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© *Mohammad T. Selah[1], Ghada A. Mohammad[2]*

Effect of Biosynthesized and commercial selenium nanoparticles on A 498 and CaCo-2 cancer cell lines

[1]Nineveh Education Directorate, Mosul, Iraq
[2]Department of Biology/College of Science/University of Mosul, Mosul, Iraq

© *Мохаммад Т. Селах[1], Гхада А. Мохаммад[2]*

Влияние биосинтезированных и коммерческих наночастиц селена на клеточные линии A-498 и CaCo-2

[1]Управление образования Ниневии, Мосул, Ирак
[2]Кафедра биологии, Научный колледж, Университет Мосула, Мосул, Ирак

Introduction. Cancer is a major cause of death worldwide, and there is a need for new treatment approaches. Nanoparticles have been proposed as a potential therapy for cancer due to their unique properties.

Aim. We conduct our study to assess the effect of selenium nanoparticles biosynthesized by *Serratia marcescens* and commercial selenium nanoparticles on the kidney cancer line (A-498) and the colon cancer line (CaCo-2) in comparison with the HdFn normal cell line.

Material and Methods. This study utilized two types of selenium nanoparticles (SeNPs) — one synthesized by the bacterium *Serratia marcescens* and the other commercially sourced from Nanoshel, USA — to assess the toxic effects on cancer cell lines. The kidney cancer (A-49.8) and colon cancer (CaCO-2) cell lines were cultured alongside a normal fibroblast control (Hdfn) using RPMI-1640 medium enriched with serum and antibiotics. The cytotoxicity of both types of SeNPs was evaluated using the MTT assay. After incubation, cell viability was measured by assessing absorbance at 570 nm, and the IC50 values were calculated to determine the concentration required for 50 % inhibition of cell growth.

Results. The results showed that the biosynthesized Selenium nanoparticles had a higher effect on the A-498 cancer line than on the normal line Hdfn. The highest lethal percentage of cancer cells for biosynthesized nanoparticles was 60.1 %, at a concentration of 400 μg/ml, while the lethal percentage for normal cells was 28.6 %. Commercial selenium nanoparticles showed a higher lethal percentage of 33.3 % for cancer cells and 28.1 % for normal cells at the same concentration. The results on colon CaCo-2 cancer cell line showed that commercial Selenium nanoparticles had a higher effect than biosynthesized ones: the lethal percentage of cancer cells with the concentration 400 μg/ml was 47.1 % vs 38.1 % respectively. Meanwhile, the lethal percentage at Hdfn was 28.1 % and 28.6 % with the same concentration, respectively. The IC50 at A-498 for biosynthesized and commercial SeNPs were 113.3 and 157.5 μg/ml respectively. The IC50 at CaCO2 for biosynthesized and commercial SeNPs were 121.6 and 102.8 μg/ml respectively. ID50 at Hdfn is 213.7 and 164.2 μg/ml respectively.

Введение. Рак является одной из основных причин смертности в мире, что обуславливает необходимость поиска новых подходов к его лечению. Благодаря своим уникальным свойствам, наночастицы рассматриваются для применения в противораковой терапии.

Цель. Мы провели исследование, чтобы оценить влияние биосинтезированных наночастиц селена, полученных с использованием *Serratia marcescens*, и коммерческих наночастиц селена на клеточные линии карциномы почки (A498), и рака толстой кишки (CaCo-2), в сравнении с нормальной клеточной линией HdFn.

Материалы и методы. В данном исследовании использовались два типа наночастиц селена (SeNPs) — для синтеза одного из них применялись бактерии *Serratia marcescens*, а другой был приобретён у компании Nanoshel, США для оценки токсичности на клеточные линии. Клеточные линии карциномы почки (A-49.8) и рака толстой кишки (CaCO-2) культивировали вместе с нормальной нормальной фибробластной контрольной линией (Hdfn) в среде RPMI-1640, обогащенной сывороткой и антибиотиками. Для оценки цитотоксичности обоих типов SeNPs применялся MTT-анализ. После инкубации жизнеспособность клеток измерялась путем оценки абсорбции при 570 нм, а значения IC50 рассчитывались для определения концентрации, необходимой для 50 % ингибирования роста клеток.

