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The Cytotoxic Effects of Gold Nanoparticles on $L_{20}B$ cell line.

Eman Ghadhban*.

Department of Biomedical engineering, College of Engineering, AL-Nahrain University, Baghdad, Iraq.

ABSTRACT

Pure gold nanoparticles were synthesized using laser ablation in liquid environment. An Atomic force microscopy (AFM) wasemployed to give the morphology of the particles and average particle size. The produced Au colloids nanoparticles have an average diameter of 29-34 nm were produced. The UV– VIS absorption spectra of Au nanoparticles exhibit a characteristic single peak around 524-525 nm, indicating the formation of gold nanoparticles. The Atomic absorption spectrophotometer (AAS) was used to estimate the concentration (50 µg/ml) of produced gold nanoparticles. This study was carried out to evaluate the cytotoxic effects of gold nanoparticles with different concentrations and sizes on nonmalignant mouse ($L_{20}B$) cell line. Significant changes were found in cell viability when they were treated with some concentrations.

Keywords: gold nanoparticles, $L_{20}B$ cell line, AFM, AAS

Corresponding author

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INTRODUCTION

The uniqueness and distinct characteristics of nanomaterials arise specifically from their higher surface to volume ratiotherefore they differ from their bulk macroscopic materials. They represent a novel class of materials in the development of new devices involved in differentbiological, biomedical and physical issues [1]. The small moleculessuch as drugs, proteins, RNA,DNA, and probes carried and bondedtonanoparticles with high efficiency. As well as,high stability, carrier capacity, and compatibility with various administration routes due to their geometry, precise size, andthe properties of their surface, which make them desirableand useful in many issues of oncology. Other applications:involvedin the carrier system (drug delivery),surface-enhanced Raman spectroscopy, photo-thermal therapy, enhance x-ray image [2], the healing inpatient with diabetes mellitus [3] and as antibacterial factor [1].

Gold nanoparticles (GNPs) properties areattributed to their localized surface plasmon resonance (LSPs), i.e., charge density oscillations that are confined to the particles whichmakes them the axis of researcheson cancer [4]. In addition, the physio-chemical and LSPs characteristics, based on thesize, geometry and the surface of nanoparticles. A wide variety of GNPs has been generated to produce those have maximum absorption in near-infrared region of spectra [5],which is not induce photochemical damage, deeply penetrates biological tissues, as well as achieved a better signal-to-noise ratio in photoimagingbecause of lackingautofluorescenceof the cell in near-infrared region [6].

Both LSPs and their interaction with the bio-environmentaffected by shape of gold NPs, i.e. shells,rods, or branched nanoparticles, and this isa critical point for the uptake and toxicity of NPs [7-10]. Also, the bio-nanointeraction influenced by the cell type itself [10].

In previous years,laser ablation synthesis in solution became a reliable alternative to the traditional chemical reduction methods for obtaining noble metal nanoparticles.Ablation process by laser gives the capability to synthesis nanoparticles in non-sterile condition and directly produce colloidal solution[11].

MATERIAL AND METHODS

GNPs solutions were synthesized using Pulsed Nd:YAG laser via ablation of a piece of gold metal plate (with purity of 99.999%),put it inatube containing 3.6 ml of deionized water. Varied laser energies were used from (600, 800 and 1000) mJ, respectively.The spot size of the laser beam was adjusted to 1 mm in diameter on the surface of the metal plate through changing the distance between the metal plate and the focusing lens. The laser wavelength was performed by using a focused beam output at 1064 nm, repetition rate of 6 Hz / second and pulse width of 10 ns.

Using uv-visible spectrophotometer, shimadzu uv-160a model for a range of (300-800) nm to measure the absorption spectra of the synthesized gold nanoparticles solutionsat room temperature.

While the size of nanoparticles,their distribution and surface roughness weredetermined by using atomic force microscopy (AFM).The test starts by putting a few drops of each sample on slide to dry at room temperature.

The concentration of each the GNPs sampleis obtained via using Atomic absorption spectrophotometer.

$L_{20}B$ cell line, genetically engineered nonmalignant mouse cell line expressing the human poliovirus receptor (CD155), was used as cell line model [12].

