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PREPARATION AND CHARACTERIZATION OF GREEN GOLD NANOPARTICLES INHIBITING K562 CELL APOPTOSIS

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ABSTRACT

Eco-friendly synthesis of nanoparticles, which is one of the most advanced evergreen branches of nanoscience for biomedical application. This synthesis is non toxic and very low cost are major features of production it further attractive prospective option for biomedical field and elsewhere. In this investigation, the anticancer potentiality of AuNPs, stabilized with *Olea europaea* L was studied in K562 cell line. Metallic AuNPs were prepared by reducing with ethanolic *Olea europaea* L extract. The green synthesis of AuNPswas characterized and evaluated by UV-visible Spectroscopic, XRD, FTIR, TEM, DLS and biological activities using various biochemical assays. Green synthesis of AuNPs was confirmed by instrument method. AuNPs were decreased the growth and viability of the K562 cell line and increased the apoptosis.

KEYWORDS: **Green gold, Nanomedicine, Cell line, *Olea europaea* L. (OL), Apoptosis.**

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INTRODUCTION

Nanotechnology, an interdisciplinary research field involving chemistry, engineering, biology and medicine, has great potential for early detection, accurate diagnosis and personalized treatment of cancer. Nanoparticles are typically smaller than several hundred nanometers in size, comparable to large biological molecules such as enzymes, receptors, and antibodies [1-5]. In recent years, gold nanoparticles (AuNps) have been widely used in diverse biomedical applications because of their efficient optical and electronic properties. Gold nanoparticles are also possesses a strong surface chemistry which renders them suitable for attachment with biomolecules. Gold nanoparticles have found wide spread applications in life sciences and attracted significant research interest. In fact, the use of AuNPs has a long history in biology, dating back to the application of "immunogold" in biological imaging. The foremost metal nanoparticles under study are gold (Au). Gold nanoparticles in particular have been extensively investigated due to their unique physical and chemical properties and wide potential applications [6] as in bioassays [7], where colloidal gold nanoparticles are used as protein tags to amplify the signal from a surface plasmon resonance biosensor [7]. Throughout the history of civilization, the olive plant, *Olea europaea* L. (OL) has been an important source of nutrition and medicine. In addition, OL has antioxidant and anti-inflammatory activities. The major active phenolic components in olive leaf are known to be oleuropein and its derivatives, such as hydroxytyrosol and tyrosol, as well as cafeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin- 7-glucoside, and diosmetin-7-glucoside [8-10]. Today, biosynthesis of silver and gold nanoparticles using plants or plant extracts have been reported, the potential of the plants as biological materials for the synthesis of nanoparticles is yet to be fully explored [11-22]. This present study deals with the synthesis of gold nanoparticles using ethanol olive leaf extracts first time. The nanoparticles have been characterized using FTIR, TEM and XRD techniques. The cell viability study has been reported against K562 cell

MATERIALS AND METHODS

All chemicals were used of analytical grade laboratory reagents. All solutions were prepared with deionised water obtained from a Millipore Milli-Q water system, excluding media, which was prepared with distilled water. The chemicals were purchased from Nobel enterprises and used for synthesis of gold nanoparticles (AuNPs). A K562 cell line was maintained in School of Biotechnology, KIIT University, Odisha, India. It was grown in medium (*i.e.*, RPMI-1640) supplemented with 10% foetal calf serum in a humidified incubator at 37°C along with 5% CO_2.

Preparation of Olea europaea L. Ethanol Preparation of Extract

Dried leaves of *Olea europaea* L. (weight-200g) were ground with the outer shells to obtain a coarse powder using an electric mixer. This was then taken in a muslin bag and subjected to Soxhlet extraction using ethanol (90%) as solvent maintained at 60^{o}C for 20 hours. The ethanol extract of *Olea europaea* L. were made free from the solvent by using a rotary evaporator. The yield obtained was 7% (w/w). Several scientific investigations are that *Olea europaea* L. contains high levels of antioxidant polyphenols.

Synthesis of Gold Nanoparticles

For the synthesis of the gold nanoparticles, a certain volume of the olive OL (0.1– 6 ml) was added to the $HAuCl_4$ $3H_2O$ solution and the volume was adjusted to 10 ml with de-ionized water. The final concentration of Au was 1.3 x 10^{-4} M. The reduction process of Au^{3+} to Au nanoparticles was followed by the change in the color of the solution from yellow to violet to dark pink and green depending on the extract concentration.

