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Pulsed laser ablation synthesized silver nanoparticles induce apoptosis in human glioblastoma cell line and possess minimal defect in mice brains

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Abstract: In this research we have assessed the antiproliferative activity of silver nanoparticles (SNP) synthesized by pulsed laser ablation in liquid (PLAL) method. Shape and particle size distribution of the synthesized nanoparticles was characterized with atomic force microscope (AFM) surface plasmon resonance was determined with UV-Vis spectroscopy. A silver nanoparticles concentration in the prepared liquid was determined by atomic absorption spectroscopy. Cytotoxic activity of three different concentrations 15, 30 and 60 µg/ml of SNP against human glioblastoma cell line (AMGM) was assessed with MTT assay. Possibilities of apoptosis induction were inspected using acridine orange/ ethidium bromide staining and DNA fragmentation assay. Toxicity of the synthesized SNP on normal brain cells was assessed *in vivo* using BALB/c mice. These results indicated that SNP was able to inhibit AMGM growth due to induction of apoptosis and their damage effect toward normal brain cells were at minimal according to histotoxopathological inspection.

Key word: silver nanoparticles, glioblastoma, apoptosis, nanotechnology, laser ablation.

دور جسيمات الفضة النانوية المصنعة بطريقة القلع بالليزر النبضي على حث الموت المبرمج في خط خلايا سرطان الأرومة الدبقية البشري (سرطان الدماغ المتعدد الأشكال) دون الحاق ضرر في دماغ الفئران في الجسم الحي

عامر طالب توفيق

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الخلاصة: قيمت فعالية جسيمات الفضة النانوية المصنعة بطريقة القلع بالليزر النبضي في الوسط السائل على تثبيط نمو وتضاعف خلايا سرطان الأرومة الدبقية الدماغية البشري . تم وصف شكل وحجم جسيمات الفضة النانوية المصنعة بإستخدام مجهر القوى الذرية وحددت نقطة طيف الامتصاص القصوى بإستخدام المطياف االماسح للطول الموجي الممتد بين طيف الأشعة فوق البنفسجية إلى الضوء المرئي وقدر تركيز الفضة النانوية في المحاليل المحضرة بواسطة طيف الإمتصاص الذري . أختبرت السمية الخلوية لثلاث تراكيز من محلول الفضة النانوية المحضرة 15 و 30 و 60 مايكرو غرام / مليلتر إتجاه خط خلايا سرطان الأرومة الدبقية الدماغي البشري (AMGM) من خلال فحص MTT. تم التحري عن إحتمال حث الموت المبرمج في الخلايا المعاملة بالتلوين بالصبغات المتفلورة الأكردين البرتقالي / وبروميد الإيثديوم (acridine orange/ ethidium bromide) وفحص تقطع مادة الخلايا الوراثية (DNA) . أختبرت سمية محلول الفضة النانوية المحضرة إتجاه الخلايا الطبيعية داخل الجسم الحي للفئران البيضاء ضرب BALB/c من خلال التحري عن التضرر النسيجي لدماغها . أظهرت النتائج أن الفضة النانوية المحضرة تمتلك قدرة عالية على تثبيط نمو وتضاعف خط خلايا سرطان الأرومة الدبقية الدماغي البشري وكان تركيز 60 مايكروغرام / مليلتر قادر على تثبيط 50% من الخلايا المعرضة خلال 24 ساعة وهذا التثبيط يرجع الى حث الموت المبرمج في الخلايا . لم يكن هناك تضرر نسيجي في دماغ الفئران المجرعة بمحلول الفضة النانوية بعد التجريع لمدة إسبوعين بمحلول تركيزه 60 مايكروغرتم / كغم من وزن جسم الحيوان . وقد يشير ذلك إلى إمكانية إستخدام الفضة النانوية في علاج سرطانات الدماغ.

