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Enhanced cytotoxic effect on human lung carcinoma cell line (A549) by gold nanoparticles synthesized from *Justicia adhatoda* leaf extract

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ABSTRACT

Objective: To synthesize bio-inspired gold nanoparticles (AuNPs) using the leaf extract of *Justicia adhatoda* and evaluate the anti-cancer activity on human lung cancer cell line (A549). **Methods:** Synthesis of AuNPs was done using an aqueous leaf extract of *Justicia adhatoda* as a green route. The bio-synthesized AuNPs were confirmed and characterized by using various spectral studies such as UV-Vis spectrum, Scanning Electron Microscope with EDAX, Transmission Electron Microscope, Fourier Transmission Infrared Spectroscope analysis and Surface Enhanced Raman Spectroscopy. The cell viability was determined by MTT reduction assay. In addition, cytomorphology and the nuclear morphological study of A549 cell line was observed under fluorescence microscope. **Results:** UV-Vis spectrum showed surface plasmon resonance peak at 547 nm, scanning electron microscope and transmission electron microscope studies showed the monodispersed spherical shape and its average size in the range of 40.1 nm was noticed. Fourier Transmission Infrared Spectroscope analysis confirmed that the C=O group of amino acids of proteins had strong ability to bind with the surface of nanoparticle. Interestingly, our results also demonstrated inhibited proliferation of A549 cell line by MTT (IC_{50} value: 80 μg/mL). Cell morphology was observed and cell death was caused by apoptosis as revealed by propidium iodide staining. **Conclusions:** The current study proves the anticancer potential of bio-synthesized AuNPs. Thus, synthesized AuNPs can be used for the treatment of human lung cancer cell (A549) and it can be exploited for drug delivery in future.

1. Introduction

Nanotechnology is expected to be a frontier technology for all sciences due to extensive applications in all fields. Accordingly related research and developments are growing rapidly in this field. Interdisciplinary nature of green chemistry proposes the environmentally benign materials and process. Now a major health issue around the world is cancer caused by an unusual growth of cells[1,2]. International Agency for Research on Cancer has reported that approximately 7.6 million deaths arise due to cancer and

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12.7 million cancer patients are diagnosed every year[3]. Cancer diagnostics and treatment costs are very high and also have side effects. Researchers have strived to attain a novel therapy and drugs for treatment of cancer. Antibiotics can kill many diseases instigated by some organisms meanwhile nanomaterials can be able to kill 650 kinds of cells, consequently nanomaterials are called as "a Wonder of Medicine"[4]. In past decades, nanomaterials were acting as a good anticancer agent[5]. In recent years, AuNPs were used for anticancer therapy for different kinds of cancers such as Hep2 cell[5,6], breast cancer (MCF-7)[7] and prostate cancer (PC-3)[8]. Almost 60% of anticancer agents are reported to be from the plant materials[9]. Many types of respiratory disorders are characterized by inflammatory reaction in air way. In humans, many causative agents are responsible for formation of lung cancer. Most of the lung cancer are carcinomas and 31% of cancers are adenocarcinomas[10]. It is the acronym of human lung adeno carcinoma epithelial cell (A549).

In this work, the synthesis of AuNPs was conducted by using *Justicia adhatoda* (*J. adhatoda*) (Acanthaceae family) leaf extract. Previous studies reported that the leaf contains phenols, tannins, alkaloids (vasicine, vasicinol, vasicinone, adhavasinone, adhatonine and peganine), anthraquinones, saponins, flavonoids (apigenin, astragalin, kaempferol, quercetin, vitexin), reducing sugars and vitamin C[11–13]. Vasine and vasinone are the major alkaloids in *J. adhatoda* leaf extract. Chanu and Sarangthem reported the phytochemical components in leaves of *J. adhatoda*, which contains total carbohydrate (16.1 mg/g), soluble proteins (7.8 mg/g), phenols (32.1 mg/g), flavonoids (37.9 mg/g) and alkaloids (1.09 mg/g)[14]. Various phytochemicals such as alkaloids, polyphenols and flavonoids have been proven to fight against various types of human diseases[15,16]. Flavonoids can prevent the activities of enzyme which are involved in breaking the carbohydrate into sugar and few flavonoids show cytotoxicity in various cancer cells. The polyphenols can maintain the level of glucose in blood by numerous mechanisms. Phenolic compounds might act as an antioxidant agent by scavenging free radicals[17]. Earlier reports have focused on biological activities of *J. adhatoda* such as antibacterial, bronchodilator, anti-asthmatic, anti-tubercular, and anti-inflammatory activities[12,15]. However, none of them have tested anticancer effect of *J. adhatoda* mediated gold nanoparticles (AuNPs) on A549 cell line. Therefore, we designed to explore the anticancer efficiency of AuNPs by measuring cytotoxicity through MTT assay and apoptosis by propidium iodide (PI) staining. To the best of our knowledge, this is the pioneer report of cytotoxicity of AuNPs synthesized from *J. adhatoda* leaf extract against lung cancer (A-549) cell line.

2. Materials and methods

2.1. Materials

Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$) Dulbecco's Modified Eagle's medium (DMEM), Fetal Bovine Serum, trypsin, streptomycin, MTT, and dimethyl sulfoxide were purchased from Hi Media and Sigma Aldrich. Human lung cancer carcinoma cell lines (A549) were obtained from Cancer Institute (Chennai, Tamilnadu).

