, the media was replaced with RPMI-1640 media containing different concentration of lycopene and Nano-lycopene. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4),

20 μ l/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl- tetrazolium bromide cells (MTT) phosphate- buffered saline solution were added. After 4h incubation, 130 μ l 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm with reference at 655nm. The concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of cancer and normal cell lines (mentioned above) were expressed as the % cell viability, using the following formula:

$$\text{Cytotoxicity \%} = 100 - \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100\% \quad [28].$$

Statistical analysis: Statistical analyses were conducted by use of CoStat for Windows version 6.45. All data were represented as mean \pm standard deviation (SD) with 3 individual experiments each in triplicate. Treatments were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Lycopene-NPs characterizations.

Transmission Electron Microscope TEM.

The results of TEM analysis showed that the particle size of lycopene-NPs was less than 50 nm (Figure 3) compared to PEG-NPs control. Drug nano-formulations technology have been involved in physical properties alteration, including particles size, solubility and shapes. Such alteration, leads to coordinate various pharmaceutical characteristics such as drug release pattern, efficiency and cytotoxicity [30-31]. However, nanoparticles enhance drug delivery to target organisms and reduce the toxic effects on non-target organisms [32].

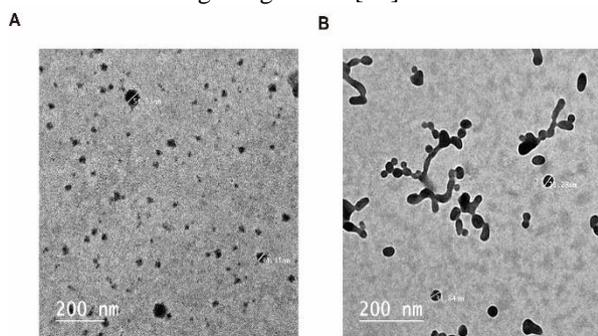


Figure 3. Transmission electron microscope TEM micrographs of PEG control (left panel A), lycopene-loaded PEG-NPs (right panel B).

Fourier-transform infrared spectroscopy (FTIR)

The associations occurred between lycopene and other materials during nano-preparation was evaluated by FTIR (Figure 4). The results indicated that, the spectrum of PEG nano-carrier bands were loaded in lycopene-NPs. In addition, spectrum of lycopene showed that four bands corresponding to lycopene spectrum were loaded in lycopene-NPs at 1102, 1238, 1454 and 2850 cm^{-1} . However, The FTIR spectrum of lycopene-NPs displayed two new bands at 1413 and 1964 cm^{-1} were observed compared to blank nano carrier. The vibration bands of lycopene FTIR spectrum corresponds to CH₂/CH₃ was appeared at 2850 and 3364 cm^{-1} with corresponding scissoring vibrations of CH₂ at 1454 cm^{-1} and CH₃ deformation at 1374 cm^{-1} [33], the R₂C=CR at 611 cm^{-1} [34], trans CH out of plane bending at 957 cm^{-1} [33], stretching vibration of C=C at 1553 cm^{-1} , stretching band of C-C at 957–1166 cm^{-1} [35], and CH₂ rocking vibration at 718 cm^{-1} [36]. The peaks overlapped between lycopene loaded nanocarriers spectrum compared with lycopene and PEG-NPs prove that, an interaction between lycopene and other nanomaterials are found. However, the presence on new beaks may be due to a chemical reaction of lycopene with other ingredient. Fourier-transform infrared spectroscopy (FTIR) spectra of lycopene loaded to PEG-NPs. Black spectrum corresponds to lycopene, Blue spectrum corresponds to lycopene-NPs, Red spectrum corresponds to Lycopene.

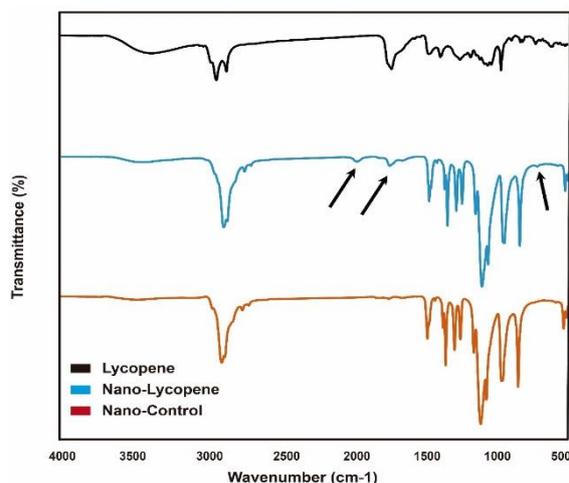


Figure 4. Lycopene-NPs FTIR characterization.

