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Study the effect of gold nanoparticles on cancer and normal cells (*in vitro* study)

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Abstract:

This study was done to evaluation the empact of gold nanoparticles on two cancer cell lines in vitro, HeLa (human cervicle cancer cell line) and AMN3 (mammary adenocarcinoma), in addition to isolated lymphocytes from peripheral blood of healthy human as normal cells, at different concentrations and incubation period.

Results shows that cancer cells more effected than normal cells, as well as AMN3 more effected than HeLa cell line, so the effect is related to cells type, concentration, incubation period and size of particles . The present study suggests that gold nanoparticles is a good candidate for medical application as anticancer and needs further studies.

Key word: *gold nanoparticles, cancer cells, normal cells, in vitro.*

Introduction:

Cancer is a major public health problem in the world, and is expected to surpass heart disease as the leading cause of death in the next few years (1). The conventional cancer treatments (surgery, radiation and chemotherapy) are not effective at eradicating cancer from other body. The urgent need to develop new and innovative technologies that could help in cancer treatment is stay.

Nanotechnology is, in part, science of synthesizing molecular sized material that can range about 1-100nm and that are invisible to the human eye, nanotechnology including fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, microfabrication,.etc.(2).

Nanoparticles and noble metal NPs (e.g. gold or silver) in particular, are versatile agents a variety of biomedical applications including their use in highly sensitive diagnostic assays (3).

Gold nanoparticles possess optical properties such as strong absorption scattering in the visible –near infrared region or green light depending on their size and shape, based on these optical properties, GNPs have been used in various types of biomedical application including photothermal therapy, biosensering, Drug delivery and gene therapy (4).

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Materials and methods:

Gold nanoparticles (GNPs) was purchased from Sigma Aldrich Company with 10nm, spherical shape and at concentration 6× 1012 particles/ml.

Cell culture:

Human cervical cancer (HeLa) and Mammary adenocarcinoma (AMN3) cell lines were provided from tissue culture unit/ Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR), the cells were cultured in 50 cm3 tissue culture flasks under humidified 5% CO2 atmosphere at 37ºC in RPMI-1640 medium (Sigma chemical) with 10% fetal bovine serum (FBS), and peniciline-streptomycin during the experiment.

Lymphocytes preparation:

Lymphocytes were prepared according to Hudson, 1980 method (5).

Cytotoxicity assay:

From stock solution of GNPs and by using free serum media, five concentrations (dilution) of GNPs (9×10^{11}, 3×10^{11}, 1×10^{11}, 36×10^{8} and 12×10^{8}) particles/ml were prepared,

then, the cell culture in microtiter plate (96 well) and during log phase of growth were exposed to five concentration of GNPs with four replicate at 200µl for each concentration, for three incubation period 24, 48 and 72 hours. While the control well were exposed to only free serum medium, the plats were sealed with self adhesive film then returned to incubator.

After incubation period, the cells staining by MTT stain and read by micro-ELISA reader at wavelength on 550nm, then, the inhibition rate was calculated as follows:

IR% = A-B/A × 100

IR=inhibitor rate, A=the optical density of control, B=the optical density of treatment.

Statistical analysis

The Statistical Analysis System, SAS (2012) was used to identify effect different factors in study parameters. Least significant difference –LSD test was used to compare between means in this study significantly.

Results and Discussion:

Effect of Gold nanoparticles on HeLa cell line

After 24, the result of inhibition rate (IR%) for HeLa cancer cell line at the first concentration revealed higher significant inhibition in comparison with the fifth concentration which showed lower inhibition rate, without significant variation between the cytotoxic effect for the other concentrations. While at incubation period 48h, the third concentration has higher inhibition rate with less significant between other concentrations, after 72h of exposure, the result revealed higher inhibition growth in the first and second concentration than others.

For effect the time on cytotoxic effect, all concentrations showed higher inhibition rate at the time 72h, with highly significantly variation for all concentrations at level (P<0.01), table (1)

Table (1): Effect of GNPs concentrations and time on growth inhibition rate of HeLa cancer cell line.

Concentration Particles/ml	Time (hours)			LSD value	P-value
	24	48	72		
9×10^{11}	45.9 ± 2.53 A b	52.2 ± 2.75 B b	87.6 ± 4.06 A a	7.812 **	0.0001
3×10^{11}	39.0 ± 1.92 B b	41.9 ± 2.38 C b	76.7 ± 3.79 B a	8.549 **	0.0001
1×10^{11}	38.3 ± 1.79 B b	59.6 ± 3.25 A a	64.2 ± 3.06 C a	7.619 **	0.0001
36×10^{8}	36.0 ± 2.06 B c	48.2 ± 2.38 B b	65.9 ± 3.41 C a	7.934 **	0.0001
12×10^{8}	30.3 ± 1.66 C c	43.1 ± 2.09 C b	65.4 ± 3.07 C a	8.217 **	0.0001
LSD value	5.612 **	4.751 **	5.702 **	---	---
P-value	0.00318	0.0107	0.00438	---	---

Effect of Gold nanoparticles on AMN3cell line

In AMN3 cell line, approximately the same thing, the effect of gold nanoparticles on growth inhibition of AMN3 cell line was higher for the first concentrations in 24, 48 and 72hrs. For effect of incubation period, all concentrations have higher inhibition rate in exposure period 72hrs with significant variation at level (P<0.01) in all three time for all concentrations as in table (2).