2.2. Collection and preparation of leaf extract

Fresh leaves of *J. adhatoda* were collected from agriculture farms in Cumbum valley, at Theni district, Tamilnadu, India. Collected leaves were washed and shade dried for 15 d and ground into coarse powder. Ten mg of coarse powder was taken in a 250 mL beaker along with 120 mL of double distilled water. The mixture was digested for 20 min at 60-70 ℃ and then the extract was filtered with Whatman No.1 filter paper. This filtrate was called as aqueous extract, and then it was stored in the refrigerator for further experiments.

2.3. Synthesis of AuNPs

2.3.1. Effect of time on synthesis of AuNPs

One mL of leaf extract was added to 7 mL of $HAuCl_4 \cdot 3H_2O$ solution. Within 20-30 min pink-ruby red color appeared due to reduction of Au^{3+} ions to Au^0. The formation of color was monitored by visual appearance.

2.3.2. Effect of temperature on formation of AuNPs

The effect of temperature on the synthesis of AuNPs was studied by 1 mL leaf extract from *J. adhatoda* added to 7 mL of $HAuCl_4 \cdot 3H_2O$ (1 mM) followed by the reaction in a water bath at 30, 50 and 70 ℃. The formation of AuNPs was monitored as a UV-Vis spectrophotometer.

2.3.3. Effect of pH on formation of AuNPs

The effect pH was experimented by 1 mL leaf extract from *J. adhatoda* added to 7 mL of $HAuCl_4 \cdot 3H_2O$ (1 mM) at different pH (4, 7 and 11). The formation of AuNPs was monitored by UV-Vis spectrophotometer.

2.4. Characterization of AuNPs

A UV-Visible spectrophotometer technique is used to ascertain stability and formation of nanoparticles. UV-Vis spectra were recorded using a Perkin-Elmer Lamda-45 spectrophotometer. Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) were performed on Hitachi S-3400N and TEM-JEOL model 1200EX. Fourier Transmission Infrared Spectroscope (FTIR) measurements were carried out using Perkin-Elmer in the range of 400 to 4 000 cm^{-1} by using KBr pellets. Surface enhanced Raman spectrum measurements (SERS) were analyzed by Nano photon-Raman 11 spectrometer.

2.5. In vitro anticancer studies

2.5.1. Cytotoxicity assay

Lung cancer (A549) cells were grown in DMEM containing 10% fetal bovine serum, penicillin and streptomycin, and subsequently incubated

at 37 ℃ in a 5% CO_2. Cultured cells (1×10^5 per well) were seeded into a 96-well plate with 1 mL of DMEM medium. Various concentrations of the AuNPs (20, 40, 60, 80, 100 and 120 μg/mL) were added to each well and incubated for 24, 48 and 72 h at 37 ℃ in a CO_2[18]. Control cells were also maintained without adding AuNPs. After the incubation period, the medium was removed and fresh medium was introduced with 10 μL of MTT solution and incubated for 4 h. The formation of purple colored formazan crystal was dissolved with dimethyl sulfoxide (DMSO), and then spectrophotometric absorbance was measured using an Elisa-Reader at 570 nm. The effect of biosynthesized AuNPs on the proliferation of A549 cells was expressed as percentage of cell viability.

$$\text{Percentage of viability} = \frac{\text{OD value of experimental samples}}{\text{OD value of experimental controls}} \times 100$$

2.5.2. Cytomorphological observation

The cells were seeded in a six well plate at 1×10^5 cells/well and incubated overnight. Thereafter the cells were maintained in DMEM medium. Biosynthesized AuNPs of IC_{50} (80 μg/mL) were added and then incubated for 24, 48 and 72 h. The cytomorphology of the exposed cells was monitored under fluorescence microscope.

2.5.3. PI staining

A549 cells were seeded into a six well plate at 5×10^4 cells/well. After that when it reached more than 90% confluence, the cells were treated with AuNPs at 80 μg/mL concentration (IC_{50} value) for 24-72 hours of incubation time. The cells were washed with PBS and CH_3OH:CH_3COOH (3:1) for 10 min and stained with 50 μg/mL PI for 20 min. Nuclear morphology of apoptotic cells with condensed or fragmented nuclei was observed by fluorescence microscope[19].

3. Results

3.1. UV-Visible spectroscopy studies

3.1.1. Effect of contact time

The present study was aimed to monitor the contact time of the reaction mixture in synthesis of AuNPs by UV-Visible spectroscopy. The *J. adhatoda* plant contains alkaloids, flavonoids, glycosides, poly phenolic compounds, reducing sugar *etc.*, Such kind of phytocompounds are beneficial to reducing the metal and also act as a capping agent as well as stabilizing agent. The surface plasmon resonance (SPR) peak of nanoparticles occurred at 547 nm within 25 min, which was steadily increasing with reaction time and without any shift in wavelength (Figure 1).

3.1.2. Effect of reaction temperatures

Several factors are responsible for synthesis of AuNPs, amongst them temperature and pH occupy important role in a reaction process. Consequently, the first factor is optimal temperature requisite to synthesis of AuNPs. Thus, the reaction mixtures were prepared and kept at various temperature *viz.*, 30, 50 and 70 ℃ which were examined under UV-Visible spectrum. As shown in Figure 2, with increasing temperature, the SPR band sharpness also increased which indicated temperature was one of the factor for affecting synthesis of AuNPs. When the temperature increased, the rapid color change of mixture was noticed.

