Iraqi Journal of Market Research and Consumer Protection
(2024) 16(1): 210-219
DOI: http://dx.doi.org/10.28936/jmracpc16.1.2024.(18)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

PREPARATION AND STUDY OF NATURAL AND NANO LYCOPENE IN INHIBITING THE GROWTH OF CANCER CELLS EX VIVO IN VITRO

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Received 21/ 6/ 2023, Accepted 30/ 11/ 2023, Published 30/ 6/ 2024

ABSTRACT

The effectiveness of both natural and nano-lycopene extracted from tomato waste powder in affecting the growth of cancer cells outside the body was studied. The study included the preparation of natural lycopene extract, using the triple mixture of hexane, acetone, and ethanol in proportions 2:1:1 and drying it, then preparing the nanocomposite using the high-energy mechanical grinding technique, and its dimensions were estimated using a Scanning Electron Microscope (SEM) and which was 78nm, and the effectiveness of the two preparations was tested in Inhibition of cancer cell lines of the human mouth and skin, during three periods of time 12, 24, 72 h and at concentrations of both natural and nano-lycopene 0.0,150, 300, 600, 1200, 2400 micrograms/ml, the results of the study showed There was a significant inhibitory effect p≤0.05. for both natural and nano-lycopene in the growth of cancer cells, and nano-lycopene was significantly superior to natural lycopene for skin and mouth models, and the inhibition effect of cancer cells increased for both natural and nano-lycopene with increasing concentration and period, and the highest percentage of inhibition for natural lycopene was 71% and 79.8% While the highest percentage of inhibition for nanoscale icon was 85.2% and 93.1% at a concentration of 2400 µg/ml for 72 h and for skin and oral cancer cell lines, respectively.

Keywords: Lycopene, Lycopene nanoparticles, Anticancer, Antioxidants, Cancer cell lines.

[*] **The research is taken from a doctoral thesis for the first research.**

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(2024) 16(1): 210-219

تحضير ودراسة اللايكوبين الطبيعي والنانوي في تثبيط نمو الخلايا السرطانية خارج الجسم الحي In vitro

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الخلاصة

تم دراسة فعالية كل من اللايكوبين الطبيعي و النانوي المستخلص من مسحوق مخلفات الطماطة في التأثير في نمو الخلايا السرطانية خارج الجسم الحي,وتضمنت الدراسة تحضير مستخلص اللايكوبين الطبيعي بأستخدام المزيج الثلاثي من الهكسان والأسيتون والأيثانول وبالنسب (2 :1: 1) وتجفيفه ثم تحضير المركب النانوي بأستخدام تقنية الطحن الميكانيكي عالي الطاقة, وتم تقدير أبعادها بأستخدام جهاز المجهر الألكتروني الماسح وكانت (78) نانومتر وأختبرت فعالية المستحضرين في تثبيط الخطوط الخلوية السرطانية للفم والجلد البشري, وخلال ثلاث فترات زمنية (12 ,24, 72) ساعة وبتراكيز لكل من اللايكوبين الطبيعي والنانوي (0.0, 150, 300, 600, 1200, 2400 مايكروغرام / مل), أظهرت نتائج الدراسة وجود تأثير تثبيطي معنوي .($p\leq0.05$) لكل من اللايكوبين الطبيعي والنانوي في نمو الخلايا السرطانية,وتفوق اللايكوبين النانوي معنويا على اللايكوبين الطبيعي لنماذج الجلد والفم,وأزدادت فعالية التثبيط للخلايا السرطانية لكل من اللايكوبين الطبيعي والنانوي بزيادة التركيز والفترة الزمنية وبلغت أعلى نسبة مئوية للتثبيط للايكوبين الطبيعي (71% و 79.8%) فيما بلغت أعلى نسبة مئوية للتثبيط للايكوبين النانوي (85.2 %و 93.1%) وعند التركيز 2400 مايكروغرام / مل ولمدة 72 ساعة ولخطوط الخلايا السرطانية للجلد والفم وعلى التوالي.

الكلمات المفتاحية : اللايكوبين , اللايكوبين النانوي,مضادات السرطان , مضادات الاكسدة,خطوط الخلايا السرطانية.

