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Formulation of ceftriaxone conjugated gold nanoparticles and their medical applications against extended-spectrum β-Lactamase producing bacteria and breast cancer

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Running title: Formulation and antimicrobial activity of Cef-AuNPs
Abstract

Gold nanoparticles (AuNPs) and their conjugates have been getting a great medical advantage. Extended-spectrum β-lactamases (ESBL)-producing bacteria demonstrate a challenging problem for health care. Aim of the study was the biosynthesis of AuNPs using *Rosa damascenes* petals extract and conjugation to ceftriaxone antibiotic (Cef-AuNPs) to inhibit ESBL-producing bacteria and study assesses *in vitro* anticancer activity. Characterization of the synthesized AuNPs and Cef-AuNPs was studied. ESBL-producing strains, *Acinetobacter baumannii* ACI1 and *Pseudomonas aeruginosa* PSE4 were used for testing the efficacy of Cef-AuNPs. The cells of MCF-7 Breast cancer were treated with previous AuNPs and Cef-AuNPs at different time intervals. Cytotoxicity effect of apoptosis and its molecular mechanism were evaluated. Ultraviolet-visible spectroscopy and Fourier transform infrared spectroscopy established the formation of AuNPs and Cef-AuNPs. Transmission electron microscope demonstrated that the formed nanoparticles were of different shapes with size ~15-35 nm and the conjugation were established by a slight increase in size. Minimum inhibitory concentration (MIC) values of Cef-AuNPs against tested strains were obtained as 3.6 and 4 µg/L, respectively. Cef-AuNPs demonstrated a decrease in the MIC of ceftriaxone down to more than 27 folds on the studied strains. The biosynthesized AuNPs displayed apoptotic and time-dependent cytotoxic effects in the cells of MCF-7 at concentration of 0.1µg/mL medium. The Cef-AuNPs have low significant effects on MCF-7 cells. These results enhance the conjugating utility an old unresponsive ceftriaxone with AuNPs to restore its efficiency against otherwise resistant bacterial pathogens. Additionally, AuNPs may be used as an alternative chemotherapeutic treatment of MCF-7 cancer cells.
Key words: Ceftriaxone conjugated gold nanoparticles, Antimicrobial activity, Extended spectrum β-Lactamase (ESBL) producing bacteria, MCF-7 Cells, MTT assay.

Introduction

The respiratory tract infections (Chronic and acute) are a cause of the inappropriate antimicrobial agents. These agents lead to the resistance development and the opportunistic emergence of bacterial pathogens that substitute the indigenous microorganisms[1]. *Acinetobacter baumannii* (*A. baumannii*) bacteria and *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria belongs to the ESKAPE pathogens group (*Enterococcus faecium, A. baumannii, P. aeruginosa, Klebsiella pneumoniae, Enterobacter species, and Staphylococcus aureus*) that are common reasons of life-threatening nosocomial infections among immune-compromised and critically ill patients[2]. The occurrence of the ESBLs is suspected in *A. baumannii* and *P. aeruginosa* diseases when resistance to one or more than one of the extended-spectrum cephalosporins (ESCs) (ceftriaxone, cefotaxime, cefepime or ceftazidime) [3, 4]. The ESBLs Production has become increasingly prevalent among *P. aeruginosa* and *A. baumannii* [5-7]. The ESBLs enzymes cause inactivating the antibiotics aztreonam, cephalosporins, penicillins and the β-lactamase inhibitors, which limits the number of effective antibiotics for treatment[3, 4]. The studies on risk factors for infectious diseases due to ESBLs-producing *A. baumannii* and *P. aeruginosa* are limited and have been related to respiratory tract infections[8, 9].

