



Anticancer potential of green-synthesized gold nanoparticles via modulation of *p53* and *p21* pathways

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The incidence and mortality of cancer have increased worldwide. The limitations of available anticancer agents in reducing mortality have encouraged researchers to develop an alternative therapy. Recently, green - synthesized nanoparticles have emerged as promising potential alternative therapeutic strategies. This study focused on the anticancer activities of gold nanoparticles (AuNPs) against two types of cancer cells: human breast cancer cell line (MDA-MB-231) and human prostate cancer (PC3), with a particular focus on the expression of the tumor suppressor genes *p53* and *p21*. Green synthesis of AuNPs from *Ziziphus spina-christi* (*Z. spina-christi*) is carried out, followed by morphological and physical characterization. Their anticancer activity against the human breast cancer cell line (MDA-MB-231) and human prostate cancer (PC3) is evaluated, along with their effect on the expression of tumor suppressor genes *p53* and *p21*. The prepared AuNPs showed significant anticancer and cytotoxic activities against both cancer cell lines. The sensitivity of the PC3 cell line is higher than that of the MDA-MB-231 cell line when the percentages of viability are 48.74%, 33.47%, and 23.15% at 200, 400, and 800 µg/ ml, respectively. 5.43- and 6.41-fold increases in the expression of the *p53* and *p21* genes, respectively, are observed in the PC3 cell line when treated with AuNPs, compared to untreated cells.

Keywords: Alternative therapy; Apoptosis; Breast cancer; Gold nanoparticles; Prostate cancer.

1. INTRODUCTION

The significant elevation in cancer incidence has urged scientists to develop novel therapeutic agents by exploring cost-effective and eco-friendly materials. Nanotechnology, which deals with small-sized matter ranging between 1-100 nanometers, is used for this purpose. In recent years, scientific research has witnessed massive progress in different fields; however, the development of effective anticancer agents with fewer side effects remains a major challenge. The term “nano” is obtained from the Greek word “nanos,” referring to “little”. Nanoparticles (NPs) have been employed in different applications, such as anticancer, anti-microbial, cosmetics, etc [1,2]. NPs can be synthesized using a variety of biological and chemical resources, such as microorganisms, plants, algae, marine organisms, and animals [3-6]. Eco-friendly biological sources have emerged as a safe alternative to the hazardous traditional chemical and physical methods, which may involve hazardous substances. Plants produce a wide range of natural products, such as alkaloids, flavonoids, tannins, and other phytochemicals. Some of these natural products have proven to have properties as inexpensive and safe nanoparticles, which are often preferred over microbial nanoparticles [7].

Nanoparticles can enhance drug delivery to the required target sites at a specific time [8]. *Z. spina-christi*, commonly known in Iraq as “Nabaq”, belongs to the family Rhamnaceae. This plant has been traditionally used for medicinal purposes due to its biological activities, including antimicrobial and anti-inflammatory. Previous works have demonstrated the effect of *Z. spina-christi* plant extract on the fertility of buck goats [9,10]. Furthermore, it is found that *Z. spina-christi* synthesizes different types of NPs, such as gold nanoparticles (AuNPs) and Ag nanoparticles (AgNPs), which show activities against malignant cells [11,12].

Recently, gold nanoparticles (AuNPs) have shown low toxicity against living organisms, such as animals and microorganisms, compared to other metal nanoparticles [13,14]. The antimicrobial activities of AuNPs have been characterized by inhibiting microbial growth and biofilm formation. Moreover, the unique physicochemical properties and biocompatibility make AuNPs a promising candidate for anticancer therapy. The property of AuNPs as small-sized matter has the ability to penetrate the cellular membrane of cancer cells and therapeutic agents. The diagnostic characteristic of nanoparticles is demonstrated by binding to cancer cells, particularly. AuNPs have different features, such as the ability to interact with different biological molecules, including nucleic acids, enzymes, fluorescent dyes, antibodies, and proteins, in addition to their biocompatibility [15,6]. One of the prominent mechanisms of elimination of defective cancerous cells and arrest of cell growth is cell apoptosis. This mechanism is controlled by two important genes: *p53* and *p21*. Protein products of these genes arrest the cells cycle. *p53* protein contributes to the activation of *p21*, which inhibits cyclin-dependent kinases, leading to cell arrest [17]. The current study aims to investigate the effect of AuNPs produced from the local plant *Z. spina-christi* on the expression of *p53* and *p21* genes as a mechanism of cancer cell apoptosis. To achieve this goal, AuNPs are prepared, and their morphological and physical properties are measured. The cytotoxicity of the AuNPs and their anticancer activities are examined against two types of MDA-MB-231 and PC3 cell lines.

2. EXPERIMENTAL

2.1 Sample collection

Fresh *Z. spina-christi* leaves are collected from local gardens in Basrah. The plant samples are transferred to the Biotechnology Lab. in the Department of Pathological Analysis - College of Science - Basrah.

2.2 Preparation of *Z. spina-christi* L. extract

Z. spina-christi leaves extract is prepared following the protocol of Abalaka et.al. [18]. Collected leaves are washed with D.W, drained, and ground into fine powder by a grinding machine. Then, 10g of leaf powder is dissolved in 100 mL of D.W, distilled, and incubated for 1 h at 100°C. Later, the mixture is cooled down to room temperature and filtered using Whatman No. 1 filter paper. The extract is stored for further analysis.