Результаты. Результаты показали, что биосинтезированные наночастицы селена оказывали более сильное воздействие на раковую линию A-498, чем на нормальную линию Hdfn. Наибольший процент летальности раковых клеток для биосинтезированных наночастиц составил 60,1 % при концентрации 400 мкг/мл, в то время как процент летальности для нормальных клеток составил 28,6 %. Коммерческие наночастицы селена показали более высокий процент летального исхода 33,3 % для раковых клеток и 28,1 % для нормальных клеток при той же концентрации. По результатам исследования для клеточной линии рака толстой кишки CaCo-2 коммерческие наночастицы селена показали более высокую эффективность, по сравнению с биосинтезированными. При концентрации 400 мкг/мл летальность раковых клеток составила 47,1 % для коммерческих наночастиц и 38,1 % для биосинтезированных. В то же время летальность нормальных клеток линии Hdfn составила 28,1 % и 28,6 % соответственно при той же концентрации. Значения IC50 для линии A-498 составили

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Conclusion. The biosynthesized SeNPs were effective in both A-498 and CaCO2 but it was more effective on the A-498 kidney cancer line than the commercial SeNPs.

Keywords: A- 498; CaCo-2; selenium nanoparticles; Hdfn; RPMI

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113,3 мкг/мл для биосинтезированных SeNPs и 157,5 мкг/мл для коммерческих. Для линии CaCo-2 IC50 составил 121,6 мкг/мл и 102,8 мкг/мл соответственно. Значения IC50 для нормальных клеток Hdfn составили 213,7 мкг/мл и 164,2 мкг/мл соответственно.

Выводы. Биосинтезированные наночастицы селена показали эффективность против линий A-498 и CaCo-2, но оказались более действенными в отношении клеток карциномы почки A-498 по сравнению с коммерческими наночастицами.

Ключевые слова: A-498; CaCo-2; наночастицы селена; Hdfn; RPMI

✉ Contact: Mohammad T. Selah, mohammed.23scp31@student.uomosul.edu.iq

Introduction

Selenium nanoparticles have anti-cancer activity. Their effect has been shown on many cancer cell lines including the prostate, breast, neck, uterus, lung, colon, rectum, and liver cancer. Neoplasms is a global health challenge. development of resistance to cancer treatment is a worrying trend. Therefore, researchers seek to understand the mechanisms behind this resistance and develop new techniques to overcome it [1]. Chemotherapy is one of the most common treatments for cancer, but the side effects during treatment are a significant concern [2]. One of the most important challenges facing researchers in the field of cancer is the lack of treatments that target cancer cells without causing collateral damage. Nanotechnology is one of the most promising technologies that has caught the attention of scientists around the world due to its potential use in cancer treatment. Its unique characteristics, such as its tiny size ranging from 1-100 nanometers, make it able to penetrate tumor tissue and destroy cancer cells while sparing healthy cells [3]. Many nanoparticles, such as gold, silver, and others, have been used to treat cancerous diseases, and selenium nanoparticles are among the best treatments used in treatment [4]. Microorganisms play an important role in reducing the oxyanion selenate and selenite to the element selenium, as these microorganisms have the ability to produce nanoparticles of different sizes and shapes and are more stable [3].

Recent studies have focused on the use of selenium nanoparticles bound to other materials for the purpose of delivering the drug to the target organ to treat many types of cancer [5]. Selenium nanoparticles (SeNPs) kill cancer cells by their oxidizing effect inside the cells [4]. They work to stimulate free radicals (ROS) inside the cells, which ultimately leads to the programmed death of cancer cells. Previous studies indicate that SeNPs prevent the growth of cancer cells by stopping cell growth in the S phase [6]. The aim of the study is to assess the effect of selenium nanoparticles biosynthesized by *Serratia marcescens* and commercial selenium nanoparticles on the kidney cancer line (A-498) and the colon cancer line (CaCo-2) in comparison with the HdFn normal cell line.

Material and methods

Selenium nanoparticles

Two types of SeNPs were used. One of them was synthesized by the bacterium *Serratia marcescens*, which was obtained from a previous study conducted by the Department of Biology at the College of Science at the University of Mosul [7]. The other one was obtained commercially from Nanoshel, USA.

Cell lines

The kidney cancer cell line (A-49.8) and the colon cancer cell line (CaCO-2) were used. A normal cell line (Hdfn), which represents newborn fibroblasts, was used as control. All of the cell lines were provided by Aims research center in Baghdad/ Iraq.