Application of nano-materials to inactivate pathogens; this is in virtue of the inability of such agents to induce resistance in bacteria. Also, nanotechnology appears a key role in
treatment of different metabolic and pathological disorders including tumor, HIV, liver
diseases, arthritis and inflammatory, in addition to the modern medicinal applications [10,
11]. Recently, the biosynthesis of metal nanoparticles (MNPs) with novel, eco-friendly,
and non-toxic, convenient plant extracts [12] is under many studies. In the MNPs
biosynthesis, the active biogenic functional groups of plant extract play a key role by
acting as biological reducing, capping and stabilizing agents [13]. The biosynthesized
MNPs can also have reduced toxicity, enhanced biocompatibility and stability, mainly
due to coating them with capping agents or biogenic surfactants. The biosynthesized
nanoparticles are used in medicinal applications, which contain drug delivery,
disinfection, an anticancer agent, imaging, and tissue repair[10]. The AuNPs appear
countless cancer treatments effects due to their amazing interactions on the cancer cell
surface [14]. Because of nontoxicity and non-immunogenicity, AuNPs are ideal for drug
delivery preparation. Also, the AuNPs act as the excellent potential vehicle and highly
attractive for the drug delivery applications due to their functionalization property [15].
On the other hand, AuNPs alone are believed to lack antibacterial efficiency; it can be
conjugated with antibiotics to enhance antibacterial efficacy [16]. AuNPs does not
contain a reactive oxygen species (ROS) mechanism, although ROS damage is the
purpose of cellular death stimulated by some bactericidal nano-materials and antibiotics
[17].

Currently, there are no studies for using Cef-AuNPs to control ceftriaxone resistance
against ESBLs-producing bacterial strains *P. aeruginosa* PSU4 and *A. baumannii* ACII1
(as shown in table 1). These strains were isolated and identified in our previous study[18].
Therefore, this study was designed to look for a new antimicrobial agent such as Cef-
AuNPs as a good alternative to the antimicrobial compounds of ESBLs-producing bacteria. Also, this work is to assess the in vitro anticancer activity of biosynthesized AuNPs and Cef-AuNPs on MCF-7 breast cancer cells. Thus, the present study is an attempt to revive an old ineffective drug into an effective drug against ESBLs-producing bacterial pathogens using AuNPs as a tool. To achieve the same, we synthesized AuNPs, conjugated them with ceftriaxone, and performed characterization of them by UV-visible spectroscopy, Transmission Electron Microscopy, Fourier transform infrared spectroscopy followed by pertinent antibacterial efficacy and anticancer assays. The strategy summarized in the current study is expected to work as a scientific scaffold based on which a series of new nano-formulations meant for “reviving” old inefficient antibiotics into potent therapeutics could be designed.

Materials and methods

Bacteria and growth conditions

*P. aeruginosa* PSU4 and *A. baumannii* AC11 strains were possessed from our previous study[18]. These strains were ESBLs positive and displayed complete resistance to antibiotic ceftriaxone. Before performing the antibacterial activity test, fresh inoculum of each bacterial strain was inoculated in nutrient broth medium and the culture was incubated at 37°C for 18 h. However, turbidity of bacterial cells was detected with the nutrient broth to the common standard (i.e., 1.5×10⁸ CFU/ml).

Preparation of *Rosa damascenes* petal extract

The fresh and healthy *Rosa damascenes* petals were cleaned several times with MilliQ water until no foreign material remained. Twenty-five grams of petals were finely cut
and stir with 100 ml of de-ionized water at 85°C for 20 min. The petal extract was filtered with Whatman number one filter paper and the filtrate was stored at 4°C for further experiments as reducing agent and stabilizer, being usable for within 2 weeks[19].

**Biosynthesis of AuNPs**

*Rosa damascenes* petal extract was prepared as above. Five ml of petal extract was added drop-wise into 50 ml of 1 mM aqueous solution of HAuCl₄ with constant stirring at different temperature and different pH. The color change was monitored [20]. After complete incubation, the AuNPs were collected by centrifuge at 15,000 g for 30 min and washed with de-ionized water. However, 50% ethanol was used to remove excess petal extract from the nanoparticles and the AuNPs sample was kept at room temperature until further characterization.

**Conjugation of ceftriaxone with gold nanoparticles**

In our study, ceftriaxone conjugated gold nanoparticles (Cef-AuNPs) were synthesized in a two-stage procedure. The previous method for synthesis of AuNPs considered the first stage procedure. In the second stage procedure, ceftriaxone (1mg/ml) were mixed with AuNPs (1mg/ml) in phosphate buffer solution, incubated for 24 h, and collected at 12,000 g by ultracentrifugation for 10 min [21]. Freshly synthesized Cef-AuNPs was dialyzed using a 10 kDa cellulose membrane against Double-distilled water over-night to remove any unreacted chemicals. Once dialyzed, the samples were freeze-dried. The Cef-AuNPs were obtained and were stored to further characterization.