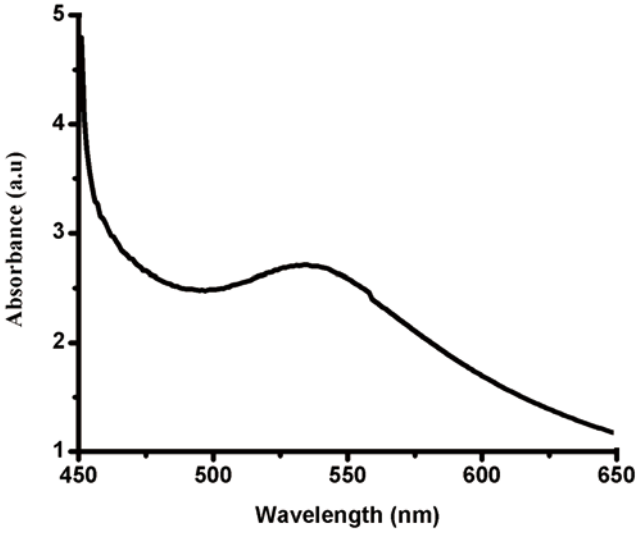

Figure 1. UV-Vis spectra of AuNPs (*J. adhatoda* leaf extract added with $HAuCl_4 \cdot 3H_2O$).

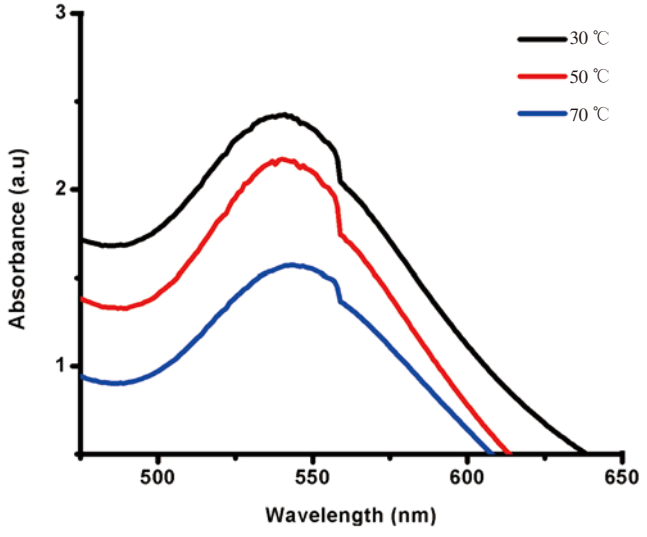

Figure 2. UV-Vis spectra of AuNPs by the addition of leaf extract with choloroauric acid at different reaction temperatures (30, 50, 70 ℃).

3.1.3. Effect of pH on stability of nanoparticles

After that, the another factor is pH which acts as a vital role in the formation of AuNPs. As above mentioned, the experimental set up was maintained in synthesis of nanoparticle with varying pH of the mixture *viz.*, 4, 7 and 11. The rate of AuNPs formation increased with increasing pH, which was visually monitored by immediate

dark color and a red shift was observed in UV-Vis. The absorption spectra showed the peaks at 538 nm (pH-4), 541 nm (pH-7) and 545 nm (pH-11). The pH 7 was the most favourable for synthesis of AuNPs (Figure 3).

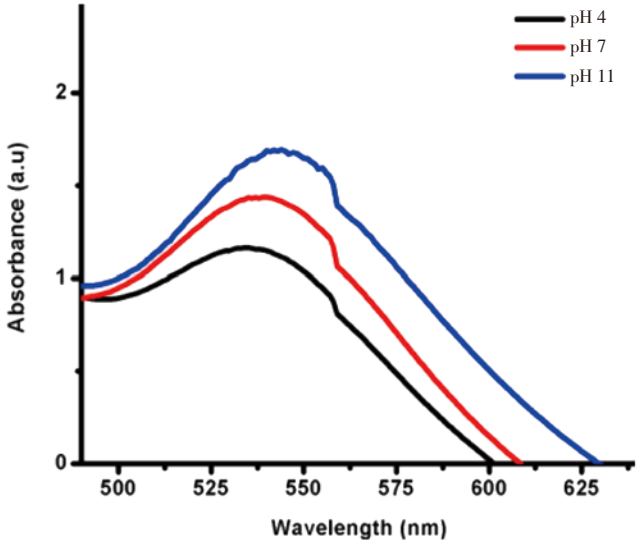

Figure 3. UV-Vis spectral studies of AuNPs at different pH values.

3.2. Morphological studies

3.2.1. SEM analysis

The SEM images of AuNPs synthesized from *J. adhatoda* leaf extract were shown in Figure 4 (a-b). SEM photographs described the morphology and distributions of AuNPs. EDAX pattern exhibited the strong signals for AuNPs (Figure 4c). The optical peak was noted at 2.3 KeV which denoted for the crystalline nano gold due to SPR.

3.2.2. TEM studies

TEM analysis discussed the morphology of biosynthesized AuNPs as shown in Figure 5. It showed that the TEM images of AuNPs were spherical shape and the average size of the AuNPs was observed at the range of 40.1 nm (Figure 5).

3.3. FTIR analysis of AuNPs

FTIR analysis stands for the molecular absorption and transmission bands or peaks which is due to the vibration frequencies between the bonds of atoms. FTIR was revealed to identify the functional groups of biomolecules that were occupied on the surface of synthesized AuNPs. The FTIR analysis of synthesized AuNPs was examined under the peaks at 3 419, 2 927, 1 631 and 1 401 cm^{-1} (Figure 6).