Roswell Park Memorial Institute-1640 (RPMI) Medium

The medium was prepared by adding 4-(2-hydroxyethyl)-1 piperazine ethane sulfonic acid (HEPES), (L-glutamine), 10 % Fetal Bovine Serum and some prepared solutions, which are Penicillin (10^3IU), Streptomycin (0.001 g), and Sodium Bicarbonate (1 %). All the ingredients were mixed, then sterilized using a filter (0.22 mm) and incubated for 72 hours at 37 °C to ensure it is free of contaminants.

Cultivation

The cells were transferred separately into culture medium (RPMI-1640) with 10 % bovine calf serum, and incubated in 5 % CO2 at a temperature of 37 °C for 24 hours, then culture medium was discarded, and Phosphate Buffer Saline was used to wash the cells, then the trypsin/EDTA enzyme

was added after that the enzyme was stopped by adding medium again, centrifuge was performed at a speed of 2000 rpm. the sediment containing the cells added it to a new medium containing 10 % of fetal bovine serum. To determine the cell count, a sample of the cell suspension was added to an equal volume of Trypan blue dye. Using a Haemocytometer slide, the number of cells was counted according to the following equation:

Total Cell Count/ml = cell count x dilution factor (sample volume) × 10^4.

Methyl Thiazolyl Tetrazolium (MTT) Cytotoxicity Assay

In this study, we determined the cytotoxicity of biosynthesized and commercially available SeNPs on two types of cancer cell lines and compared them with a normal cell line, Hdfn, using a standard method. The method followed the manufacturer's instructions. After preparing the cancer cells, we placed 200 µl of cell suspension at a concentration of 1 × 10^4 to 1 × 10^6 cells/cm^3 in a standard microtiter plate, one well for each concentration. The cells were incubated in 5 % CO_2 at 37 °C for 24 hours. We then removed the culture medium and added 100 µl serum-free medium to each well. Additionally, we added 100 µl prepared concentrations of biosynthesized SeNPs or commercial SeNP (25, 50, 100, 200 and 400 µg/ml), three wells for each concentration, as well as a control sample, and incubated them in a CO_2 incubator at 37 °C for another 24 hours. After that, 10 µl of MTT solution was added to the wells, and the microtiter plate was incubated for 4 hours at 37 °C in a CO2 incubator. Then, 100 µl of solubilization solution was added to each well, and the plate was incubated for an additional 5 minutes. The absorbance was read at a wavelength of 570 nm using an ELISA reader.

A statistical analysis was performed to determine the concentration of nanoparticles required for a 50% inhibition of cell growth, or the Inhibitory Concentration 50 (IC50), for each cell line.

The biological activity was calculated using the following equation:

$$\textbf{Viability}\ (\%) = \frac{Optical\ density\ of\ sample}{Optical\ density\ of\ control} \times 100$$

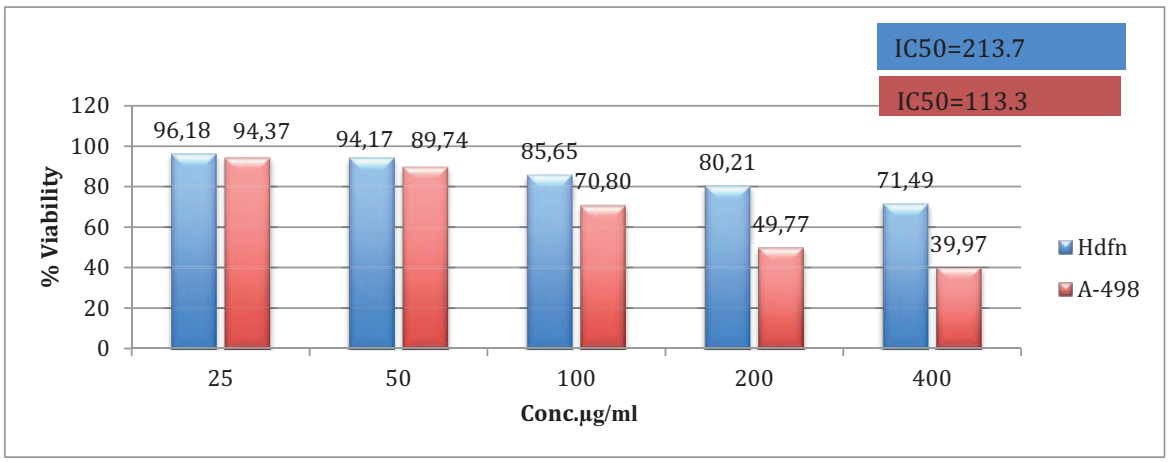

Fig. 1. Percentage of live cells A-498 and Hdfn cell line treated with biosynthesized selenium nanoparticles