Introduction

Nanotechnology is the science or technology that deals with or operates on a nanoscale ranging. This range should be only extended from 1 to 100 nm in diameter. Therefore materials thin films and devices fabricated within this scale range are belonging to nanotechnology. As a result of being at this scale new scientific principles and material properties was found (1). This is mainly because particles on the nanometer scale have unique optical, electronic, structural, physical and chemical properties that are totally different from their isolated molecules or bulk solids material. These unique size-dependent properties make these materials a good candidate to be incorporated in different applications (2). The capability of developing multifunctional nanoparticles that can perform many functions in biology considered a major advance that nanotechnology makes. Such multifunction included targeting, imaging, and therapy. Various type of nanoparticles such as magnetic iron oxide nanoparticles, quantum dots, and gold nanoparticles have been investigated for potential multifunctional uses as therapeutic agents, delivery vehicles and imaging agents (3). One of nanoparticles types that gained attention for its possible application in biology in early stage of this science development was silver nanoparticles (SNP) (4). Different methods for SNP synthesis were proposed. Chemical, physical, and biogenic synthesized nanoparticles were used in different biological applications. The pulsed laser ablation of noble metal in liquid is a physical method conduct up down procedure to synthesize noble metal nanoparticles. Laser ablation of a solid target material in a liquid environment provides with an easy, straightforward and environmentally friendly method for nanoparticles synthesis as well as with the unique possibility of directly controlling the type of the nanoparticles surface ligands through the liquid choice (5). Gold and silver nanoparticles were easily synthesized with high purity since the primary metal plate used in this process is highly pure. Nickel, cobalt, copper, platinum, aluminum and Zinc oxide as well as titanium oxide nanoparticles were obtained using this method. The laser ablation method requires less time and the nanoparticles can be obtained in a pure solvent without any additives that can contaminate their surface and the produced solutions as well. The toxicity issues during the synthesis of nanoparticles (6). This type of nanoparticles (SNP) have noticeable broad spectrum of antimicrobial activity (7, 8). This activity makes SNP good candidate to find its way to the applications in which the prevention of bacterial infections is a priority. Catheters and wound dressings impregnated with silver nanoparticles are suggested to be used in therapeutic applications (9, 10, and 11). Such applications mandate toxicity assessment of these nanoparticles toward human and environment to verify its safety use (12). In order to understand the risks of SNP, numerous studies were conducted to determine the mechanistic interaction between SNP and human cells, animal cells and environment. Most of these studies used human cancer cells as a model to investigate this toxicity (13, 14, 15, 16, and 17). Many of them conclude that different doses, shapes and sizes of SNP

has the potential to cause toxicity and genotoxicity through DNA damage, chromosomal aberrations, cell cycle arrest and inhibition of proliferation in human cancer cells as well as normal cells. They mentioned that the use of SNP as an anticancer and antimicrobial agent could be limited by the fact that SNP was equally toxic to normal cells (18, 19, and 20). At the mean time other researchers conducted a different approach using toxicity assessment for SNP *in vivo*. This was in order to have more realistic conclusions for this material toxicity and distribution in the animal organs (21). Such studies gave a general consideration and knowledge about SNP behavior *in vivo*. It appears that nanoparticle characteristics include concentration, surface area, type, chemical composition and surface modifications plays an important role in biological activity and toxicology *in vivo*. They can enter alveoli in the lungs, deposed in liver, kidney and spleen as well as pass biological barriers such as the blood–brain barrier and enter cells (22). With all these considerations, SNP remain to be an interesting material that when used in proper concentrations can be optimistically produce therapeutic effect (23). The prospective to use SNP for cancer treatment is an ongoing research interest and different scenarios were proposed to meet these requirements. Chemically and biogenic synthesized SNP was recently introduced as an efficient anticancer agents that capable of inducing apoptosis in tumor cells (24, 25, and 26). These SNP synthesizing methods conducted the down up procedures to produce SNP. In this research we synthesized silver nanoparticles by laser ablation method in liquid and assessed its diluted concentrations ability to inhibit human glioblastoma cells through induction of apoptosis and assessed its toxicity toward mice brains *in vivo* through histotoxopathological observations.

