The Effects of Photodynamic Therapy Using Green Laser and Glutathione-Capped-Gold Nanoparticles on Cancer Cells (MCF-7 Cell Line)

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ABSTRACT

Background: Nanotechnology is a discipline that manages, constructs, analyzes and deals with application of materials that have diameter from 1 to 100 nanometers. Gold nanoparticles are defined as stable colloid solutions of clusters of gold atoms with sizes at this minuscule.

Objective: The objective of current study was to treat breast cancer using gold nanoparticles capped with drug molecules in specific quantities to reduce the harmful side effects.

Method: The gold nanoparticales were prepared by method of Brust-Schiffrin. These nanoparticles were capped with type of drug (Glutathione) used to treat cancerous tumors. These gold nanoparticles were uncapped with drugs at (526nm) when capped with the glutathione drug at (589nm).

Results: The resulting evidence suggested that Glutathione-capped Gold nanoparticles were non-toxic up to the maximum recommended dosage. Therefore, the demonstrated biocompatibility offers the potentials of Glutathione-capped with Gold nanoparticles.The candidate is considered as a medicine for cancer therapy.

Keywords: *Gold nanoparticles, PDT, MCF-7 cell, glutathione, breast cancer.*

Introduction

Nanotechnology is a discipline that manages, constructs, analyzes and deals with application of materials that have diameter from 1 to 100 nanometers [1]. Gold nanoparticles (AuNps) are defined as stable colloid solutions of clusters of gold atoms with sizes at this minuscule. They possess different physicochemical characteristics when compared to the bulk gold, the most obvious example being the color change from yellow to ruby red when bulk gold is converted into nanoparticulate gold [2,4,6]. The surface plasmon resonance (SPR) peak is positioned at 520mm and this peak is responsible for the ruby red color displayed by conventional gold colloids [10,11,25]. This ruby red color of AuNps is explained by a theory called "surface plasmonics". According to this theory, when the clusters of gold particles are hit by the electromagnetic field of the incoming light, the surface free electrons (6 electrons in case of AuNps) present in the conduction band of AuNps oscillate back and forth, thus, creating a plasma band which has an absorption peak in the visible region at 530-540nm. The surface plasmon band (SPB) of AuNps is used as an indicator for formation during the synthesis of AuNps from their precursor salts. Physical properties of AuNps in turn depend on the size, shape, particle-particle distance and the nature of the stabilizer used to prevent the agglomeration of nanoparticles [12,13]. According to Mie theory, Surface Plasmon Band (SPB) is absent for AuNps less than 2nm and greater than 500nm. Gold nanorods have two SPB's, one longitudinal wavelength band at 550-600nm and one transverse wavelength band at 520nm [14]. The longitudinal wavelength band is very sensitive and changing the aspect ratio of Gold nanorods

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changes the absorption region from visible to near infra-red (NIR). This unique optical property of Gold nanorods is used in near infra-red ray therapy [26] and can be used as a therapeutic means to eradicate diseased cells which forms the basis for cancer treatment using Photodynamic therapy (PDT). It is a minimally invasive technique for cancer therapy.

The use of laser in medical applications has grown enormously from the time of its invention in 1960 to present days [15,16]. In this regard, the use of laser in photodynamic therapy (PDT) has gained increasing interest in medicine representing an experimental tool for the detection and treatment of tumors located in the lung, esophagus, colon, peritoneum, pleura, genitourinary tract, brain, eye and skin [17].

Current study was aimed to treat breast cancer using gold nanoparticles capped with drug molecules in specific quantities to reduce the harmful side effects and the use of lasers as an additional means to kill cancer cells through what is known as the Photodynamic therapy (PDT).

Materials and Method

Glutathione-capped gold nanoparticles (GSH-AuNPs) synthesis: The gold nanoparticales (AuNps) were prepared by method of Brust-Schiffrin [18]. A volume of 1ml of 0.1M $HAuCl_4$ was added in 500ml deionized water by a stirring device. Then, 0.06g $NaBH_4$ placed in 10ml deionized water. Prepared 10ml of $Nabh_4$ solution. The solution changes from yellow to dark red. The dark red disappears by stirring for 1.5h. Then, adding 0.1g of glutathione to AuNps solution with continuous stir for 2h to obtain AuNps solution to larger size of particles due to glutathione capped. The obtained nanoparticles were subjected to centrifugation at 13000rpm for 15min.

