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Green Synthesis of Gold Nano Particles VII: Green Synthesis and Characterization of Gold Nano Particles Using the Extract of Lemon (*Citrus limon*) and Study of its Cytotoxicity Properties

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Abstract: The synthesis of eco-friendly nanoparticles is evergreen branch of nanoscience for biomedical application. Low cost of synthesis and non toxicity are main features make it more attractive potential option for biomedical field and elsewhere Gold nanoparticles are traditionally synthesized by reducing metallic agents. There are a number of reducing agents reported in the literature for the synthesis of AuNps. These methods are toxic methods. In the present investigation, green synthesis of gold nano particles has been carried out using eco-friendly method such as the plant extract of Lemon. The nano particles so synthesised were characterized by Uv-visible and TEM analysis. The Cellular Internalization studies of AuNps provide new opportunities for probing cellular processes via nanoparticulate-mediated imaging. The cytotoxicity studies clearly demonstrate that the phytochemicals within these herbs provide a nontoxic coating on AuNps.

Key words: Green synthesis · Gold · Lemon · Cytotoxicity studies

INTRODUCTION

During the last decade, scientists have developed techniques for synthesizing and characterizing many new materials with at least one dimension on the nanoscale, including nanoparticles, nanolayers and nanotubes.[1] Still, the design and synthesis (or fabrication) of nanoscale materials with controlled properties is a significant and ongoing challenge within nanoscience and nanotechnology. Nanoscience is still largely in the "discovery phase" wherein new materials are being synthesized (using any means available) on small scales (lOOs of milligrams or less) for testing specific physical properties. Typically, during this phase of development given that green chemistry has been employed successfully in the preparation of highly functionalized products (e.g. pharmaceuticals) that have a strong analogy to the functionalized nanomaterials proposed for a range of future applications, one would expect successful application of this approach for these nascent materials. Application of green chemistry to nanoscience should also prove beneficial in developing production level commercial scale materials. The development of high-precision, low-waste methods of nanomanufacturing will be crucial to commercialization. In addition to providing enhanced research and development strategies, green chemistry offers an opportunity to improve public perception of nanoscience, as this approach is relatively easy to explain and can be used to convey a responsible attitude toward the development of this new technology. For these reasons, green chemistry can play a prominent role in guiding the development of nanotechnology to provide the maximum benefit of these products for society and the environment.

Recently Nayak and coworkers have extensively studied the use of plant extracts for the green synthesis of gold nano particles [1-4]. The use of phytochemicals in the synthesis of nanoparticles is an important symbiosis between nanotechnology and green chemistry [5-7]. As the nanorevolution unfolds, it is imperative to

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develop 'nano-naturo' connections between nanotechnology and green domains of the nature. Production of nanoparticles under nontoxic green conditions is of vital importance to address growing concerns on the overall toxicity of nanoparticles for medical and technological applications [8-10]. The power of phytochemicals, which initiate varieties of chemical transformations within biological systems, is well known [9, 11-13]. For example, a high level of genistein found in plant materials is both a phytoestrogen and antioxidant and has been extensively used to treat conditions affected by estrogen levels in the body [14, 15]. Polyphenolic flavonoids in Lemon of which epigallocatechin gallate (EGCG) is the major constituent, has anti carcinogenic activity [16, 17]. The tremendous health benefits of chemical cocktails present within Lemon is beyond doubt, the actual applications of the chemical reduction power of the myriad of chemicals present in herbs and spices is still in infancy. Therefore we investigated the synergistic potentials of polyphenols, flavonoids, catechins and various phytochemicals present in Lemon for the reduction reactions of gold salts to produce AuNps which have potential applications in the diagnosis and therapy of various deadly diseases including cancer.

In the present research programme, gold nano particles have been synthesised by the plant extract of Lemon. The nano particles have been characterized by using Uv-Visible and TEM studies. The cytoxicity study of the nano particles have also been studied.

a

Fig. 1: a) LEMON

b.

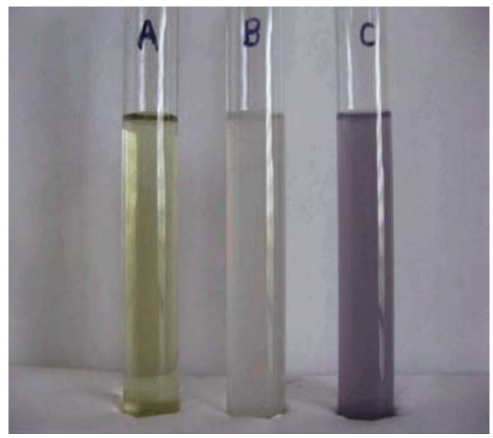

Fig. 1: b) Tube A- Auric acid, Tube B- Lemon extract, Tube C- Lemon gold nanoparticle solution.