CHARACTERIZATION

UV-vis Spectroscopy

The study of UV-vis spectroscopy measurements of the gold nanotri-angles were

carried out using Shimadzu 1600 UV-vis Spectrophotometer (Kyoto, Japan)

XRD (X-Ray Diffraction) Analysis

It was done by using BEDE D-3 system with Cu Kα radiation at a generator or voltage of 40 kV and a generator current of 100 mA. The samples were scanned from scanned from 2θ = 1 – 100° at a scanning rate of 2°/min.

FT-IR Analysis

A Perkin-Elmer Model of FTIR spectrophotometer, USA within the range of 4000 - 400 cm^{-1} was used for the samples analysis. Approximately, a sample of 5 mg was with KBr (100 mg) and condensed into pellet using the hydraulic press. The KBr pellet methods were used for all FT-IR spectra analysis.

Transmission Electron Microscopy

It was performed on a JEOL model 1200EX instrument operated at an accelerating voltage at 80 kV.

Zeta Size and Potential Analysis

A Malvern Instrument, MAL 1037088, USA was used to determine the standard size of particles and zeta potential of nanoparticles. Disposable zeta cell measurement was carried with ultra-pure water at 25°C with a −50 mV latex standard calibrated frequently. The mean zeta potential was carried out using phase analysis in light scattering technique.

Cell Viability

The cytotoxicity and viability effect of AuNPs against K562 cell line were studied by MTT assay. MTT based cytotoxic assay was carried out according to the instruction of kit providing by the manufacture. The absorbance was calculated at 490 nm using a micro plate reader. OD values were expressed as % over the control group. Each data point was calculated in triplicate and all assays were performed at least three times.

RESULTS AND DISCUSSIONS

UV–visible Absorption Spectrum

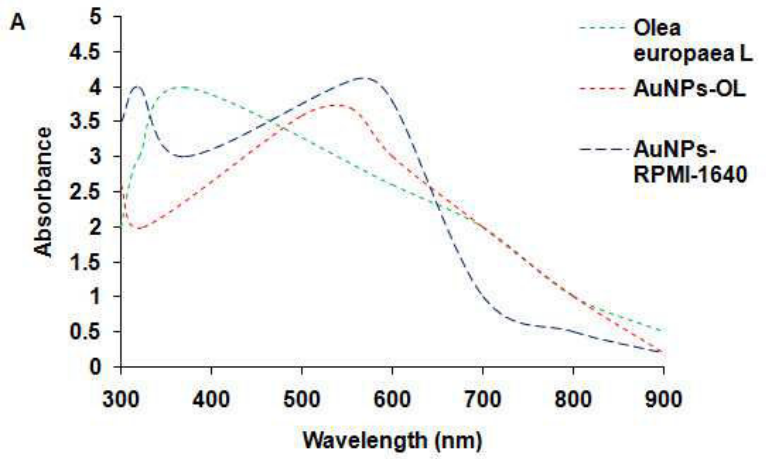

Figure 1
UV–visible spectrum of Olea europaea L extract solution, OL-AuNPs in water, and OL-capped AuNPs in cell culture media. It indicates the characteristic absorbance peak at 556 nm.

Figure 1 shows the UV–visible absorption spectrum of the *Olea europaea* L., OL-AuNPs in water and OL-AuNPs in RPMI-1640 cell culture medium. The characteristic absorbance peaks at 367, 547 and 556 nm were clearly observed in OL-embedded AuNPs in water and in cell culture medium [23, 24]. This data indicates that the biological media to be used for the cell culture studies did not change the stability of the AuNPs. The

symmetric peak indicates that the solution does not contain any aggregated particles. The absorption spectra of this system were periodically recorded in the subsequent two months. No obvious change in the shape, position, or symmetry of the absorption peak was observed, which indicates that the as-prepared gold nanoparticles can remain stable for at least two months at room temperature.

TEM

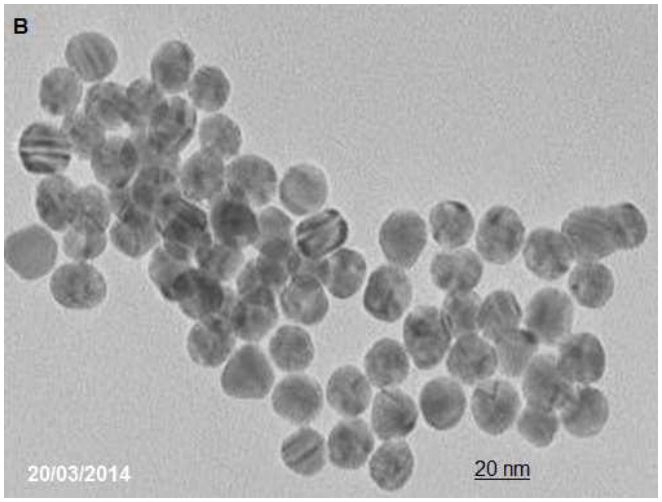

Figure 2
Transmission electron microscopy image of OL-embedded AuNPs.

