Gold nanoparticles inhibiting proliferation of Human breast cancer cell line

Zainab Ali[1], Majid Jabir[1*] and Ahmed Al- Shammari[2]
1. Department Applied Science, University of Technology. Baghdad, IRAQ
2. Iraqi Center for Cancer and Medical Genetic Research, University of Mustansiriyah. Baghdad, IRAQ
*msj_iraq@yahoo.com

Abstract

The aim of this study is to prepare Gold nanoparticles (GNPs) and investigate their role in vitro as anticancer agent against MCF-7. The prepared GNPs were observed through a color change and using SEM. MCF-7 cells were treated with GNPs at different concentration and then anti-proliferative activity of this nanoparticles was studied using MTT assay. Apoptotic cells were studied using Acrydin Orange /Ethidum bromide AO/EB daul stain. Our results refer to therapeutic role of GNPs in breast cancer. The results of this study suggest that the GNPs could be used for wide medical applications in future and offer new drug instead of chemotherapy in treatment of various types of cancer disease.

Keywords: Gold nanoparticles (GNPs), Cytotoxicity, Apoptosis.

Introduction

Cancer is not a single disease; It only represents a group of diseases characterized by uncontrolled increase and spread of abnormal cells. When the spread is not controlled; it can lead to death[25,34]. In Iraq, cancer is alarmingly at high levels and blame returns to the mutagenic and carcinogenic agent. The latest Iraqi Cancer Registry revealed that out of estimated population size of 32,500,000 a total of 21,101 new cases of cancer were registered in 2012 in men 9, 268 cases and 11,833 in women. In addition, there are several reports of apparent excess rates of cancer with birth defects in the town of Falujah[2,15].

Cancer has been treated by classical therapeutic processes like surgery; radiation; chemotherapy; immunotherapy; targeted therapy and hormone therapy[26.33]. There are therapeutic paths which use Nanotechnology for treatment of cancer cells, employed the characterization of cytotoxicity. So, nanotechnology is particularly the promising field for new applications in medicine[1,27]. Nanotechnology deals with materials in the range of size 1 - 100 nm.

Therefore, the advancement in this field, its applications to the fields of medicines; pharmaceuticals and biological have revolutionized the twentieth century. So, term nanobiotechnology was proposed[11,13]. Nanoparticles have a lot of biological applications like: biosensing; biological separation; molecular imaging; anticancer therapy[11] because their physicochemical and biological characteristics are compared with their bulk one[27]. Different kinds of nanoparticles have unique properties, that can be used to target cancer cells[15]. Gold, silver, zinc, oxide, titanium, magnetic iron oxide and quantum dots nanoparticles are considered as the one of most basic inorganic nanomaterials that have important role in the field of biological applications[18,20]. In current time gold nanoparticles are widely used in all the fields, particularly in medicine[31].

Today, gold nanoparticles (GNPs) have been considered as remarkable nanoparticles in biomedical applications due to their unrivaled optical properties, electronic and surface because of the strong and size-tunable surface plasmon resonance, fluorescence and easy-surface functionalization. It is worth mentioning that gold nanoparticles have been widely used in biosensors, cancer cell imaging, photothermal therapy and also as drug delivery. It can be useful as a novel radiosensitizer in radiotherapy because the strong photoelectric absorption and secondary electron caused by gamma or X-ray irradiation can accelerate DNA strand breaks. So, they are considered as nano-toxic particles and still not completely understood[19].

The rapid antioxidant gold nanoparticles (GNPs) technology presents great promise for future applications due to their numerous specific surface areas with high diverse surface activities than bulk gold. Physical and chemical properties have made (GNPs) or (AuNPs) of great importance in the development of excellent nano electronic chips and promising vehicle for biomedical and environmental applications. AuNPs are established both *in vitro* and *in vivo* nanotoxicities[4].