INTRODUCTION

Lycopene is a natural carotenoid pigment produced by plants and microorganisms during the process of photosynthesis to protect them from photoactivity. It is a plant chemical primarily found in tomatoes and their products, and other plant sources including watermelon, guava, papaya, apricot, and red grapefruit. Additionally, other sources such as red carrots, rosehip, and autumn olive are among the main sources of lycopene. (**Al-Tameemi *et al.*,2023; Muna *et al*., 2023; Aziz *et al*., 2023; Fordham *et al*., 2002)**

The molecular weight of lycopene is 536.89, and the melting point of lycopene is 172-175 C. Lycopene is found in ripe tomato fruits in the form of rectangular crystals resembling needles. It is responsible for the bright red color of ripe tomato fruits. Lycopene is more soluble in organic solvents such as chloroform, benzene, hexane, ether, and ethyl acetate. It dissolves in vegetable oils but does not dissolve in water, methanol, and ethanol. **(Shi & Maguer, 2000; Asaduzzaman, 2022; Al-jubouri *et al*., 2022).**

The rates of infection and death resulting from cancer are constantly increasing, which makes cancer a major global health problem and ultraviolet radiation has increased in recent decades hit the earth's surface and depleted the ozone layer, so it is necessary to protect our skin from these rays because they cause damage to human skin such as skin cancer and hyperpigmentation and skin aging. Many active natural compounds inhibit cancer, such as lycopene, which has proven effective in protecting the skin from these rays **(Khaleel *et al*., 2019; Al-Anbari *et al*., 2019).** Lycopene is one of the biologically active compounds and one of the most important antioxidant carotenoid components in tomatoes and plays important roles in maintaining and improving human health. **(Al-Anbari *et al*., 2019; Altaee *et al*., 2020; Kanyar and Karadaş, 2023)**

Consumption of lycopene from its natural sources leads to enhanced protection of human skin from ultraviolet radiation and biological activities related to the skin and anti-aging

of the skin, and a diet rich in tomatoes has been associated with a variety of health benefits including anti-cancer properties **(Collins *et al.*, 2022; Honda, 2023;Nahla *et al.*, 2018).**

Studies indicate that lycopene accumulated in the skin can provide protection against UV rays as well as protect target molecules by suppressing free radicals, inhibiting cellular inflammatory responses, and repairing damage caused by UV rays. And that balanced nutrition is necessary to maintain healthy skin and that the loss of some nutrients leads to abnormalities in the skin **(Tarshish & Hermoni, 2023; Hamdia & Ahamed, 2023; Al-Jumaily ., 2014)**

The preparation of nanoparticles of lycopene from natural lycopene leads to an increase in its antioxidant activity and its anticancer activity in the laboratory **(Shamurad *et al.*, 2019; Khaleel *et al.*, 2019; Omran & Baek., 2021).**

The study aimed to compare the effectiveness of natural and nano-lycopene extracted from dried tomato residues in inhibiting the growth of skin and oral cancer cells. **(Al-hadedee *et al.*,2021)**

MATERIALS AND METHODS

1- **Processed tomatoes** were obtained from the farms of Karbala Governorate for the fall agricultural season 2021to2022.The dried tomato waste powder was prepared using the electric vacuum oven at 40°C until the weight stabilized. Then prepare the dried natural powder by grinding it with a mill equipped by (Monolex) company.

2 – Lycopene Extraction

Lycopene was extracted from tomato waste powder by following the method described by **(Thompson *et al.*, 2000)** by taking 1g of the dried sample and mixing it with 10 ml of a solvent mixture (acetone: hexane: ethyl alcohol) in a ratio of (1:2:1) and mixing In a vortex vibrator for 10 min, then 1.5 ml of water was added to separate the hexane layer from the acetone and ethyl alcohol layer, and mixed for another 5 min. The upper layer containing lycopene was withdrawn and kept in a dark closed vial, then it was placed in the electric oven at a temperature of 30°C until a stable weight was obtained.

3 – Preparation of Nanoparticales of Lycopene

A quantity of dried lycopene was placed in a high-energy steel ball mill was used from the German company (Retsch) and in the Ibn Al-Bitar Center, which is affiliated to the Ministry of Industry and Minerals, at a speed of 400 rpm per min for 15 min, then the crushed product was collected in sterile and opaque glass bottles and kept in refrigeration 4±2 °C, and the nanoscale dimensions were estimated using the SEM **(Ali *et al.*, 2016; Slewa & Mowsowy, 2018; Murthykumar and Malaiappan 2020)**

Preparation of cancer cell lines

The inhibitory effect of the natural and nano-lycopene extract was studied on two types of cancer cell lines, namely human squamous cell carcinoma cell line and human oral squamous cell carcinoma cell line, in passages 27 and 22 respectively.and at the Biotechnology Center Al-Nahrain University At concentrations (0.0, 150, 300, 600, 1200, 2400) µg/ml, the cells were grown in medium Rosswell Park Memorial Institute -1640 supplemented with 5% Fetal Calf Serum (FCS).