The $L_{20}B$ cells were exposed to twelve concentrations of each GNPs solution [25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.098, 0.049, 0.024, 0.012] µg/mL.The procedure started with seeding the microtiter plate wells with about 10^4-10^5 cells /200µL of growth media/well for treated and control group. Then incubated at 37 ºC for 24-48h. Then pipetting 200µL from each concentration into each well of the treated group (three replicates for each concentration), while adding 200µL of maintenance medium/well for the control group.The plate was wrapped with adhesive parafilm and was reincubated at 37ºC, 5% CO_2 in humidified atmosphere. The photos(pictures) were taken after 24 h and 48 h, while the evaluation of

cytotoxicity was carried out after removing the medium followed by adding MTT solution (20 µl of 5 mg/ml) and incubating for 4 h at 37°C. Then addition of 200 µL of Dimethyl Sulphoxide (DMSO) and incubated in 37°C/ 15 min with shaking. The absorbance was evaluated by the microplate reader at wavelength of 620 nm. The inhibition of cell growth wascalculated after 48 h. of exposure to GNPs according to Betancur- Galvis*et al*., in 1999 [13] and Gao *et al*., in 2003 [14] as follows:

$$\text{Inhibition rate} = \frac{mean\ of\ control - mean\ of\ treatment}{mean\ of\ control} \times 100$$

RESULTS AND DISCUSSION

GOLD NANOPARTICLES

The different parameters of laser such as energies, wavelength, frequencies, etc., affect the formation of GNPs. Fig. 1 (A, B, and C)shows the UV spectra of GNPs samples weregenerated at variable laser ablation energies.

The duration of ablationprocess was 3.33min.,then the solutions gradually became colored.

Metal nanoparticles concentration increased in the solution as the ablation process increased. Moreover, the peak or maximum intensity was about 524-525 nm that indicates formation of GNPs. However, the absorption spectra peaks(height and width)are depend on the pulsed laser energy.

The average diameters of the generated GNPs were 29, 31, and 34 nm when the laser energies; 1000, 800, and 600mJ, respectively as obtained from AFM and manifested in figure 2 (A, B, and C). That means the size of GNPs decreasedwith an increasingin the laser energies and this is agree with **Imam et. al.,**in 2012, they recorded that decreasing in the size particles can be referred to a large energy that excited the GNPs in a solution.In a single laser pulse,the photon energy is readily converted to the internal modes of the nanoparticles and theyielded gold nanoparticle absorb consecutively more than one thousand photon leading to rise the temperature significantly which cause fragmentation of the nanoparticle. After spreading the single laser pulse in the solution, the temperature of nanoparticles returns to room temperature before diffusingthe second pulse. So, heating and cooling of nanoparticles occur during each laser pulse [15].

On the other hand,**Sasohet. al.,** in 2005 and **Nichols et. al.,** in 2006 clarified that: the variation in size distributions of the gold nanoparticles may be due to vaporization of the surface target, as well as, explosive ejection of molten droplets directly from the target which leads to abroad size distribution[16,17].

NFLUENCEOF GOLD NANOPARTICLES ON L_{20}B CELL LINE VIABILITY

The effect of different concentrations and sizes of GNPson the L_{20}B cell proliferation were mentioned as shown in Fig. 3 and table 1. The cell line was treated with [25 - 0.012]µM of 29, 31, and 34 nm gold nanoparticles for 48 h.

The Gold nanospheres (GNSs) with 34 nm showed significant ($p \leq 0.05$) reduction of L_{20}B cells proliferation, the IR of cell growth was 27% in 0.012 µM, 58.333% in 1.56µM and 72.222% in 25 µMin comparison with the control group.The inhibition ratesare concentration dependent manner, as it's increased proportionally tothe concentration of GNPs. Moreover, the receptors can receive abundant GNPs that presented in the solution more easily and faster,making the wrapping times shorter,as well asenhancing particles uptake by the cells. Hence, increasing the uptake of gold nanoparticles leading to increasethe inhibition rate for the same particle size. And this corresponds with **Trono JD *et al.,*** in 2011 they demonstrated that lesser chance for a receptor to geta fewer gold nanoparticle that presented in the solution; causing longer membrane wrapping time, anddecreasing the uptake by the cellswith less chance for growth inhibition. As well as, longer incubation time at lower concentrations will not enhance the gold nanoparticle uptake by the cells because of the few number of nanoparticles reaching the cellsreceptors[18].

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Table (1): Mean values of inhibition rate percentage (IR %) for $L_{20}B$ cell line by aqueous solution of GNPs after 48 h. of exposure.