3.4. SERS

Figure 7 showed the Raman peak intensities were at 269, 447, 503, 588, 663, 768, 774, 1 012, 1 154, 1 398, 1 444 and 1 609 cm^{-1}. The synthesized AuNPs have shown a strong SERS effect band 447 cm^{-1} with a shoulder peak at 503 cm^{-1} due to the skeletal deformation vibration of C-N-C and C-S-C. The small peaks were observed at 774 and 1 012 cm^{-1} which correspond to C-N-C and C-S-C skeletal deformation as well as N-CH_3 stretching.

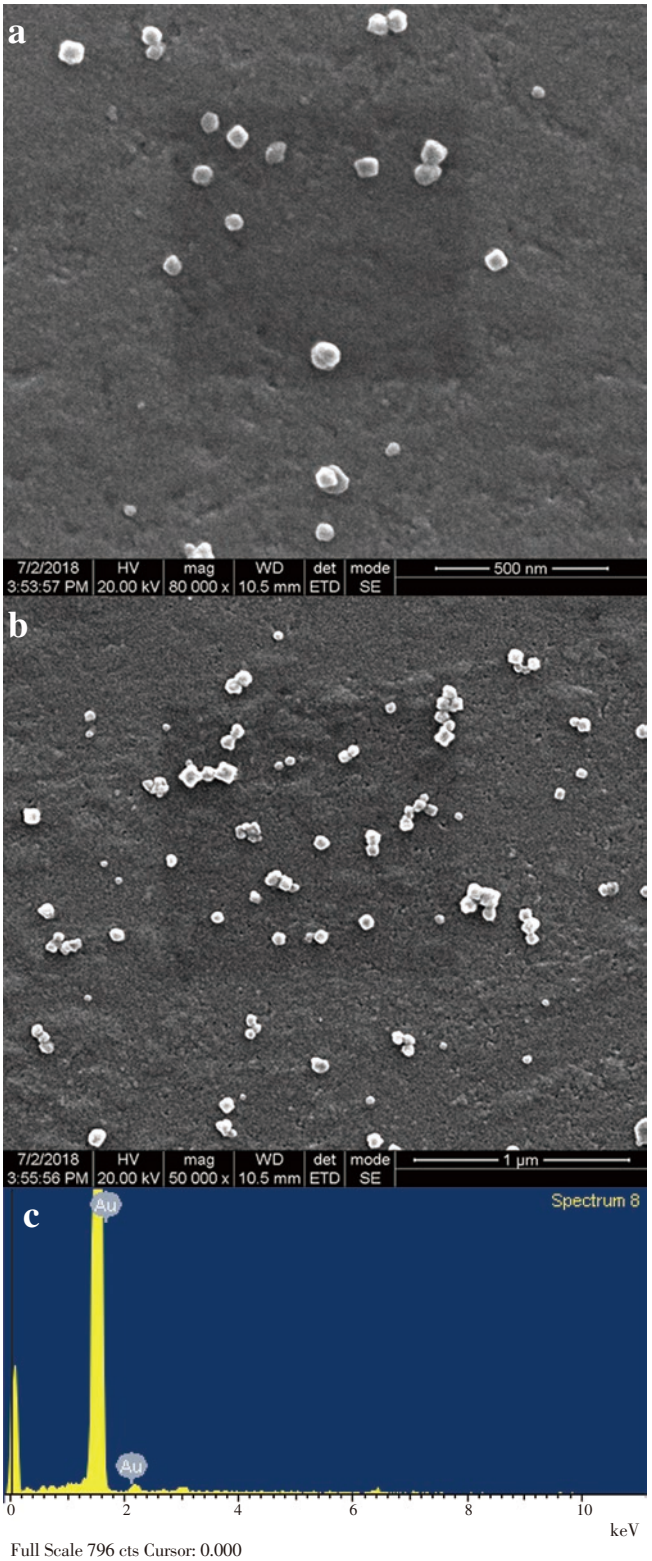

Figure 4. SEM images of AuNPs (a–b) from *J.adhatoda* leaf extract and EDAX of AuNPs (c).

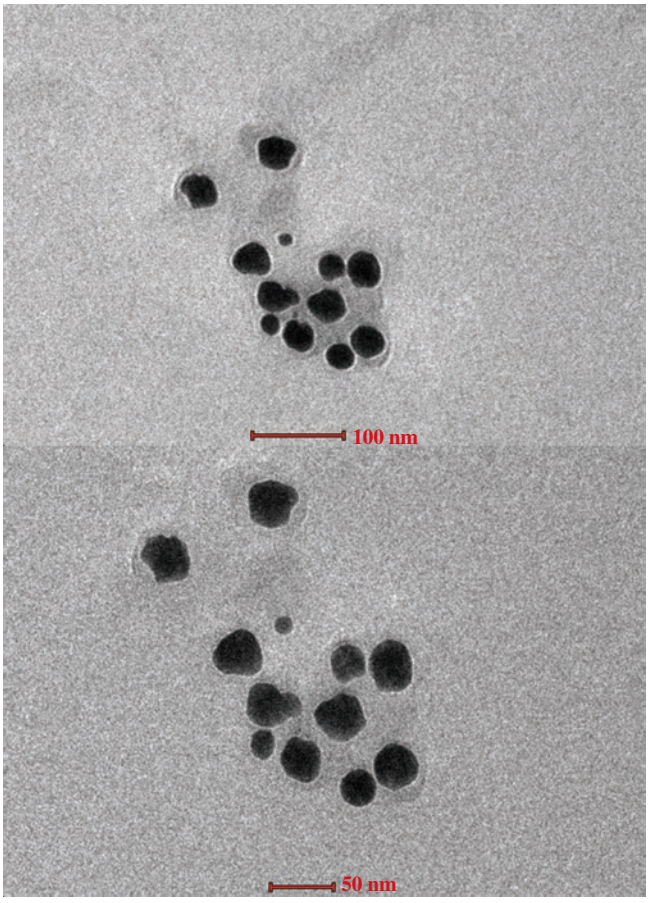

Figure 5. TEM images of AuNPs from *J. adhatoda* leaf extract.