The toxic effect of culturing cells in tissue culture dishes was studied with multiple holes (Microtiter plates) 96 and the flat bottom Flat Bottom to conduct this test. The experiment included three stages:

Cells seeding

Cancer line cells were activated and proliferated for 24 h, then the growth monolayer was treated with a Trypsin-Versen solution. 25 ml of RPMI-1640 medium prepared with serum was added to each vessel and the number of cells was adjusted to 1×10^4 using a slide count. A volume of 100 µl of cell suspension was taken and distributed to the holes of the tissue culture dish. The dishes were incubated after covering them with sterile adhesive paper at a temperature of 37 °C for 24 h to allow the cells to adhere to the glass,

Preparation of experimental samples

Several concentrations of both natural and nano-lycopene extract were prepared simultaneously using a tissue culture medium devoid of fetal calf serum, and added to pits containing adherent cancer cells. Six replications were used for each treatment. The culture medium was poured into the tissue culture dishes. Column No. 1 was considered as a negative control, and 200 µl of serum-free culture medium were added to it. As for columns from (2 to 12), graduated concentrations of 200 µl / hole were added. The dishes were covered and incubated at a temperature of 37°C, for different exposure times 24, 48, 72 h.

Cytotoxicity assay

After the end of the prescribed incubation period, the contents of the dishes (the culture medium and the suspended cells) were poured out and then washed with phosphate-buffered saline three times to ensure the removal of any trace of the test material and non-adherent cells, then a volume of 10 µl of Methyl Thiazolyl Tetrazolium MTT dye solution (0.5 mg/ml) was added to each hole then left for 4 h at a temperature of 37 ° C in a carbon dioxide incubator. The cells were washed several times with a saline phosphate buffer until the excess dye was removed. After the dishes were completely dry, 100 µl of dimethyl sulfoxide DMSO were added. The results were read using an ELISA reader using a spectrophotometer on the titration dishes. Microplate spectrophotometer (ELISA) at a wavelength of 500 nm.

The inhibition rate was calculated according to the equation below:

$$\%IR = \frac{\mathrm{A-B}}{\mathrm{A}} \times 100 - 100$$

IR= Inhibitory Rate

A= Absorbancy for Negative Control

B= Absorbancy for Test

Statistical Analysis

The Statistical Analysis System **(SAS. 2018)** was used to analyze the data to study the effect of different coefficients on the studied traits according to a Complete Random Design (CRD), and the significant differences between the means were compared with the Least Significant Difference-LSD test.

RESULTS AND DISCUSSION

Figure (1) shows the image of the prepared nanostructures. It is noted that the structures fall within the nanoscale dimensions, and the average particle size is 78nm

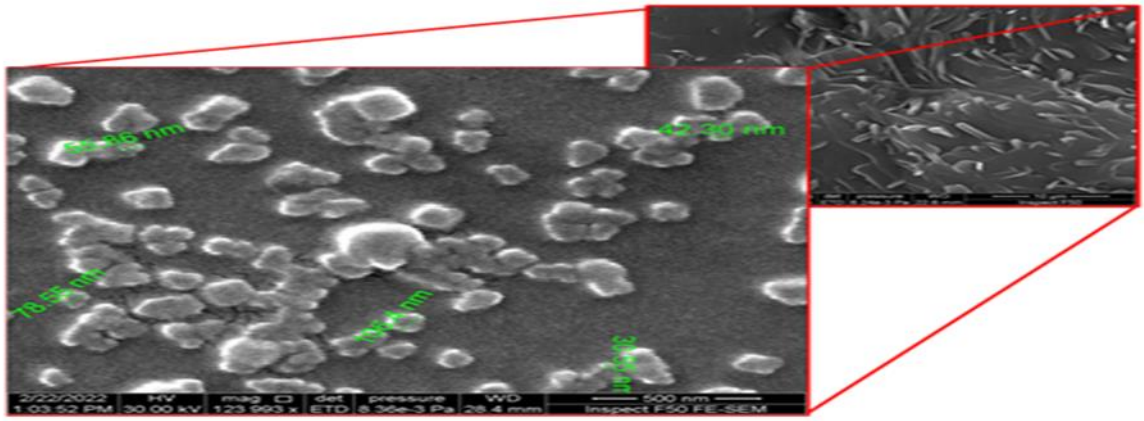

Figure (1): Lycopene nanoparticles image by SEM

As shows in the table (1) effect of adding different proportions of each natural and nano-lycopene extract in the inhibition of the human skin cancer cell line. ($p \leq 0.05$).

