

Bio fabrication of gold nanoparticles and their combination with chemotherapy and ultrasound for effective treatment of tumors

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Abstract

Background and Objective: Chemotherapy is one of the important medical options for cancer therapy, but still new combinations of therapeutic treatment methods are needed to achieve efficient anti-tumor activity. In this work, *Pueraria lobata* leaf extract was utilized as a bio-reductant for the fabrication of gold nanoparticles (Au-NPs) by an ecological approach without using any harmful chemicals. This work also evaluates whether the combined effect of chemotherapy along with Au-NPs and Ultrasound (US) is effective over the tumors or not.

Materials and methods: About 10 mL of plant extract was added to 1 mM of H₂AuCl₄ solution (20.0 mL) and pH was maintained at 12 and allowed for stirring for half an hour. The change in solution color from yellow to purple signified the Au-NPs formation.

Results: XRD results and TEM images confirmed the formation of crystalline Au-NPs with sizes ranging from 23-30 nm. Further, biological studies revealed that the combination of Au-NPs and US along with chemotherapy improved the impact of anti-cancer drug.

Conclusion: In conclusion, *Pueraria lobata* leaf extract mediated synthesis of Au-NPs by an eco-friendly approach was reported in this work. The plant biomolecules of the extract were involved in the reduction and capping of the Au-NPs formed. The major conclusion is that addition of Au-NPs with chemotherapy and ultrasound has shown more effective anti-tumor activity.

Keywords: Au-NPs; chemotherapy; ultrasound; plant bio-constituents.

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Introduction

Fabrication of gold nanoparticles (Au-NPs) has gained significant interest from researchers because of their very good thermal and chemical, along with some exceptional features such as catalytic, electronic and optical properties. Au-NPs exhibit great benefits in biological applications when compared to other NPs because of their non-toxic nature and very good cytocompatibility, resulting in their wide scope of applications in drug delivery, tumor therapy, biosensors and bio-imaging¹⁻⁴.

Various physical and chemical approaches have been reported for production of Au-NPs, which possess several disadvantages such as non-eco-friendly nature and

high cost because of the involvement of toxic reducing agents. On the other hand, the usage of natural plant biomolecules, fungi and microbes for the preparation of nanomaterials resulting in an environmental friendly, cost-effective and non-hazardous approach⁵.

In the green synthesis approaches, some biomolecules such as diastase⁶, tyrosine⁷, casein⁸, silk sericin⁹ plant extracts like *Abelmoschus esculentus*¹⁰, *Azadirachta indica*¹¹, *Maduca longifolia*¹², *Citrus sinensis*¹³ and *Hibiscus rosasinensis*¹⁴ have been reported to be used, as they act as reducing and capping agents for synthesis of various nanomaterials. Although several green methods have been reported for the fabrication of Au-NPs, still there is a requirement for the evolution of new techniques for the Au-NPs fabrication for their potential biological applications.

On the other hand, Ultrasound (US) and US contrast agents added with chemotherapy is a method that enhances the permeability of the cell membrane at the focused site and has specific advantages such as low invasiveness, low toxicity, repeated applicability and

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high tissue selectivity¹⁵⁻¹⁶. One of the major popular anti-cancer drugs, CDDP (Cis-Diamminedichloroplatinum (II)) is mostly utilized for treating various kinds of solid tumors¹⁷⁻¹⁹. The CDDP effective delivery into tumors in LNs may be greatly expected to improve its chemotherapeutic activity.

The objective of the current research is to biosynthesize Au-NPs via a bio friendly approach and to evaluate the antitumor efficiency of combining Au-NPs and US along with CDDP. We determined that Au-NPs and US combined with CDDP is efficient in producing a prominent anti-tumor activity.

Materials and methods

Materials

Chloroauric acid (HAuCl₄•3H₂O), Sodium hydroxide (NaOH) and other chemicals were purchased from sigma aldrich chemicals, shanghai. The leaves of pueraria lobata were collected from Shenlongjia forest area, china. Milli-Q water was used for all experiments.

Fabrication of Au-NPs

Initially the obtained leaves of pueraria lobata were collected and dried under sunlight. Later, 4 g of above dried powder is added to 100 ml of water and boiled for 30 min and the mixture was filtered using cellulose nitrate membrane filter paper. The filtrate was used for fabrication of Au-NPs. About 10 mL of plant extract was added to 1mM of HAuCl₄ solution (20.0 mL) and pH was maintained at 12 and allowed for stirring for half an hour. The change in solution color from yellow to purple represented the Au-NPs formation.