Synthesized AuNPs come in a different sizes and shapes ranging from 1 - 500 nm, so some rods, spheres, tubes, wires, ribbons, plate, cubic, hexagonal, triangular, tetrapods etc. are affected by Gold nanoparticles toxicity[32]. Turkevich method (Citrate reduction method) is one of the main methods used in the GNPs synthesis. They are popular and convenient methods, but the products stability and disparity are limited[17]. The toxicity of Gold nanoparticles GNPs has been manifested at the cellular level. GNPs enter cells in a size and shape dependent manner[7,8].

GNPs uptake reaches a maximum when the size is 50 nm. The uptake of GNPs is consistent with receptor-mediated endocytosis and the transport reaches to 30 min after incubation and it is installer, GNPs have ability to enter cells. Most studies have indicated that they are nearly harmless to tumor cultured cells[5,9,15,28]. GNPs do not affect on cell

viability, but affect cell proliferation and cause (DNA) damage, gold nanoparticles are used in many biomedical applications like intracellular gene regulation[30]; chemotherapy[29]; drug delivery[10,14] and optical and electronic applications[6].

Material and Methods

Preparation of Glutathione modified Gold Nanoparticles: An aqueous solution of gold salt (1 mL, 0.025 M) was mixed with Glutathione (GSH) (8 mL, 0.019 M). The pH of the combination was adjusted to be 8. $NaBH_4$ (Sodium Borohydride) solution (2 mg/ mL) was prepared and added to the mixture. The combination allowed reacting overnight and then the excess reactants have been eliminated by way of centrifugation at 10,000 rpm.[22]

Scanning Electron Microscopic: SEM was used to visualize the morphology and nanoparticle grain size of gold nanoparticles. Thin films of gold nanoparticles were prepared on a cover slide grid by dropping amount of solution on the cover slide and then allowed to dry at room temperature before being visualized under SEM.

Cytotoxicity determination using MTT assay: This assay was done according to Sulaiman et al[12]. Briefly, MCF-7 cellline were seeded at 1×10^5 cells/mL in 96 well microtiter plates in RPMI medium. The cells were incubated overnight for attachment. GNPs, were added in triplicate at different concentration and incubated for 48 hrs. Thereafter, the cells were stained with MTT at concentration 2µg/ml. The samples were incubated at 37ºC, after 3 hr DMSO (Dimethyl Sulfoxide) was added to each well; the absorbance was measured at 492 nm using microplate reader.

To visualize the shape of cells under inverted microscope, 200 µL of cell suspensions were seed in 96-well micro-titration plates at density $1x10^4$ cells mL^{-1} and incubated for 48 hrs at 37ºC. Then the medium was removed and add GNPs after exposure time, the plates were stained with 50 µL with Crystal violet and incubated at 37ºC for 10-15 min, the stain was washed gently with tap water until the dye was removed. The cell was observed under inverted microscope at 100x magnification microscope and photographed with digital camera.

Acridine orange –Ethidium bromide (AO/EtBr) dual staining: The induction of apoptosis in MCF-7 cellline was performed using AO/EtBr dual staining method. Briefly, the cells in 96- well plates were treated with GNPs and incubated for 24 hrs. The cells were washed twice using PBS. Dual fluorescent dyes (10 µL) were added into the cells at equal volumes. Finally, the cells were visualized under fluorescence microscopy.[17,23]

Statistical analysis: The obtained data were statically performed using unpaired T test with GraphPad Prism 6, values were presented as the mean ± S.D of the three replicates of each experiment.[21]