**Characterization of AuNPs and Cef-AuNPs**
Nano-materials were characterized according to Harshiny, et al.[21].

**UV–visible spectroscopy**

Analysis of UV–Vis spectra was done by using a double beam spectrophotometer (Shimadzu uv-1650 pc spectrophotometer, Osaka, Japan). The solutions of AuNPs and Cef-AuNPs were monitored by measuring the UV–Vis spectrum of the solution in the wavelength range 300–800 nm. The solution of nanoparticles was poured into a test tube and diluted four times with MilliQ water. Finally, it was analyzed at room temperature.

**Transmission electron microscopy (TEM)**

The TEM technique was used to show the size and shape of the Cef-AuNPs and AuNPs. The TEM (model JEOL 100CX II with an accelerating voltage of 100 kV, at Electron Microscope Unit, Assiut) was used to determine the TEM image. The sample was prepared by the piping of aqueous Cef-AuNPs or AuNPs sample drops on carbon-coated copper grids. Then the film on the grid was dried.

**Fourier transform infrared spectroscopy (FT-IR)**

A powder of petal extract, AuNPs, ceftriaxone, and Cef-AuNPs were prepared by centrifuging the solution of nano-materials at 15,000 g for 10 min. The residue of previous products was washed with bi-distilled water to remove any unattached biological materials from the surface of the nanoparticles. Each residue was then dried, and the powder was used for measurement of FT-IR, which was detected in the 4000–500 cm\(^{-1}\) region with a spectrometer (Shimadzu IR-470, Japan). The one hundred milligrams dried sample was mixed with 100 mg of grade KBr and pressed into discs under
hydraulic pressure. The FT-IR peaks were identified and expressed in wavenumbers (cm\(^{-1}\)).

**Determination of conjugating efficiency of Cef-AuNPs**

Conjugating efficiency of Cef-AuNPs was calculated as Shaker and Shaaban [22]. The Cef-AuNPs were centrifuged for 10 min at 12,000 g. Free ceftriaxone drug in the supernatant was quantified by the same aforementioned double beam spectrophotometer (\(\lambda\) max 330) obtained from wave scan [21]. The calibration curve of ceftriaxone antibiotic was schemed in the linear range of 10–100 mg/ml. We need to find out the amount of ceftriaxone attached to the AuNPs to calculate the percentage of ceftriaxone conjugating efficiency. For this, we took out the free drug remained in the supernatant (or the drug not attached to the AuNPs) from the initial amount of ceftriaxone added and used the following equation:

\[
\text{Conjugating efficiency (\%)} = \frac{\text{Total amount of ceftriaxone} - \text{Free ceftriaxone in the supernatant}}{\text{Total amount of ceftriaxone}} \times 100.
\]

**Antibacterial activity of Cef-AuNPs using well agar diffusion method**

The AuNPs and Cef-AuNPs solutions were tested for their antibacterial efficacy against studied ESBLs-producing bacteria using the well agar diffusion method [23]. The pathogens cultures were initially inoculated in nutrient broth medium followed by incubated at 37 °C for 18 h. The overnight grown cultures were then again sub-cultured into a nutrient broth for 2 h till 0.01 optical densities. Thus, 100 µl of each bacterial culture was distributed onto the surface of nutrient agar medium and wells of six mm were made on nutrient agar plates. Ceftriaxone, AuNPs and Cef-AUNPs with different concentration were pipetted into the wells and the plates were incubated at 37 °C for 24
hours. The inhibition zone was detected by recording the diameter of clear area around each well.

**Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Cef-AuNPs**

The MIC evaluation was performed on a 96 well plate. Two hundred μl of AuNPs or Cef-AuNPs were piped into the 6 wells in column 1. One hundred μl of broth growth medium for each strain were piped into the wells in each row. Thereafter, 100 μl of the AuNPs or Cef-AuNPs solution was taken from column 1 and was serially diluted along the row until column number 10. Thereafter, 5 μl of the bacterial cultures were piped into each wells containing the suitable medium except column number 10, which considered as blank. Moreover, 50 ml of phenol red dye (2 mg/ml) was pipetted in each well to estimate the viability of bacteria. The plate of 96-well was incubated and red color of bacterial cell viability was detected after incubation time for 24 h at 37 °C. The inhibition of growth was detected by showing no color in the well. The minimum concentration of Cef-AuNPs at which no growth or no color was observed has been considered as MIC value. However, after inoculation of five μl from each well on the surface of agar medium plates, minimum bactericidal concentration (MBC) was evaluated. The incubation of bacterial culture plates were obtained at 37°C for 24 h. Finally; the agar medium plates were examined for growth or no growth. The wells containing the minimum concentration of test solutions, showing no growth, were declared as the MBC [24].

