Iraqi Journal of Cancer and Medical Genetics

Genotoxicity of Silver Nanoparticles synthesized by Laser Ablation Method *in Vivo*

Amer T. Tawfeeq, Husam Al-Deen M. Kadhim, Nahi Y. Yaseen, Saba K. Kalil, Aseel F. Ghedan, Rasha A.Hussein, Amina Yusif Abdul-Lateaf

Iraqi Center for Cancer Research and Medical Genetics / University of Al-Mustansiriyah

Abstract:

This research was conducted to evaluate three main methods usually used to assess cellular DNA damage in genotoxicity assays. These methods were single cell gel electrophoresis (comet assay), micronucleus formation, and DNA fragmentation assay. Nanoparticles genotoxicity is a subject of compelling immediate action as a result of wide application of nanotechnology in many sectors which in contact with humane health. The mentioned methods were used to assess the genotoxicity of silver nanoparticles in vivo. Silver nanoparticles were synthesized by laser ablation of pure silver plate submerged in double distilled water. The synthesized nanosilver was characterized with UV-Visible spectroscopy and atomic force microscope. After its characterization, silver nanoparticles were injected subcutaneously in to BALB/c mice at 200 µg/ kg BW for two different periods of time, one week and two weeks in daily manner. After the end of injection the animals were sacrificed and their bone marrow cells, lymphocytes, and spleen cells were collected. DNA damage in these cells was assessed using the three mentioned methods. Results indicated that the three types of DNA damage assessment methods were capable to detect the genotoxicity of silver nanoparticles in the treated animals. Spleen cells were the less DNA damaged cells as indicated with the three assays in the first week of injection. Lymphocytes and bone marrow cells was effected in more aggressive manner.

Keywords: *genotoxicity, silver nanoparticles, comet assay, micronuclei, DNA damage*

Introduction:

Nanomaterials are invading now different consumption products and fields of applications resulting in the increment of human exposure to these newly engineered materials (1, 2). As a result of its novel chemical and physical properties, Silver nanoparticles are class of such materials that have shown interesting characteristic in biomedicine, intending to use them in diagnosis and/ or therapy (3, 4). Antibacterial activity of silver nanoparticles are encouraging to introduce them as an ingredient in the disinfectant agents or coat surfaces to prevent bacterial growth for surface sanitation (5- 11), or even to use them in the therapy of chronic bacterial infections and as synergistic factor with antibiotic to overcome the traditional antibiotic resistant of some bacteria (12, 13). All these activities mandate to assess the potential risks of these materials on both environment and humane health (14-16). Therefore different particle sizes, shapes, doses and time of exposure was used to assess the cytotoxic, genotoxic and oxidative damage induced by silver nanoparticles in different type of cells (17-22).

Toxicity of silver nanoparticles was appeared to be resulted from silver ions that released from its surface. Researchers suggested that the presence of silver nanoparticles inside the cells was behind the reactive oxygen species (ROS) generation in that cells. Thus, cytotoxicity and genotoxicity of SNP were suggested to be generated from ROS induced by the presence of silver nanoparticles in the cells (23-27). Relevantly, a need to assess this toxicity is an issue of great consideration. most of SNP toxicity assessments were conducted using cancer cell lines or normal fibroblast cells (28-30). Although the use of those types of cell lines in these assessments are very important as a criteria to have a general preliminary idea about the toxicological properties of silver nanoparticles, but stile it may not reflect precise mechanism and it needs more confirmative studies (31). Since cancer cell lines are far from being normal metabolic model, the use of normal fibroblast cell line may reflect more realis-

Corresponding Address:
Amer T. Tawfeeq
Iraqi Center for Cancer Research and Medical Genetics / University of Al-Mustansiriyah
Email: Amer.taufeaq@iccmgr.org

tic events (32-36). The choice of the cell type to be used is a critical determinant in toxicology assays (37) From this point of view the in vivo studies can give the most realistic results to a great extent.

The traditional genotoxic assay, Ames test, was not effective enough to generalize toxicological behavior for silver nanoparticles (38). Comet assay and micronucleus formation assay are more suitable to detect the genotoxicity of silver nanoparticles than Ames test (39). While the mentioned tests are capable of assessing toxicity of silver nanoparticles in vitro, there is scars knowledge about the genotoxicity behavior of silver nanoparticles in vivo. Comet assay was efficient to detect genotoxicity in a very similar manner in vitro and in vivo (40-42). This assay was widely used in the in vitro assessments of genotoxicity of different substances (43). The propidium iodide is well known staining procedure used to indicate the apoptosis properties of cell nuclei , it was mainly used in the flowcytometry procedures to indicate cell apoptosis. These fluorogenic stain is capable of intercalate with DNA when it across the cell membrane as a result of cell death due apoptosis, another use for this stain is to indicate DNA fragmentation appearance of cell nuclei under fluorescent microscope (44). Agarose gel electrophoresis can be employed to detect DNA fragmentation as cell apoptosis let event, ladder appearance of DNA on agarose characterizes this cellular phenomenon. The degradation of internucleosomal DNA by endonucleases activated by caspase-3 during apoptosis such as DNA fragmentation factor (DFF) is responsible for the degradation of internucleosomal DNA (45-47). This assay was also used to determine the genotoxicity of different types of substances (48). Micronucleus detection in mice bone marrow is one of the methods frequently used to detect the in vivo genotoxicity of several types of materials (49). The above mentioned tests as well as many other tests represent a battery of assays which may use individually or together to shade a light on the genotoxic effect of any substances. (50)

In this research we tried to assess in vivo, the genotoxicity of silver nanoparticles synthesized by laser ablation method in doubled distilled water. This assessment carried out using three different tests commonly used to assess the cellular DNA damage. These tests are comet assay, micronuclei formation assay and DNA fragmentation assay in agarose gel electrophoresis. The purpose was to determine the efficiency of each one of them to detect silver nanoparticle genotoxicity. The animals BALB/c mice were injected subcutaneously with the synthesized SNP for two different period of time. The genotoxicity of SNP was assessed in bone marrow cells, lymphocytes and spleen cells with these three methods. We hypothesized that the use of these test collectively could confirm the event of DNA damage and genotoxicity in the treated animals in more realistic way. The rational of the tissue type to be tested stemmed from two reasons, the first one, was that these types of cells can be collected easily from the animals in suspensions; the second was that these organs may accumulate SNP and be the most damaged tissues in the animals (51).

Material and methods:

Culture media preparation

RPMI 1064 culture media with 2 mM L-glutamine and HEPES (USbiological, USA) was prepared, 100 µg/mL penicillin/streptomycin and 10% fetal bovine serum (Capricorn, Germany) was added. This media was use to wash and cultivation of the cells isolated from mice (spleen cells, lymphocytes, and bone marrow cells).