2.3 Biosynthesis of AuNPs

H.AuCl₄.3H₂O is considered as the precursor material for nanoparticle synthesis, while reducing and capping agents are obtained from *Z. spina-christi* leaves extract. 0.04 g (0.001 moles) of HAuCl₄.3H₂O is dissolved in 100 mL of distilled water in a beaker under vigorous magnetic stirring to get a homogeneous solution; then 10 mL of the solution is added to 90 mL of diluted plant extract with mixing at room temperature. Once the metallic salt is added to the plant extract, a change in coloration is visible, indicating the formation of gold nanoparticles. The biosynthesized nanoparticles are centrifuged at 3000 rpm for 10 min, washed three times with D.W and dried at 45°C in the oven for 1 h. Then, the nanoparticle powder is collected and stored in a fresh container until used [19].

2.4 Gold nanoparticles characterizations

The green combined nanoparticles are labeled for miscellaneous physicochemical properties following protocol described for UV-visible spectroscopy at a wavelength of about 500–580 nm [19,20]. The magnitude of the arranged nanoparticles is examined by scouring energized matter microscope Scanning Electron Microscope (SEM).

The half maximal inhibitory concentration (IC₅₀) of the green-synthesized AuNPs is determined from the dose–response curve generated using the MTT assay. Cell viability percentages are plotted against different concentrations of AuNPs to generate a dose-response curve. Regression analysis is performed to estimate the IC₅₀ value, and the goodness-of-fit of the model is evaluated using the coefficient of determination (R²). The IC₅₀ value is defined as the concentration required to reduce cell viability by 50% compared with untreated control cells.

Scanning electron microscopy (SEM) is used to examine the size and morphological features of green-synthesized AuNPs. The dried AuNPs powder is mounted onto an SEM specimen stub and coated. The samples are scrutinized at appropriate magnifications to measure the size and morphology of the synthesized particles.

2.5 Regulatory effect of AuNPs on the p53 and p21 gene expression

2.5.1 Cell lines and culture

To explore the role of gold nanoparticles (AuNPs) on the expression of the *p53* and *p21* genes in malignant cells, two human cancerous cell lines, MDA-MB-231 (breast carcinoma) and PC3 (prostate carcinoma), are purchased at the National Cell Bank of Iran (Pasteur Institute, Iran) and frozen in liquid nitrogen before use. The cell lines are cultivated in Minimum Essential Medium via addition of 10% fetal bovine serum, 100 units/mL of penicillin, and 100 µg/mL streptomycin and incubated at 37 °C in a Sanyo CO₂ incubator (MCO-17A).

2.5.2 Cytotoxicity of AuNPs

2.5.2.1 Studying the cell viability in vitro (MTT assay)

To explore the biological effects of the AuNPs on cellular viability, cytotoxicity is measured using the MTT assay (Sigma- Aldrich). Cells are plated at a density of 1.4×10^4 cells/well and allowed to incubate under a humidified environment at 37 °C with 5% CO₂. After that, the cells are washed with phosphate-buffered saline (PBS), incubated with AuNPs at a concentration of 25-800 µg/mL, and incubated further for 48 and 72 hrs. at 37 °C. The cells are washed with PBS after removing the MTT solution, and 100 µL of dimethyl sulfoxide per well is added to dissolve the formed formazan crystals. An ELISA reader (model Wave xs2, Bio-Tek, USA) is used to record absorbance at 570 nm. In order to achieve reproducibility and reliability of the data, experimental conditions are triplicated. Cell proliferation is inhibited at the index as calculated using the equation below [15]:

$$\text{Inhibition rate (\%)} = \left[\frac{(A-B)}{A} \right] \times 100 \quad (1)$$

where A is OD of control and B indicates OD of treated sample. IC₅₀ is evaluated using Linear regression (dose-response curves). The cytotoxicity graph plotted the concentrations of AuNPs against cell viability. IC₅₀ indicated that the concentration of AuNPs inhibited 50% of cell growth (viability).

2.5.3 Examination of apoptotic efficacy of AuNPs against human prostate cancer (PC3)

2.5.3.1 AO/EB staining method

Fluorescent staining with ethidium bromide (EB)/acridine orange (AO) (Sigma-Aldrich) assessed the rates of cellular viability. 1.4×10^4 cells/well of PC3 cell lines are seeded in 6- well plates, treated with 200 µg/ml of the AuNPs, and incubated overnight. Then, wells are washed with PBS and stained with 1µg/ml of EB/AO stain solution. Subsequently, the cells are scrutinized under a fluorescence microscope (Axioskop 2 plus, Zeiss, Germany), and images are captured. The apoptotic (necrotic cells) are characterized as viable cells based on the cell's color appearance. Green cells are viable cells, which are stained with AO, compared with green and orange cells with condensed chromatin indicated early and late apoptotic cells stained with both AO and EB (with moderate alteration in membrane permeability). Necrotic cells appeared as orange cells owing to staining with EB [21].

2.5.3.2 Effect of gold nanoparticles on p53 and p21 genes expression in PC3 cells

Total RNA is extracted from gold nanoparticle-treated PC3 cells using *TRIzol*® *Reagent* according to the manufacturer's instructions (Invitrogen, USA). The integrity of the extracted RNA is evaluated by urea denaturing agarose gel electrophoresis. One microgram, 1 µg of total RNA is reverse-transcribed

into complementary DNA (cDNA) using a random hexamer PrimeScript™ Reverse transcription kit (TaKaRa, Japan) according to the manufacturer's instructions. The resulting cDNA samples are diluted and stored at -20°C until used.