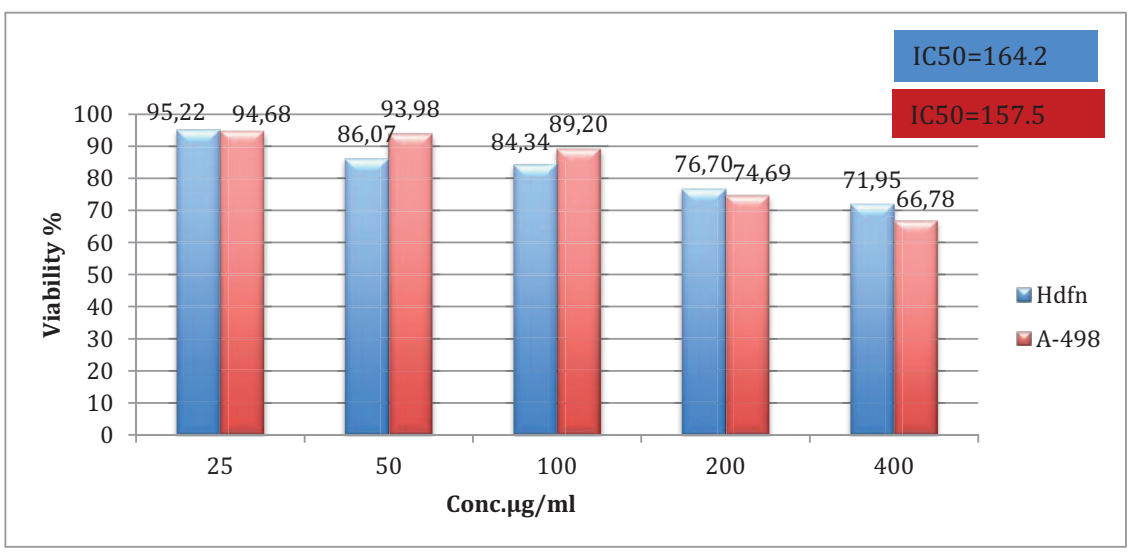

Fig. 2. Percentage of live cells A-498 and Hdfn cell line treated with commercial selenium nanoparticles

Results

The results showed that biosynthesized SeNPs at the first three low concentrations (25, 50, and 100 µg/ml) had approximately similar effects on the percentage of live cells in both normal Hdfn and cancer A-498 cell lines. However, the percentage effect of biosynthesized SeNPs on cancer cell line A-498 was greater than its effect on normal cell line Hdfn at concentrations of 200 and 400 µg/ml. At these concentrations, the percentage of living cancer cells was only 49.7 % and 39.9 % respectively, while the percentage of normal cells Hdfn was 80.2 % and 71.4 %, respectively, as shown in fig. 1.

Meanwhile, the effect of commercial SeNPs on the cancer cell line A-498 and the normal cell line Hdfn was small at the first four concentrations (25, 50, 100, 200 µg/ml), and the percentage of effect increased at the concentration of (400 µg/ml), as the percentage of live cancer cells (66.7 %), while the percentage of live normal cells was (71.9 %), as shown in fig. 2.

Based on the data from Figures 1 and 2, we calculated the lethal percentage for the A-498 cancer cell line and the Hdfn normal cell lines for both biosynthesized and commercial SeNPs. It appears that the biosynthesized SeNPs have a higher lethal percentage of cancer cells than the commercial SeNPs. The highest lethal percentage was 60.1 % at a concentration of 400 µg/ml, while the lethal percentage for the normal Hdfn line was 28.6 %. The lethal percentage for the commercial SeNPs at the same concentration was 33.3 % for the normal Hdfn line.