**Anticancer activity**

**Maintenance of cell cultures**
The MCF-7 cells (ATCC HB8065, Manassas, VA) used in this study were obtained from Collection of American Type Culture by National Research Center-Egypt. The human breast cancer MCF-7 cell line was kindly provided by Dr. Khaled Fekry -National Research Center-Egypt. The MCF-7 cells were propagated and maintained in Eagles Minimum Essential Medium (EMEM) supplemented with (10%, v/v) Fetal Bovine Serum at 37 °C in a CO₂ incubator (100% relative humidity, 5% CO₂, and 95% air). The cells were harvested after brief trypsinization.

**Trypan blue dye exclusion assay**

The influence of biosynthesized AuNPs and Cef-AuNPs on the viability of MCF-7 cancer cells were evaluated using the trypan blue dye exclusion assay. The cells were seeded (0.5 × 10⁵ cells/ml) in six-well plates in complete medium. The MCF-7 cells were mixed with 0.1μg/ml of each treatment (AuNPs and Cef-AuNPs). Additionally, different times were used for incubation the MCF-7 cells, these times was 24h, 48h, and 72h. After the periods of incubation, the cultures of MCF-7 human cancer cell line were harvested, washed and suspended with phosphate buffered saline (PBS and 0.4% trypan blue) for preparation t next step. Finally, the hemocytometer apparatus were used for counting the MCF-7 cancer cells [25] and each experiment was carried out in three replications.

**MTT assay**

In order to evaluate the cytotoxicity of AuNPs and Cef-AuNPs, the MCF-7 cells were collected in the exponential phase of growth followed by seeded into 96 well tissue culture plates. Additionally, the adherent of cells was allowed for 24 h. Thereafter, 0.1 μg/ml of each AuNPs or Cef-AuNPs concentration was piped to the desired wells and incubated for 48 h. After incubation, 20 μl of the Eagle's Minimum Essential Medium
(EMEM) containing MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (5 mg/mL) was piped to each well and incubated for 4 h at 37 °C. Then, the EMEM medium was changed with 100 μl of dimethyl sulfoxide (DMSO) [26]. The viability of MCF-7 human cancer cells was detected by appearing the purple color of the formazan crystals formation. The optical densities were measured at wavelength 570 nm. All experimental measurements of optical densities were determined in triplicate and expressed as the mean standard deviation.

**DNA Fragmentation Assay**

The fragmentation assay of DNA was carried out using the agarose gel electrophoresis. After 72 h, AuNPs and Cef-AuNPs treated cells were harvested by centrifugation. The cell pellets were mixed with lysis buffer (100 ml) followed by incubation for 1 hour at 50°C. Proteinase K (10 μl) was added and incubated for 30 min at 50°C. Three microliters of RNase enzyme was then added and incubated for 2 h at 50°C. The DNA was extracted using isoamyl alcohol: chloroform: phenol (25:24:1) and 2.5 volumes of absolute ethanol. Two percentage of agarose gel was used and stained with ethidium bromide dye. The fragmented was observed using UV white light transilluminator.

**Gene Expression Analysis for Apoptotic Related Genes**

Using the manufacturer’s protocol, TRIZOLA reagent (Invitrogen, CA, USA) was used for extraction the total RNA from control and treated MCF-7 cultured cells. The first strand cDNA formation was performed using the reverse transcription Maxime RT PreMix kit. Bax and TNF-a gene expression was determined using reverse transcription-polymerase chain. Then, the PCR amplification reactions were performed using the PXE 0.5 Thermo.
thermal cycler apparatus. The initial denaturation step of PCR amplification reactions were performed for 5 min at 95 °C; then 35 cycles at 95 °C for 30 sec (denaturing); at 55 °C for 40 sec (annealing), and at 72 °C for 1 min (extension); and a further extension at 72 °C for 7 min. The gel bands were stained by ethidium bromide dye, scanned and the signal intensities were quantified by a densitometer. The ratio between the levels of the GAPDH (housekeeping gene) and target genes-amplification product was calculated to normalize for initial variation in sample concentration as a control for reaction efficiency [27, 28].