Lasers for heating Gold nanoparticles: The lasers used as a source of heating for AuNps with Glutathione and AuNps with cisplatin in the cell lines, were two continuous lasers; model MGL-III-532-110mW (532nm), and MRL-III-650 and 41mW output power.

Methyl Thiazolyl Tetrazolium (MTT) solution: MTT is a yellow-colored water soluble tetrazolium dye. Mitochondrial lactate dehydrogenase, produced by metabolically active cells, reduces MTT to water-insoluble Formosan crystals. When dissolved in appropriate solvent, these Formosan crystals exhibit purple color. The intensity of the purple color is directly proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. In order to prepare a 5mg/ml concentration of the dye according to [24], 0.5g of MTT was dissolved in 100mL of PBS. The solution was filtered through 0.2µm syringe filter to remove any blue Formosan product and then stored in sterile, dark, screw-capped bottles at 4°C. The solution was used within no longer than 2 weeks of preparation.

Characterized gold nanoparticles: The particle size and zeta potential of AuNps were measured using transmission electron microscope (TEM) before being capped with glutathione by transmission electron microscopy (TEM). Light absorption measured by UV spectrophotometer at wavelength 400-800nm before and after glutathione capped.

Cells viability assay

MCF-7Cells:

Organism: Homo sapiens, human Tissue of mammary gland, breast; derived from metastatic site: pleural effusion, Cell Type of epithelial**,** Product Format of frozen**,** Morphology: epithelial, Cultural Properties: adherent, Disease**:** adenocarcinoma, Age: 69 years adult, Gender: female, Ethnicity of Caucasian**,** Complete Growth Medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01mg/ml human recombinant insulin; fetal bovine serum to a final concentration of 10%. Culture conditions: Atmosphere: 95% air, 5% carbon dioxide and 37°C temperature.

The viability of melanoma cells treated with chemical agents was determined via the MTT assay. The cell viability was determined before assessing the cytotoxic effect of AuNPs, AuNPs+GHS, GHS, and Laser green time on cell lines. Seeding of trypsinized and suspended cells for any cell line in a microtiter plate should be in the range of 21–22 cell/well for the cytotoxicity assay as mentioned by [23]. Viable cell counting was accomplished by using trypan-blue exclusion. Dead cells take up the dye within a second making them easily distinguishable under the microscope from viable cells which remain unstained. The following protocol was conducted:

Cell suspension was prepared MCF-7 cancer cell. The hemocytometer with its cover slip fixed on its place was prepared (for counting soon). Adding 0.2ml of cell suspension to 0.2ml trypan-blue and 1.6ml PBS. Then, adding 20µl from the mixture to the edge of the cover

slip, allowed to run into the counting chamber. After 1-2 minutes viable and dead cells counting started with light microscope at 40X magnification. Cells concentration (cell/ml), total cell count and cell viability (%) were calculated as in the following equations:

$$C = n \times d \times 10000 \quad \ldots(1)$$

Where C= Cell concentration (cell/ml), n= number of counted cells and d= dilution factor= 10.

Total cell count= C (cell/ml) × the original volume of fluid from which the cell sample was taken.

$$\text{Cell viability (\%)} = \frac{\text{Total viable cells (unstained)}}{\text{Total cells counted (stained \& unstained)}} \times 100 \quad \ldots(2)$$

Treatment conditions and experimental groups: To investigate the potential effect of hyperthermia on cell growth and survival, cell viability was assessed by the MTT assay. In brief, the cells were seeded into 6-well plates at a density of 5-105 cells/well and were exposed to MW exposure for 24h. Then they were allowed.

For each exposure time (5, 10 and 15 minutes) the irradiation experiments (6 replicate wells for each group) were done in dark room including the:

Experimental groups for *in vitro* study including the following groups:

Group (A): this group was considered as control group.

Group (A1):This group was treated with AuNps.

Group (A2): PDT group was treated with AuNps.