Materials and Methods

Synthesis and characterization of silver nanoparticles

Syntheses of silver nanoparticles carried out in The Center of Nanotechnology and Advanced Material Research (CNAMR), University of Technology during 2011. The method was according to Albakri *et al*. (27) with some modification briefly a pure and polished metal plate of silver (ounce: 99.999%) placed in glass beaker containing 10ml of double distilled deionized water (DDDW). The ablation source was 1064 nm Nd-YAG laser (type HUAFEI), with a lens having a focal length of 100 mm. Lasers pulse duration and repetition rate were 10 ns and 10 Hz respectively. Laser energy was 600 mJ/pulses. Number of pulses utilized to produce the silver nanoparticle solution was 1000 pulses. During the laser ablation procedure the silver plate target was rotated manually to ensure uniform ablation, homogeneous solution and to avoid aggregation. The solution color started to change to faint yellow during the ablation process. Atomic force microscope examination (AA2000 Angstrom Advances Inc, USA) was employed to take topography and three dimension (3D) nanoscaled photograph. Surface roughness analysis and particle size distribution was carried out with CSPM software (Angstrom Advances Inc, USA). Sample preparation for AFM examination was carried out according to Rao *et al*. (28) with modification. Briefly a glass slide 10×10 mm was cleaned with D.W then absolute ethanol and dried in ear forced

oven. A drop of the sample solution of proper dilution was placed on the cleaned glass slide, the drop was dried in an argon stream chamber then examined in AFM. Absorbance spectra of the nanoparticles solution were measured by UV-visible double beam spectrophotometer (CECIL, C. 7200, UK). Atomic absorption spectroscopy (AAS model GBS 933, Australia) was carried out for the estimation of silver concentration in the prepared silver nanoparticles solution.

Cancer cell line

Cancer cell line used in this study was human glioblastoma (AMGM), provided from the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR). The cell line was originally derived from Iraqi patient with brain tumor it was adapted to *in vitro* cultivation as a stable cell line by Dr. Ahmed Majeed Al-Shimmari in the department of experimental therapy ICCMGR. This cell line was maintained in RPMI-1064 medium with 10% fetal calf serum and supplemented with 2 mM glutamine, 100 Uml^{-1} penicillin, and 100 µg ml^{-1} streptomycin (SDI, Iraq) in a humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C (29).

MTT assay

Cell viability assay was carried out using Cell Viability QMTT Assay kit (USBiological, USA) as specified by the manufacturer. The cells grown in 96-microwell plate (Falcon, flat bottom, Becton Dickinson, Franklin Lakes, NJ, USA) and were treated for 24 hours with 15, 30, and 60µg/ml of the pulse laser ablation synthesized silver nanoparticles solutions. Control treatment contained RPMI medium and without silver nanoparticles. Absorbance at 570 nm of the solubilized formazan (which reflects the relative viable cell number) was then determined by microplate reader (Asays, UK). Viability was determined by the following equation: % viable cell = OD of control cells- OD of treated cells/ OD of control cells × 100. From the dose-response curve, the 50% cytotoxic concentration (CC50) was determined.

Acridine orange and ethidium bromide(AO/Eth)

In order to confirm the event of apoptosis, the treated and control cells was stained with fluorescent dyes to determine the cells morphology and nucleus shape. Cells were grown in 3 cm diameter Petri dishes until monolayer was achieved. Cells was exposed to 15, 30, and 60µg/ml silver nanoparticles in serum free media for 24 hr and incubated at 37 °C with 5% CO_2. After the time of incubation was over media was discarded and cells was washed with PBS, AO/Eth stain mixture (5µg/ml, 10 µl) was added over the cells and cover slip was laid. Cells were observed under fluorescent microscope (Olympus, Japan) at 400× magnification. Microscopic fields were photographed with digital camera (Lumenira corporation, Austria).

DNA fragmentation Assay

Cells were grown in 3 cm Petri dishes until confluent monolayer formation in the suitable media. After that cells were exposed to 60µg/ml of silver nanoparticles for 24 hr. cells were harvested with scraping and DNA was extracted with KAPA Extra Extract Kit(KAPA Biosystems, South Africa, Johannes berg) as manufacturer indicated. The extracted DNA was dissolved with DNA loading buffer, and then applied to 0.8% agarose gel electrophoresis. After staining with ethidium bromide (USBiological, USA), the DNA was visualized by UV irradiation and photographed by gel

documentation system (Sci-Plus, UK). (30).