**Statistical Analysis**

All experiments were carried out three times. Results were represented as means ± standard deviation (S.D). The Statistical Package for Social Science (SPSS, version 11.0) was used to perform statistical analyses. One-way ANOVA (Analysis of variance) was used to compare different time intervals of exposure to the same dose of treatment and P<0.05 was considered as statistically significant.

**Results and discussion**

**Biosynthesis of AuNPs:**

The *Rosa damascenes* petal extract was added to gold chloride solution. After addition, the mixture color changed from colorless to wine-red within 30 min. This is indicating the formation of the AuNPs, as shown in Figure 1 due to the fabrication of AuNPs with the biological reducing agents present in the *Rosa damascenes* petal extract. The biological reducing agents in *Rosa damascenes* petal extract reduced the gold ions to AuNPs. The *Rosa damascenes* petals are known to be a rich extract of phenolic compounds that are used as biological reducing agents. The AuNPs aqueous solution
appeared wine-red color due to the surface Plasmon vibrations excitation. Samples of AuNPs were removed and analyzed by using UV visible-spectroscopy for the confirmation of AuNPs formation at regular time intervals. It was noticed that the complete color change took about 1 hour, thereafter no further color of the reaction mixture changed. This is indicating the complete reduction of HAuCl₄ in the reaction mixture. The protection, stability, and reductive activity of plant biomolecules are accounted for the reduction of gold ions [29]. They have advantages over other metal nanoparticles (MNPs) for being biocompatible and non-toxic nature [30].

**The formation of biosynthesized AuNPs conjugated with ceftriaxone**

Ceftriaxone has carboxylate group through which it can adsorb to the amino group of AuNPs surface[31]. UV-Vis absorption was also studied after the formation of AuNPs and ceftriaxone conjugates; Cef-AuNPs were dispersed and absorbance was measured by using UV–visible spectrophotometer between 300 and 800 nm. The Cef-AuNPs showed peaks at 330 nm and 530 nm while The UV–visible peak at 530 nm was for pure AuNPs (as shown in Figure 1).

**Transmission electron microscope analyses**

The AuNPs, as well as the Cef-AuNPs were characterized using TEM analysis (Figure 2A and B). Using TEM analysis, the size of AuNPs and Cef-AuNPs were determined to be at 14.2–25.3 nm and 26.6–45.8 nm, respectively. These results suggested that there was an increase in the size of AuNPs due to the attachment of ceftriaxone to AuNPs and these results agree with Shaikh et al.[32]. TEM analysis appeared that all these AuNPs were spherical hexagonal and triangle shape.
Comparison of FTIR spectra of Rosa damascenes petal extract, AuNPs, ceftriaxone, and Cef-AuNPs:

The FTIR were displayed in the mid-infrared region (MIR) within the range (500–4000 cm$^{-1}$), as shown in Figure 3. The FTIR analysis further confirmed the presence of functional groups representing bioactive compounds of Rosa damascenes extract through the absorption bands at 3438 cm$^{-1}$, implying OH stretching, 1631 cm$^{-1}$, denoting a carbonyl group, 1409 cm$^{-1}$, representing the C-N bond and 1042 cm$^{-1}$ of C–N stretch as shown in Figure 3A. Au metal ions caused a reduction in the absorbance intensities at 3437 cm$^{-1}$, representing OH stretching. Absorbance peak at 1631 cm$^{-1}$ (carbonyl groups), 1425 cm$^{-1}$ (C–N stretching vibrations), 1019 cm$^{-1}$ (C–O) and 543 cm$^{-1}$ (–CH– stretch) have shown that organic compounds in the plant extract have stabilized AuNPs, thereby preventing agglomeration (figure 3B). Ceftriaxone conjugation was also confirmed by FTIR spectroscopy (Figure 3C). The absorption peaks appeared in the FTIR spectrum of ceftriaxone was 3425 cm$^{-1}$, 3255 cm$^{-1}$, and 2796 cm$^{-1}$ which could be assigned to the stretching vibrations of N–H, O–H, and C–H groups, respectively. The stretching vibrations of carbonyl groups, C–N, C–O and =C–H appeared at 1657 cm$^{-1}$, 1356 cm$^{-1}$, 1030 and 750 cm$^{-1}$, respectively. The vibration modes of C–O and C–C groups were represented by the absorption peaks in the region from 600 cm$^{-1}$ to 500 cm$^{-1}$, respectively [21].