Quantitative real-time PCR (qRT-PCR) is performed using the Rotor-Gene Q 6 plex real-time PCR Platform (Qiagen, Germany) using primers pairs as listed in Table 1. The PCR reaction mixture and conditions are carried out according to the protocol provided by the kit manufacturer. Each sample was analyzed in duplicate. Relative gene expression levels are calculated following the $\Delta\Delta\text{Ct}$ method, and expressed as fold changes relative to the control (untreated cells).

Table 1 Oligonucleotide Primer pairs of the *p21*, *p53*, and housekeeping gene (*GAPDH*) used in the current study.

Primers	Sequences	References
<i>p21</i> (CDKN1A) (NM_000389)	(F) 5'AGGTGGACCTGGAGACTCTCA G-3' (R) 5'TCCTCTTGGAGAAGATCAGCCG -3'	OriGene qPCR Primer Pair (HP200369), validated experimentally [origene +1] and Mol Cell Biol 2006 [pmc.ncbi.nlm.nih]
<i>p53</i> (TP53) (NM_000546)	(F) 5'CCTCAGCATCTTATCCGAGTGG -3' (R) 5'TGGATGGTGGTACAGTCAGAG C-3'	OriGene qPCR Primer Pair, validated commercially and reported in multiple studies [origene]
<i>GAPDH</i> (NM_002046)	(F) 5'GTCTCCTCTGACTTCAACAGCG -3' (R) 5'ACCACCCTGTTGCTGTAGCCAA -3'	OriGene qPCR Primer Pair, widely used as housekeeping gene, validated in literature [origene]

3. RESULTS AND DISCUSSION

3.1 Biosynthesis of AuNPs

This study aimed to evaluate the anticancer activity of AuNPs synthesized using the local plant *Z. spina-christi* against two cancer cell lines. The formation of gold nanoparticles is initially indicated by a visible color change in the reaction mixture, from light brown to brownish-black, following the reaction between gold ions and *Z. spina-christi* extract [22].

3.2 Identification of AuNPs

3.2.1 UV- Visible spectrophotometer

Based on this observation, the formation of nanoparticles is detected by UV–vis spectrophotometer as shown in Figure 1. Surface plasmon resonance (SPR) peak at 520 nm demonstrates the successful

biosynthesis of Zi-AuNPs. Hosny et al. have synthesized Zi-AuNPs and reported a strong absorption peak at 540 nm [23]. Similarly, green synthesis of gold nanoparticles using different precursors, including *Z. spina-christi*, resulted in an SPR peak at 530 ± 2 nm [24]. These obtained results indicated that *Z. spina-christi* is an efficient source used to synthesize AuNPs at a low cost, available in local area and saving time. Another study conducted by Aljabali et al., where suggested that the change in color from light-yellow to ruby-red refers to the formation of AuNPs with a maximum peak of SPR range of approximately 527–535 nm [25].

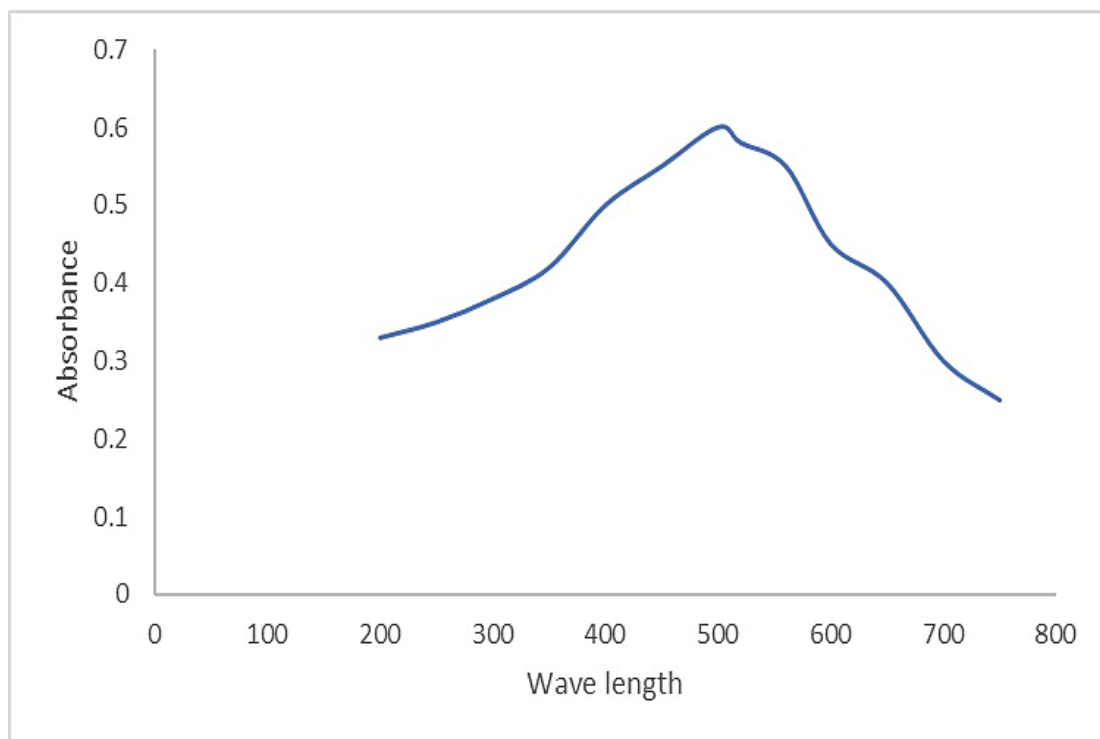


Figure 1 UV-visible absorption spectrum of AuNPs produced by adding gold salt to *Z. spina-christi* extract. The surface plasmon resonance (SPR) peak is observed at 520 nm.