In vivo experiment

Two groups of BALB/c mice each contain five individuals was caged in the ICCMGR animal house with free reach of food and water. Injections were carried out for two weeks in one day and another. The first group was injected with D.W and considered as control group, the second group was injected with 60µg/Kg BW of silver nanoparticles solution. After the experiment ended mice were sacrificed and brain was preserved in 10% formalin in phosphate buffer solution. Week after that the organs were paraffin embedded 5 µm sliced with microtome (Hestion, Australia). The slides was observed and investigated under light microscope (Human, USA) and photographed with digital camera (Lumenira Corporation, Austria) (29).

Statistical analysis

One way analysis of variance (ANOVA) was determined and the significant differences were calculated on probability level of 0.05 and 0.01 (31).

Results and Discussion

Shape and size characterization of silver nanoparticles considered to be one of the most important steps to understand its biological effect. Figure 1, A. shows an atomic force microscope (AFM) image for the prepared SNP and the imager software analysis produced by the AFM device. The image field was 1000×1000 nm, the roughness average of the prepared nanoparticles deposited on the glass surface was 4.46 nm and the root mean square was 5.8 nm. The color scale of the image which represent the heights and valleys of the image extended from 0 to 35 nm (representing darkest and lightest color of the image respectively). All these results indicated that the prepared silver particles are in nano-range (1- 100 nm). The three dimension image of AFM emphasized the nanostructure of the prepared particles (figure 1, B).

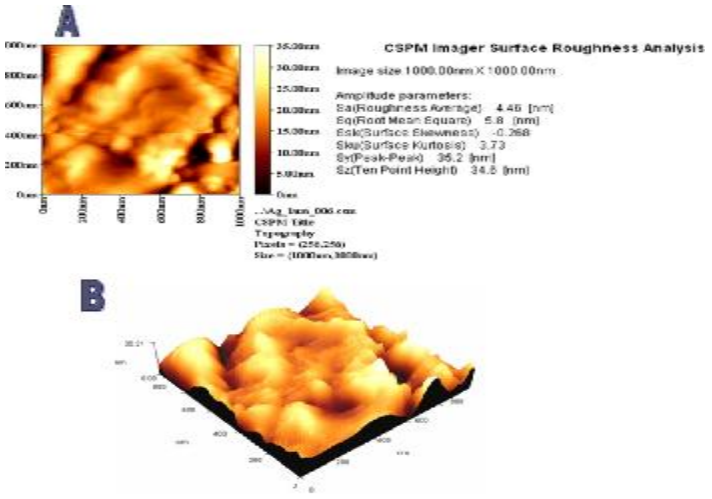


Figure 1: Atomic Force Microscope study of silver nanoparticles synthesized by PLA method in liquid. A, topography image with colored scale of highest and lowest points. Surface roughness analysis in the right panel. B, three dimensional image

The particle size distribution of SNP in the prepared solution ranged from 8nm to 110nm (figure 2). Although overall particle size distribution in the prepared solution extended widely, the particle size of 8nm and 16nm represent the highest accumulative percentage in the solution, 9.7 and 9.8 % of total accumulative percent respectively. The particle size which exceeded 100nm in the solution represented only a slight percentage of the total accumulative percentage of the nanoparticle size (0.2%). Collectively the average diameter of the synthesized SNP was 26.77nm as stemmed from the AFM data analysis (figure 2).

All these result indicated that the prepared solution was with particle size of nano-range as directed by the American National Nanotechnology Initiative (NNI) (32).

Silver nanoparticle solution produced in this study was definitely in range between 8 to 66 nm and 26.77nm in average as concluded from AFM study (figure 1 and 2).

In order to study the shape of the SNP synthesized by laser ablation method, UV-Vis spectrum was determined. Figure 3 shows the peak absorbance spectrum of the prepared solution in the UV-Vis spectrum, it was 411nm. This absorbance indicated the spherical shape of the prepared SNPs.

Synthesizing Silver nanoparticles by laser ablation method in liquid considered to be one of the easiest way and lowest cost to collect such a material. As a result of using high chemically pure target metal plate, the resulted silver nanoparticles are totally pure with less contamination.

The produced nanoparticles are distinct from those produced by other chemical methods, in which the presence of residual ions is a serious disadvantage. Water has been the primary choice as a medium of laser ablation-based nanoparticles formation, possibly because of its inert and nonflammable nature (33).