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, a Q-switched Nd-YAG laser (type HUAFEI) operating at 1064 nm wavelength was employed. After laser-based setup was constructed, silver particles from metallic silver plate (1 mm thick and high purity) submerged in double distilled water was ablated. The plate target was fixed on a magnetic holder at the bottom of a glass cell, and was rotated to avoid deep ablation. The maximum laser pulse energy delivered was 320 mJ/plus with pulse width of 10 ns. The distance between target plate and laser source was 10 cm, diameter of laser spot on a targeted plate was 1 mm and the liquid volume was 10 ml. After firing of 500 laser pulses, a yellowish colloidal solution of silver nanomaterial was obtained.

Nonmaterial characterization

Ultra violate-visible optical absorption measurements were performed directly after silver nanopraticles synthesis to avoid the effect of growth and aggregation of nanoparticles. The optical absorption spectra were recorded by UV-visible double beam spectrophotometer (CECIL 7200, UK). The average particle size and shape of synthesized nanoparticles were characterized with an Angstrom Advanced Inc AA3000 Typing mode 200 FEG atomic force microscope (Angstrom Technologies, USA). Concentration of the synthesized nanoparticles was determined using atomic absorption spectroscopy (AAS model GBS 933, Australia).

***In vivo* experiment design**

Thirty male BALB/c mice (28-32 g) were housed five per cage with free access to food and water and were maintained on a 12hr light/dark cycle (lights on at 7:00 am) at 25 °C. All experimental procedures were performed in accordance with the recommendations of the Iraqi Center for Cancer and Medical Genetic Research for the Care and Use of Laboratory Animals. Animals were divided into three groups, ten mice in each group. Subcutaneous administration of silver nanoparticles was conducted in the back dorsal of the animals. The first group served as control and injected with PBS, the second group received daily injection of 200 µg/ kg BW of silver nanoparticles for one week and the third group was injected daily with 200 µg/ kg BW of silver nanoparticles for two successive weeks. At the end of the experiment (injection time), animals were sacrificed and blood samples were drawn by cardiac puncture and collected in heparinized tubes. Lymphocytes were isolated from the collected blood samples utilizing the density gradient centrifugation standard method.

Bone marrow cells isolation

The isolation of mice bone marrow cells was carried out

according to the following steps. After mouse was sacrificed tissue was removed from legs with scissors and dissects away from the mouse body. Remaining tissue was cleaned from the pelvic and femoral bones and separated at knee joint. Both end of femoral bone was cut off,

Using a 25g needle and a 5 ml syringe filled with RPMI medium, the bone marrow was expelled from both ends of the bone with a jet of medium directed into a 10ml screw cup tube. Collected cells were Centrifuge at 1500 rpm for 10 minutes. Cells pellet was re-suspended in 10ml RPMI (10 % FCS). To disintegrate cells aggregates, cells were gently aspirate and expelled for more than once using an 18g needle attached to a 5 ml syringe.

Spleen cell isolation

Carried out according to the protocol described by Czuprynski and Brown (1998) (52). Anesthetized mouse placed on its back on the support platform. The mouse limbs were fastened to rubber bands fixed to the platform. Mouse abdomen was swabbed with 70% ethanol. A longitudinal ventral midline incision was made. The connective tissue underneath the skin was separated from the dermis and the cutaneous margins were fixed to the platform with pins. Spleen was drawn with a dissecting forceps and extracted carefully with scissor. The ectomized spleen was transferred to a Petri Dish contain 10 ml of RPMI-1640 media with glutamine, HEPS and 10% bovine calf serum . The spleen tissue was mechanically disrupted by cutting the spleen into conveniently sized fragments. These fragments were tore and squeezed using sterile forceps and scissors to release spleen cells from normal tissue architecture. The cell suspension produced in the previous step was passed through 5 ml syringe cylinder packed with sterile gauze and transferred directly to centrifuge tubes. Tubes were centrifuged at 200 Xg (1500 rpm) for 7 min. the supernatant was gently decanted and the cell pellet was re-suspended with 5 ml of ACK lysing buffer (NH4Cl (0.15 M), KHCO3(1 mM), Na 2EDTA (0.1 mM) pH 7.4, Roberts et al., (1998) (53) per spleen in order to remove red blood cells from the spleen cell population. The cells were incubated for 5 min at room temperature, with occasional shacking. After ACK incubation was completed, the tubes was filled to 10 ml with RPMI-1640(10%FBS) media and centrifuged again for 7 min. this wash process was repeated one additional time with 10 ml of the same media after that cells was utilized in the assays.

Single cell electrophoresis (Comet assay)

Comet assay was performed for the organs isolated cells according to Collins et, al (1996) (54). Ethedium bromide was used instead of SYBR green dye to detect DNA damage. Slide was viewed by fluorescence microscopy (Micros MCX 500, Austria), each slide was divided to 50 microscopic filed and each field was photographed with digital microscopic camera (LW scientific, USA). The genomic damage in the cells nucleoid was assessed visually in each image photographed collectively from each slide. Total number of cells counted from each slide was between 80 to 120 cells, the number of tailed nucleoid was scored and calculated as a percentage of total number of cells counted in each slide according to the method of Collins et, al (1996) (55).

Micronucleus induction in cells isolated from organs

After organs cells isolation they separated over a microscopic slide, air dried and fixed with -20 °C absolute ethanol (schulds, Spain). Fixed cells were stained with propidium iodide (5µg/ml) (sigma-alderch). The stained slides were observed under fluorescent microscope (Micros MCX 500, Austria). Each slide was divided to 50 microscopic fields; each filed was photographed with digital camera (LW Scientific, USA). One thousand cells from each animal were scored for the appearance of micronucleus structure and their frequencies were recorded.

Agarose gel electrophoresis assay for DNA (ladder pattern).

Isolated cell's DNA extraction was carried out using KAPA Express Extract kit (KAPA Biosystem, South Africa) according to the manufacturer instruction. Agarose gel electrophoresis for the extracted DNA was carried out according to Narendra P. Singh (2000) (56) with 1.8% Agarose (USBiological, USA) in Tris borate, EDTA (pH8) buffer. Gels were imaged with Scie-Plas gel documentation system (Scie-Plas, England). Molecular weight marker ladder (KAPA Biosystems, south Africa) was used to characterize the DNA fragmentation patron

Statistical analysis

Analysis of variance (ANOVA) of the results was calculated in one and two way factorial design experiment. Statistically significant differences limits were (1% and 5%). These calculations were carried out according to program statistical package for social sciences SPSS, version 10 (57).