3.2.2 Scanning electron microscope (SEM)

The size and shape of the AuNPs synthesized through the interaction of gold salt with *Z. spina-christi* plant extract are evaluated using Scanning Electron Microscope (SEM). Our finding agrees with Aljabali et al., when the particles are securitized; the results revealed the spherical appearance with high dispersability [25]. The average size of particles is 39, 42 nm, as shown in (Figure 2). Our finding is close to Abed et al., who has measured AuNPs ranging from 31.26–58.06 nm, while (56.49–89.38) nm from *Cordia myxa L.* extracts [26]. It is stated that there are different shapes and sizes of photosynthesized AuNPs observed. This variation is attributed to the variation of plant components present in the plant extract [23,26].

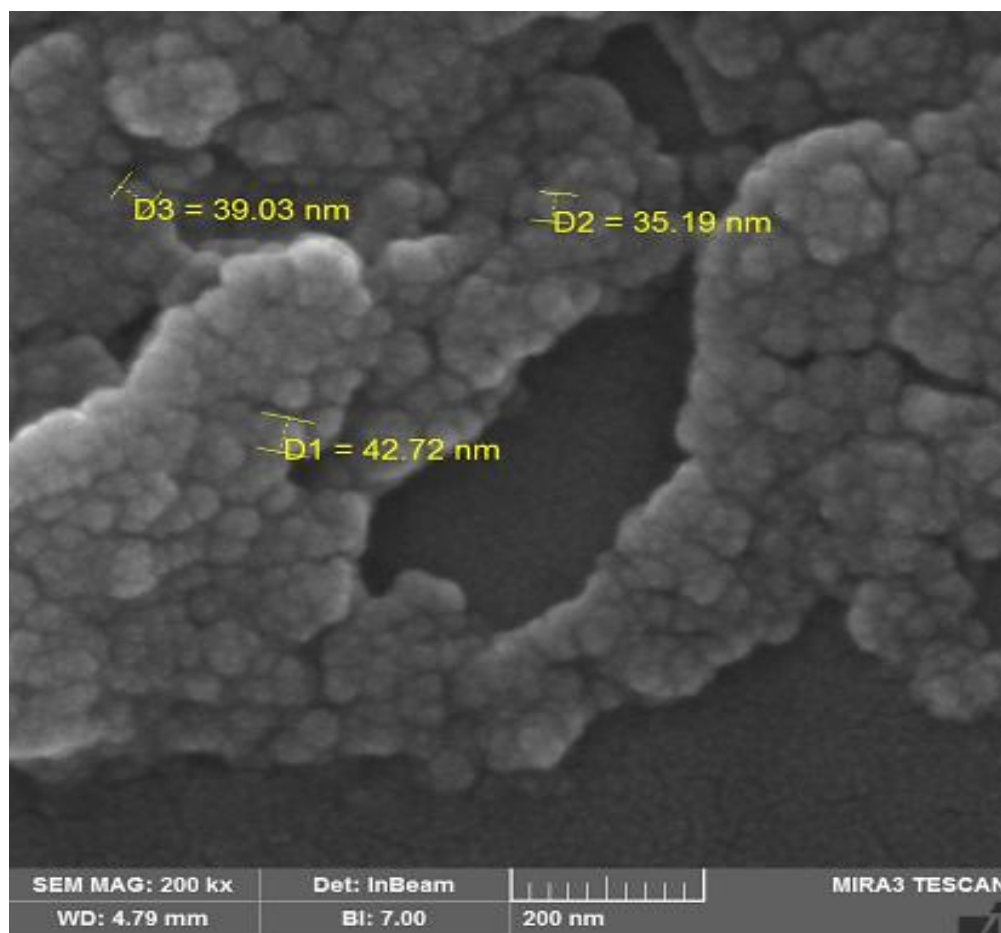


Figure 2 SEM of green synthesized AuNPs produced from *Z. spina-christi*. The results show that the AuNPs spherical shape with size ranging from 39.03 to 42.72 nm. Labels D1, D2, and D3 indicate particle diameter measurements.