Using the condition described in this research, silver nanoparticles were produced successfully in water as a colloidal and it was stable for reasonable period of time. Since no capping agent was used in this method, silver nanoparticles aggregation is not eliminable or avoidable. Atomic force microscope images (figure 1, A and B) indicated such aggregations, though the produced particles still possess nano-rage dimensions.

Particle size and shape represents one of the most effective factors related to nanomaterial behavior. Cytotoxicity and *in vivo* bio-distribution depend totally on nanoparticle size distribution. The more nanoparticles are small in size the more they can penetrate into the cell and induce more toxicity (34).

Avg. Diameter:26.77 nm	**<=10% Diameter:8.00 nm**
<=50% Diameter:18.00 nm	**<=90% Diameter:52.00 nm**

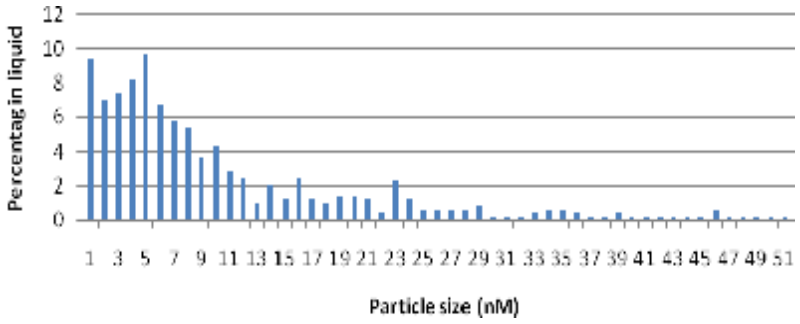


Figure 2: particle size distribution of silver nanoparticles synthesized by pulse laser ablation in liquid method as configured from C scanning probe microscope software analysis

Particle size of nanosilver is one of a significant parameters related to its toxicity. Therefore the determination of particle size in the synthesized silver nanoparticles solution is a crucial parameter to be determined in every study dealing with this material (35). Using pulse laser ablation in liquid method to synthesize nanoparticle would produce nanoparticles with different range of particle size. In this method, the rate of nanoparticles formation, the average of particle size and nanoparticle colloidal stability could be controlled by proper selection of the laser parameters such as laser energy and laser wavelength (36). In this study the specified parameter of the used laser energy and wavelength and whole ablation conditions produces a range of different particle size and shape. All of the produced particles were in a nano-range (figure 2) indicate successful investment of the parameters to produce silver nanoparticles. Pulsed laser ablation method was easy method for the preparation of SNP with well-defined nanoparticle size distribution. No additives, such as solvents, surfactants or reducing agents, are needed in this procedure (37, 38). Another important parameter of silver nanoparticles characterization was the peak absorbance under UV-Vis spectra. This parameter reflects the surface plasmon resonance (SPR) of the generated nanoparticles. Each type of noble metal nanoparticles synthesized by pulse laser ablation would have a specific SPR depend entirely upon its shape and particle size. Thus, characterized by its peak absorbance wavelength under UV-Vis spectra (190-800nm) and band width of that peak, the SPR would refer to the optical properties of nanoparticle colloidal.

Shifting of PSR peak of noble metal nanoparticles solution toward visible area of the spectra indicates nanoparticles glomeration and aggregation (39). Silver nanoparticle solution prepared in this study has a peak absorbance totally equal to the one that indicates a specific character of spherical shape particles with narrow nanoparticle diameter (figure 3). This result confirms the result of particles size distribution obtained with AFM. Conclusively this study was successful in prepare spherical shape silver nanoparticles solution as a colloidal with nanoparticle size.