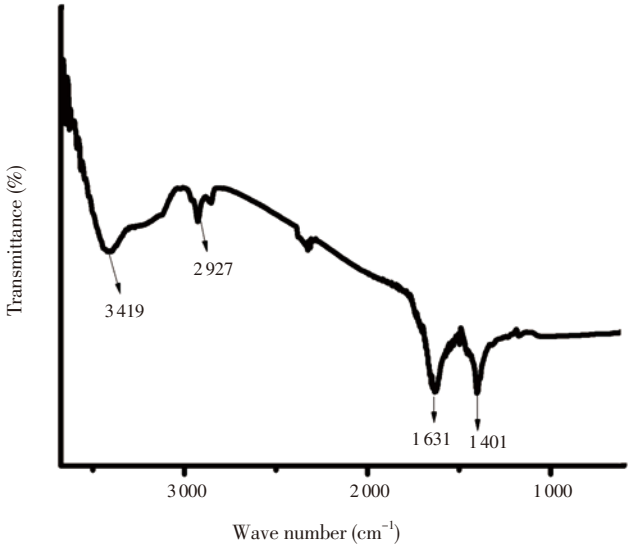

Figure 6. FTIR analysis of AuNPs from *J. adhatoda* leaf extract.

3.5. Study of anticancer activity

3.5.1. Cell viability assay

The cytotoxic effect of AuNPs was determined against A549 cancer cell line. Synthesized AuNPs were added with A549 cell line and later were monitored for their cell viability using the MTT. There was low rate of metabolism which occurred in cells treated by AuNPs compared with untreated cells (control). The viability of cells depended upon the dosage of nanoparticles and incubation period (24-72 h). Significantly, reduction of viability was observed by the concentration of AuNPs and incubation period (20-120 μg/mL & 1-3 days) which was shown in Figure 8.

3.5.2. Cytomorphological observation

Human lung cancer cells were treated with freshly synthesized AuNPs at the IC_{50} (80 μg/mL) concentration for 24-72 h incubation period (Figure 9). Cytomorpholgy of treated cells clearly exhibited the apoptotic mechanism leading to cell death which was observed at 24 h incubation. The apoptotic cells increased with increasing incubation time. Morphological changes including uniform detachment of cells from 6-well plate, inhibition of cell growth, membrane integrity and cell shrinkage were observed (Figure 9). The results indicated that the biosynthesized AuNPs treated A549 cells underwent cell death compared to untreated cells. Treated cells showed distinct cellular morphological changes.

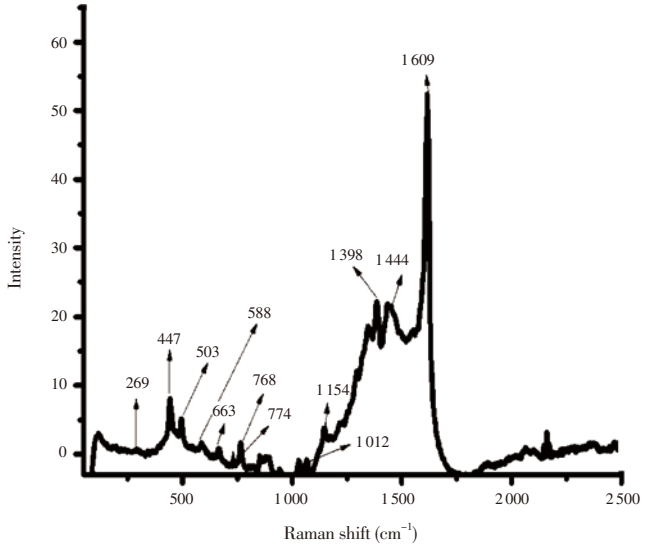

Figure 7. SERS analysis of green synthesized AuNPs using *J. adhatoda* leaf extract.

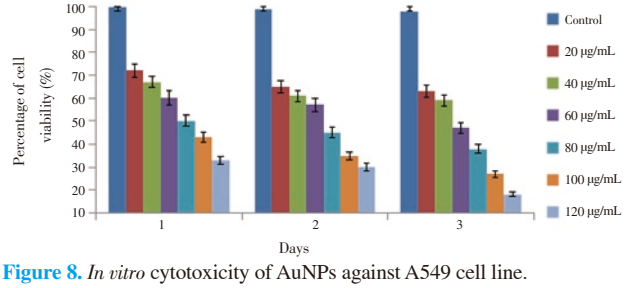

Figure 8. *In vitro* cytotoxicity of AuNPs against A549 cell line.

3.5.3. PI staining

To confirm the cytotoxic effect encouraged by biosynthesized AuNPs from leaves extract of *J. adhatoda*, apoptotic changes were monitored. In control cells, negligible number of PI positive cells was observed. In the case of treated cells at 80 μg/mL concentration

(IC_{50}) of biosynthesized AuNPs with exposure period of 24-72 h, an increase in the number of PI positive cells was also monitored during increasing incubation period (Figure 10). The results were shown in Figure 10. Nuclear compactness and apoptotic cell changes were identified by the PI staining method.