Table (2): Effect of GNPs concentrations and time on growth inhibition of AMN3 cell line at different time.

Concentration (particles/ml)	Time (hours)			LSD value	P-value
	24h	48h	72h		
9×10^{11}	64.9 ± 3.64 A c	77.5 ± 2.51 A b	90.0 ± 4.62 A a	9.438 **	0.0001
3×10^{11}	55.1 ± 2.79 B c	71.6 ± 2.09 A b	81.6 ± 3.75 B a	8.712 **	0.0001
1×10^{11}	34.6 ± 2.05 C c	56.0 ± 2.14 B b	83.0 ± 3.82 B a	8.653 **	0.0001
36×10^{8}	32.4 ± 1.94 C c	51.9 ± 2.66 B b	81.0 ± 3.75 B a	8.506 **	0.0001
12×10^{8}	30.2 ± 1.28 C c	55.4 ± 2.39 B b	82.2 ± 3.51 B a	9.469 **	0.0001
LSD value	8.027 **	6.742 **	6.509 *	---	---
P-value	0.0001	0.00316	0.0275	---	---

Effect of Gold nanoparticles on lymphocytes

As normal cell line, lymphocytes were isolated from healthy human blood and exposed to the effect of GNPs, the results showed minimum inhibition rate for five concentrations. When the lymphocytes were exposed to GNPs for 24 h, the results showed reverse effect with the concentrations, while in 48 and 72hrs, the third concentration has higher inhibition rate than others with highly significant variation at level ($P<0.01$). In time dependent manner, the table (3) referred to higher effect of GNPs for all concentrations at time 72hrs with highly significant variation for the first fourth concentrations at level ($p<0.01$) and non- significant variation for last concentration.

Table (3): Effect of GNPs concentrations and time on growth inhibition rate of lymphocytes at different time.

Concentration (particles/ml)	Time (hours)			LSD value	P-value
	24h	48h	72h		
9×10^{11}	9.4 ± 0.55 B b	12.2 ± 0.61 C ab	15.5 ± 0.54 C a	4.356 **	0.001
3×10^{11}	14.4 ± 0.72 A b	20.7 ± 0.82 B b	37.0 ± 1.25 A a	6.439 **	0.0001
1×10^{11}	15.4 ± 0.61 A b	34.8 ± 1.05 A a	38.2 ± 2.06 A a	6.754 **	0.0001
36×10^{8}	17.1 ± 0.57 A b	23.4 ± 0.84 B a	26.2 ± 1.26 B a	5.786 **	0.0001
12×10^{8}	18.8 ± 0.48 A a	21.0 ± 0.79 B a	21.9 ± 0.74 BC a	4.73 NS	0.275
LSD value	4.591 **	5.613 **	5.894 **	---	---
P-value	0.0001	0.0001	0.0001	---	---

The comparison between two cell lines and normal cells was illustrated in table (4).

Table (4): Comparison between the effect of GNPs on HeLa , AMN3 cell line and lymphocytes at different time and concentrations.

Concentration (particles/ml)	24h			48h			72h		
	HeLa	AMN3	lymphocytes	HeLa	AMN3	lympho-cytes	HeLa	AMN3	lympho-cytes
9×10^{11}	45.9 ±2.91	64.9 ±3.65	9.4 ±0.76	52.2 ±2.6	77.5 ±3.1	12.2 ±0.6	87.6 ±3.6	90.0 ±4.2	15.5 ±0.53
3×10^{11}	39.0 ±2.07	55.1 ±2.69	14.4 ±0.45	41.9 ±1.9	71.6 ±2.7	20.7 ±0.42	76.7 ±3.1	81.6 ±3.7	37.0 ±1.6
1×10^{11}	38.3 ±2.65	34.6 ١,٩±	15.4 ±0.71	59.6 ±2.0	56.0 ±3.0	34.8 ±1.5	64.2 ±2.6	83.0 ±3.3	38.2 ±1.5
36×10^{8}	36.0 ±2.91	32.4 ±1.4	17.1 ±0.35	48.2 ±2.5	51.9 ±2.8	23.4 ±0.94	65.9 ±2.5	81.0 ±4.0	26.2 ±0.94
12×10^{8}	30.3 ±2.30	30.2 ±1.6	18.8 ±0.51	43.1 ±1.8	55.4 ±2.0	21.0 ±0.67	65.4 ±2.1	82.2 ±3.5	21.9 ±0.68
LSD value	4.67 *	6.99 **	3.75 **	3.82 *	6.12 **	3.94 *	5.35 **	5.06 *	5.84 **
P-value	0.034	0.004	0.001	0.039	0.001	0.051	0.001	0.041	0.001

The growth inhibition rate is depend on type of cell line, exposure period and concentration, but in the nanoparticles experiment, the growth inhibition rate is also depend on type of cancer cell lines, size and shape of nanoparticles and surface chemistry (6).