The cytotoxic effect of the synthesized silver nanoparticles on cancer cell line was conducted against glioblastoma cell line (AMGM). Figure 4 represents the growth inhibition percentage induced by three different concentrations of silver nanoparticles the prepared solution were 15, 30 and 60µg/ml. Growth inhibition of AMGM cells was13.49%, 14.41% and 51.69% of the control treatment respectively for the used concentration of SNP. There was no significant differences between 15µg/ml and 30µg/ml in inhibition rate ($p<0.01$), whereas the differences were significant ($p<0.01$) when the concentration of SNP increased to 60µg/ml. The visualization of AMGM cells damage as a result of its exposure to silver nanoparticles was carried out using fluorescent stains mixture of acridine orange and ethidium bromide. The cells suffered from aggressive membrane disintegration when exposed to 60µg/ml of silver nanoparticle for 12hr (figure 5, A and B). These cells kept green staining of their nucleus indicating the early stage of apoptosis induction. Cells treated with 15 and 30µg/ml did not show such membrane destruction but they suffered of necrosis as indicated from the staining of their nucleus with orange color (fig. 5. C and D).

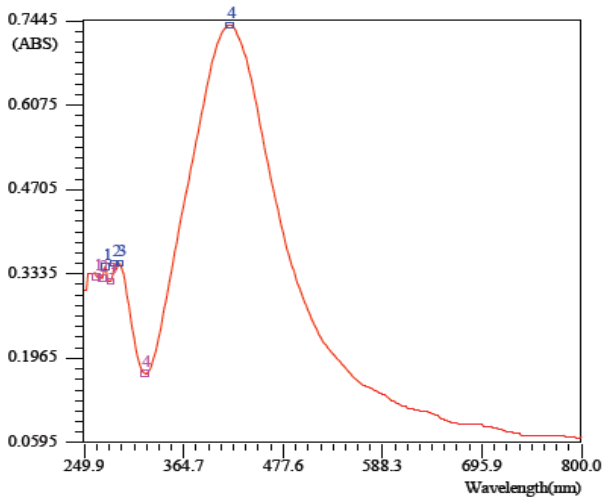


Figure 3: Ultraviolet-Visible spectrum of silver nanoparticles synthesized by pulse laser ablation in liquid method

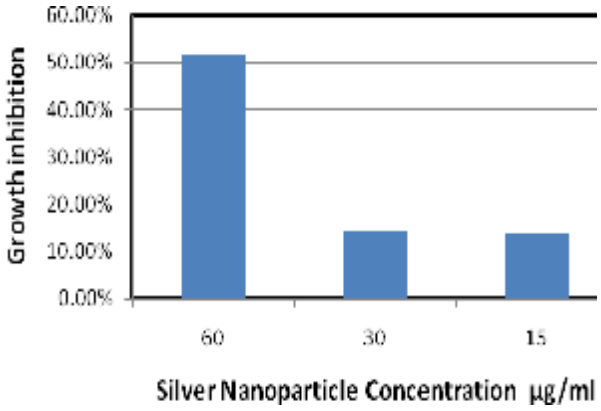
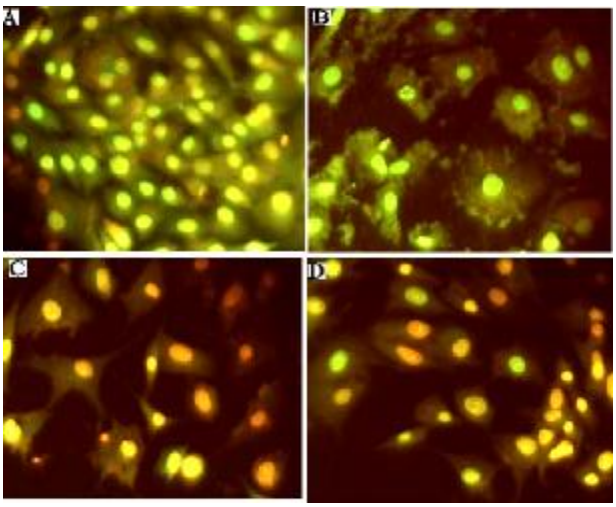


Figure 4: Growth inhibition effect of three different concentrations of silver nanoparticles synthesized by pulse laser ablation (PLA) method in liquid against human glioblastoma cell line (AMGM) as detected with dimethylthiazol diphenyl tetrazolium (MTT) assay



Figurer 5: Glioblastoma cell line (AMGM) incubated for 12hr without silver nanoparticles (A) or with 60µg/ml (B), 30µg/ml (C) and 15µg/ml (D) of silver nanoparticles synthesized by pulse laser ablation in liquid method. After incubation adherent cells were stained with acridine orange/Ethedium bromide fluorescent stains