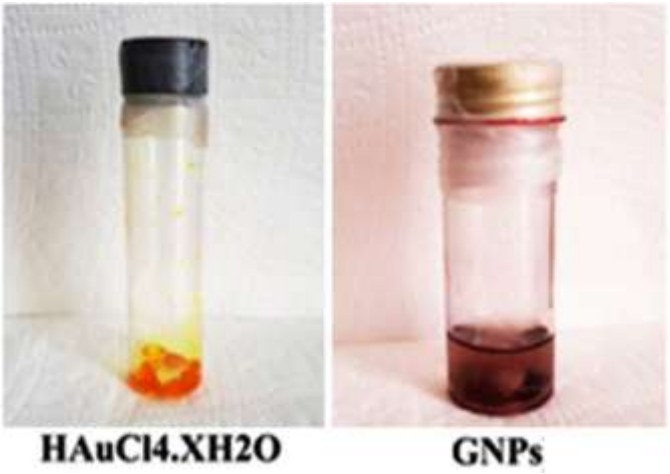

Figure 1: Preparation of GNPs

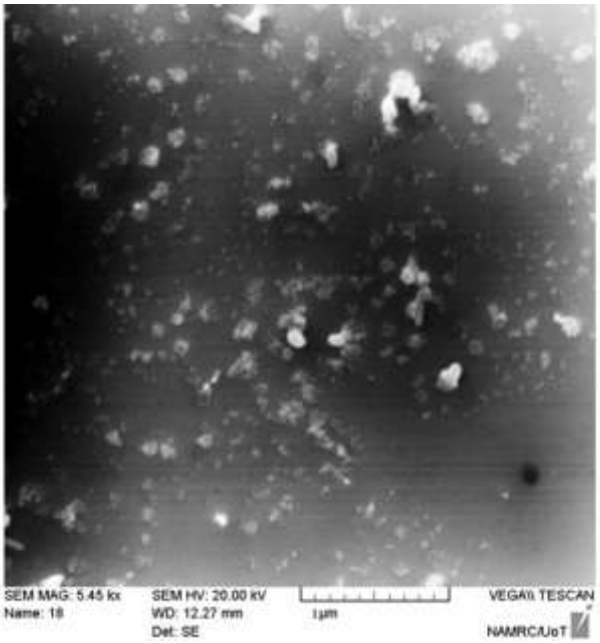

Figure 2: SEM image of GNPs

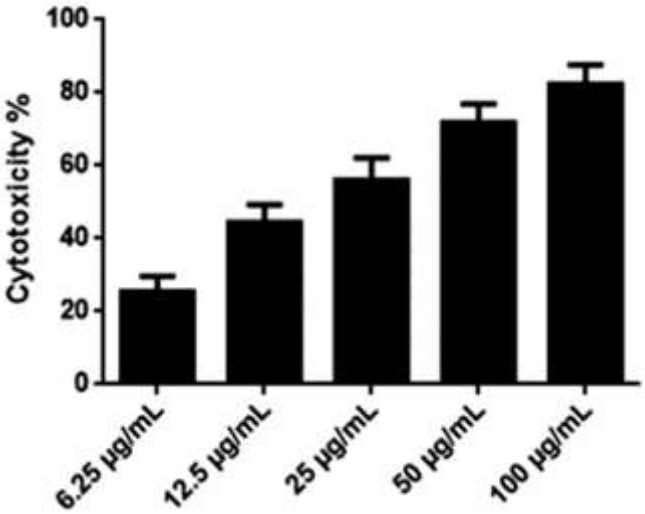

Figure 3: Cytotoxic effect of GNPs in MCF-7 cell. IC50=13.56µg/mL

Results and Discussion

Preparation of Gold: The GNPs were synthesized employing three main steps. The first step involved the reaction between theiGSHiandiHAuCl$_4$ and then followed the second step that involved the adjustment of pH to be 8. Lastly the AuNPs formed by additioniofiNaBH$_4$. Gold salt had yellow color solution which changed into the red wine color after adding of NaBH$_4$ as in figure 1.

Scanning electron microscope: SEM technique was used to visualize the size and shape of GNPs. The SEM image of the synthesized Gold nanoparticles was shown in figure 2. The image of SEM exhibited spherical shape that formed with diameter in the range less than 20 nm.

GNPs inhibits growth of MCF-7 cells: The cytotoxic effects GNPs on the viability of human cancer cell lines MCF-7 for 48 hrs were examined as shown in figure 3. The results showed significant inhibition of cell proliferation in MCF-7 cell line after 48 hrs. The cell proliferation was significantly lower when compared to untreated control cells. After 48 hrs treatment with GNPs at concentrations 6.2, 12.5, 25, 50, and 100 µg/mL, the cytotoxicity effect of GNPs showed good cytotoxic effect on cell line. The results indicate that GNPs are considered to be a particularly valuable source of effective anti-proliferative and cytotoxic substances. The apoptogenic property was investigated through morphological changes in MCF-7 cell line using inverted phase contrast microscope. As seen in figure 4, the control (untreated) cells showed that the cells maintained their original morphology form that most of the control cells were adherent to the tissue culture dishes. In contrast, MCF-7 cells treated with GNPs exhibited high efficiency on the proliferation and morphology.