Results:

Characterizations of the synthesized silver nanoparticles

In pulsed laser ablation method, the synthesized silver nanoparticles generated will posses different shape and size and that would depend upon the ablation process conditions and parameters (58). All the experiments in this research carried out with a single preparation of the synthesized nanoparticles, sufficient quantity was produced. Atomic force microscope (AFM) images for the synthesized silver nanoparticles, both topography and three dimension images, shows the globally spherical shape of the prepared particles (Fig. 1A and B), nanoparticles agglomeration was also indicated. Granularity distribution chart obtained from AFM software (CSPM Angstrom Technologies, USA) indicated that 90% of the synthesized NPs diameter was 58nm and 50% of them have 20nm diameter. The overall particle diameter range for the preparation was 28.09nm (Fig.2). The UV-Vis spectrum for the synthesized NPs has a maximum absorbance at 417.40 nm (Fig.3), the narrow peak width at half maximum indicating that nanoparticles generated in the liquid was spherical (16).

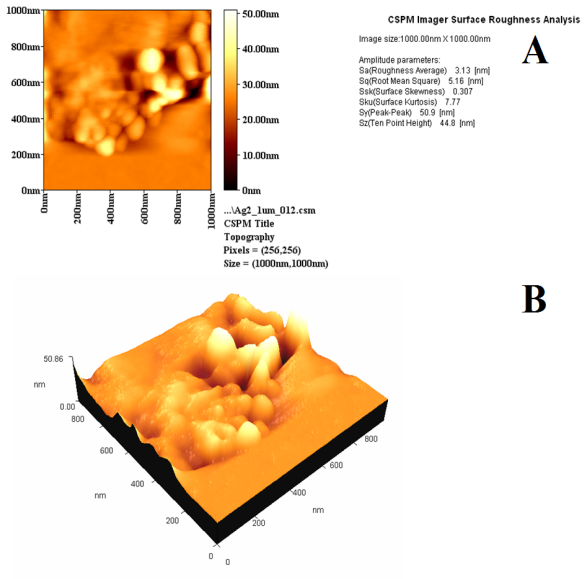

Figure 1: Atomic force microscope image for the silver nanoparticles synthesized by laser ablation method. A, topography image; B, three dimension image. Image field is 1000nm. The image analyzed by C scanning probe microscope software for surface roughness and root mean square to indicate the nanoparticles formation.

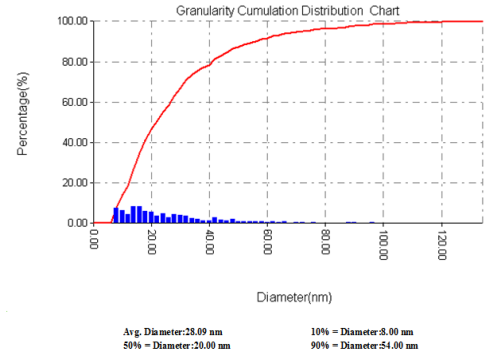

Figure 2: Particle size distribution silver nanoparticles synthesized by laser ablation method in double distilled water, analyzed by the C scanning probe microscope software.

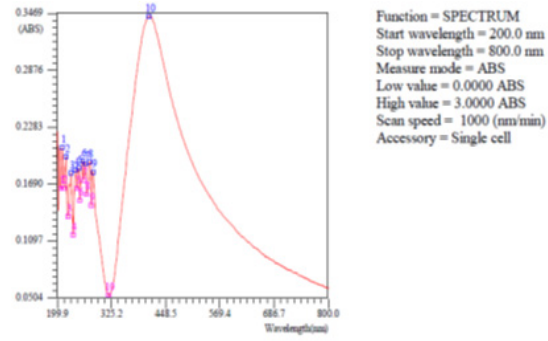

Figure 3: surface plasmon resonance for silver nanoparticles synthesized by laser ablation method in double distilled water indicating peak absorbance of the nanoparticles.

Single cell electrophoresis (comet assay)

We have detected the genotoxicity of the synthesized nanoparticles in different types of organ cells derived from mice injected with SNP. In comet assay, the tailed cells were calculated visually as percentage of total counted cells that derived from each animal and each organ of the animal. The bone marrow cells showed the highest percentage of tailed cells of total cells counted, they were 43.55% and 51.51% in one week and two weeks period of injection time respectively (Fig. 4 and 5). This increment was significantly different from control group ($p<0.01$), also there was a significant different ($P<0.05$) among them. Tailed lymphocytes reached 35.88% and 40.17% in the injected animals after one and two weeks, these percentages differ significantly ($P<0.05$) between them (Fig. 4 and 6). Spleen cells produced the lowest DNA damage in the first week of injection, its tailed cells consist 12.35% with no significance to control treatment. After two weeks of SNP injection this percentage increased to 37.86% of total cells counted after ($P<0.01$) (Fig 4 and 7).

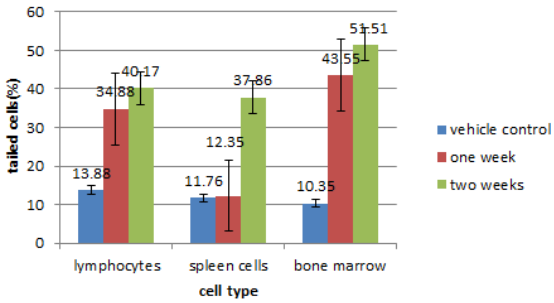

Figure 4: Percent of tailed cells appeared in single cell gel electrophoresis of spleen cells, lymphocytes and bone marrow cells for vehicle control, one week and two weeks period of time of mice injected silver nanoparticles.

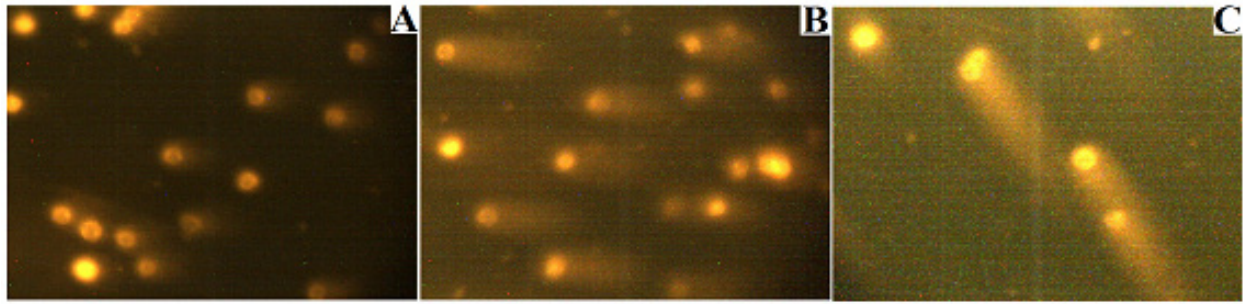

Figure 5: Comet assay for the bone marrow cells extracted from untreated control, one week, and two weeks treated animals with SNP. A, untreated control; B, one week treated; C, two weeks treated.