Conc. µg/mL	IR% of different GNPs sizes		
	29 nm	31 nm	34nm
25	65.926 ± 0.980 a B	66.111 ± 0.642 a B	72.222 ± 1.604 a A
12.5	64.444 ± 0.642 a B	65.000 ± 0.321 a B	68.889 ± 0.641 a A
6.25	59.444 ± 0.962 ab A	60.556 ± 4.170 ab A	66.111 ± 0.321 ab A
3.125	57.778 ± 0.321 abc B	59.444 ± 0.642 abc B	64.444 ± 0.962 ab A
1.563	55.000 ± 1.604 bc A	55.000 ± 2.887 bcd A	58.333 ± 0.962 bc A
0.781	50.000 ± 0.962 c A	51.667 ± 1.283 cd A	52.222 ± 0.000 cd A
0.391	48.889 ± 5.453 cd A	48.889 ± 5.132 de A	50.000 ± 3.528 cde A
0.195	37.778 ± 1.283 de A	41.667 ± 3.208 ef A	43.889 ± 8.660 def A
0.098	34.444 ± 3.528 e A	37.222 ± 1.604 f A	41.667 ± 3.528 ef A
0.049	34.444 ± 3.208 e A	36.111 ± 3.528 f A	38.889 ± 4.811 f A
0.024	34.444 ± 1.604 e A	34.444 ± 1.604 f A	36.111 ± 1.604 fg A
0.012	18.889 ± 7.377 f A	25.000 ± 3.849 g A	27.778 ± 1.604 g A

Note: Data are expressed as mean ± S.E.; values with different small characters superscript (a, b, c, d, e, f, and g) on the same column differ significantly (P≤ 0.05); values with variable capital characters(A and B) on the same row significantly different (P≤ 0.05).

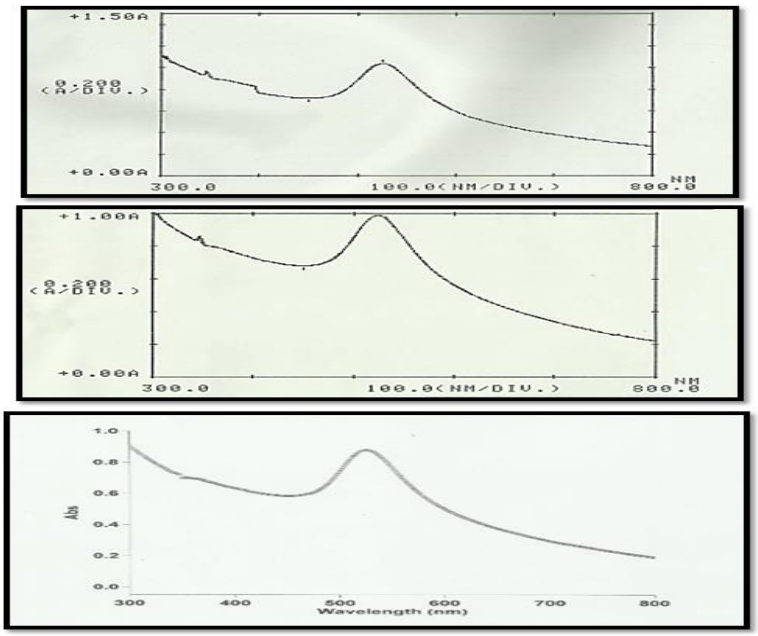

Figure 1: Absorption spectra of the colloidal gold nanoparticles at laser energy (A) 1000mJ, (B) 800mJ, (C) 600mJ.