Cell preparation

GFP/KM-Luc cells, expressing stably a fusion of green fluorescent and luciferase protein genes, were formed using transfection of fatal fibrous histiocytoma such as the (N/MRL-1) cells²⁰ along with pEGFP-Luc with the help of lipofectin transfection reagent. N/MRL-1 cells were a sarcoma cell line determined from the spleens of MpTn-gld/gld/MRL rats²¹. GFP/KM-Luc cells were stored in the medium, Dulbecco's modified Eagle added with fetal bovine serum of 10%, geneticin (0.5 mg/mL, G418 sulfate) and L-glutamine-penicillin-streptomycin of 1%. The cells are incubated at a temperature of 37°C in a solution of carbon dioxide 5% and air 95% until achieving 80% confluence.

In vitro US treatment

GFP/KM-Luc cells are trypsinized using the solution of EDTA-trypsin (EDTA 0.02% and trypsin 0.25%)

and dissolved at a 4.0×10^4 cells/mL concentration in the same medium utilized in the cell formation. Later, samples of 500-μL were aliquoted in 48-well plates and then incubated at a temperature of 37°C in the solution of carbon dioxide 5% and air 95%. After 24 h, the medium in every well plate was extracted and suspended in a 450 μL of similar medium, which was utilized for the preparation of cell. Moreover, PBS of 50 μL was added to the US control group; PBS and Au-NPs of 25 μL each was added to the Au-NPs + US group; and for the CDDP + Au-NPs + CDDP groups, CDDP of 25 μL in PBS and Au-NPs of 25 μL were added, maintaining the overall CDDP concentration as 250 or 50, 10, 1 μM. In every group, the samples were placed 100 mm overhead a 30 mm diameter US transducer dissolved in the tap water (degassed followed by heating to a temperature of 38°C) and later may be exposed to US (1.0, 0.5, 0.1 W/cm²; 60 s) or may not be exposed. After exposing to US, the samples are incubated at a temperature of 37°C in a solution of carbon dioxide 5% and air 95%. After 24 h, we determined the cell viability with the help of a MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) as demonstrated earlier²². The samples that were not exposed to CDDP, Au-NPs and US were utilized as the controls. Six samples were used to perform every experiment. For every experiment, the results achieved by the treated samples were made to be divided by the average of the control samples for obtaining a normalized cell viability factor.

Characterization

UV-visible spectrophotometer (Thermo Scientific) was used to obtain the optical absorbance spectrum of the Au-NPs at a wavelength from 400 to 700 nm. A Rigakumini Flex 11, XRD instrument was used to know the crystalline nature of Au-NPs with Cu Kα ($\lambda = 1.5406$ Å), 15 mA current at an operating voltage of 30 kV. Tecnai G2 spirit BioTWIN, TEM instrument was utilized to analyse the size and shape of Au-NPs. Sample for TEM analysis was prepared by dispersing the purified centrifuged product in water under ultrasonication conditions. The as prepared dispersion was loaded and dried on a copper grid and allowed for evaporation and then visualized under TEM microscope. A Perkin Elmer FTIR instrument was used to know the surface capping of Au-NPs with plant polyphenols and the measurements were carried out at 400 cm⁻¹ to 4000 cm⁻¹ wavelength range.

Statistical analysis

All the statistical information provided were represent-

ed as the mean \pm standard deviation (SD). Data was conducted with ANOVA. To determine the source of significant variations we utilized Dunnett range post-hoc comparisons, wherever applicable. The value of P less than 0.05 was statistically considered as significant.

Results and discussion

From the UV-Vis spectral analysis (shown in Fig.1), it is found that no optical absorption peak is detected for plant extract. Whereas absorption band is observed at 529 nm indicated the generation of Au-NPs. This absorption peak of Au-NPs is due to phenomenon of surface plasmon resonance of Au-NPs.