It is directly proportional to the increase in lycopene concentrations, and the percentage of inhibition of natural lycopene was 8.5% for 24 h and at the lowest concentration of 150 µg /ml, while it reached 71% at the highest concentration of 2400 µg /ml for 72 h. Nano-lycopene was superior to natural lycopene, as it recorded the highest level of inhibition by 85.2% at a concentration of 2400 µg/ml for 72 h. **(Haider *et al.*,2024)**

(Table, 2) also shows an increase in the percentage of inhibition of the oral cancer cell line by increasing the added concentrations, and the percentage of inhibition of natural lycopene was 7.9% at a concentration of 150 µg / ml for 24 h, while it reached 79.8% at a concentration of 2400 µg / ml for 72 h, and lycopene was superior nanoparticles to natural lycopene, as it recorded an inhibition rate of 93.1% for 72 h and at the highest concentration. **(Yaaqoob, 2022; Al-Jubouri *et al.*, 2022)**

The results indicate the effectiveness of both natural and nano-lycopene extracts in inhibiting the growth of cancer cells and the superiority of nano-lycopene over natural in both skin and oral lines **(Jasim & AI-Obaidi, 2022; Al-Anbari *et al.*, 2019; Mula and Alrubeii, 2024)**

The results agreed with what was found by **(Soares *et al.*, 2017; Doosh and Al-Mosawi, 2010)** in the inhibitory activity of lycopene extracted from tomato paste on human prostate cancer cells, at different concentrations from 500 to 5000 µg/ml and for exposure periods (24, 48, 72, 96) h.

Table (1): Comparison of the effect of natural and nanoscale lycopene on the human skin cancer cell line at different concentrations during 72 h

concentration µg/mL	inhibition%					
	Nano-Lycopene			natural lycopene		
	24 h	48h	72h	24 h	48 h	72 h
150	15.6	22.4	27.3	8.5	6.6	19.7
300	21.6	25.9	46.9	17.5	32.6	39.7
600	33.6	41.4	63.3	29.4	49.1	45.4
1200	54.6	60.7	79.1	45.5	57.1	65.3
2400	67.7	74	85.2	54.4	62.1	71
LSD	8.04 *	8.79 *	8.05 *	6.51 *	7.48 *	7.93 *
* (P≤0.05).						

Table (2): Comparison of the effect of natural and nanoscale lycopene on oral cancer cell line at different concentrations during 72 h.

concentration µg/Ml	inhibition%					
	Nano-Lycopene			natural lycopene		
	h 24	48h	72h	h 24	h 48	72 h
150	29.3	42.5	53.8	7.9	16.3	28.4
300	39.4	50.8	59.8	18.1	33.6	56.5
600	53	62.6	67.2	27	56.3	68.4
1200	66.5	77.5	86.9	50.1	63.8	75
2400	88.4	84.7	93.1	61.2	72.6	79.8
LSD	9.92 *	9.01 *	10.42 *	11.08 *	9.66 *	8.37 *
* (P≤0.05).						

(**Teodoro *et al.*, 2012**) found an inhibitory effect of lycopene on many types of cancer. The inhibitory effect depends on the type of cancer and the concentration of lycopene used.

(**Hussein *et al.*, 2023 ; Campos *et al.*, 2022**) indicated that the toxic effect of lycopene against cancer cell lines is one of the most powerful antioxidants because it contains a large number of double bonds that absorb free oxygen molecules and inhibit free radicals and is characterized by its ability High inhibition of cancer cells through its antioxidant activity, as it is toxic to cancer cells, through the mechanism of removing free radicals generated when cancer cells form (**Masoud *et al*., 2022 ; Al-Taweel *et al*., 2022**).

The inhibitory effect of lycopene is attributed to possible mechanisms represented in affecting the effectiveness of cell division through its effect on DNA replication, or one of the enzymes important in replication, or through fragmentation of the DNA strand and inducing cells to programmed death. (**Faddagh *et al.*, 2020 ; Al-jubouri *et al*., 2022 ; Usui *et al.*, 1**998). It can also work to inhibit multiple divisions of some types of human cancer cell lines and induce cells towards programmed cell death (apoptosis) and thus its role in protecting the genetic material from the effect of environmental mutagens and the ability of its components to correct genetic errors. (**Khalid *et al*., 2021 ; Shamurad *et al*., 2019; Lopus & Panda., 2006**).

The superiority of nanoparticles lycopene over natural lycopene in the toxic effect and inhibitory effectiveness against cancer cell lines could be due to its characteristics and properties in interacting with different mechanisms and methods compared to its interactions when it is in its normal dimensions due to its low size, the increased surface area of the nanoparticles and the spread of surface charges, which allows it to have a greater increase in cell activity. The number of atoms and molecules involved in the reactions. (**Sridhar *et al.*, 2021; Yaaqoob., 2022; Abdulsada *et al.*, 2023**)

CONCLUSIONS

The importance of natural lycopene extract extracted from dried tomato residues as a biologically effective compound for its inhibitory ability to inhibit the growth of cancer cells ex vivo and by increasing concentrations and exposure time. Its presence in its nano form doubles its inhibitory effectiveness against cancerous lines of the skin and mouth. Nano-lycopene achieved superiority over natural lycopene with concentrations equivalent to half the concentrations of natural lycopene in inhibiting the growth of cancer cells for both lines.

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