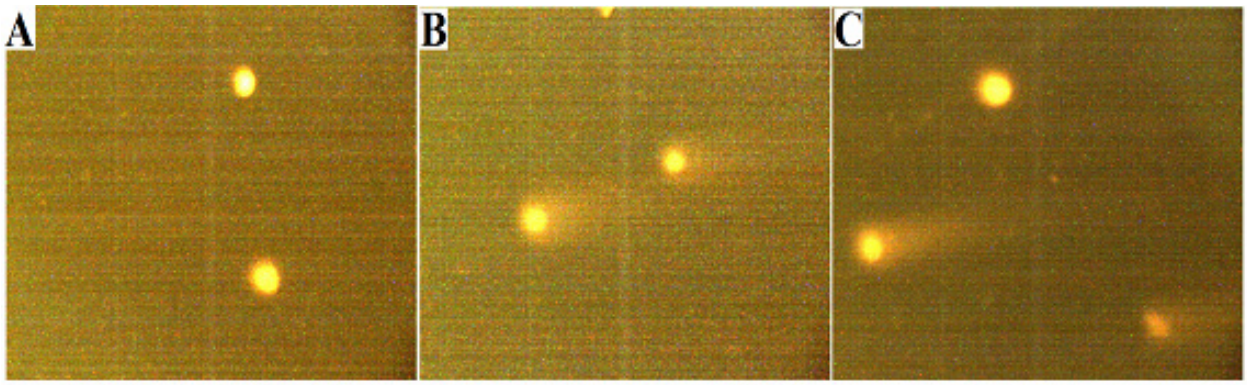

Figure 6: Comet assay for the peripheral blood lymphocytes extracted from untreated control, one week, and two weeks treated animals with SNP. A, untreated control; B, one week treated; C, two weeks treated.

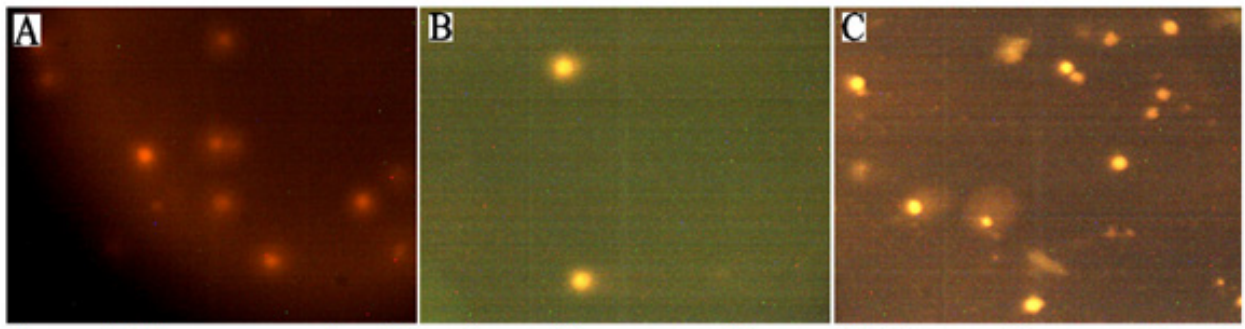

Figure 7: Comet assay for the spleen cells extracted from vehicle control, one week, and two weeks treated animals with SNP. A, vehicle control; B, one week treated; C, two weeks treated.

Micronucleus induction in cells isolated from organs:

In order to have a better judgment for the genotoxicity of SNP in vivo we conducted another familiar genotoxic specific assay. Micronuclei are good markers of genotoxic exposure in humans and their scoring has been extensively used to identify potential genotoxic agents (59). Micronucleus frequencies in three cell types isolated from treated and vehicle control animals were calculated for each animal in each group. In bone marrow cells, micronucleus frequency in one week period of time did not increase significantly from vehicle control indicating no genotoxicity. After two weeks of daily injections, the micronucleus formation increased 1.67 fold than the frequency on vehicle control animals (Table 1 and Fig 8). This significant increment ($P < 0.05$) indicate genotoxicity.

Micronucleus formation showed in lymphocytes significant increment from vehicle control animals up to 1.4 fold after one week ($P<0.05$), and the micronucleus frequency increased significantly after two weeks ($P<0.05$) up to 1.5 fold (Table 1 and Fig 9). However, in spleen cells the situation was not the same, spleen cells showed significant micronuclei formation reached 2.1 fold of control after one week of injection. The level was significantly increased up to 2.7 fold in spleen cells after two weeks of daily SNP injections ($P<0.001$) (Table 1 and Fig 10).

Table 1: Percent of micronucleus in the spleen cells, lymphocytes and bone marrow cells of silver nanoparticles injected animals for one and two weeks period of time.

group	Number of treated animals	Number of counted cells from each animal of the group	(Percent of Micronucleus in (mean ±S.E		
			Bone marrow	Lymphocytes	Spleen cells
Vehicle control	5	1000	0.950±0.031	0.730±0.045	0.421±0.011
One week	5	1000	±0.007 1.0	*1.08±0.100	*0.903±0.044
Two weeks	5	1000	**1.594±0.112	*1.15±0.125	**1.147±0.088

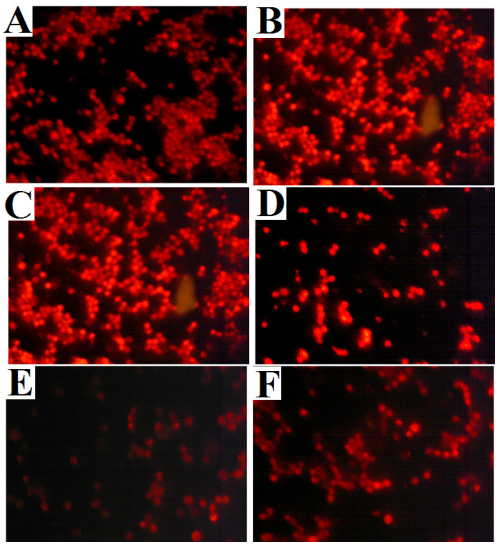

Figure 8: Bone marrow cells of vehicle control, one week, and two weeks SNP treated animals. A & B vehicle control animals; C & D one week treated animals; E & F two weeks treated animals.

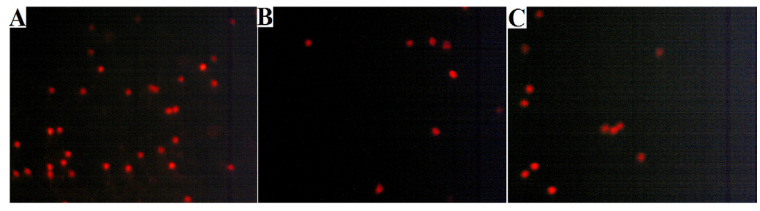

Figure 9: Micronucleus formation assay for lymphocytes of control untreated, A; one week, B; and two weeks, C; SNP treated animals. Cells was stained with propidium iodide fluorescent dye.