As shown in (Figure 3D), the conjugation of ceftriaxone with AuNPs resulted in merging and the reduction of bands N-H 3425 cm$^{-1}$, 1657 cm$^{-1}$ carbonyl groups, 1356 cm$^{-1}$ C–N and 1030 C–O cm$^{-1}$ [21].
Calculation of conjugating efficiency

Conjugating efficiency is estimating important parameter for the characterization of nanoparticles conjugates. The ceftriaxone conjugating of efficiency AuNPs was found to be 81.5 %. During preparation of ceftriaxone conjugated AuNPs, the lesser amount is needed for its therapeutic use because the conjugating efficacy could be high so that ceftriaxone is not lost [22, 31].

Antibacterial activity

The antibacterial activities of the biosynthesized AuNPs and Cef-AuNPs were studied by a qualitative well agar diffusion assay on both bacteria. Table 2 appeared the inhibition zone (ZOI) for each pathogenic strain. After 18 h of incubation at 37 °C, the nutrient agar plates containing ceftriaxone, AuNPs and Cef-AuNPs conjugates exhibited a ZOI around 6–29 mm. The control plates and ceftriaxone antibiotic devoid of AuNPs did not exhibit any inhibition zones. *P. aeruginosa* and *A. baumannii* showed zones of inhibition of 6 mm for ceftriaxone, 6 and 7 mm respectively, for AuNPs, and 27 mm and 29 mm inhibition zones respectively, for Cef-AuNPs. The results suggested that the Cef-AuNPs were highly effective against bacterial strains compared to ceftriaxone and AuNPs alone. Cef-AuNPs conjugate appeared greater antimicrobial efficiency against both bacteria strains.

Minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC) assays

MIC of Cef-AuNPs was found as 3.6 and 4 µg/mL against the tested strains of *P. aeruginosa*, and *A. baumannii* respectively (Table 2). Whereas, MBC of Cef-AuNPs was
determined to be 16 µg/mL for each strain. Ceftriaxone-AuNPs significantly decreased the MICs of ceftriaxone against *P. aeruginosa* and *A. baumannii* by more than 27 folds. Because of the unique physiochemical properties of AuNPs such as biocompatibility and non-cytotoxicity, they were used as drug delivery tools in various studies [33]. Additionally, conjugation ampicillin and carbapenems with AuNPs retained its activity and showed potent effect against multi-drug resistant bacteria while AuNPs alone were non-toxic [22, 34]. In the same way, our data also implicate that ceftriaxone retain their activity after conjugation to AuNPs because unconjugated gold nanoparticles were inactive against the tested drug resistant strains.

**Mechanism of overcoming resistance by ceftriaxone-AuNPs (Hypothesis)**

Figure 4 showed TEM of *P. aeruginosa* which treated with 3.6µg/ml Cef-AuNPs. Conjugated nanoparticles accumulated in the cell wall of bacteria and inside the cell. Regarding the overcoming resistance mechanism in this particular piece of work, we could propose the following hypothesis. The effective concentration of ceftriaxone delivered to the bacterial cell was increased due to its binding to AuNPs. It was a two-way damage to bacteria. The first way could be genuinely expected to be due to the increased presence of ceftriaxone molecules per unit volume of the system, that is, the ceftriaxone molecules which retained untouched by the bacterial enzyme. Ceftriaxone works by inhibiting the mucopeptide formation in the bacterial cell wall [35, 36]. The cell membrane disruption due to previous drug results in leakage of the cytoplasmic content, lysis and death of the bacterial cell. Increased porosity of the membrane attributed to The second damage of cell membrane [17]. Once Cef-AuNPs enter the bacteria they easily interrupt all of the membrane regulatory functions, effectively inactivate the bioactive
blockage of protein synthesis, sulfur containing proteins, and interact with the phosphorous in the nucleic acid (i.e. RNA and DNA) structure [35, 36].