MATERIAL AND METHODS

Synthesis of Dhania Gold Nanoparticles (Dhania-AuNps):

Lemon or Lembu (Citrus Limonium): Belongs to citrus family *Rutaceae* (Fig. 1. l). Along with ascorbic acid, flavonoids (hesperidoside, limocitrin), Caffeine, essential oils like isopulegol, alpha-bergamotene, alpha-pinene, alpha-terpinene, alph-thujene, beta-bisolobene, beta-bergamotene, beta-phelandrene, citral, limonene and sabinene are the main components,. Because of high ascorbic acid (Vitamin C) content, it shows antibacterial and astringent properties, used in herbal medicine to build immunity against colds, influenza and other viral infections; Lemon shows antiescorbutic, antimigraine, anticancerigenous [34].

Step 1: Lemon Extract Preparation 100mg of Lemon were added to 6 ml of distilled water and the reaction mixture was stirred continuously at 25°C for 15 min.

Step 2: To the stirring mixture, 100 µl of 0.1 M NaAuCl4 solution (in DI water) were added. The color of the mixture slowly turned Light violet from White within 10minutes, which indicates the formation of gold nanoparticles (Lemon-AuNps).

Step 3: The reaction mixture was stirred for an additional 15 minutes and the gold nanoparticles thus formed were separated from residual Lemon juice extract immediately using a 5µ filter and analyzed using UV-Visible spectroscopy and TEM.

Cytotoxicity Studies (MTT assay):
Cytotoxicity evaluation of Lemon-AuNps was performed using MTT assay as described by Mosman [20]. Approximately 1 × 105 ml-1 cells (MCF-7 and PC-3) in their exponential growth phase were seeded in a flat-bottomed 96-well polystyrene coated plate and were incubated for 24 hrs at 37°C in a 5% CO2 incubator. Series of dilutions (10, 30, 50, 70, 90, 110 and 150 µM) of AuNps in the

medium was added to the plate in hexaplates. After 24 hrs of incubation, 10 µl of MTT reagent was added to each well and was further incubated for 4 hrs. Formazan crystals formed after 4 hrs in each well were dissolved in 150 µl of detergent and the plates were read immediately in a microplate reader (Spectramex, 190 Molecular Devices Inc., USA) at 570 nm. Wells with complete medium, nanoparticles and MTT reagent, without cells were used as blanks. A control experiment with series of dilutions of NaAuCl4 was performed using the same MTT kit to validate the assay.

RESULTS AND DISCUSSION

Synthesis of Green gold Nanoparticles: Our new *green* process for the production of gold nanoparticles uses direct interaction of sodium tetrachlroaurate (NaAuCl4) with Lemon juice extract in the absence of man-made chemicals and thus, satisfies all the principles of a 100% green chemical process. Various phytochemicals present in Lemon is presumably responsible for making a robust coating on gold nanoparticles and thus, rendering stability against agglomerations. Absorption measurements indicated that the plasmon resonance wavelength, ëmax of Lemon-AuNps, is 535 nm respectively. The size of Lemon-AuNps is in the range of 16±5 nm; respectively as measured from TEM techniques (Figures 1).

XRD of Gold nano Particles: Figure 2. Shows the XRD patterns obtained for gold nanoparticles synthesized in present research work. The crystalline nature of the gold nanoparticles is clearly shown in XRD pattern. Bragg reflections corresponding to lattice planes (111), (200), (220), (311), (222) are observed in XRD pattern.

Cellular Internalization Studies: Results of cellular internalization studies of AuNps solutions are key to providing insights into their use in biomedicine. Their selective cell and nuclear targeting will provide new pathways for their site-specific delivery as diagnostic/ therapeutic agents. A number of studies have demonstrated that phytochemicals present in Dhania have the ability to penetrate the cell membrane and internalize within the cellular matrix [21, 22]. Cancer cells are highly metabolic and porous in nature and are known to internalize solutes rapidly compared to normal cells [22]. Therefore, we hypothesized that Dhania derived phytochemicals, if coated on AuNps, will show internalization within cancer cells. TEM images of prostate (PC-3) and breast tumour (MCF-7) cells treated with AuNPs unequivocally validated our hypothesis. Significant internalization of nanoparticles via endocytosis within the MCF-7 and PC-3 cells was observed (Figures 2; 3). The internalization of nano particles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor