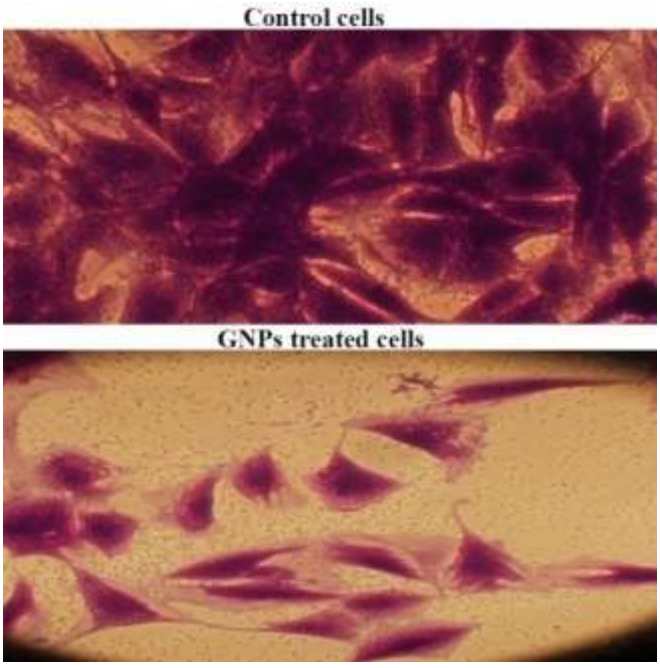

Figure 4: Anti-proliferative activity of GNPs against MCF-7 cells

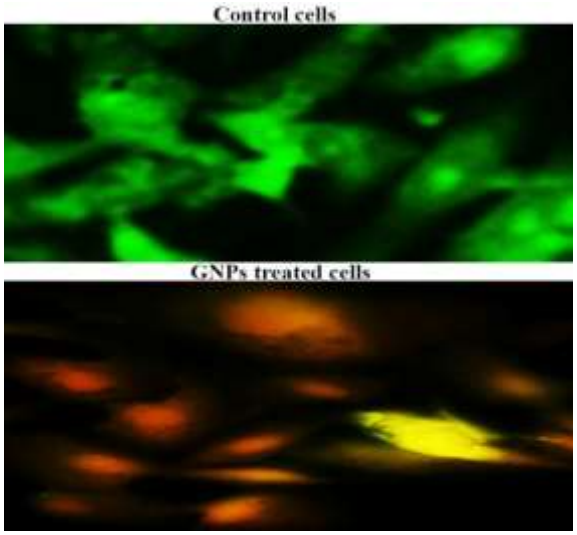

Figure 5: GNPs induces apoptosis in MCF-7 cells

Acridine orange –Ethidium bromide (AO/EtBr) dual staining: The apoptotic cells were evaluated based on DNA damage. In this study, the efficiency of GNPs, at concentrations 13.56 µg/mL was studied. The using of AO-EB dual staining was used to distinct apoptotic signs characteristics to nucleate alternations. Viable and nonapoptotic cells were shown green and apoptotic cells were shown orange or red as they stained with AO – EtBr figure 5. Exposure of cells to GNPs caused an increase in membrane disruption and formation of lysosomes vacuoles compared to untreated control cells. The results investigated the high ability of GNPs to cause death to the cell due to the ability of this nanoparticles to penetrate through the cell membrane and effect on the mRNA expression level.