3.3 Examination of AuNPs activity against cancer cells

3.3.1 Cytotoxicity of the AuNPs on MDA-MB-231 and PC3 cancer cell line

Cytotoxicity and biological activity of gold nanoparticles on two types of cancer cell lines, human breast cancer cell line (MDA-MB-231) and human prostate cancer (PC3), are evaluated. The previously mentioned cell lines are incubated with different concentrations (25, 50, 100, 200, 400, and 800 $\mu\text{g/ml}$) of the synthesized AuNPs for 72 h. The cytotoxicity effect of AuNPs against cancer cells is investigated by an MTT assay. Our results indicate that the percentage of cell viability in treated cells is inversely proportional to particle concentrations (Figure 3). In other words, AuNPs had anticancer activity against the human breast cancer cell line (MDA-MB-231) and human prostate cancer (PC3), with the IC_{50} equal to 184 $\mu\text{g/ml}$ for PC3 cell lines. Different cell viability of up to 31.63% and 23.15 % is obtained for MDA-MB-231 and PC3, respectively, at 800 $\mu\text{g/mL}$ of AuNPs. Alafaleq et al. have suggested high toxicity of AuNPs against MCF-7 and HCT-116 cell lines. On the other hand, cell morphology and migrations show alteration after incubation with 2 $\mu\text{g/mL}$ of AuNPs in MCF-7 cells after 48 h compared with the control [27]. The present results are consistent with the anticancer activity of AuNPs against A549 with the IC_{50} concentration equal to 24.2691 $\mu\text{g/mL}$ [14].

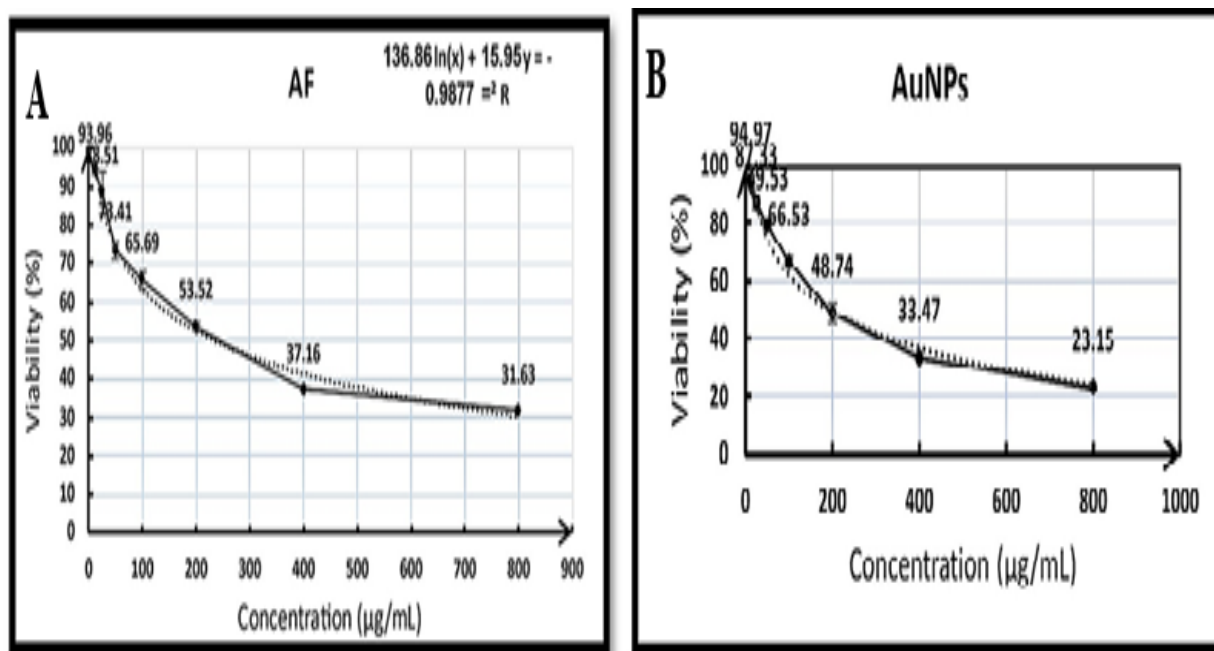


Figure 3 Viability percentages of both a- MDA-MB-231 and b- PC3 cancer cells after 48 and 72 hours of incubation with various concentrations of AuNPs synthesized using aqueous extracts of *Z. spinachristi*. AF indicates the sample symbol. A) represents MDA-MB-231 breast cancer cells, and B) represents PC3 prostate cancer cells. The cells are exposed to different AuNP concentrations for 72 hours, and cell viability decreased as the concentration increased.

3.3.2 Impact of gold nanoparticles on p53 and p21 genes expression in PC3 cells

The results showed that the anticancer activity affected both cell lines, but the effect is more pronounced on PC3 cells, even at low concentrations. Consequently, the expression of apoptotic and tumor suppressor genes is examined in the PC3 cells. The differences in cell sensitivity might be due to variations in the cells' ability to take up AuNPs or differences in cell signaling pathways. It can be concluded that AuNPs specifically target cancer cells and induce apoptosis. Our findings suggest that the small size of AuNPs (39-42 nm) enhances cellular uptake, which may explain the increased sensitivity of PC3 cells to AuNPs. As a result, AuNPs attract attention to be selected as a promising alternative cancer therapy. Small particles size enables AuNPs to move through natural barriers, reaching target cells [28].

Figure 4 shows the fluorescent microscopic images of PC3 cells labelled with ethidium bromide (EB)/acridine orange (AO) used to measure cellular viability (Live/Dead) after incubating cells with the IC₅₀ concentration of gold nanoparticles after a period of 24 hours. The findings reveal that differentiation of viable, apoptotic, and necrotic cells is grounded on the disparity in the permeability of dye through intact plasma membranes. Viable cells appear as green because of exclusive AO staining; cells with condensed chromatin that are green and orange are in the early and late apoptotic state, respectively, and show staining of both AO and EB, respectively, due to intermediate changes in membrane permeability. The cell which has only absorbed EB is necrotic and is orange. To make sure that the data obtained is representative as compared to the control sample, images are obtained randomly on chosen regions of the stained slides.