The biosynthesized SeNPs began to double the lethal percentage of cancer cells compared to normal cells from a concentration of 100 µg/ml. For commercial SeNPs, it was almost equal to the normal cell concentration starting from a concentration of 200 µg/ml, as shown in tab. 1. In addition, the IC50 value in A-498 for biosynthesized and commercial SeNPs were 113.3 and 157.5 µg/ml respectively, while the IC50 value in Hdfn were 213.7 and 164.2 µg/ml respectively. This is a promising result, and we look forward to using the biosynthesized SeNPs in the treatment of kidney cancer.

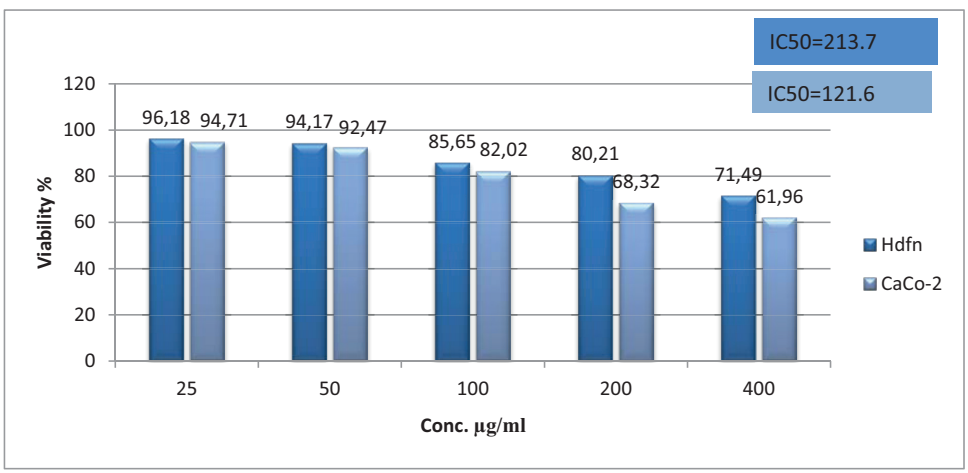

Fig. 3. Percentage of live cells of CaCo-2 and Hdfn cell line treated with biosynthesized selenium nanoparticles

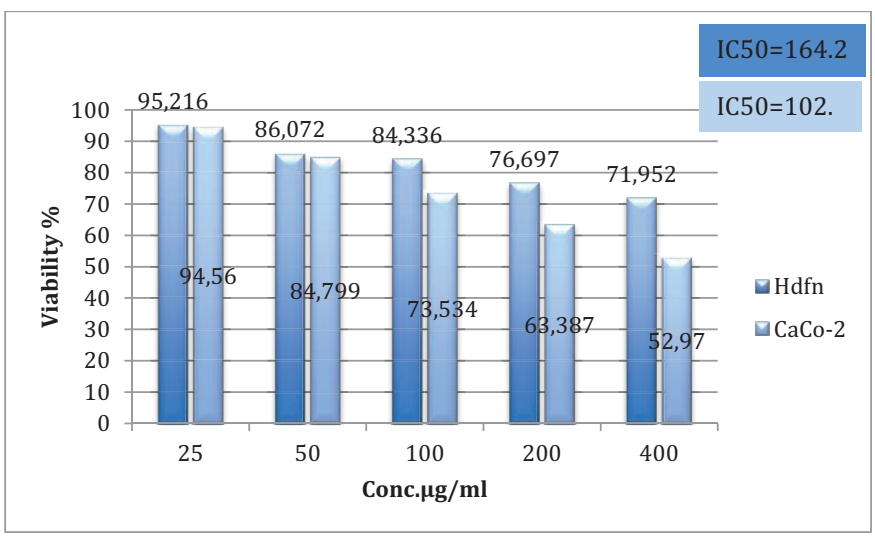

Fig. 4. Percentage of live cells of CaCo-2 , Hdfn treated with commercial selenium nanoparticles

Table 1. The cytotoxicity of biosynthesized and commercially available SeNPs tested on the kidney cancer cell line A-498 and a normal cell line Hdfn

SeNPs concentration, μg/ml	Cytotoxicity effect, %			
	commercial SeNPs		biosynthesized SeNPs	
	Hdfn	A-498	Hdfn	A-498
25	4.8	5.4	3.9	5.7
50	14.0	6.1	5.9	10.3
100	15.7	10.9	14.4	29.3
200	23.4	25.4	19.8	50.3
400	28.1	33.3	28.6	60.1