Particle size distribution.

Particle size and zeta potential are limiting factor of nanomedicine effectiveness at drug delivery and stability levels. The Zeta Potential gives an indication about particles surface charge [37]. As a results, particle size (nm), PDI, and zeta potential (mV) analysis revealed that the diameter of produced

lycopene-NPs and PEG-NPs were in range 360–793 nm (Table 1). Thus, the larger particle size formation may be due to greater viscosity of the dispersed phase during ultrasonication [38]. In another hand, PDI value of lycopene-NPs was lower than 0.116 points to a narrow size distribution [39]. Lycopene-loaded NPs exhibited a positive surface charge of about + 4.79 mV, while the unloaded nanoparticles were + 4.50 mV (Table 1). However, it has been reported that ZP ranged from 0-5 mV indicated a rapid coagulation or flocculation of produced nanoparticles [40].

Table 1 Particle size, PDI and Zeta potential of PEG-NPs and lycopene-NPs.

	Lycopene-NPs	PEG-NPs
Partial size (nm)	793.7	360.9
Pdi	0.116	0.588
Zeta potential (mV)	4.79	4.50

Antioxidants activity on DPPH and ABTS.

Previous studies confirmed that plant-derived substances, displayed antioxidant activities by different mechanisms, such as phenols, flavonoids and tannins that [41].

The antioxidant activity was measured by the potential scavenging of two artificial radicals ABTS and DPPH. In this assay we tested the potential of lycopene-NPs to improve its antioxidant activity using ABTS and DPPH assays. The concentration that scavenge 50% IC₅₀ of the radicals was calculated as observed in Table 2. The native lycopene showed the highest antioxidant activity, with IC₅₀ 0.055 and 0.057 µg/ml for DPPH and ABTS, respectively, followed by the lycopene-NPs, with IC₅₀ values 0.115 and 0.128 for DPPH and ABTS respectively. While, antioxidant activity of PEG-NPs control IC₅₀ from 2.50 to 4.59 µg/ml. However, the lycopene loaded to PEG was one third of PEG (one gram of lycopene nanoparticles contain 33.3% of native lycopene). For that a correction factor was calculated according to the next equation: Corrected IC₅₀= Obtained IC₅₀ of lycopene-NPs / 3. The results indicated that, Lycopene-NPs enhances the potential antioxidant activity due to their nano-formulation. In this context, nano-formulations has been altering some physical properties, and therefore increasing the antioxidants activity [30-31]. Lycopene derived from tomato are higher in the lipophilic fraction [42]. In this regards, the antioxidant activity of the liposoluble fraction of tomato poems corresponds to lycopene contents was tested after loading to PEG according to (Raffo *et al.*, 2002 [43]). This was confirmed by the high correlation coefficient value between its content and the antioxidant activity of this fraction. According to the study by (Wawrzyniak *et al.* 2005 [44]), the lycopene content of tomato plays a role in many factors regulation, such as the degree of processing, ripeness and harvest

period of tomato. Previous studies were shaded light on the structure function relationship of lycopene as antioxidants. They found that, the conjugated double-bond system of lycopene reflexes a strong antioxidant activity.

Table 2. Antioxidant activity (IC₅₀) of lycopene and lycopene NPs.

	DPPH	ABTS
	IC ₅₀ (µg/ml)	
Lycopene	0.055 ^b ± 0.0010	0.057 ^c ± 0.0011
Lycopene-NPs	0.115 ^b ± 0.0123	0.128 ^b ± 0.0014
PEG-NPs	2.500 ^a ± 0.1277	4.590 ^a ± 0.0066
LSD	0.148	0.008

All experiments were performed in triplicate; all data are expressed as the mean ± SD.

Means with different letters (at the same Colum) are significantly different at p ≤ 0.05.

DNA damage protection.