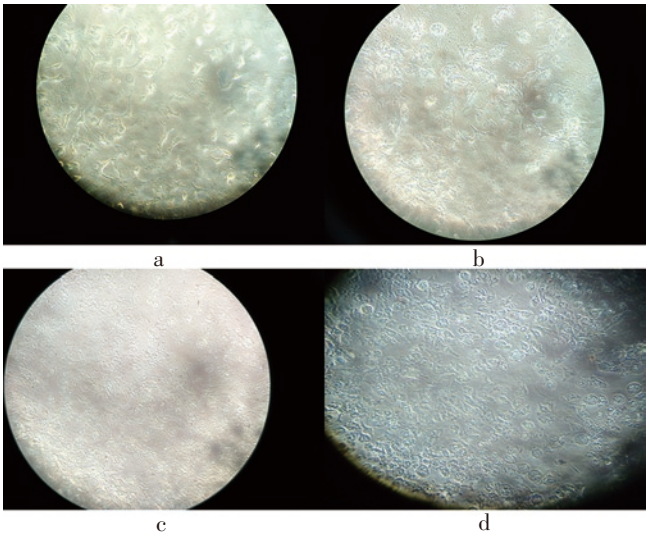

Figure 9. Morphology of control and AuNPs treated A549 cells (a) Control; (b) (IC_{50}: 80 μg/mL) 24 h; (c) 48 h; (d) 72 h.

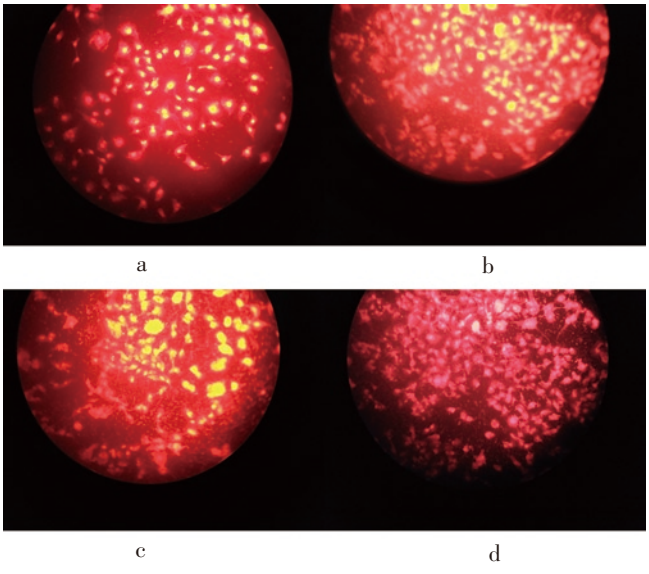

Figure 10. PI staining of A549 cells treated with AuNPs (a) Control; (b) 24 h; (c) 48 h; (d) 72 h.

4. Discussion

In recent years, the application of metallic nanoparticle is increasing due to their economy, growing interest of biological field and eco-friendly benign method of their production. Phytochemical compounds of *J. adhatoda* are the foremost sources for several biological activities. Synthesis of AuNPs using *J. adhatoda* leaf was conducted in this study, and it was identified by the color changes of mixture from pale yellow to pink-ruby red color. The color of the nano gold is ascribed to the combined oscillation of free electron induced by electromagnetic field that is called as SPR[20,21]. Previous reports demonstrated the SPR band of AuNPs appeared almost in this region (500-600 nm), but the position of SPR range may vary which depends on the size as well as shape of the nanoparticles[16]. Lokina *et al*. also reported that the sharpness of the peak of SPR elevates with the increase of reaction time[16]. The formation of stable aggregation of the AuNPs in a solution or anisotropy nature of particles was pointed out by shoulder peak of SPR[22].

At high temperature, faster rate of the reduction enhances the nucleation rate, utmost number of nanoparticles is formed, thereby stopping the secondary reduction process and providing minimal possibility for particle size-growth[23,24]. Interestingly, at low temperature, the particle size is large due to overgrowth of nuclei which is possible to form the anisotropic nature of nanoparticles. With increasing temperature, the SPR band sharpness also increased, the same result was also observed in the *Coleus aromaticus* leaf[23]. Above 30 ℃, SPR peak decreases due to overgrowth of crystal around the nucleus[25,26]. Therefore the optimal temperature for AuNPs synthesis was preferred to be 30 ℃ which is the best result compared with other results[26–28].

In pH parameter, the absorption band shows the red shift due to localized SPR of nanoparticles. In general, acidic medium suppresses the growth of nano particles but the basic condition boosts the growth of nanoparticles[26,27]. At lower pH, larger size of nanoparticle may be possible to form this that is due to over nucleation, besides higher pH, utmost number of functional groups is bound on surface of nanoparticle; as a result, maximum number of nanoparticle is obtained with small size[24]. At lower pH, synthesized AuNPs were bound with -SH group (cysteine), and $-NH_2$ (glutamic acid) groups which is confirmed by FTIR analysis[28]. Verma *et al*. also reported that the red shift (longer wavelength) of SPR indicates that size of the nanoparticle increases along with increasing pH[28]. The optimal pH for AuNPs synthesis was preferred to be pH 7 which is in agreement with the available literature[26]. SEM and TEM studies revealed spherical shape of AuNPs without aggregation. It was obviously illustrated that a spherical and triangle shape of the nanoparticle was capped with the biomolecules. The capping and stabilization of AuNPs are due to the *J. adhatoda* leaf extract which contains flavonoids, glycosides and proteins. The broad band at 3 419 cm^{-1} is due to the O-H stretching vibration[22]. The band at 2 927 cm^{-1} corresponds to C-H groups[22]. A sharp peak at 1 631 cm^{-1} is attributed to primary amide bond Ⅰ in FTIR analysis[29].