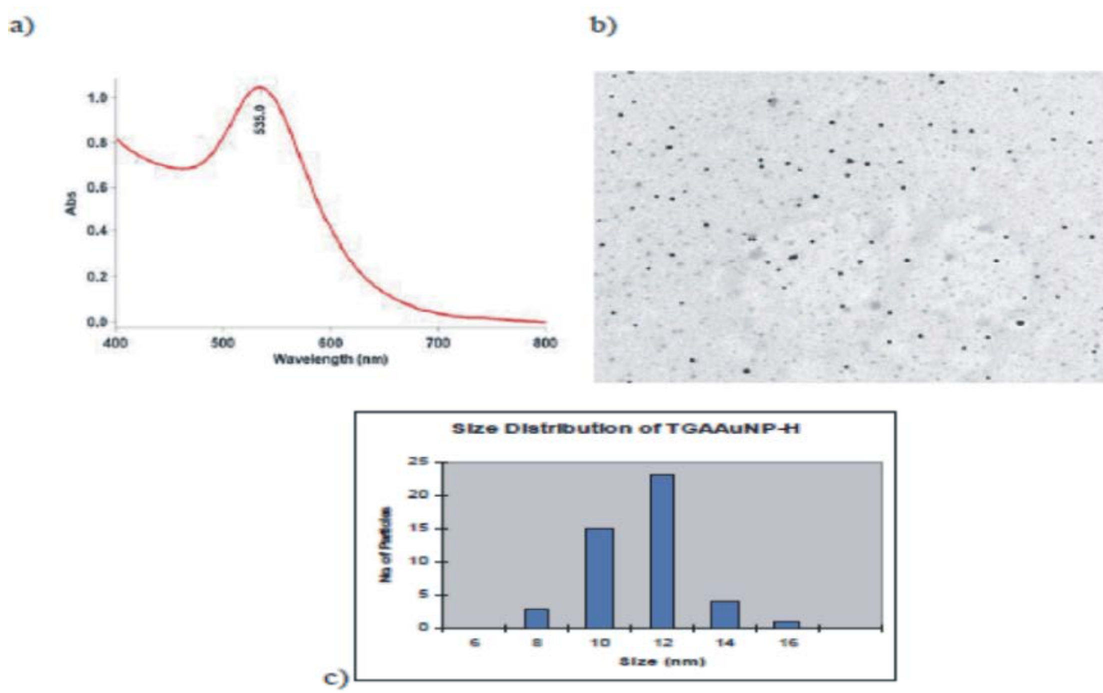

Fig. 1: a) UV-Visible absorption spectrum, b) TEM Image c) size distribution of Lemon Gold Nanoparticles.