Table 2. The cytotoxicity effect of commercial biosynthetic SeNPs on CaCo-2 kidney cancer line

SeNPs concentration, μg/ml	Cytotoxicity, %			
	SeNPs commercial		SeNPs biosynthesized	
	Hdfn	CaCO-2	Hdfn	CaCO-2
25	4.8	5.5	3.9	5.3
50	14.0	15.3	5.9	7.6
100	15.7	26.5	14.4	18.0
200	23.4	36.7	19.8	31.7
400	28.1	47.1	28.6	38.1

When we tested the efficacy of biosynthesized SeNPs on colon cancer cell line CaCo-2 using the MTT method and compared it with the normal line Hdfn, we found that the effect of SeNPs on cancer cells was greater than on normal cells. The inhibitory effectiveness increased with increasing concentration, and at a concentration of 200 μg/ml, the percentage of live cancer cells was 68.3 %, while the percentage of live normal cells was 80.2 %. When the concentration increased to 400 μg/ml, the percentage of cancer cells decreased to 61.9 %, but the percentage of normal cells remained high at 71.4 %. For the first three concentrations (100, 50, and 25 μg/ml), the effect of biosynthesized SeNPs was similar on both normal and cancer cells, as shown in fig. 3.

While the results of commercial SeNPs showed a higher effect than bio-synthesized SeNPs, at a concentration of 200 μg/ml, the percentage of live cancer cells CaCo-2 was 63.3 % and the percentage of live normal cells was 76.6 %. At a concentration of 400 μg/ml, the percentage of live cancer cells decreased to 52.9 % and the percentage of live normal cells remained at 71.9 %, as shown in fig. 4.

The cytotoxicity of biosynthesized and commercial SeNPs on normal and cancer cell lines is summarised in in tab. 2.

The commercial SeNPs showed a higher effect on the cancer cell line than on the normal lines, and the commercial SeNPs also had a higher lethal percentage compared to their biologically synthesized counterparts. The lethal percentage was 47.1 % at a concentration of 400 μg/ml for the commercial SeNPs, while it was 28.1 % for the normal Hdfn line. For the biosynthesized SeNPs, the highest lethal percentage was 38.1 %, with a lethal percentage of 28.6 % for the Hdfn normal line at the same concentration. Additionally, the IC50 value in $CaCO_2$ for the biosynthesized and commercial SeNPs were 121.6 and 102.8 μg/mL, while the IC50 values at Hdfn were 213.7 and 164.2 μg/mL, respectively.

Discussion

The results of our study were in agreement with the study of R.Freshny, where biosynthesized SeNPs by Halophilic Bacteria had an effect on MCF7 breast cancer cells and Ht-29 colon cancer cells at concentration 100 μg/ml [7]. Similar results were obtained by M. Tabibi, who found that SeNPs biosynthesized by *Acintobacter* sp sw30 have an lethal effect on breast cancer cell lines MCF-7 and 4T1 [8]. Another research team found that SeNPs biosynthesized by *Streptomyces griseoruber* showed good cytotoxic activity against HT-29 cell line with 40.5 %, 33 % and 23.7 % of cell viability at 2 μg/ml, 4 μg/ml and 30 μg/ml concentration respectively [9, 10].

Cancer cells have an acidic environment and an imbalance in oxidation and reduction. This leads to the oxidation of SeNPs, which increases the production of free radicals. This, in turn, causes a defect in the mitochondrial membrane, leading to the leakage of mitochondrial proteins. On the other hand, the stress caused by the imbalance also works

to cause defects in the endoplasmic reticulum membrane, leading to the efflux of various proteins. This also stimulates programmed cell death by activating caspases, a group of protease enzymes that are essential for programmed cell death [4, 11–13].

Conclusion

The biosynthesized SeNPs were effective in both A-498 and $CaCO_2$ but it was more effective on the A-498 kidney cancer line than the commercial SeNPs. However, for the CaCO-2 colon cancer cell line, the commercial SeNPs were more effective than the biosynthesized SeNPs. However, biosynthesized SeNPs are considered preferable from our point of view, as commercial SeNPs are highly toxic.

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Conflict of interest
There is no conflict of interest.

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Author information / ORCID

Mohammad T. Selah / ORCID ID: https://orcid.org/0009-0002-2679-4876.
Ghada A. Mohammad / ORCID ID: https://orcid.org/0000-0003-1298-3350.