FTIR analysis showed that the C=O group of amino acids of proteins, these molecules had strong capability to bind with AuNPs and it also acted as a capping agent for AuNPs. Hence, the capping agents can prevent agglomeration and thereby stabilize the AuNPs. The biological molecules are reducing and stabilization agents of AuNPs.

SERS is a very efficient technique to determine the enhanced effect of surface metal nanoparticles which is associated with efficient capping biomolecules. A small peak that appeared at 269 cm^{-1} due to vibrations between Au^0 and Cl, S, N or C confirms that the biomolecules are at the surfaces of AuNPs[30]. Nuntawong *et al*., have also reported that the SERS intensity increases with decreasing the concentration of methylene blue[31]. Several Raman bands are found and in some peaks are splitting in SERS[32].

The splitting and shifting of few Raman bands pointed out that the methylene blue molecule is chemisorbed on AuNPs[32]. This study also revealed that various peaks were found in SERS spectrum, such as 1 012 and 1 398 cm^{-1} which correspond to histidine and cysteine respectively[33]. In general, such kind of protein molecules can bind on the surface of AuNPs. SERS analysis also verified that the protein molecules acted as a capping agent with the AuNPs.

The percentage of cell viability decreased by increasing concentration of AuNPs as well as incubation period. The synthesized AuNPs has shown 50% of cell death at 80 μg/mL concentration[16] which is considered as IC_{50} value, while at 120 μg/mL, concentration of nanoparticle, cell viability reduced to 33% at 24 h. Cell viability became almost 20% for maximum concentration at 72 h. Similar results were observed by Pernodet *et al*., in AuNPs on dermal fibroblasts[34]. Control cells were shown regular shape and AuNPs treated cells exhibited spherical shape and cell spreading patterns compared to control cells[16]. Biosynthesized AuNPs can travel into the ion channels through cell membranes and interact with DNA molecules, impeding replication and other nuclear works thus suppressing the metabolic activity[35–37]. The PI staining method has shown that plant mediated AuNPs caused cell death which occurred by apoptosis.

In conclusion, the AuNps were synthesized from *J. adhatoda* leaf extract in this study. The UV-Visible spectroscopy, SEM with EDAX, TEM, FTIR and SERS confirmed the monodispersed AuNPs which were capped with protein molecules. The synthesized AuNPs showed a potent anti-proliferation effect on the A549 lung cancer cell. Thus, the present study highlighted the biomedical properties of *J. adhatoda* mediated synthesis of AuNPs, which could form significant baseline information for development of a new nano-anticancer drug.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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References

[1] Kanchana A, Balakrishna M. Anticancer effect of saponins isolated from *Solanum trilobatum* leaf extract and induction of apoptosis in human larynx cancer cell lines. *Int J Pharm Pharm Sci* 2011; **3**(4): 356-364.

[2] Unno Y, Shino Y, Kondo F, Igarashi N, Wang G, Shimura R, et al. Oncolytic viral therapy for cervical and ovarian cancer cells by Sindbis virus AR339 strain. *Clin Cancer Res* 2005; **11**(12): 4553-4560.

[3] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**(2): 69-90.

[4] Gunalan S, Sivaraj R, Rajendran V. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Prog Nat Sci* 2012; **22**(6): 693-700.

[5] Rosarin FS, Arulmozhi V, Nagarajan S, Mirunalini S. Antiproliferative effect of silver nanoparticles synthesized using amla on Hep2 cell line. *Asian Pac J Trop Med* 2013; **6**(1): 1-10.

[6] Devi JR, Thangam EB. Mechanisms of anticancer activity of sulforaphane from *Brassica oleracea* in HEp-2 human epithelial carcinoma cell line. *Asian Pac J Cancer Prev* 2012; **13**(5): 2095-2100.

[7] Muthukumar T, Sudhakumari, Sambandam B, Aravinthan A, Sastry TP, Kim JH. Green synthesis of gold nanoparticles and their enhanced synergistic antitumor activity using HepG2 and MCF7 cells and its antibacterial effects. *Process Biochem* 2016; **51**(3): 384-391

[8] Ghagane SC, Puranik SI, Kumbar VM, Nerli RB, Jalalpure SS, Hiremath MB, et al. *In vitro* antioxidant and anticancer activity of *Leea indica* leaf extracts on human prostate cancer cell lines. *Integr Med Res* 2017; **6**(1): 79-87.

[9] Cragg GM, David J. New man plants as a source of anti-cancer agents. *J Ethnopharm* 2005; **100**(1-2): 72-79.

[10]Kaplan HG, Malmgren JA, Atwood MK. Triple-negative breast cancer in the elderly: Prognosis and treatment. *Breast J* 2017; **23**(6): 630-637.

[11]Maurya S, Singh D. Quantitative analysis of total phenolic content in *Adhatoda vasica* Nees extracts. *Int J PharmTech Res* 2010; **2**(4): 2403-2406.

[12]Singh TP, Singh OM, Singh BH. *Adhatoda vasica* Nees: Phytochemical and pharmacological profile. *Nat Prod J* 2011; **1**(1): 29-39.

[13]Dhankhar S, Kaur R, Ruhil S, Balhara M, Dhankhar S, Chhillar AK. A review on *Justicia adhatoda*: A potential source of natural medicine. *Afr J Plant Sci* 2011; **5**(11): 620-627.