Group (A3): this group was treated with AuNpsGHS.

Group (A4): this group was treated with AuNpsGHS + Laser Green.

After the irradiation, the plates were sealed with self-adhesive transparent film then incubated at 37ºC.

Results and Discussion

Characterization of Gold Nanoparticles (AuNps): The volume drawn from AuNps stock solution was 125µl. From the curves in Figure (1) when the volume ratio is increased, the surface Plasmon resonance shifts to the right or red shift and increased intensity occurred. These results agree with [63] and become more broadening which indicate a different size or the size of AuNps increased, while the size of AuNps become monodisperse when the volume ratio decreased. This was in agreement with [43, 47].

Table (1) showed the value of the wavelength where the peak of the observance of surface plasmon resonance of gold nanoparticles for different volumes of HAucl4 solution, represents SPRs of Glutathione capped-gold nanoparticles (GSH-AuNPs). From the UV–visible spectra of both AuNPs and nanoglutathione drug capped the gold nanoparticles made the wavelength peak of surface Plasmon resonance slight shift to higher wavelength (red shift). These results are in agreement with [63] and indicated that the size of the gold nanoparticles is increased [37].

Table 1: Wavelengths of SPR for AuNps solutions

Molarity of AuNPs (µM)	SPR wavelength of AuNPS λ (nm)
0.354	526
0.443	523
0.531	520

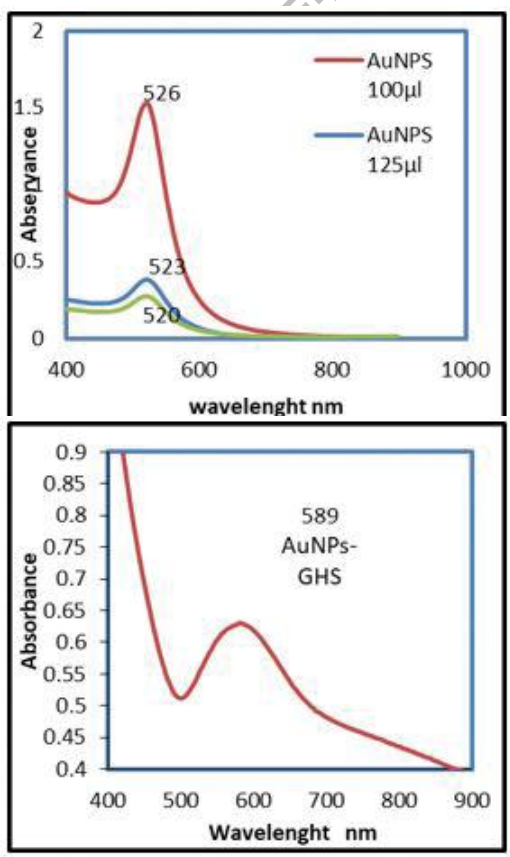

Figure 1: Effect of Glutathione-capped gold nanoparticles (GSH-AuNPs) on Surface Plasmon Resonance results revealed the shift of maximal absorption peak to 589nm after surrounding gold nanoparticle surface with glutathione.

Shape and size of Gold nanoparticles (TEM and SEM): Figure (2) showed a transmission electron microscopy (TEM) which was used to take an image to the gold nanoparticles, with or without coating by nano and GHS, used for measuring the size of gold nanoparticles. The 0.333µM of Chloroauric acid solutions were used to synthesized the gold nanoparticles with or without coating by nanoparticles. The TEM device showed a monodisperse and spherical shape. These results were in agreement with those reported by [20] for the solutions of 0.333µM of AuNps prepared by Brust-Schiffrin.