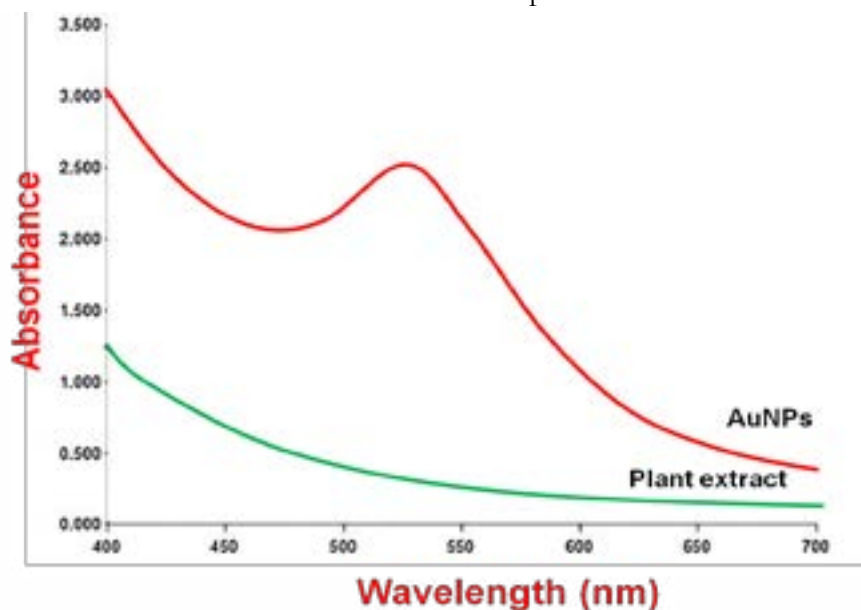


Fig.1. UV-Vis absorption spectrum of plant extract and Au-NPs

From the Fig. 2, it is found that XRD pattern of Au-NPs exhibited 2θ values at 77.300° , 64.400° , 43.825° , 37.875° , with corresponding crystal planes at (311), (220), (200), and (111) respectively. The XRD pattern agrees with face centered cubic structure of Au-NPs. The obtained XRD pattern is similar with the AuNPs prepared by using green reductants such as diastase 14. Fig. 3. shows the TEM microscopic images of the

formed Au-NPs. From the TEM images, it is observed that the formed Au-NPs were in hexagonal shape and the particle size ranges from 23–30 nm. Fig.4 showed the EDS spectrum of Au-NPs, which confirmed the presence of dominant Au elemental signals representing the preparation of Au-NPs. On the other hand, the presence of other elemental peaks may arise from plant extract bio constituents.