[14]Chanu WS, Sarangthem K. Phytochemical constituents of *Justicia adhatoda* linn. found in Manipur. *Indian J Plant Sci* 2014; **3**(2): 2319-3824.

[15]Murugan R, Parimelazhagan T. Comparative evaluation of different extraction methods for antioxidant and anti-inflammatory properties from *Osbeckia parvifolia* Arn.-An *in vitro* approach. *J King Saud Univ Sci* 2014; **26**(4): 267-275.

[16]Lokina S, Stephen A, Kaviyarasan V, Arulvasu C, Narayanan V. Cytotoxicity and antimicrobial activities of green synthesized silver nanoparticles. *Eur J Med Chem* 2014; **76**: 256-263.

[17]Maurya S, Singh D. Quantitative analysis of total phenolic content in

Adhatoda vasica Nees extracts. *Int J PharmTech Res* 2010; **2**(4): 2403-2406

[18]Krishnan V, Kalayanraman R, Fathima G, Sheriff K, Illiyas V, Rather HA. Green synthesis of silver nanoparticles using *Piper nigrum* concoction and its anticancer activity against MCF-7 and Hep-2 cell lines. *World J Pharm Pharamaceutl Sci* 2015; **4**(4): 723-733.

[19]Jayaseelan C, Ramkumar R, Abdulrahuman A, Perumal P. Green synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and its antifungal activity. *Ind Crop Prod* 2013; **45**: 423-429.

[20]Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. *Langmuir* 1996; **1**: 788-800.

[21]Noruzi M, Zare D, Khoshnevisan K, Davoodi D. Rapid green synthesis of gold nanoparticles using *Rosa hybrida* petal extract at room temperature. *Spectrochim Acta A Mol Biomol Spectrosc* 2011; **79**(5): 1461-1465.

[22]Umamaheswari C, Lakshmanan A, Nagarajan NS. Green synthesis, characterization and catalytic degradation studies of gold nanoparticles against congo red and methyl orange. *J Photochem Photobiol B* 2018; **178**: 33-39.

[23]Vanaja M, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Malarkodi C, Annadurai G. Kinetic study on green synthesis of silver nanoparticles using *Coleus aromaticus* leaf extract. *Adv Appl Sci Res* 2013; **4**(3): 50-55.

[24]Fayaz AM, Balaji K, Kalaichelvan PT, Venkatesan R. Fungal based synthesis of silver nanoparticles-An effect of temperature on the size of particles. *Colloids Surf B Biointerfaces* 2009; **74**: 123-126.

[25]Veerasamy R, Xin TZ, Gunasagaran S, Xiang TF, Yang EFC, Jeyakumar N, et al. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *J Saudi Chem Soc* 2011; **15**: 113-120.

[26]Mittal AK, Bhaumik J, Kumar S, Banerjee UC. Biosynthesis of silver nanoparticles: Elucidation of prospective mechanism and therapeutic potential. *J Colloid Interface Sci* 2014; **415**: 39-47.

[27]Khalil MMH, Ismail EH, EI-Magdoub F. Biosynthesis of Au nanoparticles using olive leaf extract. *Arabian J Chem* 2012; **5**(4): 431-437.

[28]Verma B, Ghosh SK, Kundu S, Panigrahi S, Praharaj S, Pande S. Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. *J Colloid Interface Sci* 2007; **313**: 724-734.

[29]Arumai Selvan D, Mahendiran D, Senthil Kumar R, Kalilur Rahiman A. Garlic, green tea and turmeric extracts-mediated green synthesis of silver nanoparticles: Phytochemical, antioxidant and *in vitro* cytotoxicity studies. *J Photochem Photobio B* 2018; **180**: 243-252.

[30]Lahr RH, Vikesland PJ. Surface-enhanced raman spectroscopy (sers) cellular imaging of intracellulary biosynthesized gold nanoparticles. *ACS Sustainable Chem Eng* 2014; **2**(7): 1599-1608.

[31]Nuntawong N, Horprathum M, Eiamchai P, Wong-ek K, Patthanasettakul V, Chindaudom P. Surface-enhanced Raman scattering substrate of silver nanoparticles depositing on AAO template fabricated by magnetron sputtering. *Vacuum* 2010; **84**(12): 1415-1418.

[32]Xiao GN, Man SQ. Surface-enhanced Raman scattering of methylene blue adsorbed on cap-shaped silver nanoparticles. *Chem Phys Lett* 2007; **447**(4): 305-309.

[33]Schwartzberg AM, Grant CD, Wolcott A, Talley CE, Huser TR, Bogomolni R, et al. Unique gold nanoparticle aggregates as a highly active surface-enhanced raman scattering substrate. *J Phys Chem B* 2004; **108**(50): 19191-19197.

[34]Pernodet N, Fang XH, Sun Y. Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts. *Small* 2016; **2**(6): 766-773.

[35]Rajeshkumar S. Anticancer activity of eco-friendly gold nanoparticles against lung and liver cancer cells. *J Genetic Eng Biotechnol* 2016; **14**: 195-202.

[36]Martins D, Frungillo L, Anazzetti MC, Melo PS, Durán N. Green synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and its antifungal activity. *Ind Crop Prod* 2013; **45**: 423-429.

[37]Manikandan R, Manikandan B, Raman T, Arunagirinathan K, Prabhu NM, Jothi Basu M. Biosynthesis of silver nanoparticles using ethanolic petals extract of *Rosa indica* and characterization of its antibacterial, anticancer and anti-inflammatory activities. *Spectrochim Acta A Mol Biomol Spectrosc* 2015; **138**: 120-129.