**Anticancer activity**

The metal nanoparticles have confirmed their applications in the medical field to diagnose, treat various types of tumor and other common diseases. The AuNPs that it based on biological *Rosa damascenes* extract molecules, are new and revolutionized to treat malignant deposit and without interfering the normal cells. In the present study, the cytotoxic effect of AuNPs, and Cef-AuNPs on MCF-7 human breast cancer cells showed that the biosynthesized AuNPs appeared the cytotoxicity and a direct time-response relationship against the MCF-7 cells. It was enhanced by increasing the exposure time. The maximum inhibition of growth and decreased in viability were appeared in MCF-7 cells treated with AuNPs for 72 h at a dose 0.1 μg/ml (as shown in Table 4). This exposure time was selected for further studies. The results obviously expose that AuNPs induce cytotoxicity in the MCF-7 cancer cells in a time dependent manner. This is reflecting effectively inhibits the MCF-7 cells growth by 20.3, 33.2 and 48.3 % for 24h, 48h and 72 h, respectively and the loss of viability.

There were also, morphological changes in MCF-7 presented by cell shrinkage, blebbing and pyknotic nuclei when the cell treated with AuNPs for 72 h compared to cells treated with Cef-AuNPs and a significant decreased in the cellular crowding was seen in comparing to the normal attached MCF-7 human breast cancer cells as shown in Figure 5. However, a typical apoptotic appearance of MCF-7 breath cancer cells, such as apoptotic bodies, fragmented nuclei, and chromatin condensation, were observed in a time-dependent manner and the changes in appearance were most obvious after 72 h of both
treatments. In this concern, Mohseni et al.[37] reported that AuNPs were significantly
toxic to the MCF-7 cells at the concentration of 2.50 mg/mL and higher and this result
was in agreement the present result. Various studies reported that plant derived AuNPs
have a potency to control the tumor cell growth. The improved AuNPs cytotoxic efficacy
is due to biological capping composition and other secondary metabolites in the
synthesizing medium [38, 39].

The results displayed that the level of DNA fragmentation became more prominent when
the MCF-7 cells treated with AuNPs in compared to control untreated cells (as shown in
Figure 5 and 6); while Cef-AuNPs treatment induced a moderate anticancer activity and
cytotoxic effects in compared to the AuNPs treated cells. The previous results was
confirmed by Selim and Hendi [40] who reported the AuNPs possess anticancer activity
and cytotoxic effects against MCF-7 cancer cells. The moderate concentration of AuNPs
stimulated the apoptosis mechanism in the malignant cells[41].

Finally, AuNPs were prepared from Rosa damascenes petals extract with a small-sized
hexagonal triangle and spherical shapes. In this study, we examined the efficacy of using
AuNPs as ceftriaxone delivery system for the control of multi-drug resistant respiratory
tract infections. These Cef-AuNPs showed greater antimicrobial activity against ESBLs-
producing bacteria strains, P. aeruginosa and A. baumannii bacteria isolated from the
sputum. In this study, antibacterial assays displayed that ceftriaxone after attachment with
AuNPs got “revived” and ESBL producing bacterial strains which were completely
resistant to ceftriaxone turned sensitive to the Cef-AuNPs. The ceftriaxone-AuNPs might
be used as an alternative of antimicrobial agents to ESBL producing bacteria. In future
study we will come up with fresh nano-dugs of old antibiotics to control drug resistance
bacteria, that is, “regeneration” of old antibiotics so as to keep pace with the decreasing stock of antibiotics. Our data confirmed that biosynthesized AuNPs decrease the development of MCF-7 cancer cells in vitro through a remarkable increase in cell apoptosis. AuNPs would be an amazing invention in the scope of nanomedicine.

Acknowledgement

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Conflict of Interests

The authors claim no conflict of interest to declare and this is stated in the manuscript.

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Table 1: Resistance patterns of ESBLs-producing bacteria.