Oxidative stress induced by nanoparticle formulations is a limiting factor of one of their clinical use at cellular level [45]. Thus we tested the effects of lycopene-NPs on the DNA damage protection compared to respective controls. In this context, three distinct forms of *RHNI* plasmid DNA are separated on agarose gel electrophoresis. The open circular appear as first band followed by linearized form and the supercoiled circular DNA appear as last band. Upon oxidative stress by Fenton reaction induces single or double-stranded DNA breaks, thus an alteration in the bands density are formed [46]. The DNA damage protection induced by Fenton's reagent using different concentrations (0, 1, 2.5 and 5 µg/ml) of lycopene-NPs compared to respective control were analyzed. The results showed that, *RHNI* supercoiled circular form was slightly degraded and the linear band was observed (Figure 5; lane 3). The lycopene-NPs treatments showed a DNA damage protection (lanes 4–6), indicating that lycopene loaded to NPs gains the scavenging activity against OH[•] compared to lycopene.

Surprisingly, the obtained results showed that lycopene has pro-oxidant activity under the three tested concentrations (Figure 5; lanes 7–9). However, no significant difference was observed with PEG-NPs control treatment (Figure 5; lanes 10–12). Altogether, the results indicated that Lycopene loaded NPs enhances the protection capacity of DNA damage induced by Fenton's reagent compared to lycopene. In agreements with our antioxidant obtained data, nano-formulation gained the radicals quenching activity and therefore protects DNA damage. Thus, the physical modifications occurred by nono-formulation in the size and shape and surface positive charged may be led to fast interaction with DNA negative charged plus

radical quenching of OH[•] generated by fenton's reagents. It has been reported that, lycopene displayed antibacterial activity by inducing reactive oxygen species (ROS)-mediated DNA damage in *Escherichia coli* [47]. The results indicated that, Lycopene-NPs

have the potential to protect damage DNA in *RNHI* plasmid due to their nano-formulation. The antioxidant and DNA damage-protective potencies of Lycopene-NPs still behave as antibacterial agents.

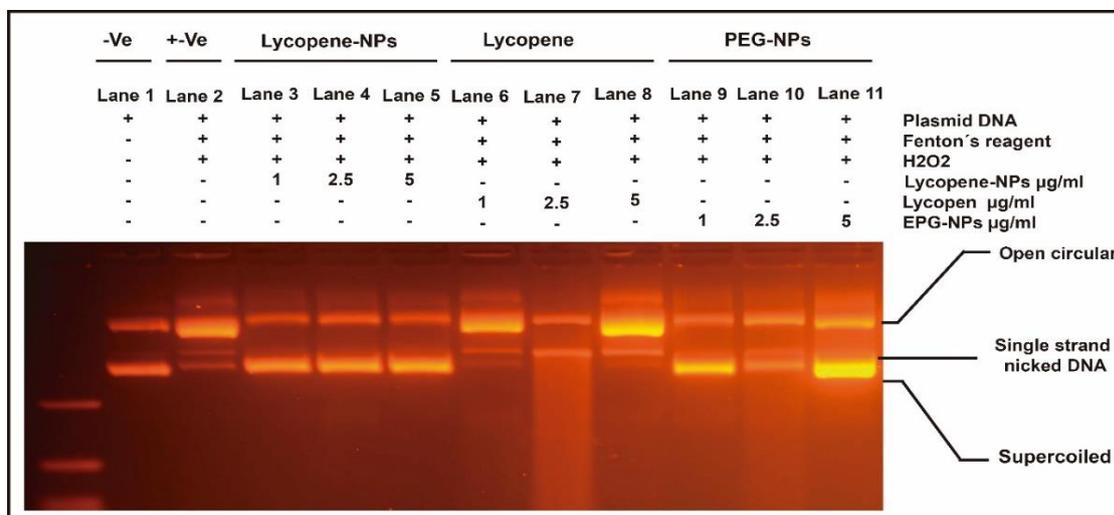


Figure 5. In vitro analysis of DNA damage protection capacity of lycopene-NPs, lycopene and PEG-NPs control. Lane 1: DNA ladder, Lane 2: *RNHI* DNA Plasmid, Lane 3: *RNHI* DNA Plasmid treated with Fenton's reagent, Lane 4-6: lycopene-NPs, Lane 7-9: lycopene and Lane 10-12: PEG-NPs control 1,2.5 and 5 µg/ml, respectively. All the reaction mixtures were incubated for 20 min at 37°C.