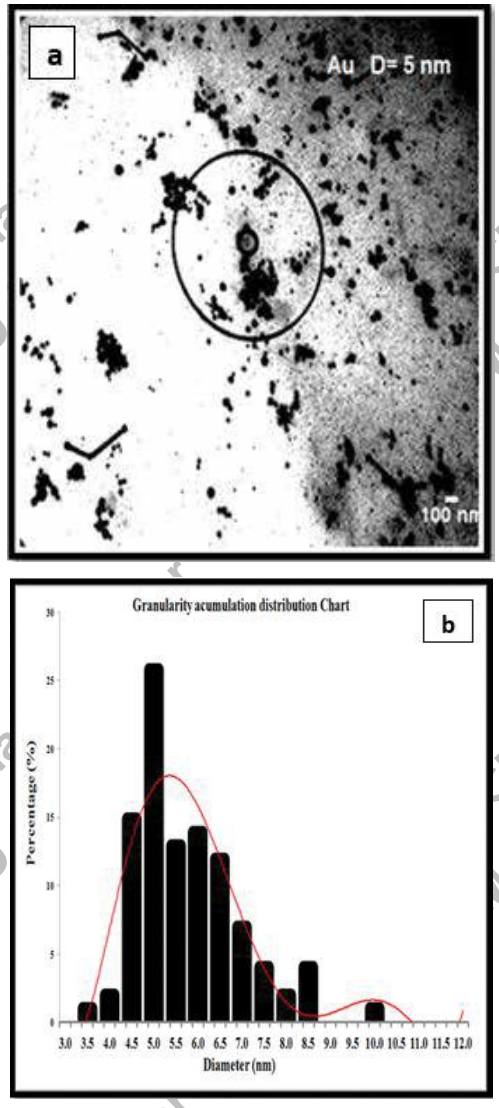

Figure 2: a: TEM image with X92000 magnification power and b: the granulate accumulation distribution chart for AuNps

Figure 3 showed gold nanoparticles Glutathione-capped (AuNps-GSH) prepared by Brust-Schiffrin. It can be noticed the increase in the size of nanoparticles from 5nm to 15nm.

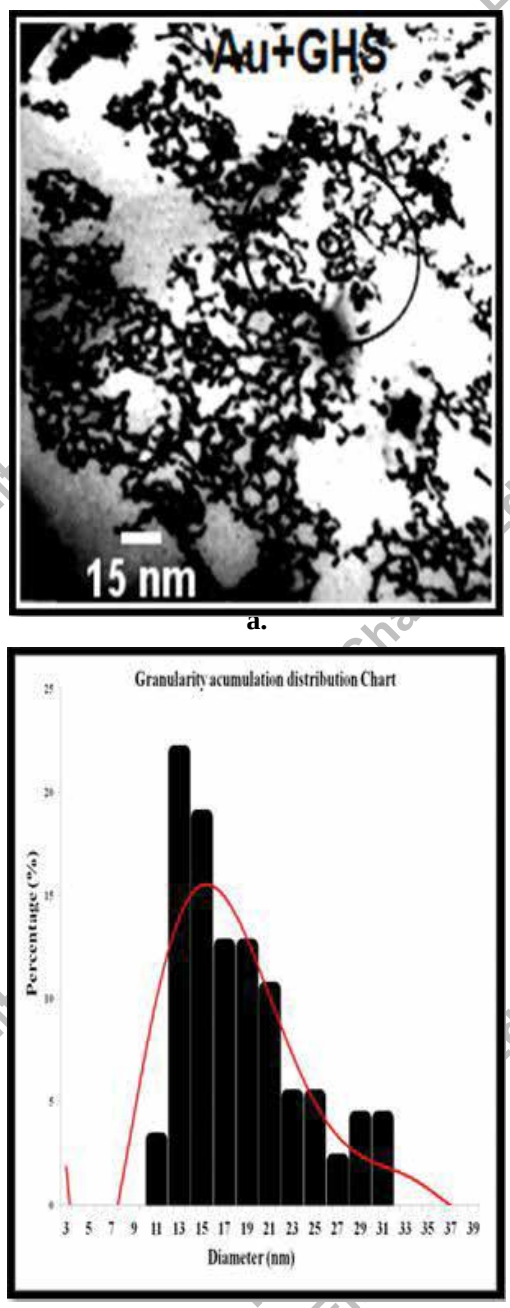

Figure 3: a: Represents Glutathione-capped gold nanoparticles TEM image with ×250000 magnification power and b: The granulate accumulation distribution chart for AuNps and capped glutathione (GHS).