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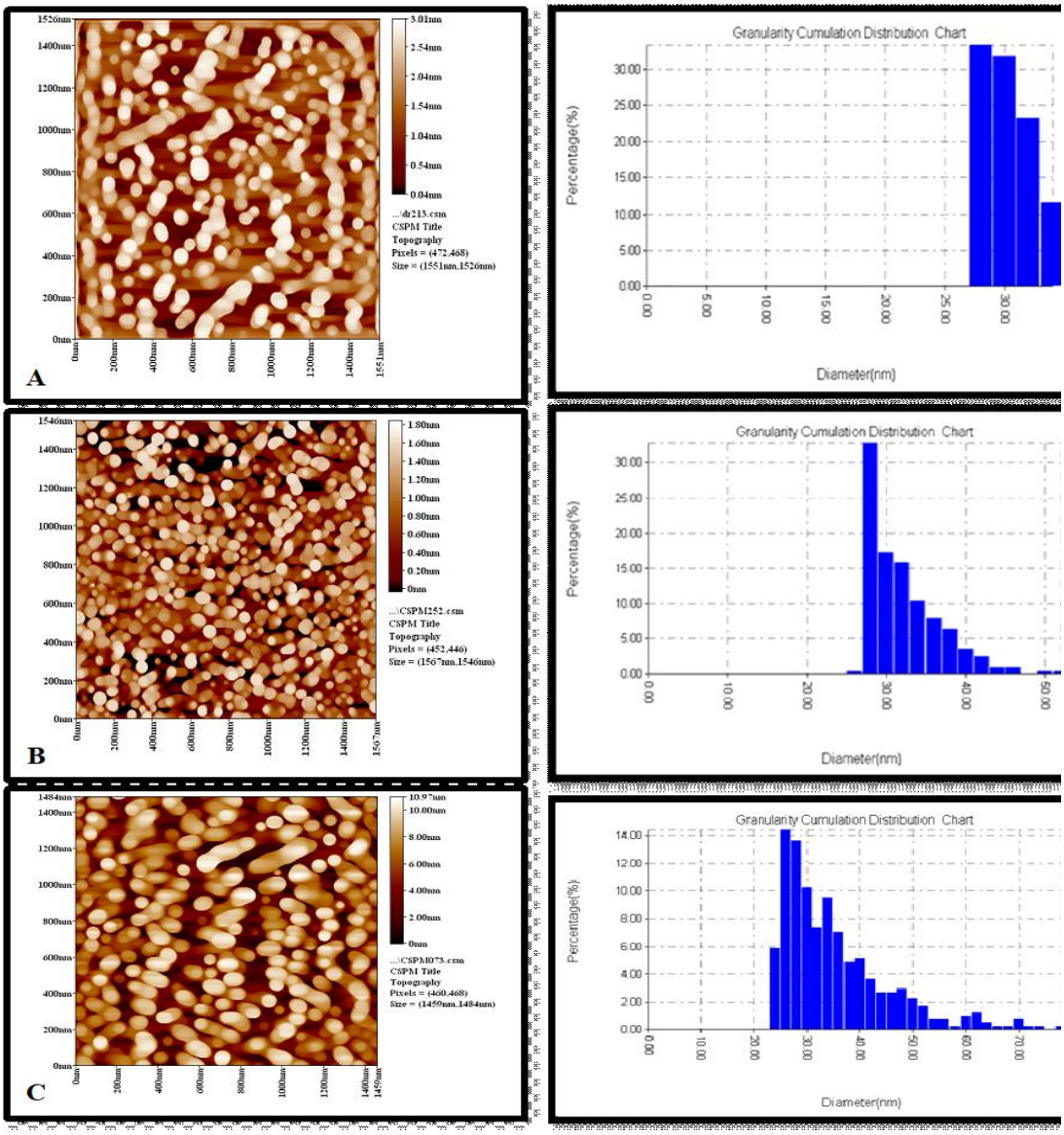

Figure 2:AFM images of gold nanoparticles deposited on slide and its size distributions prepared by laser energy (A) 1000mJ, (B) 800mJ, (C)600mJ with λ=1064 nm and PRR =6 Hz

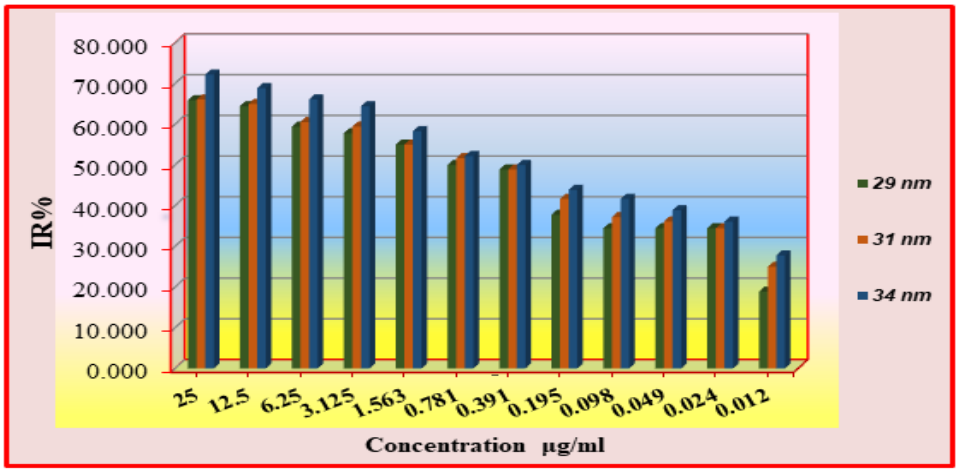

Fig. 3. Effect of different concentrations and sizes of gold NPs on the viability of L20B cell line. 48 h. after treatment with the same concentrations. The viability of the cells in the control group was considered arbitrarily 100%. The data plotted are mean, n = 3. *p <0.05 vs. the control group.