References

1. Zainal I.G., Al-Shammari A.M. and Kachi W., Characterization of the modified nickel-zinc ferrite nanoparticles coated with APTES by salinization reaction, In AIP Conference Proceeding (AIP Publishing), **1968(1)**, 030008 **(2018)**

2. Al-Shammari A.M., Salman M.I., Saihood Y.D., Yaseen N.Y., Raed K., Shaker H.K., Ahmed A., Khalid A. and Duiach A., In vitro synergistic enhancement of newcastle disease virus to 5-fluorouracil cytotoxicity against tumor cells, *Biomedicines*, **4(1)**, 3 **(2016)**

3. Alwan N.A.S., Breast Cancer among Iraqi women: Preliminary Findings from a Regional Comparative Breast Cancer Research Project, *J. of Glob. Oncology, ASCO*, **2(1)**, 1-4 **(2016)**

4. Banerjee S., Uppal T., Strahan R., Dabral P. and Verma S.C., The Modulation of Apoptotic Pathways by Gammaherpesviruses, *Front. Microbiol.*, **7**, 585, doi:10.3389/fmicb.2016.00585 **(2016)**

5. Becker M.L., Bailey L.O. and Wooley K.L., Peptide-Derivatized Shell Cross Linked Nanoparticles, 2, Biocompatibility Evaluation, *Bioconjug. Chem.*, **15**, 710-717 **(2004)**

6. Chen Y.S., Hung Y.C., Liau I. and Huang G.S., Assessment of the in vivo toxicity of gold nanoparticles, *Nanoscale Research Letters*, **4(8)**, 858 **(2009)**

7. Chithrani B.D. and Chan W.C., Elucidating the Mechanism of Cellular Uptake and Removal of Protein Coated Gold Nanoparticles of Different Sizes and Shapes, *Nano Lett.*, **7**, 1542-1550 **(2007)**

8. Chithrani B.D., Ghazani A.A. and Chan W.C., Determining the Size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells, *Nano Lett.*, **6**, 662-668 **(2006)**

9. Connor E.E., Mwamuka J., Gole A., Murphy C.J. and Wyatt M.D., Gold Nanoparticles Are Taken Up by Human Cells but Do Not Cause Acute Cytotoxicity, *Small*, **1**, 325-327 **(2005)**

10. Daniel M.C. and Astruc D., Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size- Related Properties and Applications Toward Biology, Catalysis and Nanotechnology, *Chem. Rev*., **104**, 293-346 **(2004)**

11. de Morais M.G., Martins V.G., Steffens D., Pranke P. and Costa J.A.V., Biological Applications of Nanobiotechnology, *Journal of Nanoscience and Nanotechnology*, **14**, 1007–1017 **(2014)**

12. Sulaiman G.M., Jabir M.S. and Hameed A.H., Nanoscale modification of chrysin for improved of therapeutic efficiency and cytotoxicity, *Artificial Cells, Nanomedicine and Biotechnology*, **46**, 1-13 **(2018)**

13. Rajeshkumar S., Synthesis of Zinc oxide nanoparticles using algal formulation (Padina tetrastromatica and Turbinaria conoides) and their antibacterial activity against fish pathogens, *Res. J. Biotech*, **13(9)**, 15-19 (**2018)**

14. Han G., Ghosh P. and Rotello V.M., Multi-Functional Gold Nanoparticles for Drug Delivery, *Adv. Exp. Med. Biol.*, **620**, 48-56 **(2007)**

15. Hauck T.S., Ghazani A.A. and Chan W.C., Assessing the Effect of Surface Chemistry on Gold Nanorod Uptake, Toxicity and Gene Expression in Mammalian Cells, *Small*, **4**, 153-159 **(2008)**

16. Iraqi Cancer Board Results of the Iraqi Cancer Registry 2012, Baghdad, Iraq, Iraqi Cancer Registry Center, Ministry of Health **(2015)**

17. Jabir M.S., Taha A.A. and Sahib U.I., Linalool loaded on glutathione-modified gold nanoparticles: a drug delivery system for a successful antimicrobial therapy, *Artificial Cells, Nanomedicine and Biotechnology*, **46**, 1-11 **(2018)**