AMN3 cell line more effect than HeLa cell line, in the same time, cancer cell lines more sensitive to GNPs than normal cell, this mean that GNPs has selective effect towards cell lines. Patra (7) suggested that cell death was selective induce by GNPs dependent on type of cell lines until in absence of any specific functionalization, they found that human carcinoma lung cell line would selectively absorb GNPs while the baby hamster kidney and human hepatocellular liver carcinoma showed no response to treatment with GNPs.

GNPs bind to cancer cells 600 more than with noncancerous cells because that cancer cells express different protein groups on their surface, such as breast cancer overexpress seprase and Her2 which have corresponding antibodies that can be bound to GNPs especially conjugated GNPs (8).

As normal cells, lymphocytes revealed minimum effect to the GNPs exposure in comparison with cancer cell lines. According to Al-Hasnawi (9) study which referred to that the effect of spherical GNPs on lymphocyte was only 7%. Normal cells induce autophagy as cellular defense mechanism against oxidative stress toxicity, this mechanism induce by gold particles, in addition to generated of lipid hydroperoxides as positive indication of lipid peroxidation. In contrast, some study revealed that spherical gold nanoparticles in various size 4-15nm can enter both of theme, HeLa cancer cells and normal cell line within 6hrs and localized in cytoplasm and nucleus, but cannot induce any toxic effect even up 48hrs(11)

In concentration dependent manner, the study shows that highly inhibition rate when the concentration was increased, Qu and his team(6) emphasized on the same thing, when they pointed to that low concentration after incubation 24hrs did not increase in cell viability and high concentration lead to high inhibition. As well as the spherical GNPs were lethal only at highly concentration(12).

In time dependent manner, the effect of GNPs on the cancer via induce apoptosis by increase the number of cells in S phase and decrease it in G0/G1, G2/M phase with morphological changes such as membrane blebbing and fragmented nuclei, this is increase with the incubation time (13). In lymphocytes the lowest concentration has higher inhibition level, as in the study of Rahman (14) on bovine endothelial aortic cells that reported that low nanoparticles concentration can also cause significantly decrease cell viability may be reach to 30%.

In size and shape dependent manner and For cancer treatment, the internalized number and time of GNPs are related to the size and shape (15). Small size of nanoparticles have

a surface curvature too great to provide necessary conformational rigidity to allow for multivalent binding with receptors (16). However, the experiment by Tsai and his team (17) recorded that different size of GNPs can enter cells such as leukemia cells and detected in various organelles, then, inhibited cells growth at different time by induce apoptosis and necrotic phenotype.

Due to the small size of GNPs, it can able to enter into cells by transport mechanism like diffusion around the center and edge of the culture wall or by accumulated in the extracellular space between the basal plasma membrane for insert into cells, but rare in the nucleus (18).

Little effect of GNPs on normal cells than cancer cell lines in this study, this is may be is related to that spherical shape have been reported to demonstrate less toxicity than their rode counterparts (19).

This study suggest that gold nanoparticles have ability to inhibit the growth of cancer cells in different types of cancer cell lines, and the inhibition was different according to type of cell line with less effect on normal cells.

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دراسة تأثير دقائق الذهب النانوية على الخلايا السرطانية والطبيعية

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الخلاصه:

أجريت هذه الدراسة لتقييم كفاءة دقائق الذهب النانوية على خطين من الخطوط الخلوية السرطانية النامية في الزجاج، خط خلايا سرطان عنق الرحم وخط خلايا سرطان الغدد اللبنية، اضافة الى عزل الخلايا اللمفاوية من الدم المحيطي لاشخاص اصحاء كخلايا طبيعية، لأوقات حضن وتراكيز مختلفة.

أظهرت نتائج هذه الدراسة ان الخلايا السرطانية اكثر تأثرا من الخلايا الطبيعية وان خلايا سرطان الغدد اللبنية اكثر تأثرا من خلايا سرطان عنق الرحم. وان هذا التأثير يتبع نوع الخلايا و التركيز ومدة الحضن وحجم الدقائق النانوية. تقترح هذه الدراسة ان دقائق الذهب النانوية مرشح جيد كتطبيق طبي ضد السرطان وتحتاج الى دراسات اضافية.