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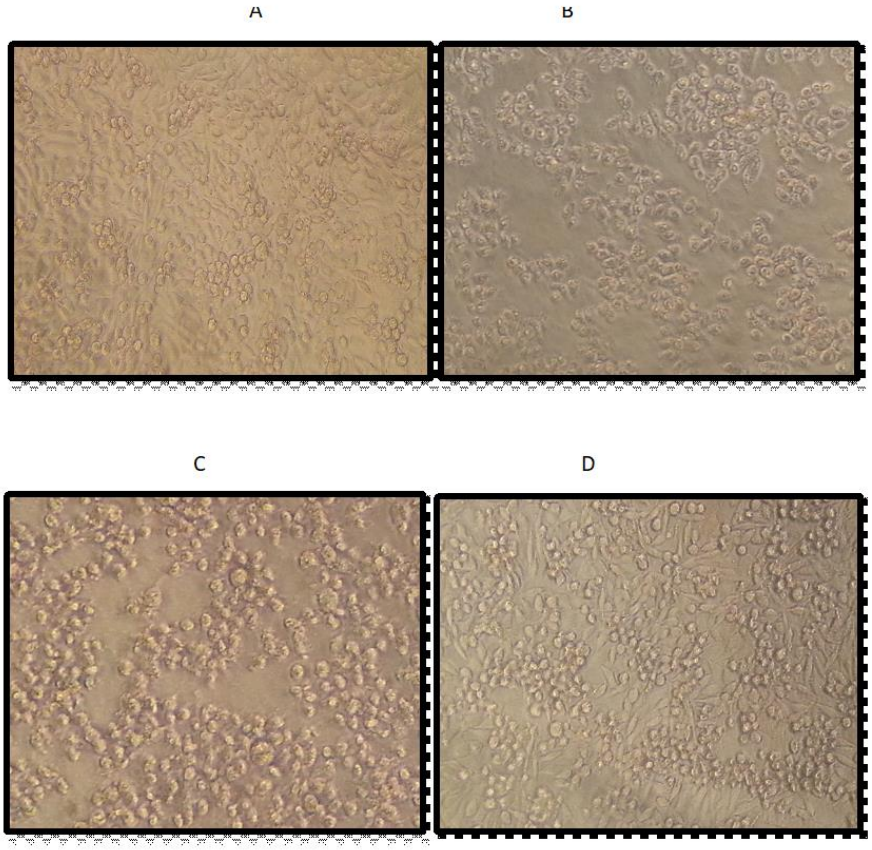

Figure (5): Effects of GNPs on $L_{20}B$ cells morphology. With different concentrations and after 48 h-treatment with the same size (34 nm). (A) Control (untreated) (B) 25µg/ml (C)1.563µg/ml(D) 0.012 µg/ml (10X).