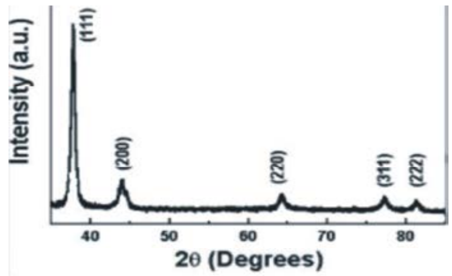

Fig. 2: XRD of Gold particle

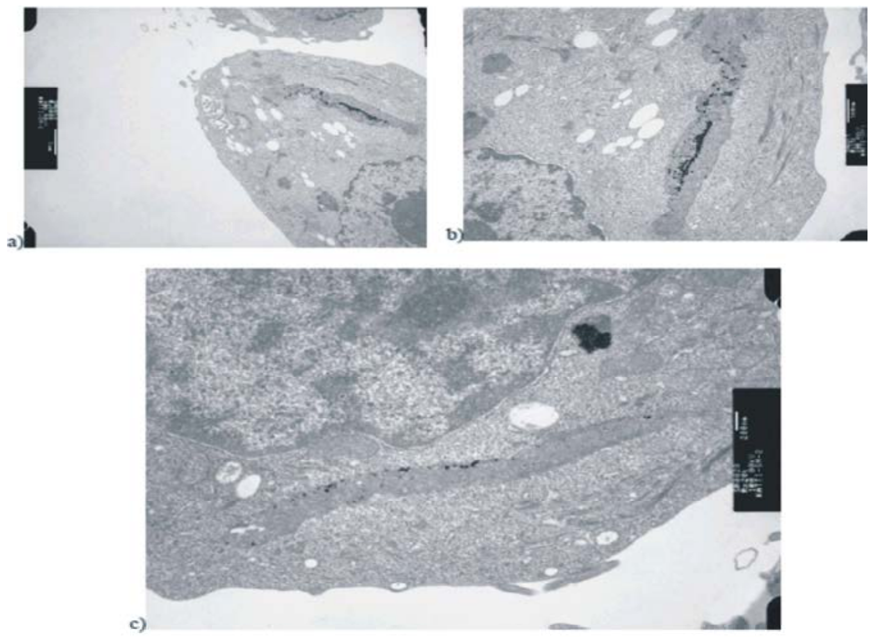

Fig. 3: a, b, c: TEM Images of different MCF-7 cells showing uptake of Lemon-AuNps in to the lysosomes.

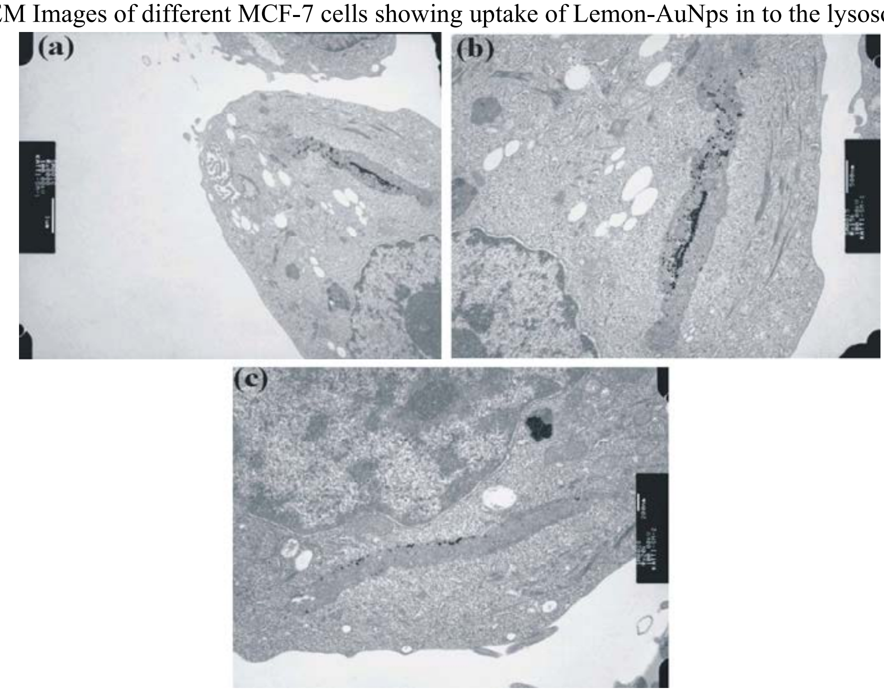

Fig. 4: a, b, c: TEM Images of different MCF-7 cells showing uptake of Lemon-AuNps in to the lysosomes.

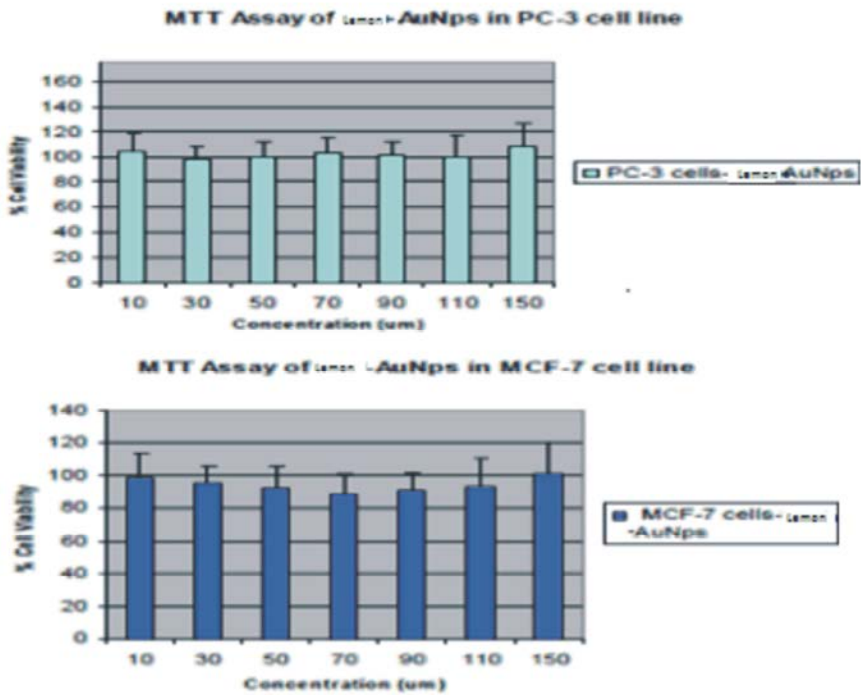

Fig. 5: a, b: Dose dependent cytotoxicity of Lemon-AuNPs in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay.