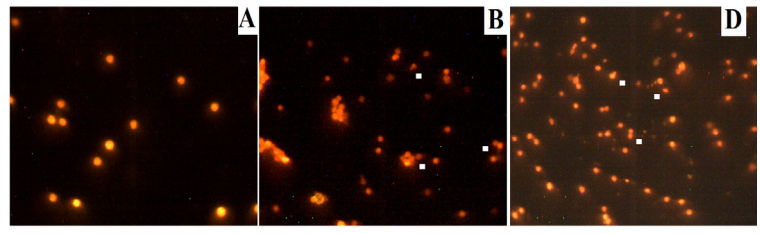

Figure 10: Representative of micronucleus formation assay for spleen cells extracted from, (A), untreated animals; (B), one week; and (C), two weeks SNP treated animals. White arrows indicate some of the MN in the pictures field.

Agarose gel electrophoresis assay for DNA (ladder pattern).

In this assay, bone marrow cells indicated a DNA ladder profile (Fig. 11). The DNA ladder formation in one week treated animal did not have differences in this type of profile compared to two weeks treated animals. Both of time treatments induce considerable genotoxicity effect in bone marrow cells as indicated in this assay. In lymphocytes the DNA ladder profile was less intense. After the end of the electrophoresis, there was a single band formation in one and two weeks period of time treatment in SNP injected animals. This may indicate the lowest events of genotoxicity induced by SNP in lymphocytes. This result correlates with the result obtained from comet assay and micronucleus formation in lymphocytes when they showed a minimum genotoxicity in both mentioned assays. Lymphocytes are an essential toll to assess the genotoxicity of different type of materials in vitro (55). They have been used by many researchers to determine the genotoxicity of different types of silver nanoparticles in both comet and micronucleus formation assay in vitro (41). In spleen cells the results of this assay was the same as observed in the case of bone marrow cells (Fig. 13). The DNA ladder profile was obviously indicated in spleen cells in this assay. This may indicate that this assay is sensitive for testing the genotoxicity in vivo in comparison with single cell gel electrophoresis and micronucleus formation assay.

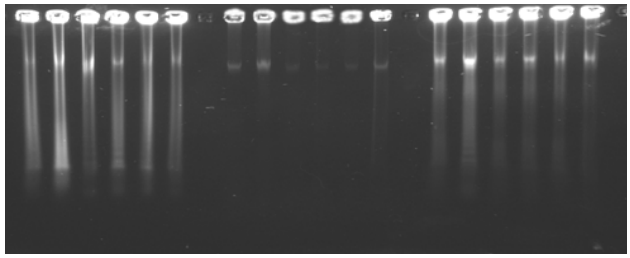

Figure 11: A representatives of DNA fragmentation assay profile for bone marrow cells extracted from control, one week, and two weak SNP treated mice. Lane 1,2,3,4,5, and 6 one weak treated; lanes 7, 8, 9, 10, 11, and 12 control animals; lanes 13, 14, 15, 16, 17 and 18 two weeks treated animals.

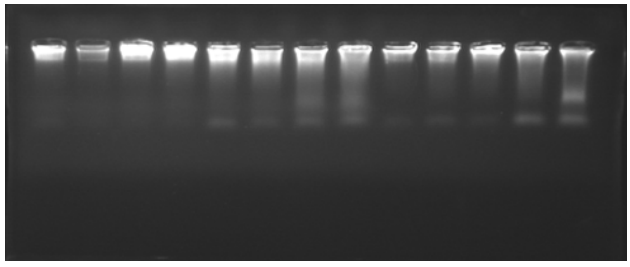

Figure 12: A representatives of DNA fragmentation assay profile for lymphocytes extracted from control, one week, and two weeks SNP treated mice. Lane 1, 2, 3, and 4, vehicle control animals; lanes 5, 6, 7, and 8 one weak treated; lanes 9, 10, 11, and 12 two weeks treated animals.

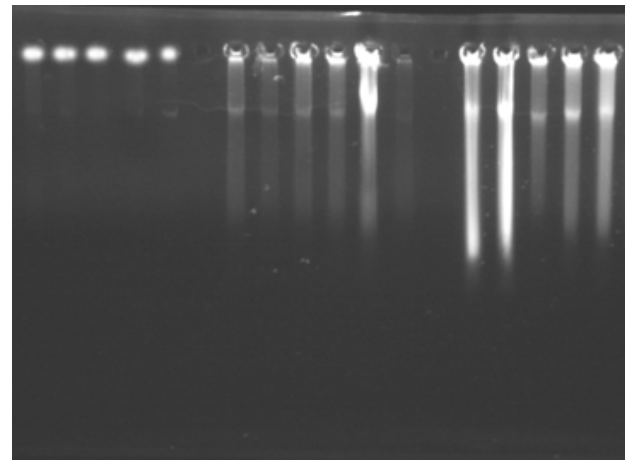

Figure 13: A representatives of DNA fragmentation assay profile for spleen cells extracted from control, one week, and two weak SNP treated mice. Lane 1, 2, 3, 4 and 5 control animals; lanes 6, 7, 8, 9 and 10 one weak treated; lanes 11, 12, 13, 14, and 15 two weeks treated animals.

Discussion:

After the nanotechnology was recognized as a revolution in material science a new approach was conducted to introduce its intervention in many applications. This new technology was capability to enhance the efficiency of industry, energy, and health sectors to promote them to be more effective and economical. All these sectors are in direct contact with humane behavioral attributes, since the introduction of this technology to these applications was so fast; we think there is an urgent need to put essentials for an accurate and precise risk assessment system. Many researches tried to establish and confirm criteria to have such risk assessment. Many of them proposed to use the already known toxicology assays. These assays have been used successfully in many protocols to test the toxicity of different chemical compounds used for the development of new pharmaceuticals (60). In vitro assays to determined genotoxicity of different types of nanoparticles were carried out, one time using immortalized cancer cell lines other time using peripheral blood lymphocytes as normal cells. Although these tests were so beneficial to reach basic understanding of the effect of these materials on cellular DNA, however the direct contact between the cells and the test material would be fare from being realistic as in the in vivo situation(61). This assumption was clearly indicated when the toxicity of cadmium and gold nanoparticles tested in both 2D and 3D spheroid cultures. Results disclosed directly that toxicity was significantly reduced in the 3D culture when compared to the 2D culture. The authors' interpreted that tissue-like morphology of the conducted 3D culture was the major factor in diminishing nanoparticles toxicity. This 3D culture could be acted as an intermediate stage bridging in vitro 2D and in vivo (62). Accordingly the considerable realistic fact which could be obtained from in vivo assays is substantial. Therefore we have demonstrated the genotoxicity of silver nanoparticles in vivo in different animal organs that may came in direct contact with the administrated nanoparticles. We used the most common and widely used assays to detect the DNA damage in the nanoparticles administrated animals (63).