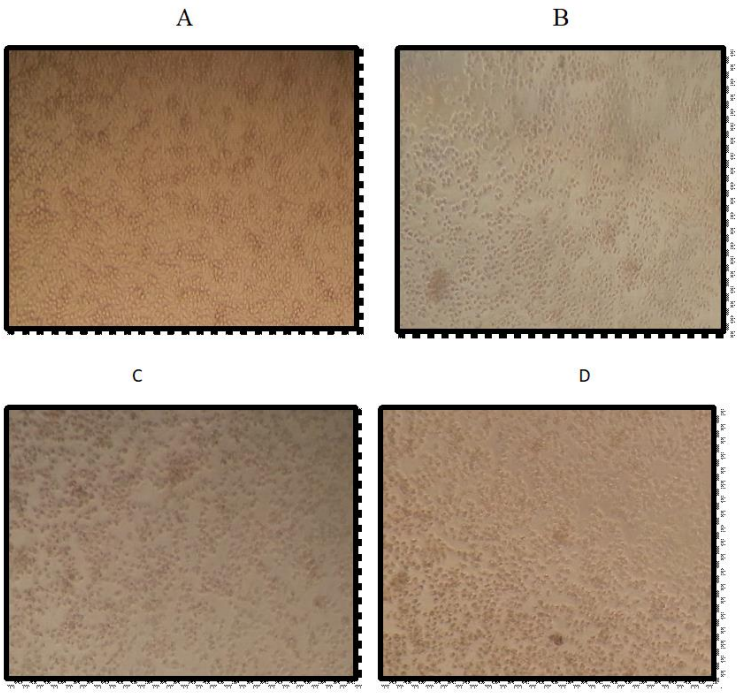

Figure (6): Effects of GNPs on $L_{20}B$ cells morphology at 25 µg/ml,post 24 h. of exposureto different particles sizes. (A) Control cells (untreated), (B) cells exposed to or treated with 34 nm, (C) cells exposed to or treated with 31nm, and (D)) cells exposed to or treated with 29 nm(10X).

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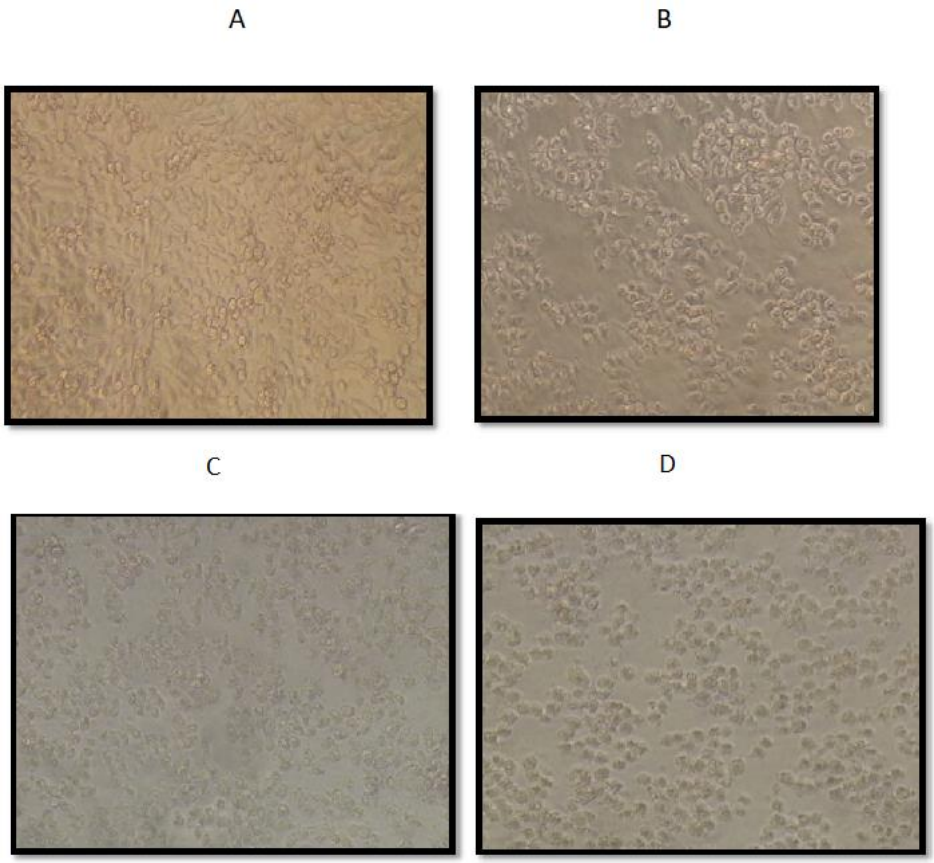

Figure (7): Effects of GNPs on $L_{20}B$ cells morphology at 25 µg/ml,post48 h. of treatment with different particles sizes. (A) Control cells (untreated), (B) cells exposed to or treated with 34 nm, (C) cells exposed to or treated with 31nm, and (D)) cells exposed to or treated with 29 nm (10X).

Microscopically the morphology of incubated cells with nanoparticles of 34 nm for 24 and 48 h revealed cellular death were observed in the treated$L_{20}B$ cells with 25, 1.563, 0.012 µM GNSs which is evident byinhibition of cell growth as shown in fig. (4 and 5).