18. Kim Y.J., Yang S.I. and Ryu J.C., Cytotoxicity and genotoxicity of nanosilver in mammalian cell lines, *Mol Cell Toxicol*., **6(2)**, 119-125 **(2010)**

19. Kroemer G., El-Deiry W.S., Golstein P., Peter M.E., Vaux D., Vandenabeele P. and Zhivotovsky B., Classification of cell death: recommendations of the Nomenclature Committee on Cell Death, *Cell Death Differ*., **12**, 1463-1467 **(2005)**

20. Lima E., Guerra R., Lara V. and Guzmán A., Gold nanoparticles as efficient antimicrobial agents for Escherichia coli and Salmonella typhi, *Chem. Central J.*, **7(11)**, 1-7 **(2013)**

21. Jabir M.S., Sulaiman G.M., Taqi Z.J. and Li D., Iraqi propolis increases degradation of IL-1β and NLRC4 by autophagy following Pseudomonas aeruginosa infection, *Microbes and Infection*, **20**, 89-100 **(2018)**

22. Jabir M.S., Taha A.A., Sahib U.I., Taqi Z.J., Al-Shammari A.M. and Salman A.S., Novel of nano delivery system for linalool loaded on gold nanoparticles conjugated with CALNN peptide for application in drug uptake and induction of cell death on breast cancer cell line, *Materials Science and Engineering* C, **94**, 949-964 **(2019)**

23. Jabir M.S., The interactions between inflammasome activation and induction of autophagy following Pseudomonas aeruginosa infection, University of Glasgow **(2014)**

24. Radhi M.M., Abdullah H.N., Jabir M.S. and Al-Mulla E.A.J., electrochemical effect of ascorbic acid on redox current peaks of $CoCl_2$ in blood medium, *Nano. Biomed. Eng*., **2**, 103-106 **(2017)**

25. Morelli V., Zoorob R. and Heidelbaugh J.J., Primary Care of the Medically Underserved, An Issue of Primary Care: Clinics in Office Practice, E-Book, The Clinics, Internal Medicine, 44-1 **(2017)**

26. National Cancer Institute Types of cancer treatment, The National Institutes of Health **(2017)**

27. Nikalje A.P., Nanotechnology and its Applications in Medicine, *Med Chem*, **5(2)**, 081-089 **(2015)**

28. Paciotti G.F., Myer L., Weinreich D., Goia D., Pavel N., McLaughlin R.E. and Tamarkin L., Colloidal Gold: Novel Nanoparticle Vector for Tumor Directed Drug Delivery, *Drug Deliv.*, **11**, 169-183 **(2004)**

29. Podsiadlo P., Sinani V.A., Bahng J.H., Kam N.W., Lee J. and Kotov N.A., Gold Nanoparticles Enhance the AntiRamakrishna D, Pragna Rao Nanoparticles: Is toxicity a concern? Leukemia Action of a 6-Mercaptopurine Chemotherapeutic Agent, *Langmuir*, **24**, 568-574 **(2008)**

30. Rosi N.L., Giljohann D.A., Thaxton C.S., Lytton-Jean A.K., Han M.S. and Mirkin C.A., Oligonucleotide Modified Gold Nanoparticles for Intracellular Gene Regulation, *Science*, **312**, 1027-1030 **(2006)**

31. Tkachenko A., Xie H., Franzen S. and Feldheim D.L., Assembly and Characterization of Biomolecule Gold Nanoparticle Conjugates and Their Use in Intracellular Imaging, *Methods Mol. Biol*., **303**, 85-99 **(2005)**

32. Webster K.A., Mitochondrial membrane permeabilization and cell death during myocardial infarction: roles of calcium and reactive oxygen species, *Future Cardiol*, **8(6)**, 863–884 **(2012)**

33. World Health Organization Cancer Fact Sheet N°297, Chapter 5.12, ISBN 9283204298 **(2014)**

34. Zhang G., Liu Z., Qin S. and Li K., Decreased expression of SIRT6 promotes tumor cell growth correlates closely with poor prognosis of ovarian cancer, *European Journal of Gynaecological Oncology*, **36(6)**, 629-632 **(2015)**.