We have noticed that each organ tested responded drastically different among the test used and other organs and. Using comet assay, it was clearly indicated that bone marrow cells DNA were effected intensively in both period of experiment time than lymphocytes and spleen cells, whereas the later produced the lowest DNA damage. This could be due to the accumulation of silver nanoparticles in bone marrow (64). Another explanation for the elevated percentage of DNA damage in these cells could be referred to that these cells is continuously under go cellular divisions in order to produce different types of blood cells. This property makes the DNA in these cells more venerable to damage compared to lymphocytes and spleen cells. The Micronucleus formation in the bone marrow cells was higher after one week and two weeks injection. Whereas lymphocytes

and spleen cells formed micronuclei with less percentage compared to bone marrow cells as a result of their DNA damage by silver nanoparticles. In our case, Comparing with comet assay, micronucleus formation assay seems equally sensitive in detecting cellular DNA damage in the situation of this study, in the cells of the organs under investigation. DNA ladder formation assay was very indicative in this case to detect genotoxicity. Since this test is cost effective and takes less time and effort than both single cell gel electrophoresis and micronucleus formation assay, we recommend using this test to detect the genotoxicity of other types of nanoparticles on the cells selected in this study. In summary this study recognized that whether using single cell gel electrophoresis or micronucleus formation assay and DNA ladder formation assay, to detect genotoxicity of silver nanoparticles in vivo, all these assays would be suitable in serving this purpose.

References:

1. Yu, I. J.; Ji, J. H. and Ahu, K. H. (2010). Development of international standards for nanotechnology and risk assessment of nanomaterial. Proceedings of the 10th IEEE international conference on nanotechnology joined symposium with nano Korea. 201-204. risk assessment of SNP
2. Rai, M.; Yadav, A. and Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 27: 76–83.
3. Rai, M.; Yadav, A. and Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 27: 76–83.
4. Lara, H. H.; Garza-Treviño, E. N.; Ixtepan-Turrent, L. and Singh, D. K. (2011). Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. J. Nanobiotechnol. 9:30-38.
5. Mohanty, S.; Mishra, S.; Jena, P.; Jacob, B.; Sarkar, B. and Sonawane, A. (2012). An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy ofstarch-stabilized silver nanoparticles. Nanomed. Nanotechnol, Bio, Med. 8: 916–924.
6. Hernández-Sierra, J., F.; Ruiz, F.; Pena, D. C. C.; Martínez-Gutiérrez, F.; Martínez, A. E.; Guillén, A. de J. P.; Tapia-Pérez, H. and Castañón, G. M. (2008). The antimicrobial sensitivity of Streptococcus mutans to nanoparticles of silver, zinc oxide, and gold. Nanomed. Nanotechnol. Bio. Med. 4: 237–240.
7. Roe, D.; Karandikar, B.; Bonn-Savage, N.; Gibbins, B. and Jean-Baptiste, R. (2008). Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. J. Antimicrobial Chemotherapy. 61: 869–876.
8. Avila Jerley ,A. and A. Anita Margret. (2012).Mycosynthesis of silver nanoparticles and antibacteral effects in wound dressing. Inter. J. pharmaceu. Sci. Res. 3: 1698-1704.
9. Chamakura, K.; Perez-Ballestero, R.; Luo, Z.; Bashir, S. and Liu, J. (2011). Comparison of bactericidal activities of silver nanoparticles with common chemical disinfectants. Colloids and Surfaces B: Biointerfaces 84: 88–96.
10. Yakub, I. and Soboyejo, W. O. (2012) .Adhesion of E. coli to silver- or copper-coated porous clay ceramic surfaces. J. Appl. Phys. 111:124324-124324-9.
11. Gutarowska, B., Skora, J.; Zduniak, K. and Rembisz, D. (2012). Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles. Int. Biodeteriora. Biodegradation 68: 7-17.
12. Thangapandiyan, S. and P. Prema. (2012). Chemically fabricated silver nanoparticles enhances the activity of antibiotics against selected human bacterial pathogens. Int. J. pharmaceu. Sci. Res. 3: 1415-1422.
13. You, C.; Han, C.; Wang, X.; Zheng, Y., Li, Q.; Hu, X.; and Sun H. (2012). The progress of silver nanoparticles in the antibacterial mechanism, clinical application and cytotoxicity. Mol. Biol. Rep. 39:9193–9201.
14. Patlolla, A. K.; Berry, A.; May, L.; and Tchounwou, P. B. (2012). Genotoxicity of Silver Nanoparticles in Vicia faba: A Pilot Study on the Environmental Monitoring of Nanoparticle. Int. J. Environ. Res. Public Health. 9: 1649-1662.
15. Panyala, N. R.; Pena-Mendez, E. M.; and Havel, J. (2008). Silver or silver nanoparticles: a hazardous threat to the environment. J. Appl. Biomed. 6:117-129.
16. Chia-Pin, C.; Wei-Yu, C.; Wei-Chun, C.; Nan-Hung, H.; Min-Pei; L.; Chung-Min, L. (2012). Assessing the potential risks to zebrafish posed by environmentally relevant copper and silver nanoparticles. Sci. Total Environ. 420: 111–118.
17. Park, M.V.D.Z.; Neigh, A. M.; Vermeulen, J. P.; Fonteyne, L. J.J.; Verharen,H. W.; Briedé, J. J.; van Loveren, H; de Jong, W. H. (2011). The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32: 9810-9817.
18. Wise Sr, J. P.; Goodale, B. C.; Wise, S. S.; Craig, G. A.; Pongan, A. F.; Walter, R. B.; Thompson, W. D.; Ng, A.; AbouEl-Makarim, A.; Mitani, H.; Spalding, M. J. and Mason M. D. (2010). Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquatic Toxicol. 97: 34–41
19. Koch, M.; Kiefer, S.; Cavelius, C. and Kraegeloh, A. (2012). Use of a silver ion selective electrode to assess mechanisms responsible for biological effects of silver nanoparticles. J. Nanoparticle Res. 14: 1-11.
20. Mahl, D.; Diendorf, J,; Ristig, S. and Greulich, C. (2012) Silver, gold, and alloyed silver--gold nanoparticles: characterization and comparative cell-biologic action J. Nanoparticle Res. 14 : 1153-1165.
21. Gorth, D. J.; Rand, D. M. and Webster, T. J. (2011). Silver nanoparticle toxicity in Drosophila: size does matter. Int. J. of Nanomedicine : 343-350.
22. Wu, Y. and Zhou, Q. (2012). Dose- and time-related changes in aerobic metabolism, chorionic disruption, and oxidative stress in embryonic medaka (Oryzias latipes): Underlying mechanisms for silver nanoparticle developmental toxicity. J. Aquatic Toxicol. 125: 238-246.
23. Lok, C.; Ho, C.; Chen, R. and He, Q. (2007). Silver nanoparticles: partial oxidation and antibacterial activities : J. Biological Inorganic Chem. 12: 527-534
24. Di, H.; Adele, M. J.; Shikha, G. and Ninh, A. P. (2011). Silver Nanoparticle−Reactive Oxygen Species Interactions: Application of a Charging−Discharging Model. J. Phys. Chem.115: 5461-5468.
25. Beer, C.; Foldbjerg, R.; Hayashi, Y. and Sutherland, D.S. (2012). Toxicity of silver nanoparticles-Nanoparticle or silver ion . Toxicology Lett.208: 286–292.
26. Liu, Y. ; Guan, W. ; Ren, G. and Yang, Z. (2012). The possible mechanism of silver nanoparticle impact on hippocampal synaptic plasticity and spatial cognition in rats. Toxicol. Lett. 209: 227-231.
27. Banerjee, M.; Mallick, S.; Paul, A. and Chattopadhyay, A. (2010). Heightened Reactive Oxygen Species Generation in the Antimicrobial Activity of a Three Component Iodinated Chitosan−Silver Nanoparticle Composite. Langmuir. 26: 5901-5908.
28. Asare, N.; Instanes, C.; Sandberg, W. J.; Refsnes, M.; Schwarze, P.; Kruszewski, M. and Brunborg, G. (2012). Cytotoxic and genotoxic