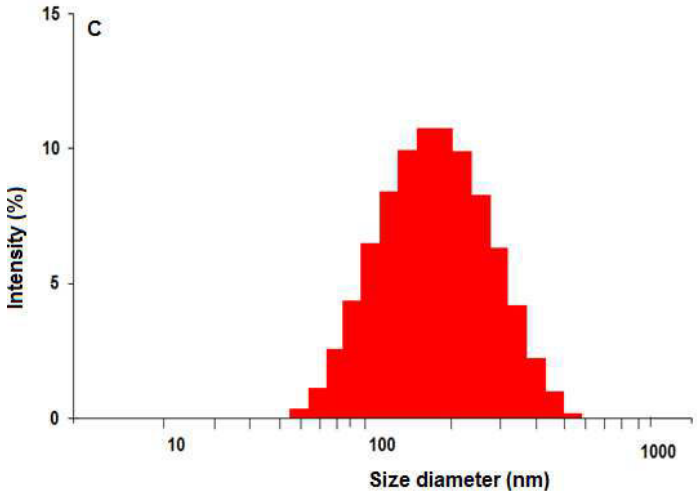

Figure 3
Size-distribution analysis by dynamic light scattering

Figure 2 shows the TEM of gold nanoparticles to visualize their morphology. The images demonstrated that particles are spherical and polydispersed with a size ranging from 20 - 100 nm (Figure 2). TEM analysis was carried out at 1.4 Å resolutions, which is optimal for NP size analysis [25]. Next, DLS analysis revealed that the formulated AuNPs had an average diameter of 145.4 ± 1.5 nm (Figure 3).

FTIR Analysis

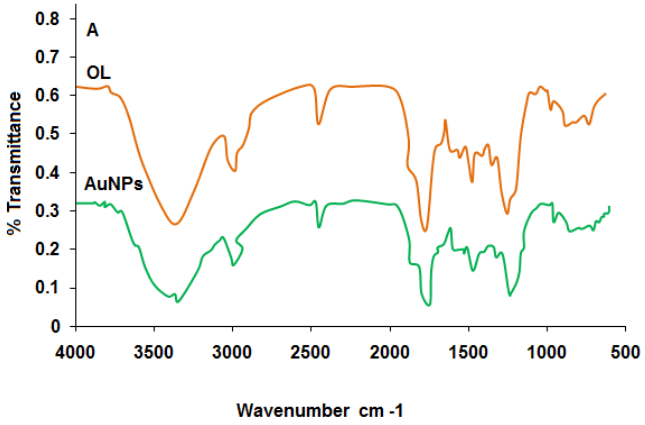

Figure 4
FTIR spectra of (a) a plain olive leaf extract and (b) capped AuNPs.

FTIR analysis was performed to characterize any chemical changes that occurred during the synthesis of NPs. Figure 4 shows the FTIR spectra of *Olea europaea* L. extract, OL-capped AuNPs. Importantly, the characteristic peaks seen for OL alone are also evident in the sample of OL-embedded AuNPs (Figure 4). The IR bands observed at 3409 and 1733 cm^{-1} in dried olive leaf extract are characteristic of the O-H and C=O stretching modes for the O-H and C=O groups possibly of oleuropein, apigenin-7-glucoside and/or luteolin-7-glucoside present in it. The very strong band at 1077 cm^{-1} could be assigned to the C-OH vibrations of the protein in the olive leaf. The IR spectrum of AuNPs exhibit a medium intense band at 1721 cm^{-1} assigned to the C= O stretching mode. The shift of C=O stretching frequency indicates a bounding of the biomolecules to the Au nanoparticles through this group. The band at 1624 cm^{-1} in the olive leaf extract assigned as amide I vibrations has become more prominent in the spectrum of gold and split into two bands at 1622 and 1648 cm^{-1}. It is well known that proteins can bind to Au nanoparticles through the free amine groups or carboxylate ions of the amino acid residues in it. The presence of the IR bands due to C=O stretching vibrations at 1721 cm^{-1} and the appearance of amide I bands with a shift from that of the plain leaves indicate the possibility that gold nanoparticles are bound to proteins and antioxidant molecules through free amine groups and C=O, O-H groups, respectively [23].