Medical applications of silver nanoparticles are numerous, the use of these nanoparticles as antibacterial agent was significant in many researches. Their use as surface disinfectant and in coating of different surfaces in hospitals finds its way for commercial application (40). After our success in the preparation of silver nanoparticle solution we investigate the

anti-proliferation activity of the synthesized silver nanoparticles (diluted concentrations) against locally established glioblastoma cell line. The prepared nanoparticles induced obvious cytotoxicity toward this cell line, the highest concentration used (60 µg/ml) was able to inhibit 50% of the cell, whereas the others (30 and 15µg/ml) inhibited these cells minimally (figure 4). Our result confirming the previous reported results regarding the ability of silver nanoparticles to inhibit cancer cells proliferation (41), but it's different in the source of the used SNP.

These studies used chemically synthesized nanoparticles caped with different types of chemical agents but not laser ablation method to challenge cancer cell lines. Also the previous studies did not use diluted solutions of silver nanoparticles; instead they employed higher concentrations (42). This study can also be recognized as the first to use SNP to challenge glioblastoma cancer cells as many of other studies did not explore such system. Many studies has declared the formation of reactive oxygen species (ROS) as general mechanism of cytotoxicity this hypothesis supported by studies indicated the reduction in GSH, increased lipid peroxidation and expression of ROS responsive genes when cancer cells in vitro exposed to silver nanoparticles. This increment in cellular ROS levels was associated with DNA damage, apoptosis and necrosis (43, 44, 45, and 46). Another study investigated the uptake and cytotoxic effects of well-characterized SNP in the human lung carcinoma cell line A549, suggested the up taking of nanoparticles by the cells leading to increased production of ROS and ultimately apoptotic and necrotic cell death (47). Although this study did not investigate the generation of ROS, the apoptosis induction in AMGM cells was evident in the disintegration of cells membrane which is an early sign of apoptosis (figure 5) (48, 49). In order to differentiate between the two type of cell death (necrosis and apoptosis), acridine orange and ethidium bromide differential staining was invested. These two fluorescent stains interchelate with DNA and refer to the genetic material condensation during necrosis and/or apoptosis. The nucleus of necrotic cells stained orange while the nucleus of apoptotic cells stained green under fluorescent microscope (50).Staining of AMGM cell's nucleus with acridine orange (green color) and membrane disintegrated appearance indicated the state of apoptosis as resulted from the treatment with 60µg/ml of silver nanoparticles solution. The other two concentrations 15 and 30µg/ml induced necrosis since the cell's nucleus stained with ethidium bromide (orange to red color) (figure 5). To examine the mechanism behind growth inhibition properties of silver nanoparticle toward AMGM cells was due to induction of apoptosis, genomic DNA fragmentation assay was conducted. Figure 6 represents genomic DNA agarose gel electrophoresis of AMGM cells after exposure to 60µg/ml of SNP for 24hr (figure 6 lane B) and untreated control (figure 6 lane A). In the exposure time the cells genomic DNA suffered from obvious fragmentation it produces a smear of DNA fragments extended from 900bp to 5000bp. That positively indicates the event of apoptosis after 24hr of cells exposure to SNP. Due to apoptosis induction the genetic material of cell would be condensed and then fragmented as a final stage of apoptosis induction. This cell genetic material fragmentation can be assessed in

agarose gel electrophoresis. The apoptosis induction in AMGM cells treated with 60µg/ml of silver nanoparticle solution was further clarified with DNA fragmentation assay. The DNA damage represented in the formation of long smear appearance with ladder pattern in agarose gel for the treated cells whereas the untreated cells showed minimal DNA damage as a very shallow smear appeared with no ladder pattern in the gel after electrophoresis (figure 6) .