Antimicrobial activity of lycopene and lycopene-NPs

The antimicrobial activity of lycopene and lycopene-NPs was illustrated in Table 3, 4. The obtained data showed that lycopene and lycopene-NPs showed antibacterial and antifungal activity against all tested microorganisms. Gram positive bacteria found to be more susceptible than gram negative ones (Table 3). *Bacillus cereus* recorded the highest inhibition zones among all tested bacteria with inhibition zone 15.3 and 12.3 mm for lycopene and lycopene-NPs respectively. While there is no significant difference between lycopene inhibition zone (15.3 mm) and the inhibition zone (16.0 mm) that developed by the control positive ceftriaxone at 1 mg/ml. Also, lycopene and lycopene-NPs showed inhibition zone against gram negative bacteria ranged from 9 to 9.6 mm for *E. coli* and *P. aeruginosa* in the case of lycopene, respectively. While the inhibition zone was from 8.3 to 8.6 mm for *B. cereus* and *S. typhi* for lycopene NPs, respectively. The inhibition zones developed by lycopene-NPs were lower than that obtained by lycopene because one gram of lycopene-NPs contain 33.3% native lycopene.

Antifungal activity of lycopene and lycopene-NPs.

The antifungal activity results against six fungal species were presented in Table 4. Both lycopene and lycopene-NPs showed antifungal activity against all the tested fungi with no significant difference between them except in *A. niger* which showed 13.3 and 10.8 mm inhibition zone for lycopene and lycopene-NPs, respectively. *A. Flavus* found to be most sensitive fungal species towards lycopene and lycopene-NPs which showed inhibition zone 14.8 and 14.1 mm respectively. The obtained inhibition zones developed by lycopene and lycopene-NPs are significantly different with that developed by Miconazole (1 mg/ml) in all tested fungi. Similar observation on the antimicrobial activity of tomato extract have been reported by (Al-Oqaili et al., 2014 [48]). The mode of action of antibacterial effects of the extract has been well studied by several reports. In this regards, the alteration of physicochemical properties induced by nano-formulation are targeting different antibacterial mechanisms, including cell wall or membrane penetration, bacterial proteins and DNA inhibition

synthesis inhibition, formation bacterial biofilms inhibition, and gene expression regulations [49-51].

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration values were presented in figure (6 A, B). MIC of lycopene for tested bacteria (Figure 6A) was in the range 0.092 to

0.50 mg/ml for *S. aureus* and *P. aeruginosa* respectively. While, lycopene-NPs recorded highest MIC with range from 0.167 to 0.667 for *S. aureus* and *P. aeruginosa* respectively. The MIC for fungi was showed in (Figure 6B). Hence, lycopene recorded MIC ranged from 0.208 to 1.33 mg/ml for *P. verrucosum* and *A. niger* respectively. In this regards, several studies for the antibacterial of tomato extract was due to the presence of lycopene [52, 53].

Table 3. Antibacterial activity of lycopene-NPs and lycopene.

Bacteria	Zone Inhibition (mm)				
	Lycopene (100 mg/ml)	Lycopene-NPs (33.3 mg/ml)	PEG (100mg/ml)	Miconazole (1mg/ml)	LSD
<i>B. cereus</i>	15.3±0.57 ^a	12.3±0.58 ^b	0.0 ^c	16±1.00 ^a	1.21
<i>St. Aureus</i>	12.3±0.56 ^b	10±0.58 ^c	0.0 ^d	17.6±1.15 ^a	1.33
<i>E.coli</i>	9.0±0.0 ^b	8.3±0.57 ^b	0.0 ^c	28±2.00 ^a	1.95
<i>S.typhi</i>	9.3±0.58 ^b	8.6±0.53 ^b	0.0 ^c	28.6±1.53 ^a	1.63
<i>P. aeruginosa</i>	9.6±0.58 ^b	8.3±0.58 ^c	0.0 ^d	26.0±2.36 ^a	1.21

All experiments were performed in triplicate; all data are expressed as the mean ± SD. Means with different letters (at the same row) are significantly different at $p \leq 0.05$.