XRD (X-ray Diffraction) Analysis

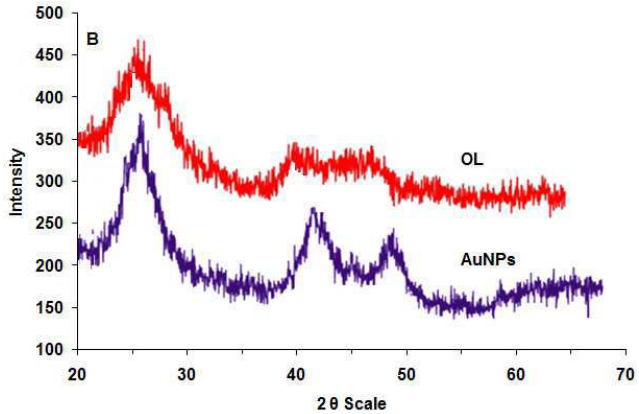

Figure 5
XRD pattern of dried powder of OL extract and gold nanoparticles

In Figure 5, the XRD pattern of OL and OL-embedded AuNPs are demonstrated. The diffraction peaks for OL-capped AuNPs was found at 2θ= 38.31^0 (111), 44.46^0 (200), 64.67^0 (2 2 0) and 77.45^0 (311). The zeta-potential or net surface charge, of the OL-embedded AuNPs was also measured. The data in Figure 5 shows the zeta-potential to be positive, with a value of 2.293 ± 5.1 mV. The neutral OL effectively coats the positively charged surface of the AuNPs, but the particles retain their positively charged characteristic. This data confirms that the OL-embedded AuNPs are homogeneously coated. The capping agent plays an important role in determining the stability and property of an NP. OL was chosen as the reducing and capping agent in these studies because less agglomeration is observed compared with other common capping agents, such as polymers and other harmful agents. Once synthesized, it was important to characterize the AuNPs by several means. Earlier reports showed that the stability of AuNPs was measured by a characteristic absorbance at 548 nm, which has been supported by spectrophotometric, idiometric and XRD analysis [23].

Effect of AuNPs on Cell Proliferation

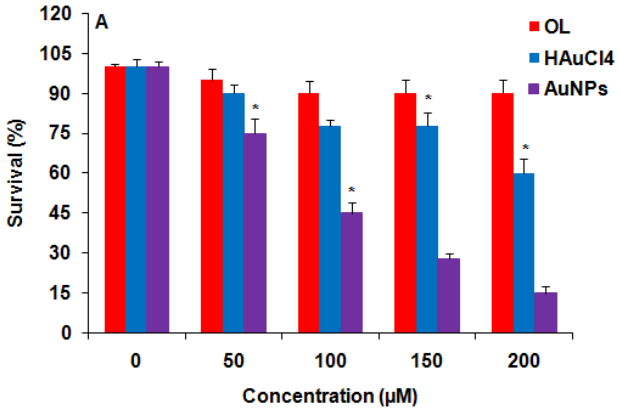

Figure 6

Cells were treated with increasing concentrations of OL, $HAuCl_4$ and AuNPs for 72h and the clonogenic assay. The graph shows percentage survival against concentration.

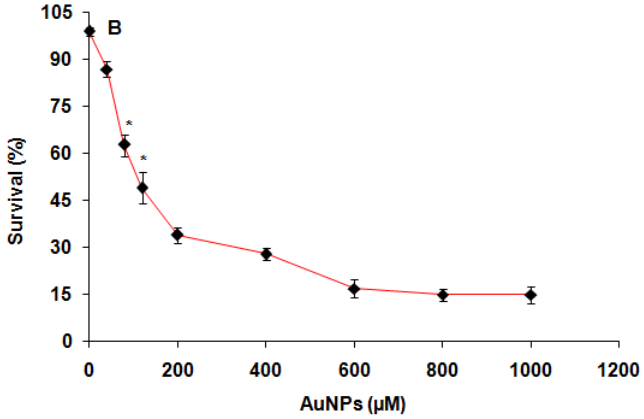

Figure 7

Percentage survival of clonogenic cell growth after treatment with increasing concentrations of AuNP for 72 h. Anchorage-dependent cell growth was decreased by AuNPs *p < 0.05.