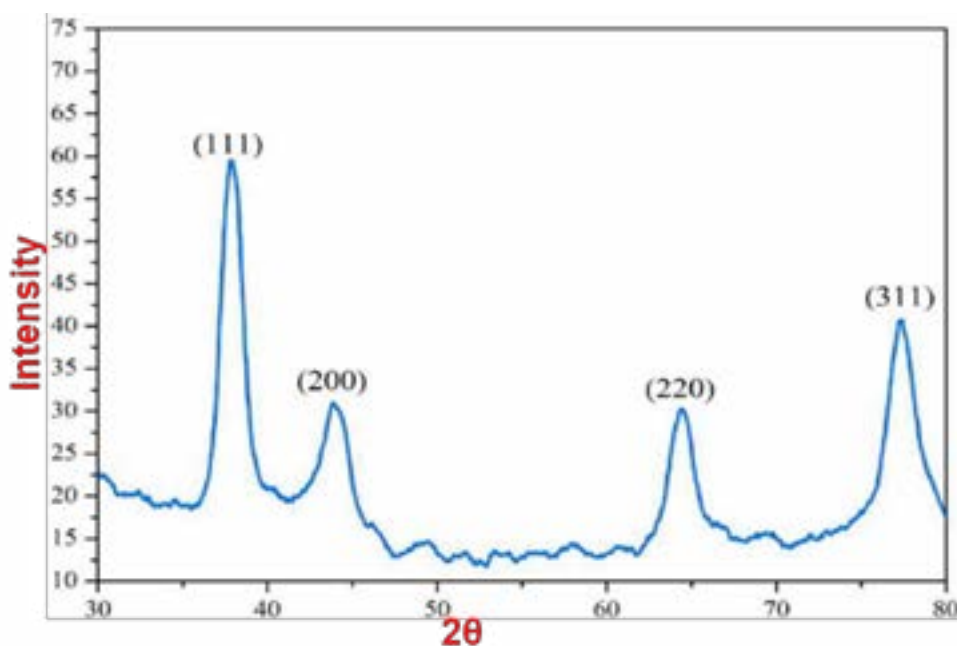


Fig.2. Au-NPs XRD pattern

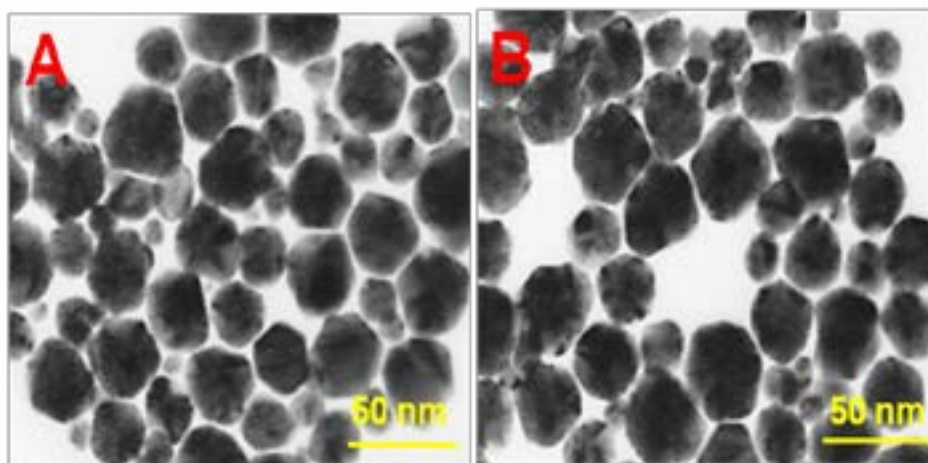


Fig.3. TEM images of prepared Au-NPs

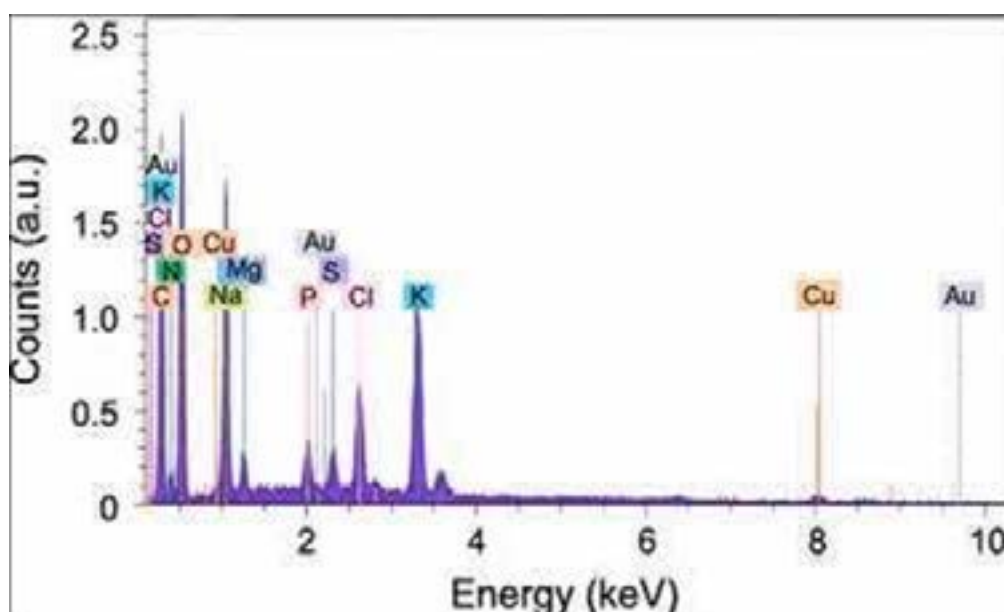


Fig.4. EDS spectrum of Au-NPs

Fig.5 represented the FTIR spectrum of plant extract and Au-NPs prepared. It is exhibited from FTIR spectrum that the -OH and aldehyde functionalities are adsorbed on the surface of Au-NPs while the reduction process continued. It is noted that the vibrational bands of alcoholic groups at 1255.00 cm^{-1} , and phenolic functionalities of plant extract and -CHO groups at 1628.72 cm^{-1} and 542.85 cm^{-1} are considerably weaker in Au-NPs when compared to plant extract. It is also

known from FTIR spectrum that there is a blue shift in bands of Au-NPs spectrum, signifying the change in the chemical environment of plant bio-constituents, indicating that -OH, alcoholic and -CHO functionalities of plant extract are involved in capping of Au-NPs formed. Also, the presence of similar functionalities of green reagent used for reduction on the surface of prepared AuNPs further indicated the capping of plant extract polyphenols onto AuNPs¹⁴⁻¹⁸.