effects of silver nanoparticles in testicular cells. Toxicol. 291 : 65–72
29. AshaRani, P. V.; Mun, G. L. K.; Hande, M. P. and Valiyaveettil, S. (2008). Cytotoxicity and genotoxicity of silver nanoparticles in human cell. ACS NANO.3: 279–290
30. Foldbjerg, R.; Dang, D. A. and Autrup, H. (2011). Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch. Toxicol 85:743–750.
31. Fabian, C.; Koetz, L.; Favaro, E.; Indraccolo, S.; Mueller-Klieser, W. and Ulrike G. A. Sattler. (2012). Protein profiles in human ovarian cancer cell lines correspond to their metabolic activity and to metabolic profiles of respective tumor xenografts. FEBS Journal 279: 882–891.
32. Arora, S.; Jain, J.; Rajwade, J.M. and K.M. Paknikar (2008). Cellular responses induced by silver nanoparticles: In vitro studies. Toxic. Let. 179: 93–100.
33. Lee, Y.J.; Cho, A.; Cho, B.C.; Yun, M.; Kim, S.K. and Chang, J. (2009). High tumor metabolic activity as measured by fluorodeoxyglucose positron emission tomography is associated with poor prognosis in limited and extensive stage small-cell lung cancer. Clin. Cancer Res.15:2426–2432.
34. Kim, H. R.; Kim, M. J.; Lee, S. Y.; Oh, S. M. and K. H. Chung. (2011). Genotoxic effects of silver nanoparticles stimulated by oxidative stress in human normal bronchial epithelial (BEAS-2B) cells. Mutation Res. 726: 129– 135.
35. Lee, Y. J.; Cho, A.; Cho, B. C.; Yun, M.; Kim, S. K. and Chang. J. (2009). High tumor metabolic activity as measured by fluorodeoxyglucose positron emission tomography is associated with poor prognosis in limited and extensive stage small-cell lung cancer. Clin Cancer Res. 15:2426–32.
36. Park , J. C.; Lee, J. H.; Cheoi, K.; Chung, H.; & Yun, M. J.; Lee, H. & Shin, S. K.; Lee, S. K. and Lee, Y. C. (2012) Predictive value of pretreatment metabolic activity measured by fluorodeoxyglucose positron emission tomography in patients with metastatic advanced gastric cancer the maximal SUV of the stomach is a prognostic factor. Eur. J. Nucl. Med. Mol. Imaging 39:1107–1116.
37. Johnson, M. D.; Schilz, J.; Djordjevic, M. V.; Rice, J. R. and Shields, P. G. (2009). Evaluation of In vitro Assays for Assessing the Toxicity of Cigarette Smoke and Smokeless Tobacco. Cancer Epidemiol Biomarkers Prev 18:3263–304.
38. Li, Y.; Chen, D. H.; Yan, J.; Chen, Y.; Mittelstaedt, R. A.; Zhang, Y. Biris, A. S. ; Heflich, R. H. and Chen, T. (2012). Genotoxicity of silver nanoparticles evaluated using the Ames test and in vitro micronucleus assay. Mutation Research 745: 4– 10.
39. Beer, C.; Foldbjerg, R.; Dang, D.A. and H. Autrup. (2011) Genotoxicity and toxicity of silver nanoparticles and silver ions Abstracts / Toxic. Let. 205:36–59.
40. Tavares, P.; Balbino,F. t.; de Oliveira, H. M.; Fagundes, G. E.; Vena^ncio, M.; Ronconi, J. V. V.; Merlini, A.; Streck, E. L., Paula, M. M. S. and Andrade V. M. (2012). Evaluation of genotoxic effect of silver nanoparticles (Ag-Nps) in vitro and in vivo. J. Nanopart. Res. 14:791-798
41. Flowera N. A. L.; Brabua, B.; Revathyb, M.; , Gopalakrishnana, C.; Rajaa, S. V. K; . Muruganb, S. S and Kumaravelb, T. S. (2012). Characterization of synthesized silver nanoparticles and assessment of its genotoxicity potentials using the alkaline comet assay. Mutation Res. 742: 61– 65.
42. AshaRani, P. V.; Mun, G. L. K.; Hande,M. P.; and Valiyaveettil, S. (2009). Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. 3: 279–290.
43. Syed, H. C. and Dubreuil, D. (2012). Escherichia coli STb toxin induces apoptosis in intestinal epithelial cell lines. Microbial Pathogenesis. 53: 147-153
44. Deitch, A. D; Law, H. and deVereWhite, R. (1982). A stable propidiumiodide staining procedure for flow cytometry. J. Histochem. Cytochem. 30: 967-972.
45. Cariddi, L.; Escobar, F.; Sabini, C.; Torres, C.; Reinoso, E.; Cristofolini, A.; Comini , L.; Nunez S. Sabini, M. L. (2012) Apoptosis and mutagenicity induction by a characterized aqueous extract of Baccharis articulata (Lam.) Pers. (Asteraceae) on normal cells. Food and Chem. Toxic. 50: 155–161.
46. Chenni, H.; Ghosh, D.; Ali, K. M.; Chatterjee, K. and Trabelsi, M. M. (2011). Spermicidal Effects of Methanolic Extract of Cestrum parqui Leaves on Human Spermatozoa: A View through DNA Breakage and Disruption of Membrane Ultrastructure. J. Reproduc. Contracep. 22: 223-232.
47. Cariddi, L.; Escobar, F.; Sabini, C.; Torres, C.; Reinoso, E.; Cristofolini, A.; Comini , L.; Nunez S. Sabini, M. L. (2012) Apoptosis and mutagenicity induction by a characterized aqueous extract of Baccharis articulata (Lam.) Pers. (Asteraceae) on normal cells. Food and Chem. Toxic. 50: 155–161.
48. Escobar, F. M.; Cariddi, L. N.;a, Sabini, M. C.; Reinoso, E.; Sutil, S. B.; Torres, C. V.; Zanon, S. M. and Sabini, L. I. (2012) Lack of cytotoxic and genotoxic effects of Minthostachys verticillata essential oil: Studies in vitro and in vivo Food. Chem. Toxic. 50 3062–3067
49. Shahrim,Z.; Baharuddin, P. J. N. M.; Yahya, N. A.; Muhammad, H.; Bakar, R. A. and Ismail Z. (2006). The in vivo rodent micronucleus assay of Kacip Fatimah (Labisia pumila) extract. Tropical Biomedicine 23: 214–219.
50. Kumari, S.; Rastogi, R.P.; Singh,K. L.; Singh S. P. and Sinha R. P.. (2008). DNA damage: detection strategies. EXCLI Journal. 7:44-62.
51. AshaRani,P. V.; Mun, G. L. K.;, Hande, M. P. and Valiyaveettil, S. (2009). Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells. ACSNano. 3: 279–290.
52. Czuprynski, C. J. and Brown, J. F. (1998). Isolation and preparation of lymphocytes from infected animals. In. Methods in microbiology. Volum 25. Kaufmann, S. H. E. and Kabelitz (Eds.) P. 189-204. Academic Press. NY. USA.
53. Roberts, A.D.; Belisle, J. T.; Cooper, A. M. and Orme, I. M. (1998). Murine model of tuberculosis. In. Methods in microbiology. Volum 25. Kaufmann, S. H. E. and Kabelitz (Eds.) P. 389-415. Academic Press. NY. USA.
54. Collins, A. R.; Dusinska, M.; Gedik, C. M. and Stetina, R. (1996). Oxidative Damage to DNA: Do We Have a Reliable Biomarker. Environ. Health Perspec. 104, Suppl: 3:465-469.
55. Collins, A. R.; Dušinská, M. and Horská, A. (2001). Detection of alkylation damage in human lymphocyte DNA with the comet assay. Acta Biochimica Polonica.48: 611-614.
56. Singh, N. P. (2000). A Simple Method for Accurate Estimation of Apoptotic Cells. Experimental Cell Res. 256: 328–337.
57. Susan,G.B.; Voelki, K. E; Anderson, T. W. and Finn, J. (1997): SPSS Guid to the New Statistical Analysis of Data. Springer. New York. USA.
58. Abdulrahman K. Ali and Dayah N. Raouf. (2011). Preparation of Silver Nanoparticles by Pulsed Laser Ablation in Liquid Medium. Eng. Technolo. J. 29: 3058-3066.
59. Terradas, M.; Martín, M.; Tusell, L. and Genescà, A. (2010). Genetic activities in micronuclei: Is the DNA entrapped in micronuclei lost for the cell. 705: 60–67.
60. James, M. and McKim, Jr. (2010). Building a tiered approach to in vitro predictive toxicity screening: A focus on assays with in vivo relevance. Combinatorial Chemistry & High Throughput Screening. 13: 188-206.
61. Ghosh, M. ; Chakraborty , A. and Mukherjee, A. (2013). Cytotoxic, genotoxic and the hemolytic effect of titanium dioxide (TiO2) nanoparticles on human erythrocyte and lymphocyte cells in vitro. J. Appl. Toxicology. 33: 1097–1110.
62. Jungwoo Lee, G. Daniel Lilly, R. Christopher Doty, Paul Podsiadlo, and Nicholas A. Kotov. (2009). In vitro Toxicity Testing of Nanoparticles in 3D Cell Culture. Small. 5: 1213–1221.
63. Love, S. A.; Maurer-Jones, M. A.; Thompson, J. W.; Lin, Y.S.; and Haynes C. L. (2012). Assessing Nanoparticle Toxicity. Annu. Rev. Anal. Chem.5:181-20.
64. Garnett, M. C. and Kallinteri, P. (2006). Nanomedicines and nanotoxicology: some physiological principles. Occupational Medicine.56:307–311