To determine the effect of AuNPs on the colony-forming ability of cancer cells, clonogenic cell survival was measured. K562 cells were treated with increasing concentrations of OL, $HAuCl_4$ and AuNPs separately for 72 h. OL did not cause any significant growth inhibition in the cells at any concentration. $HAuCl_4$ caused significant cell death in a concentration-dependent manner, with a maximum cell death of 42% observed at 200 µM. Interestingly, dose-dependent cell death was noted, with AuNPs attaining maximum cell death of 85% at 200 µM (Figure 6). To determine the IC50 value (the concentration required to kill half the cells), a broader range of concentrations was used in the same cell line, and the IC50 obtained was 150 µM (Figure 7). This data clearly indicates a decrease of clonogenic cell survival in K562 cells with AuNP exposure [26].

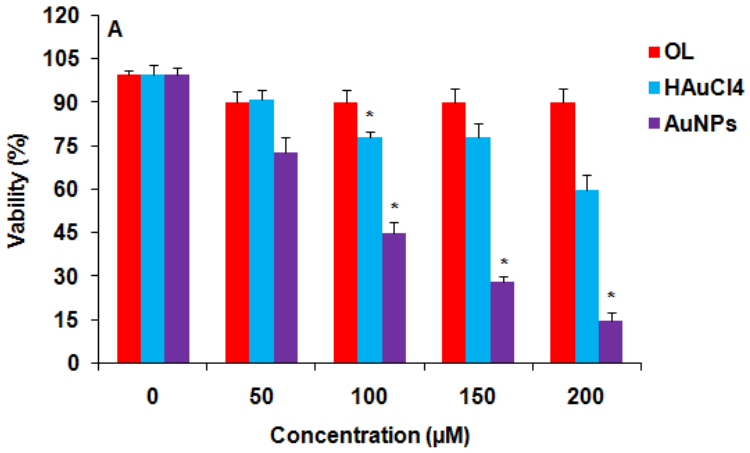

Figure 8

Cells were treated with increasing concentrations of OL, $HAuCl_4$ and AuNPs for 48h. The graph was plotted as percentage viability against AuNP concentration.

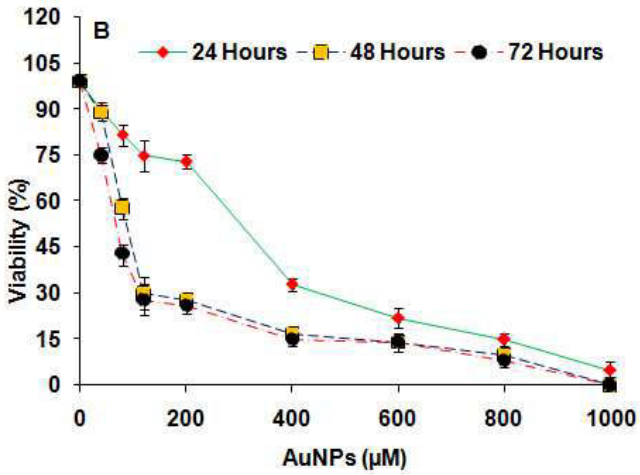

Figure 9

Percentage viability of cells after treatment with increasing concentrations of AuNPs for 24 h, 48 h and 72 h.

To further confirm the above results from the clonogenic assay, a MTT cell viability assay was performed. Figure 4A illustrates the effect of OL, $HAuCl_4$ and AuNPs on the viability of K562 cells by MTT assay. In agreement with the data from clonogenic cell survival assay, OL did not show any killing effect on these cancer cells. $HAuCl_4$ alone caused a drop in survival of 20%. At the same concentration (200 µM), AuNPs caused a dramatic drop in cell viability of more than 85% (Figure 8). Figure 9 demonstrates the

time-dependent killing of cancer cells by AuNPs. Dose-dependent killing was observed at all three time points examined, and the lowest IC50 was observed at 72 h (80 µM) in comparison to 48 h (100 µM) and 24 h (310 µM). Thus, from the above data (Figures 6, 7, 8 & 9) it was confirmed that AuNPs have anticlonogenic and antiproliferative activity against K562 cells [26, 27].

CONCLUSION

Functionalized and biomolecule gold nanoparticles will play an indispensable role in the overall design and development of AuNps-based nano pharmaceuticals. A completely green route has been developed to synthesize Au nanoparticles in a solution of OL at a slightly elevated temperature. The route proposed in this work, it seems possible that people can easily make the Au nanoparticles at home. The OL was used as a reducing agent and AuNPs are bona fide anticancer agents against K562 cell, this concept could also be extended to provide strong evidence that AuNPs should be studied further as potential novel anticancer agents to treat K562.

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