The Effect of Gold Nanoparticles concentration on cancer cells (MCF-7): Different concentrations of uncoated gold nanoparticles of sizes 5nm and 15nm were used to determine the lethal or cytotoxic concentration.

Effects of AuNps cytotoxicity on MCF-7cell line (MTT assay): Figure 4 showed that the viability percentages

of MCF-7 cell line in the presence of different concentrations of AuNps (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125mg/ml) after 24h incubation, ranged from 58.9% to 92.5%. It can be seen that a significant decrease in cell viability was observed when cells were incubated with 0.03125mg/ml GNPs (survival rate= 92.5%). This effect increases as GNPs concentration increased to 1mg/ml (survival rate = (58.92%)). In Figure (4), GNPs with consecrations (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125µ/ml) did not show any significant difference compared to GNPs with consecrations (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125µg/ml) (P= 0.0527). We can calculate the IC50 (the concentration that induced 50% viability) of AuNps for 24h to be 0.253µg/ml. Data revealed that the viability percentage of MCF-7 cell line in the presence of different concentrations of GHS (Glutathione) (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125mg/ml) ranged from 95.95% to 102.6% after 24h incubation. We can calculate the IC50 to be 0.664µg/ml. Cells viability after treatment with Glu-AuNPs (1µg/ml) was reduced to 38.7% after 24h incubation. We can calculate the IC50 to be 0.174µg/ml.

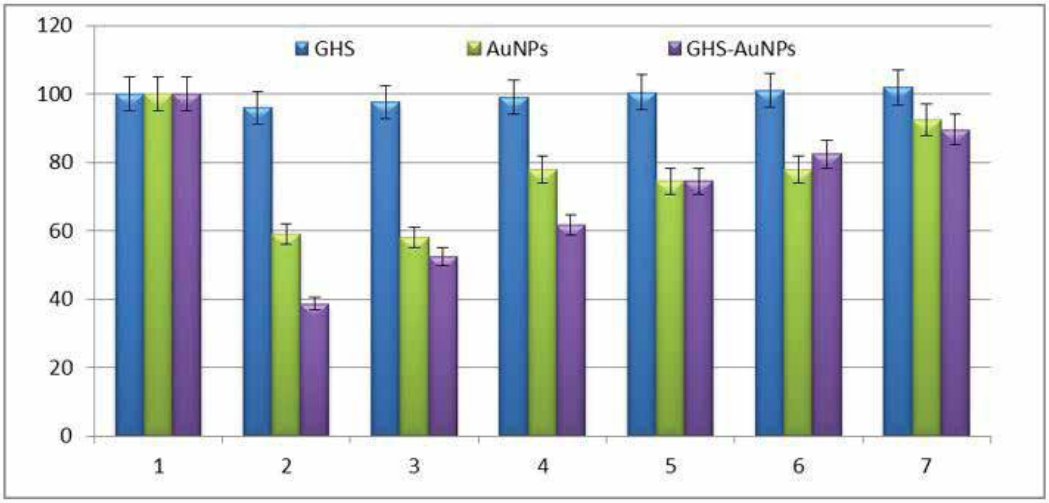

Figure 4: Effect of different concentrations on GHS-AuNps and capped AuNps and GHS on cancer cells

Effect of laser Exposure time on cancer cells: Using green laser of the wavelengths 532nm and output power of 110mw, but the laser exposure times were used in minutes (5, 10 and 15). Plano Convex lens at focal length 3cm at distance 17.5cm from laser aperture has been used as a beam expander to produce a beam diameter of 15mm to cover the area for each well (24 wells). Plates were seeded with MCF-7 cell line. The results showed that the viability percentage of MCF-7 cell line in the presence of different concentrations of AuNps and Glutathione (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125mg/ml) was ranged from 23.06% to 56.3% at 15-minute time, while at 10- and 5-minute times, it ranged from 16.83% to 65.6% and from 26.2% to 85.8%, respectively (Figure 5).