التأثير السام لجسيمات الفضة النانوية المصنعة بطريقة الإزالة بالليزر النبضي على المكنون الوراثي داخل الجسم الحي

عامر طالب توفيق، حسام الدين محمد كاظم، ناهي يوسف ياسين، سبأ كامل خليل، أسيل فايق غيدان، رشا عبد الأمير حسين، أمنة يوسف عبد اللطيف

المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية

الخلاصة:

جرى هذا البحث لتقيم ثلاث طرائق رئيسة تستخدم قياسيا لتحديد مدى تظرر الدنا الخلوي في إختبارات السمية الوراثية (تسمم المكنون الوراثي) , هذه ثلاث طرق هي الهجرة في المجال الكهربائي للخلايا المنفردة (فحص الكومت) وتكون النوى الدقيقة وفحص تقطع الحامض النووي DNA المنقى من الخلايا المعزولة. ونظرا لدخول الجسيمات الناوية العديد من التطبيقات العملية التي لها مساس مباشر بحياة الإنسان فإنه من الضروري بمكان إيجاد ألية للتحري عن تظرر المكنون الوراثي الناتج عن المعاملة بالجسيمات النانوية . صنعت جسيمات الفضة النانوية بطريقة الإزالة بالليزر النبضي من قطعة فظة عالية النقاوة مغمورة في ماء مقطر لمرتين وجرى توصيف الجسيمات المصنعة بإستخدام المطياف الممتد من الضوء المرئي إلى الفوق البنفسجية وكذلك بمجهر القوى الذرية . بعد التعرف على مواصفاتها حقنت الجسيمات النانوية المصنعة في الفئران البيضاء نوع BALB/c تحت الجلد بجرعة مقدارها ٢٠٠ مايكروغرام لكل كيلوغرام من وزن الجسم ولمدتين زمنيتين مختلفة الأولى يوميا لمدة إسبوع والثانية يوميا لمدة سبوعين . بعد إنتهاء فترة التجريع جرى التضحية بالحيوانات وعزلت منها خلايا نخاع العظم والخلايا اللمفاوية وخلايا الطحال . قيم تظرر المادة الوراثية DNA في الخلايا المعزولة من الحيوانات بإستخدام الطرق الثلاث المذكورة أنفا . أشارة النتائج إلى أن الطرائق الثلاث المستخدمة قادرة على تبيان حجم الظرر الحاصل في المكنون الوراثي للخلايا المعزولة من الفئران المحقونة بالفضة النانوية . كما أشارةالنتائج إلى أن المكنون الوراثي لخلايا الطحال كانت الأقل تظررا من بين الخلايا تحت الفحص خصوصا في الإسبوع الأول من الحقن عند إستخدام الفحوص الثلاثة في حين تظرر المكنون الوراثي لكل من خلايا نخاع العظم والخلايا المفاوية بشكل أكثر حدة بإستخدام الفحوص الثلاثة .