<table>
<thead>
<tr>
<th>Names of isolates</th>
<th>Source</th>
<th>Accession number</th>
<th>Antibiotic resistance profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em> AC11</td>
<td>Sputum</td>
<td>LC325252</td>
<td>AK, AMP, AMC, CEC, FOX, FEP, CXM, CAZ, <strong>CRO</strong>, CTX, CIP, LVX, DO, SXT, CN, MEM, TZP, IPM</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> PSE4</td>
<td>Sputum</td>
<td>LC189106</td>
<td>AK, AMP, AMC, CEC, FOX, FEP, CXM, CAZ, <strong>CRO</strong>, CTX, CIP, LVX, DO, SXT, CL, IPM, CN, MEM, CLR, TGC</td>
</tr>
</tbody>
</table>

AK, Amikacin; AMC, Amoxy / Clavulanic acid; CEC, Cefaclor; FEP, Cefepime; CTX, Cefotaxime; FOX, Cefoxitin; CAZ, Ceftazidime; CRO, Ceftriaxone; CXM, Cefuroxime; DO, Doxycycline / HCL ; CIP, Ciprofloxacin; LVX, Levofloxacin; CLR, Clarithromycin ; AMP, Ampicillin; MEM, Meropenem; SXT, Sulpha / Trimethoprim; TGC, Tigecycline; CN, Gentamicin; IPM, Imipenem; TZP, Piperacillin-tazobactam; CL, Chloremphenicol.
Table 2: Antimicrobial activity of ceftriaxone drug, gold nanoparticles and ceftriaxone–AuNPs conjugates.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Zone of inhibition for 1 µg/mL sample</th>
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<tr>
<td></td>
<td>ceftriaxone</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> AC1</td>
<td>6 mm</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> PSE4</td>
<td>6 mm</td>
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</tbody>
</table>
Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ceftriaxone-AuNPs against ESBL Positive *P. aeruginosa* and *A. baumannii*.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Ceftriaxone</th>
<th>Ceftriaxone-AuNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MIC</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> ACI1</td>
<td>&gt;100 µg/mL</td>
<td>4 µg/mL</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> PSE4</td>
<td>&gt;100 µg/mL</td>
<td>3.6 µg/mL</td>
</tr>
</tbody>
</table>
Table 4: The Effect of AuNPs and Cef-AuNPs treatment on the proliferative and viability percentage of MCF-7 cultured cells at different exposure times

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Proliferative percentage of MCF-7 cells at different times</th>
<th>Viability % of MCF-7 Cells at different exposure times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24h</td>
<td>48 h</td>
</tr>
<tr>
<td>PBS</td>
<td>93.0 ± 1.73 a</td>
<td>92.4 ± 0.67 a</td>
</tr>
<tr>
<td>AuNPs</td>
<td>79.7 ± 1.54 c</td>
<td>66.8 ± 2.86 c</td>
</tr>
<tr>
<td>Cef-AuNPs</td>
<td>85.8 ± 2.34 b</td>
<td>80.4 ± 1.73 b</td>
</tr>
</tbody>
</table>

Means with different superscripts (a, b, and c) between groups in the same column are significantly different at P< 0.05. Cell numbers were counted and data are expressed as the percentage of untreated control.
Fig. 1. UV-Vis spectrophotometry analysis of AuNPs and Ceftriaxone–AuNPs.
Fig. 2. FTIR analysis of *Rosa damascena* petals extracts (A), AuNPs (B), Ceftriaxone (C) and Ceftriaxone–AuNPs (D).
Fig. 3. TEM analysis of (A) AuNPs and (B) Ceftriaxone-AuNPs.
Fig. 4: TEM analysis of *P. aeruginosa* after exposed to Ceftriaxone-AuNPs, and its accumulation in cell wall (A) and inside the cell (B).
Fig. 5: Effect of various treatments of biosynthesized gold nanoparticles and Ceftriaxone-AuNPs on MCF-7 cells treated for 72 h showing (1); Morphological changes of MCF-7 cells using inverted microscope (2) DNA fragmentation evaluated by agarose gel electrophoresis and (3) Agarose gel electrophoresis of Bax, TNF and GAPDH RT-PCR products.
Fig. 6: Histograms represent the effect of various treatments of biosynthesized nanoparticles for 72 h on: (A) DNA fragmentation percentage in MCF-7 treated cells (B) Agarose gel electrophoresis of Bax, TNF and GAPDH RT-PCR products in MCF-7 cells after different treatments.