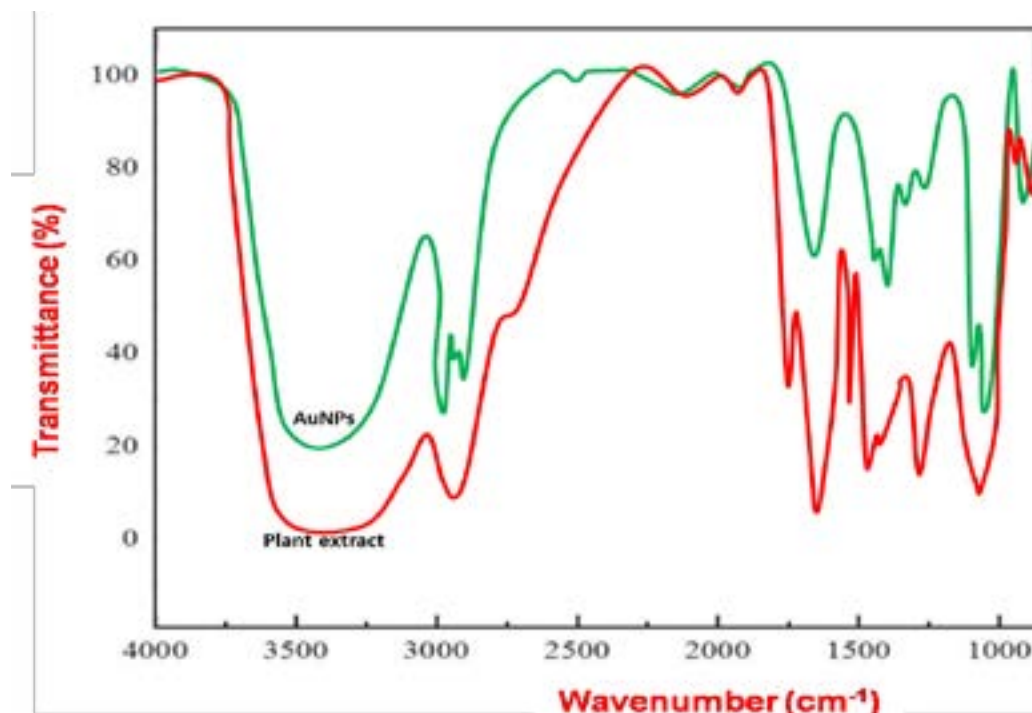


Fig. 5 FTIR spectrum of plant extract and Au-NPs prepared

In vitro US treatment

To determine whether a CDDP anti-tumor effect was enhanced by adding Au-NPs and US, each experimental group's cell viability was recorded using the MTT assay (Fig. 6). Evaluation of the results for the US + Au-NPs + CDDP (1 μ M), Au-NPs + US and US control groups exposed an unique approach towards a normalized cell viability decrease depending on the intensity of US. A

statistically substantial variance in normalized cell viability in-between the Au-NPs + CDDP and US + Au-NPs + CDDP was noticed only when the concentration of CDDP used was at 10 μ M and the intensity of US was 1.0 W/cm² (where p less than 0.05) (Fig. 7). Au-NPs along with US did not considerably improve the anti-tumor activity of high (250 and 50 μ M) or low (1 μ M) levels of CDDP injected.