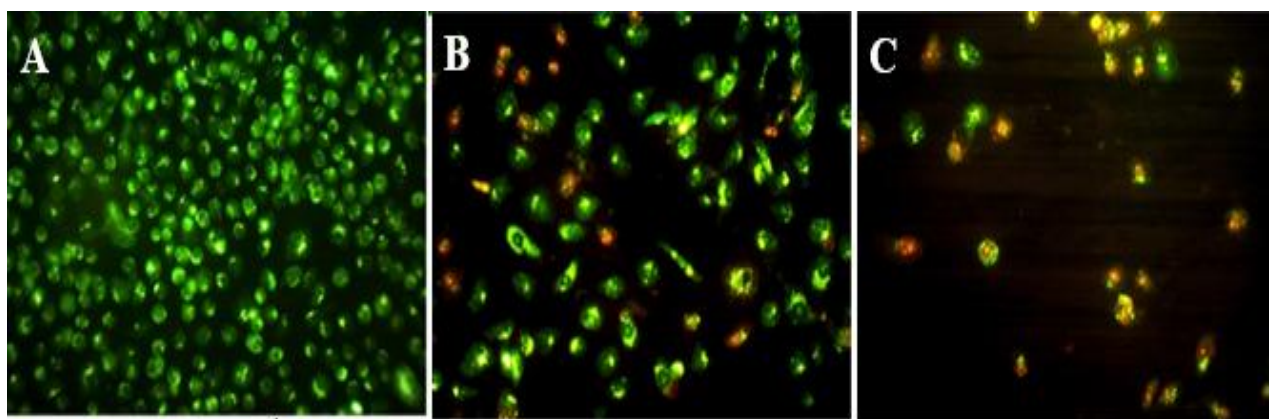


Figure 4 Fluorescent microscope analysis of PC3 cell line stained with AO/EB staining. Cell color refers to the viability of cells. A. Untreated cells (control) are green fluorescence cells referring to viability. B. AuNP- treated cells early and late apoptotic cells are indicated by their green and orange cells with condensed chromatin stained with both AO and EB. C. Necrotic cells are orange stained with EB due to loss membrane integrity.

Figure 5 illustrates the separated RNA samples of PC3 cells in the presence of gold nanoparticles and unstimulated control samples all of which are stained by the electrophoresis technique in 2% urea denaturing agarose gel. Two separate bands, the 28S and 18S ribosomal RNAs, are indicative of high-quality RNA, and the *p53* gene, a tumor-survival program, coordinates the process of destroying cells with defects by regulating the apoptotic suicide program. Based on our results, in which our study highlights the anticancer activity of AuNPs against PC3 cell line, we hypothesized that cell death of PC3 cell line may be related to alteration in gene expression and arrested cell cycle by apoptosis. In this study, the gene expression levels for both *p53* and *p21* genes of PC3 cells treated with IC_{50} concentration of gold nanoparticles are quantified by quantitative real-time PCR.

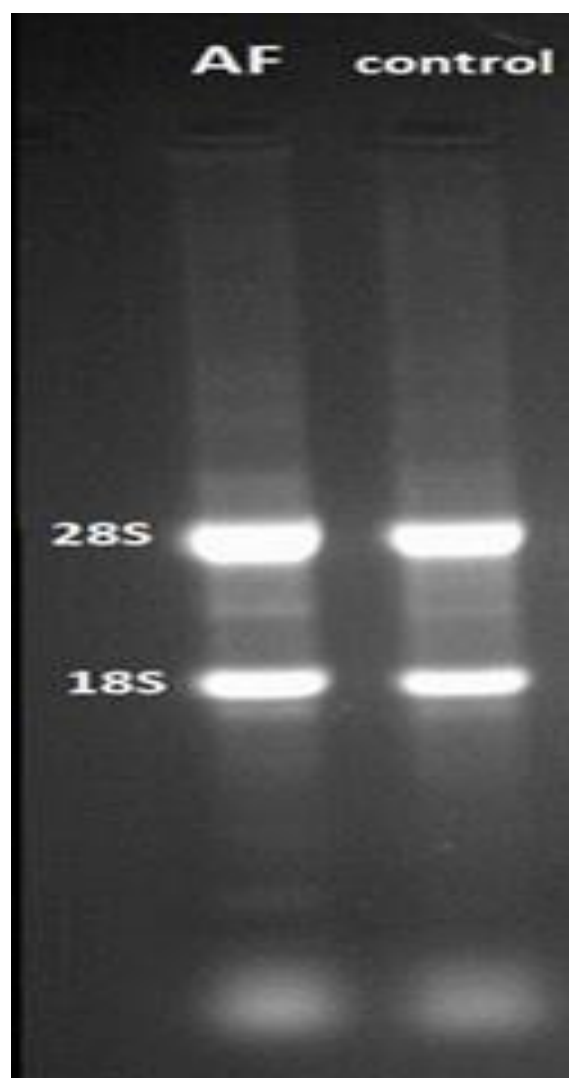


Figure 5 RNA gel electrophoresis from PC3 cancer cell lines treated with AuNPs. Ribosomal RNA appears as two sub-unit bands at 28s and 18s. The bands demonstrate high-quality RNA.