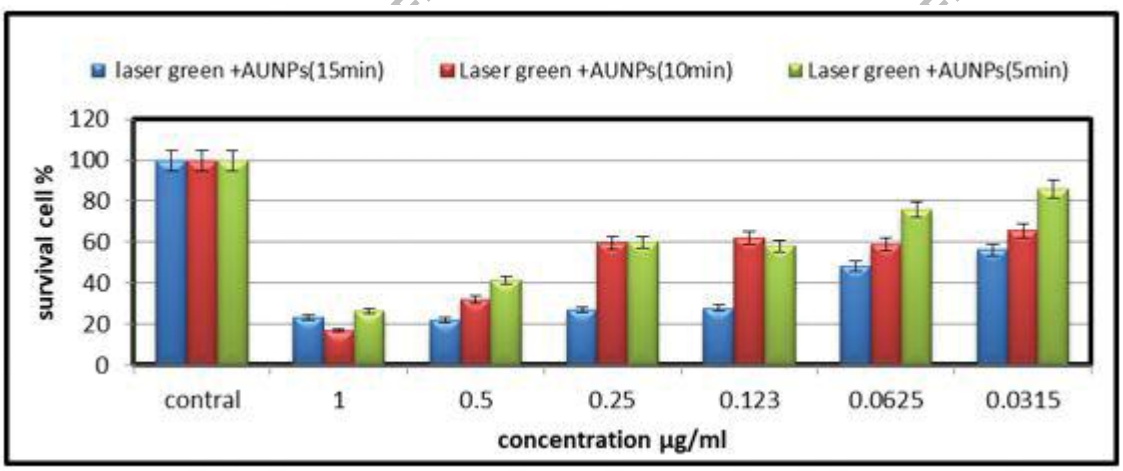

Figure 5: Effects of different concentrations and Laser green and AuNps on cancer cells

Moreover, Figure (6) showed that the viability percentage of MCF-7 cell line in the presence of different concentrations of AuNps and Glutathione (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125mg/ml) ranged from 18.51% to 40.4% at 15-minute time, while at 10- and 5-minute times, it ranged from 23.5% to 82.6% and 45.4% to 83.8%, respectively.

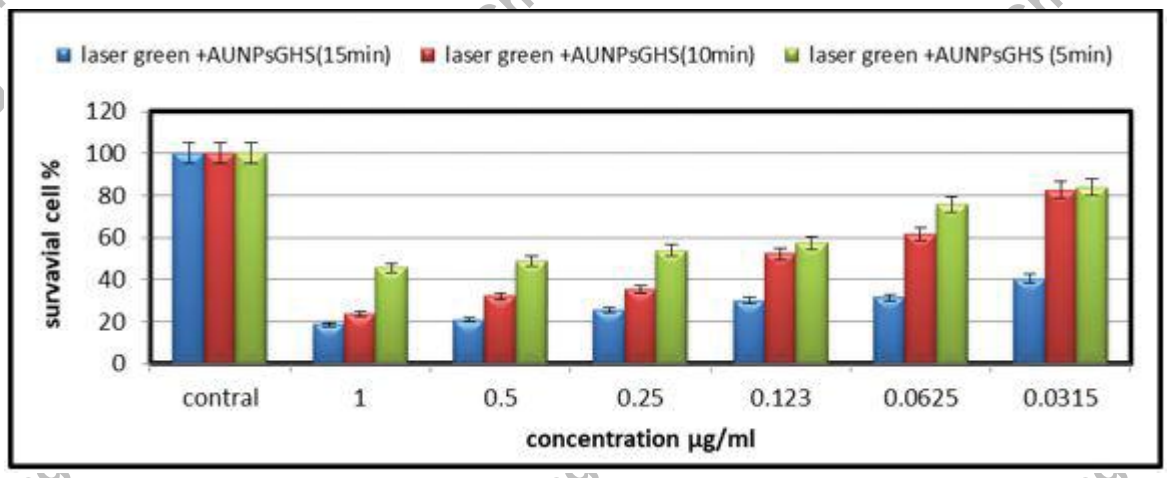

Figure 6: Effects of different concentrations and Laser green-capped AuNpsGHS on cancer cells

Conclusions

The resulting evidence suggested that Glutathione-capped AuNps were non-toxic up to the maximum recommended dosage. Therefore, the demonstrated biocompatibility offers the potentials of Glutathione-capped AuNps as a medicine for cancer therapy.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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