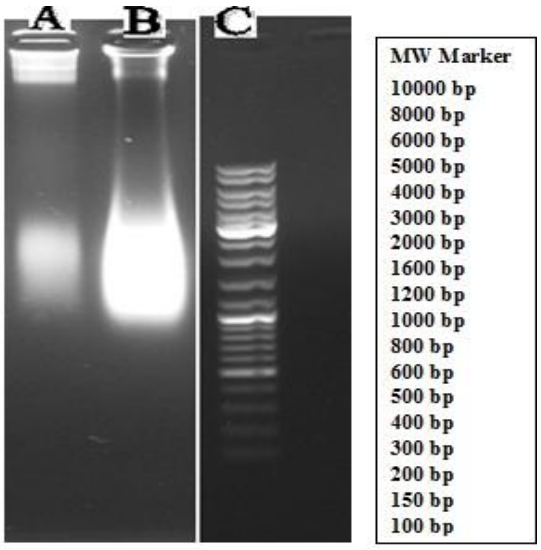


Figure 6: Genomic DNA fragmentation of AMGM cells after exposure to silver nanoparticle synthesized by pulse laser ablation method in liquid. A, untreated cells control. B, cells treated with 60µg/ml for 24hr. C, molecular wight marker ranged from 100 bp to 10,000 bp.

Many researchers proposed that silver nanoparticles induce cellular DNA they used different methods to assess such activity. Comet assay, TUNAL assay, and agarose gel electrophoresis was implemented and was recommended as useful method to detect such effect of silver nanoparticles (51, 52). The evaluation of the prepared SNP toxicity toward normal nerve cells was assessed *in vivo* in BALB/c mice. After two weeks of subcutaneous injection, histopathological inspection for the injected animal's brains was conducted. Figure 7 shows a representative histopathology photographs for the treated animals with different magnification power. No significant pathological events were seen in all prepared slides, indicating that the used concentration of PLAL-SNP is totally safe and not toxic *in vivo.*

In order to indicate the possible toxicity of the used dose (60µg/ml) of laser ablation method synthesized silver nanoparticles which were able to inhibit the proliferation of AMGM cells *in vitro*, we used *in vivo* model. Since histotoxopathological assessment consider to be one of many methods regularly used in such studies, this method was conducted to observe the adverse effect of silver nanoparticles on normal cell of mice brain. Due to its nano-size, noble metal nanoparticles such as gold nanoparticles and silver nanoparticles are accumulates in

different organs of mice and rats. This observation was indicated through different root of nanoparticles administration *in vivo* (53, 54, and 55). The result indicated that the concentration under investigation of silver nanoparticle was totally harmless to normal brain cells. No lesion was detected in all brains of the animals used in this experiment though it was extended for two weeks.

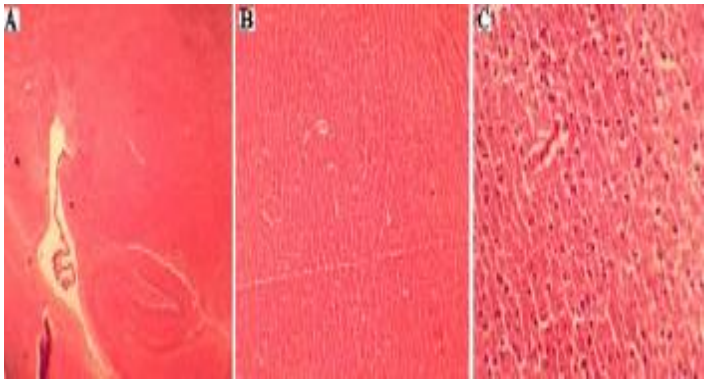


Figure 7: A representative histopathological photographs of mice brain. A, group of mice was injected subcutaneously with 60µg/ml/kBW of silver nanoparticles synthesized by laser ablation in liquid method for two weeks one day and another schadual.A,4×;B,10×;C,40×

In conclusion the results of this study propose that using laser ablation method to synthesize silver nanoparticles in liquid was successful to produce nanosilver in average diameter of 26.77nm. The produced nanosilver was able to inhibit glioblastoma cancer cell line proliferation as well as induction of apoptosis in this cancer cells when used in 60µg/ml concentration. In the mean time this concentration of the synthesized nanosilver did not induce any adverse effect in normal brain cells as investigated in an *in vivo* model of mice for two weeks of subcutaneous administration. These results imply that silver nanoparticles synthesized by laser ablation method in liquid can possibly be used to treat brain tumors.

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