As expected with our hypothesis, the data show that the expression of *p53* and *p21* is increased in PC3 cells, respectively, by 5.43 and 6.41 fold and more than in the normal control samples. The levels of *p53* and *p21* gene expression are notably higher in PC3 cells treated with AuNPs than in untreated control cells ($p < 0.001$). The $\Delta\Delta CT$ s analyses are conducted after subtraction of every gene expression of the PC3 cell lines as shown in (Table 2). As per our observations, Ramalingam et al. have obtained an up-regulation of the two tumor suppressor genes in the cells of the lung that are treated with the PVP-AuNPs [28]. Previous work by Yu et al. [29] observed a 4.3-fold upregulation of *P53* gene human nasopharyngeal carcinoma (CNE1) cells to following treatment with 50 $\mu\text{g/mL}$ of green synthesized AuNPs for 48 h [29]. The effect of AuNPs synthesized using *Moringa oleifera* leaf Extract against Dalton's Lymphoma Cells are evaluated. The evidence suggested that treatment with green synthesized AuNPs induced cell cycle arrest and upregulation of *p21* gene expression, indicating that these nanoparticles may trigger apoptosis in Dalton's lymphoma cells [30].

Table 2 The gene expression levels for both *p53* and *p21* genes of PC3 cells treated with IC₅₀ concentration of gold nanoparticles.

Name	<i>p53</i> average Ct	GAPDH average Ct	$\Delta Ct = Ct_{p53} - Ct_{GAPDH}$	SD	$\Delta \Delta Ct = \Delta Ct_{treated} - \Delta Ct_{control}$	$2^{-\Delta \Delta Ct} =$ Fold change	p- value
AuNPs	14.51	14.59	-0.08	0.26	-2.44	5.43	<0.001
Untreated control	27.69	14.32	13.37	0.36	11.01	0.00	
Name	<i>p21</i> average Ct	GAPDH average Ct	$\Delta Ct = Ct_{p21} - Ct_{GAPDH}$	SD	$\Delta \Delta Ct = \Delta Ct_{treated} - \Delta Ct_{control}$	$2^{-\Delta \Delta Ct} =$ Fold change	p- value
AuNPs	14.27	14.59	-0.32	0.14	-2.68	6.41	<0.001
Untreated control	27.32	14.32	13	0.08	10.64	0.00	

4. CONCLUSIONS

Our study demonstrated the anticancer activity of AuNPs against two types of cell lines, PC3 and MDA-MB-231. The observed alterations of gene expression of both *p53* and *p21* genes, together with the findings of AO/EB staining, indicate preliminary evidence for the involvement of apoptosis in the cytotoxic effect of AuNPs. It could be considered AuNPs as a promising alternative therapeutic anticancer. These results suggested that green synthesized AuNPs might represent promising candidates for further evaluation as alternative anticancer agents. However, the precise molecular mechanisms illustrating their anticancer activity remained unclear. The increased expression of both *p53* and *p21*, together with the findings of AO/EB staining, supported the involvement of apoptosis in the effect of AuNPs. Future research should include the association between *p53/p21* expression and other molecular apoptotic pathways. This aim could be achieved through protein level analysis by Western blot, Annexin V/PI flow cytometry, caspase 3/9 activity and ROS calculation.

Recommendations

Researchers may extend their work and use animal models to investigate the anticancer activity of AuNPs *in vivo*. Further analysis is needed to evaluate the selectivity of AuNPs toward cancer cells. Future research should include normal (non-cancerous) human cell lines to determine the selectivity index and further evaluate the potential therapeutic efficacy and biocompatibility of the green synthesized AuNPs.