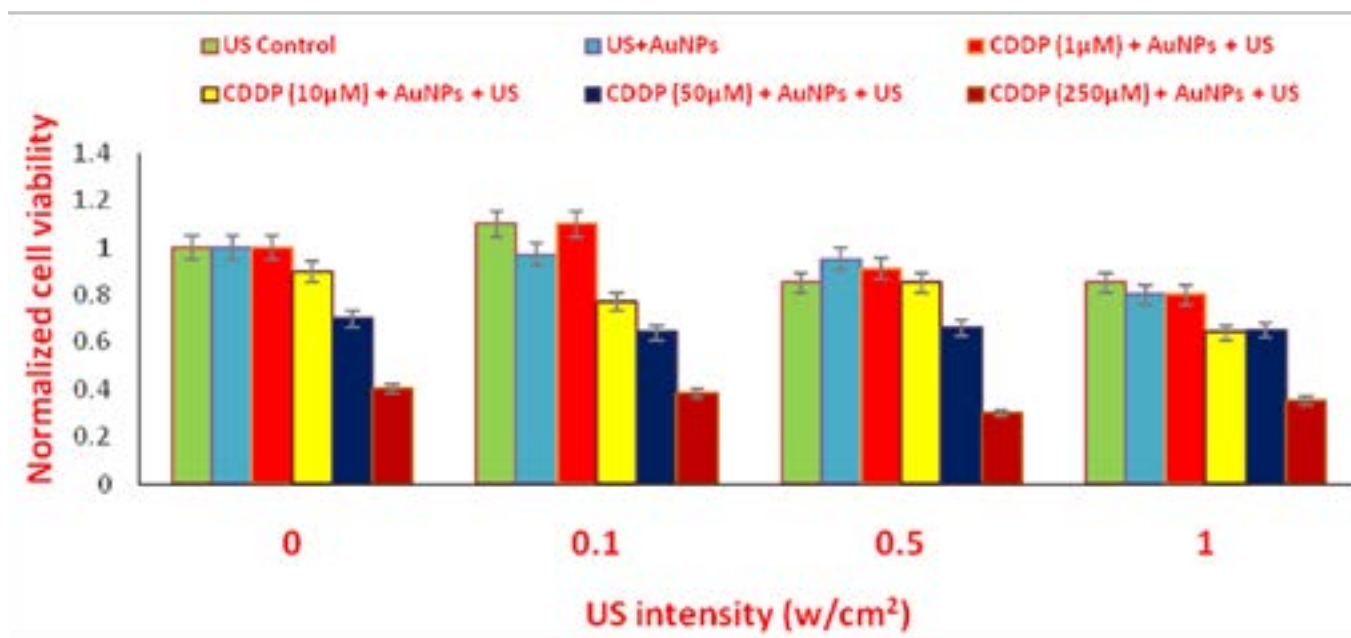


Fig. 6. Ultra sound intensity-dependent decrease in normalized cell viability of the US control, US +Au-NPs and CDDP+ Au-NPs (where CDDP is 1, 10, 50, 250 micro meter

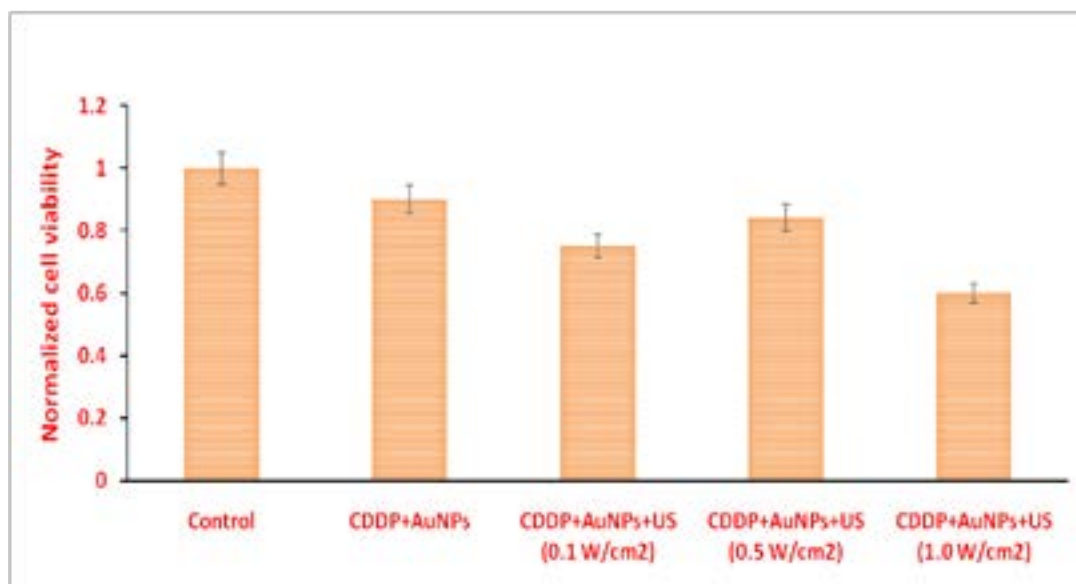


Fig.7. Normalized cell viability of the CDDP+Au-NPs+US (10 micro molar CDDP concentration), where the US intensity changes from 0.1, 0.5 and 1.0 W/cm²

Conclusion

Pueraria lobata leaf extract mediated synthesis of Au-NPs by an eco-friendly approach was reported in this work. The plant biomolecules of extract are involved in reduction and capping of the formed Au-NPs, which was confirmed by FTIR spectroscopic results. XRD results and TEM images confirmed the formation of crystalline Au-NPs with size ranges from 23-30 nm. Further, biological studies revealed that the combination of Au-NPs and US along with chemotherapy improved the impact of anti-cancer drug. The major conclusion is that addition of Au-NPs with chemotherapy and ultrasound has shown more effective anti-tumor activity.

Conflict of interest

None declared.

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