mediated endocytosis. The viability of both PC-3 and MCF-7 cells post-internalization suggests that the phytochemical coating renders the nanoparticles non-toxic to cells. Such a harmless internalization of AuNps will provide new opportunities for probing cellular processes via nanoparticulate-mediated imaging.

Cytotoxicity Studies: Untreated PC-3 and MCF-7 cells as well as cells treated with 10, 30, 50, 70, 90,110 and 150 µM concentrations of various AuNps for 24 hrs were subjected to the MTT assay for cell- viability determination. In this assay, only cells that are viable after 24 hexposure to the sample are capable of metabolizing a dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) efficiently and produce a purple colored precipitate which is dissolved in a detergent and analyzed sphectrophotometrically. After 24 hrs post-treatment, PC-3, MCF-7 cells showed excellent viability even up to 150 µM concentrations of Dhania -AuNps (Figures 5 a, b;). These results clearly demonstrate that the phytochemicals within these herbs provide a nontoxic coating on AuNps and corroborate the results of the internalization studies discussed above. It is also important to recognize that a vast majority of Gold (I) and Gold (III) compounds exhibit varying degrees of cytotoxicity to a variety of cells (Figure 4). The lack of any noticeable toxicity of Dhania-AuNps provides new opportunities for the safe application in molecular imaging and therapy.

Cellular Internalization Studies: Results of cellular internalization studies of AuNps solutions are key to providing insights into their use in biomedicine. Their selective cell and nuclear targeting will provide new pathways for their site-specific delivery as diagnostic/ therapeutic agents. A number of studies have demonstrated that phytochemicals present in Lemon have the ability to penetrate the cell membrane and internalize within the cellular matrix [21, 22]. Cancer cells are highly metabolic and porous in nature and are known to internalize solutes rapidly compared to normal cells [22]. Therefore, we hypothesized that Lemon derived phytochemicals, if coated on AuNps, will show internalization within cancer cells. TEM images of prostate (PC-3) and breast tumour (MCF-7) cells treated with AuNPs unequivocally validated our hypothesis. Significant internalization of nanoparticles via endocytosis within the MCF-7 and PC-3 cells was observed (Figures 4; 5; 6). The internalization of nano particles within cells could occur via processes including phagocytosis, fluid-phase endocytosis and receptor mediated endocytosis. The viability of both PC-3 and

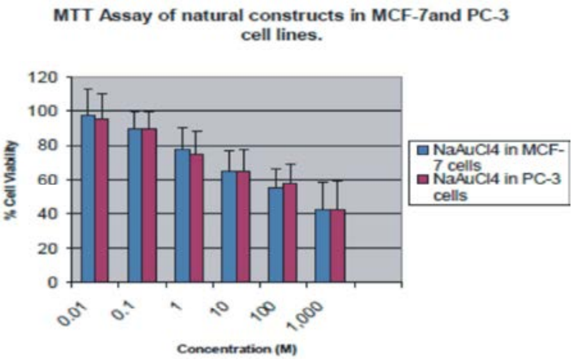

Fig. 6: Dose dependent cytotoxicity of NaAuCl4 in cultured PC-3 and MCF-cells after 24 hrs of exposure using MTT assay.

MCF-7 cells post-internalization suggests that the phytochemical coating renders the nanoparticles non-toxic to cells. Such a harmless internalization of AuNps will provide new opportunities for probing cellular processes via nanoparticulate-mediated imaging.

CONCLUSION

Green synthesis of metallic nanoparticles is a successive alternative to chemical synthesis protocols for synthesizing gold nano particles. Gold nanoparticles are defined as stable colloid solutions of clusters of gold atoms with sizes ranging from 1-100 nm. At this nanoscale, AuNps possess different physicochemical characteristics when compared to the bulk gold, most obvious example being the colour change from yellow to ruby red when bulk gold is converted into nanoparticulate gold. This ruby red colour of AuNps is explained by a theory called "surface plasmonics". Gold nano particles have been synthesised successfully by using green chemistry with the help of the plant extract like Lemon.

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