References

- [1] G. Karunakaran, K. G. Sudha, S. Ali, and E. B. Cho, *Molecules* 01 (2023) 28
<https://doi:10.3390/molecules28114527>
- [2] M. Q. Mohammed, A. H. M. Shugran, *Experimental and Theoretical Nanotechnology* 10 (2026) 13
<https://doi:10.56053/10.1.13>
- [3] N. Kh. Fadhil, N. S. Dawood, M. H. Al-Khalisy, *J. Fac. Med. Baghdad* 67 (2025) 182
<https://doi:10.32007/jfacmedbaghdad2464>
- [4] M. H. Zaboon, A. A. Saleh, H. S. Al-Lami, *J. Mex. Chem. Soc.* 65 (2021) 178
<https://doi:10.29356/jmcs.v65i2.1265>
- [5] S. S. Salman, A. T. Al-Dour, H. M. Abbas, *Experimental and Theoretical Nanotechnology* 10 (2026) 1325 <https://doi:10.56053/10.3.1325>
- [6] Z. S. Kadhim, H. Y. Hammoud, *Experimental and Theoretical Nanotechnology* 9 (2025) 395 <https://doi:10.56053/9.S.395>
- [7] J. Singh, T. Dutta, K. H. Kim, M. Rawat, P. Samddar and P. Kumar, *J. Nanobiotechnology* 16 (2018) 1 <https://doi:10.1186/s12951-018-0408-4>
- [8] J. K. Patra, J. K. Patra, G. Das, L.F.Fraceto, E. V.R. Campos, M.del P. Rodriguez-Torres, L.S.Acosta-Torres, L. A.Diaz-Torres, R. Grillo, M. K.Swamy, Sh.Sharma, S. Habtemariam and H.S. Shin, *BioMed Central Ltd.* 19 (2018) 8 <https://doi:10.1186/s12951-018-0392-8>
- [9] Aysha Sh. Hasan, Aysha Sh. Hasan, Mustafa N. Taifor, Nawzad J. Mahmood, Beyza Gavcar, *Experimental and Theoretical NANOTECHNOLOGY* 10 (2026) 427
<https://doi.org/10.56053/10.2.427>
- [10] Gulboy Abdolmajeed Nasir, Zahraa Hasan Raheem, Huda Musleh Mahmood, Suhayla Khalid Mohammed, *Experimental and Theoretical NANOTECHNOLOGY* 10 (2026) 441
<https://doi.org/10.56053/10.2.441>
- [11] M. F. Zayed, W. H. Eisa, Y. K. Abdel-Moneam, S. M. El-kousy and A. Atia, *J. Industrial and Engineering Chemistry* 23 (2015) 50 <https://doi:10.1016/j.jiec.2014.07.041>
- [12] A. S. Abed, Y. H. Khalaf, A. Mishaal, *Results Chem.* 5 (2023) 100848
<https://doi:10.1016/j.rechem.2023.100848>
- [13] Areej A. Hussain, Sami I. Al-rubiai, Hussein A. Hussein, *Experimental and Theoretical NANOTECHNOLOGY* 10 (2026) 485 <https://doi.org/10.56053/10.2.485>
- [14] A. Al-Laeiby, A. A. Saleh, E. Q. Jasim, *Experimental and Theoretical Nanotechnology* 10 (2026) 1177 <https://doi:10.56053/10.3.1177>
- [15] X. Hu, Y. Zhang, T. Ding, J. Liu and H. Zhao, *Front. Bioeng. Biotechnol.* 8 (2020) 1
<https://doi:10.3389/fbioe.2020.00990>
- [16] N. Elahi, M. Kamali and M. H. Baghersad, *Elsevier* (2018)
<https://doi:10.1016/j.talanta.2018.02.088>
- [17] S. Al Bitar, H. Gali-Muhtasib, *Cancers* 11 (2019) 1475 <https://doi:10.3390/cancers11101475>
- [18] M. E. Abalaka, S. Y. Daniyan, and A. Mann, *Afr. J. Pharm. Pharmacol.* 4 (2010) 135
<https://www.academicjournals.org/ajpp>
- [19] P. E. Cardoso-Avila, *RSC Adv.* 11 (2021) 14624 <https://doi.org/10.1039/d1ra01448j>
- [20] T. Mosmann, *J. Immunol. Methods* 65 (1983) 55 [https://doi:10.1016/0022-1759\(83\)90303-4](https://doi:10.1016/0022-1759(83)90303-4)
- [21] M. F. Hameed, I. A. Mkashaf, A. A. A. Al-Shawi, K. A. Hussein, *Asian Pacific Journal of Cancer Prevention* 22 (2021) 3533 <https://doi:10.31557/APJCP.2021.22.11.3533>
- [22] Roz Q. Rashid, Kamal M. Abood, *Experimental and Theoretical NANOTECHNOLOGY* 10 (2026) 501 <https://doi.org/10.56053/10.2.501>

Exp. Theo. NANOTECHNOLOGY 10 (2026) 1655-1668

- [23] M. Hosny, A. S. Eltaweil, M. Mostafa, Y.A. El-Badry, E. E. Hussein, A. M. Omer, M. Fawzy, ACS Omega, 7 (2022) 3121 <https://doi:10.1021/acsomega.1c06714>
- [24] I. J. Ibraheem, K. T. Hassan, H. H. Ali, A. S. Obaid, Nano Biomed. Eng 12 (2020) 331 <https://doi:10.5101/nbe.v12i4.p331-337>
- [25] A. A. A. Aljabali, Y. Akkam, M. S. Al Zoubi, Kh. M. Al-Batayneh, B. Al-Trad, O. A. Alrob, A.M. Alkilany, M. Benamara, D. J. Evans. Nanomaterials 8 (2018) 73 <https://doi:10.3390/nano8030174>
- [26] A. S. Abed, Y. H. Khalaf, A. M. Mohammed, Results Chem 5 (2023) 55 <https://doi:10.1016/j.rechem.2023.100848>
- [27] N. O. Alafaleq, A. Alomari, M.Khan, G. Shaik, A. Hussain, F. Ahmed, I. Hassan, M. Alhazza ,M.Alokail , A. Alenad , N.Jabir and S. Tabrez, Nanotechnol. Rev 11 (2022) 3292 <https://doi.org/10.1515/NTREV-2022-0502>
- [28] M. Hosny, M. Fawzy, O. M. El-Borady, A. E. D. Mahmoud, Advanced Powder Technology 32 (2021) 2268 <https://doi.org/10.1016/j.appt.2021.05.004>
- [29] T. Yu, L. Liu, Y. Liu and W. Yin, Alexandria Engineering Journal, 104 (2024) 614 <https://doi:10.1016/j.aej.2024.07.130>
- [30] S.Kumar, A.Shukla, S.P. Singh, R. K. Singh, A. K. Patel, P. K.Verma, S. kumar, N.Kumar, V. Singh, K. Wasnik and A. Acharya, Cell Biochem. Biophys 82 (2024) 1043 1043 <https://doi.org/10.1007/s12013-024-01257-1>