The inhibition rate versus different sizes of the gold nanoparticles as manifested in Fig.3 which reveals the cytotoxic effect is extremely dependent on the size. The smaller sizes of gold nanoparticles (29 and 31 nm) have a significant low cytotoxic effect on *$L_{20}B$ cell line* growth in comparison to 34 nm at 25, 12.5, and 3.125 concentrations. As well as, there is a relation between the particle size and the cell uptake efficiency.Itemslikeadherencerate and membrane expansion or stretching, the membrane's curvature orbending energy, may also affect the size electionand this agree with **Gao et al.** in 2005 proposed that the wrapping time is based on particle size. Wrapping time clarifying the process of enclosingand surrounding the particle by the cell membrane. The receptors need to be occupied and filled-upwith smallerparticles sizeprior theysurroundingand intake via the cell membrane. Lower uptake occurs when the receptors are empty or not occupied with the particles, this leads to delay of the cell signal to surround or wrap around the molecules (nanoparticles), and finally longer wrapping time.Also prolong wrapping time and decrease uptake happens where there are larger nanoparticles size, because the uptake requires more receptorsto trigger the signal for the cell to allow its membrane to enclose around the large particles [19].

Figure (6 and 7)refer to the cell viability it decreased when the particle sizes increased, it maybe concerned withthe surface charge of GNPs regarding to their concentrations. The toxic effect of GNPs possess size-dependent manner since the cell uptake the larger particle sizes more than the smaller sizes and this is agree with **Zeng et al.** in 2014 and **Tan et al.** in 2010 they revealedthat any change in the particle's size, geometry and/or surface functional group leading to a significant change in cellular interaction, as well as, increasing in particle size and surface charge would often enhancing the nonspecific uptake of NPs by the cell [20 and 21], also, **Alkilany et. al.** in 2009 and **Hauck et. al.** in 2008 they recorded that larger particles had more

toxic effect in compression with the smaller one at the same concentration, as well as, the surface charge perform a significant role during the uptake of NPs by the cell [22 and 23]. Moreover, **Zeng et al**. in 2014 treated HeLa cell line with GNPs, they demonstrated that the NPs size has a significant effect in thecell toxicity, because the bigger sizeswithelevated surface chargeof NPsmanifesteda relative clear interaction with the cell

Kodiha et al. in 2014 noted that the severity of cellular alteration and damage with multi nuclear changes can be determined or related to particle size. GNPs cause stress-sensitive regulators redistribution of nuclear biology, nuclear morphologywith nuclear laminae alteration and inhibit nucleolar functions.So the GNPs reducethe biosynthesis of RNA in the nucleoli. Depending on GNPs size, the smaller gold nanospheres, but not the large gold nanospheres depends on the cell type, inducenucleardamageat normal growth temperature. The toxic effect of gold nanoparticles associated with alterations in the organization and function of the nucleus. The results confirm that the nucleus of the cell is a notable goal or aim for gold nanoparticles of different shapes [24].The activity ofcultured cells (growth and proliferation)is linked with protein synthesis and thus depends on theability of cells to produce ribosomes. The nucleus has specializedstructures calledNucleoli that transcribe ribosomalRNA genes and assemble ribosomal subunits [25].

According to **Ashokkumar et al.**in 2014 gold nanoparticles cause cell cyclearrestas well as, DNA damagevia generation of Excessive reactive oxygen species (ROS) that may play a dominant role in cancer cells' apoptosis [26].

Furthermore, other studies recorded that the generation of intracellular reactive oxygen species, elevated expression of cleaved caspase proteins and p53 activation all entered the process of killing the cell (apoptosis)via NPs as mentioned in [27, 28, 29].

However, the initiators and signaling pathways of apoptosis are variable, they are based on the characteristic nature of NPs. In 2011 **Gao et al.**recorded that gold NPs caused hydrogen oxygen accumulation by cytosolic glutathione (GSH) depletion and therefore mitochondrial apoptosis pathwayactivated then leading to cell death [30]. While **Kang et al.** in 2010 mentioned that localization of gold NPs in thenucleusinduced an injureto the DNA in addition to cytokinesis arrest [31].

CONCLUSIONS

In conclusion, the gold nanoparticles synthesized by laser ablation in liquid environment,were significantly less cytotoxic effect on $L_{20}B$ cell line at 29 nmwhen compared with the larger size.

Also the cells showed more influence toward gold nanoparticles at higher concentration in comparison to the control group.

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