Table 4. Antifungal activity of lycopene-NPs and lycopene.

fungi	Zone Inhibition (mm)				
	Lycopene (100 mg/ml)	Lycopene-NPs (33.3 mg/ml)	PEG (100mg/ml)	Ceftriaxone (1mg/ml)	LSD
<i>A.flavus</i>	14.8±1.04 ^b	14.1±2.84 ^b	0.0 ^c	20.3±1.03 ^a	3.01
<i>A.parasiticus</i>	13.8±2.93 ^b	13.3±2.52 ^b	0.0 ^c	20.6±2.36 ^a	4.26
<i>A.niger</i>	13.3±1.15 ^b	10.8±1.04 ^c	0.0 ^d	20.3±0.76 ^a	1.63
<i>A.ochraceus</i>	12.3±0.76 ^b	11.6±0.76 ^b	0.0 ^c	18.5±0.50 ^a	1.12
<i>F.proliferitum</i>	14.3±0.67 ^b	13.8±1.15 ^b	0.0 ^c	17.3±1.04 ^a	1.63
<i>P.verrucosum</i>	12.0±2.00 ^b	12.3±3.1 ^b	0.0 ^c	17.2±2.57 ^a	4.17

All experiments were performed in triplicate; all data are expressed as the mean ± SD. Means with different letters (at the same row) are significantly different at $p \leq 0.05$.

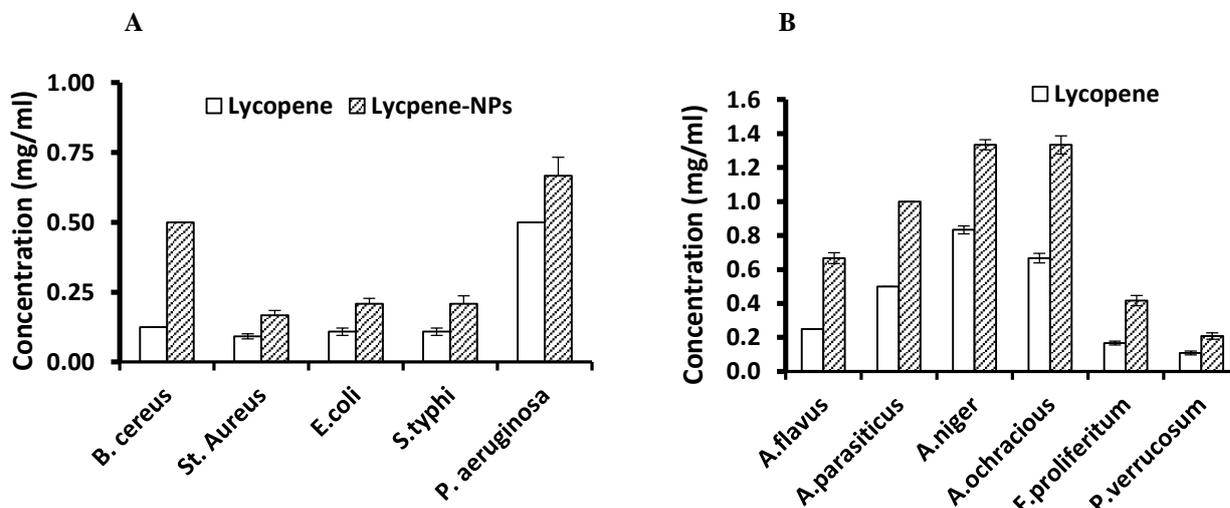


Figure 6. The minimal inhibitory concentration (MIC) of lycopene and lycopene-NPs against some bacteria (A) and fungi (B).

Anticancer activity of lycopene and lycopene-NPs

Cancer therapy is a global health challenge worldwide [54]. In this study, lycopene and lycopene-NPs were tested for their anti-proliferation activity against three cancerous and two normal human cell lines as showed in Table 5. Results showed that, lycopene at 100

µg/ml had the highest potential cytotoxic activity against HepG-2, MCF-7 and HCT-116 with 85.56, 69.53 and 58.34 %, respectively (Table 5). Whereas no significant cytotoxic activity was observed against MCF12F and Bj-1 normal cell line with 8.59 and 1.37%, respectively. The cytotoxic effects of lycopene-NPs against HepG-2, MCF-7 and HCT-116 were 69.06, 54.03 and 66.66 % cell death,

respectively. Our results indicated that, Nano-formulation of lycopene reduces the cytotoxic effect on MCF-12F to 6.65 % and similar effect against Bj-1 with 1.38% compared to lycopene. Taking in our consideration that each one gram of lycopene-NPs contains 33.3% of native lycopene our findings showed that lycopene-NPs has also improved the anticancer activity, which may be due to nano-formulations which altering the free drug delivery inside the cells at the site of action, therefore accumulate more lycopene inside the cells [55].

In addition, no significant cytotoxic activity was observed in PEG-NPs treatments. The concentration causing 50% inhibition of growth of cells (IC_{50}) by regression analyses was calculated. The IC_{50} of lycopene was 58.43, 71.92 and 85.70 $\mu\text{g/ml}$ against HepG-2, MCF-7 and HCT-116, respectively. Whereas, The IC_{50} against MCF12F and Bj-1 normal cell lines was 581 and 3624 $\mu\text{g/ml}$, respectively. Based on the obtained IC_{50} values, Lycopene-NPs was 72.40, 92.54, 75.01, 751 and 3619 $\mu\text{g/ml}$ on HepG-2, MCF-7

and HCT-116, MCF12F and Bj-1 cells respectively. It has been reported that lycopene reduces the risk of several cancers. Among them, prostate, breast, colon, gastric and liver cancerous were well studied [56].

It has been previously reported that potential mode of action of lycopene as anticancer was through cell cycle arrest, modulation of immune system, and apoptosis induction [57]. It was shown that consumption of carotenoids such as lycopene and loaded native lycopene to nanoparticles enhances its anticancer activity. The effect is due to the induction of apoptosis of cancer cells and inhibition of proliferation [58]. More precisely, lycopene induces cell cycle arrest at G0/G1 or by blocking S phase, and enhances apoptosis induction via increase the levels of p53, bax and caspase-3 pro apoptotic markers in addition to reduce the level of Bcl-2 anti-apoptotic marker [59]. Our results revealed that, Nano-formulation of lycopene improves lycopene pharmaceutical properties, including the antioxidants, antibacterial and anticancer activities.

Table 5. Anticancer activity and IC_{50} of lycopene-NPs and lycopene at 100 $\mu\text{g/ml}$.

Cell line	Cytotoxicity % at 100 $\mu\text{g/ml}$				IC_{50} $\mu\text{g/ml}$			
	Lycopene	Lycopene-NPs	PEG-NPs	LSD	Lycopene	Lycopene-NPs	PEG-NPs	LSD
MCF-7	69.5 ^a ±1.4	54.03 ^b ±0.4	9.2 ^c ±0.05	1.70	71.9 ^c ±1.46	92.5 ^b ±0.68	543.4 ^a ±2.9	3.88
MCF12F	8.6 ^a ±0.40	6.65 ^b ±0.03	2.05 ^c ±0.01	0.037	581.6 ^c ±2.7	751.1 ^b ±3.4	2439 ^a ±11.8	14.62
HepG2	85.5 ^a ±0.87	69.06 ^b ±0.67	8.06 ^c ±0.02	1.26	58.4 ^c ±0.6	72.4 ^b ±0.7	619.8 ^a ±1.17	1.72
HCT-116	58.3 ^b ±0.74	66.7 ^a ±0.77	6.05 ^c ±0.01	1.23	85.7 ^b ±1.1	75.01 ^c ±0.87	826.4 ^a ±1.36	2.26
BJ-1	1.4 ^b ±0.023	1.38 ^c ±0.05	1.86 ^a ±0.06	0.0088	3624.2 ^a ±5.9	3619 ^a ±13.2	2678.5 ^b ±8.3	19.25

All experiments were performed in triplicate; all data are expressed as the mean \pm SD. Means with different letters (at the same row) are significantly different at $p \leq 0.05$.

CONCLUSION

Based on the results even though nanoformulations enhance the bioavailability and increase the stability of individual active ingredients, all nanoscale materials applied in food industry should be used advisedly and only after in-depth investigation of cytotoxicity due to possible increased nanosize-based toxicity effects (e.g., surface reactivity of NPs), which could result in unspecified toxic effects also in humans or animals. Therefore, an increased attention should be paid to the influence of risk factors associated with their applications and possible adverse/hazardous effects to humans and animals. The present study showed that lycopene extracted from tomato's pomace in traditional and as nano-particles (nano-form) exhibited antioxidant activity. Also, it had antimicrobial activity against all tested bacteria and fungi. Additionally, the anticancer activity against different cell line (HepG-2, MCF-7 and HCT-116) was observed. The obtained results revealed that lycopene and lycopene-NPs would be used in treatment of infection caused by pathogenic bacteria as well as anticancer drug.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the

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