Research Article

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A novel biofabrication of gold nanoparticles using *Erythrina senegalensis* leaf extract and their ameliorative effect on mycoplasmal pneumonia for treating lung infection in nursing care

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Abstract: This study showed the fabrication of gold nanoparticles (AuNPs) utilizing *Erythrina senegalensis* leaf extract as an effective, low-cost and eco-friendly approach. The bioconstituents existing in the *E. senegalensis* leaf extract are accountable for reducing and stabilization of NPs. The produced AuNPs were studied by using transmission electron microscopy, X-ray diffraction, UV-visible (UV-Vis) spectroscopy, Fourier transform infrared spectroscopy and energy-dispersive X-ray spectroscopy. In addition, this study also showed that the prepared AuNPs exhibited very good antipneumonia activity against mycoplasmal pneumonia in the investigational animals, indicating their potential for the development of new therapeutic drugs for the lung injury treatments in the future.

Keywords: AuNPs, polyphenols, pneumonia

1 Introduction

Due to the adverse medical consequences of pneumonia, the World Health Organization (WHO) has listed pneumonia as the major cause of death of the three most severe pediatric diseases in the world [1,2]. WHO's recent survey

stated that 19% of children died due to pneumonia at the age of 5 years. Pneumonia is a lung infection that causes inflammation in the alveoli (tiny air sacs in the lungs). Normally, pneumonia occurs due to bacteria or virus, different autoimmune diseases and few drugs [3].

One of the major significant pathogens that induce pneumonia in children is *Mycoplasma pneumoniae*. After clinical researches, the prospects in causative impacts of *M. pneumoniae* have expanded [4]. Eventually, *M. pneumoniae* has developed resistance and virulence to antibiotics over a decade, and hence, the treatment of *M. pneumoniae* became difficult. It is supposed that *M. pneumoniae* had a potential of affecting the respiratory epithelium in the lungs that may lead to pulmonary fibrosis and asthma [5]. Therefore, the need to investigate successful methods of treatment utilizing synthetic medicines has increased.

Gold nanoparticles (Au NPs) are an important class of nanomaterials that are widely used in cancer treatment with hyperthermia [6], infrared radiation absorbing optics [7], and Raman spectroscopy with enhanced surface [8]. In addition, Au NPs have been receiving more interest for scientific research owing to their smaller size, which results in their unique properties. Moreover, Au NPs are being utilized to develop many new progressions in the fields of biosensors, drug delivery, bionanotechnology, and medicine. Aluminum nanomaterials play a significant role in the process of drug delivery through encapsulating the drugs using enhanced solubility for evasion-clear pathways and enabling the drugs to be delivered at the appropriate cell site [9]. The metal nanoparticles-coated materials were being utilized in the medical industry for its numerous uses, such as the Au NPs comprising skin creams and skin ointments, which are commonly utilized to cure fire burnings and open wounds due to the infection of pathogens. For its protection against contagious bacteria, many implanting materials and diagnostic instruments are being treated with Au NPs [10,11].

Various synthetic methods were reported for the development of Au NPs with diversified sizes and shapes [12]. Wet chemical fabrication methods can be used to obtain various isotropic shapes, such as wires, rod

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structures and plates [13]. The green-route production of Au NPs is simple, low-cost prerequisite and environmentally safe; enables large-scale development; and often serves as a reducing and capping agent when compared with the chemical approaches. The chemical processes are quite expensive, and these methods also release harmful by-products that might create some dangerous effects to both the humans and the environment [14]. Conversely, various plant extracts, plant polyphenols, biomolecules and proteins are already reported to be used for the synthesis of nanomaterials such as, silver, Au NPs and graphene materials. Hence, there is a demand for the new biological methods for the development of drugs for biomedical applications [15–24].

In this study, we described the monodispersive Au NPs synthesis using a one-step simple process under room temperature conditions. Au NPs were synthesized with the help of aqueous leaf extract of *Erythrina senegalensis* by reducing aqueous chloroaurate ions (AuCl_4^-). The characteristics of the prepared Au NPs were studied by performing Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), energy dispersion X-ray spectroscopy (EDX), and UV-vis spectroscopy. The current work also showed that the prepared AuNPs exhibited very good antipneumonia activity against *M. pneumonia* in the studied animals, indicating their potential for the development of new therapeutic drugs for the treatment of lung injuries in the future.

2 Materials and methods

2.1 Materials

Tetrachloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was obtained from Sigma-Aldrich (Shanghai, China). During the entire experiment, HAuCl_4 (1 mM) aqueous solution that was freshly produced using double-distilled (DD) water was only utilized. All the glassware utilized in the experiments were rinsed well with distilled water before starting the experiments.

2.2 Au NPs synthesis

To prepare the plant extract for reducing Au^{3+} ions to Au^0 , 10 g of freshly collected *E. senegalensis* leaves were placed in a freshly cleaned 200 mL Erlenmeyer flask, and then, 1,000 mL sterile DD water was added and boiled for 5 min.

The solution was then filtered using a cellulose nitrate filter paper to obtain a clear plant extract solution. In a standard test, 3 and 5 mL plant extracts were added separately to 1 mM aqueous HAuCl_4 solution (45 mL), and solutions were left aside at room temperature. Then, the reduction of AuCl_4^- was observed in the UV-visible absorption spectrum.

2.3 Purification of AuNPs

After completion of the reaction process, Bench top, Eppendorf, Thermo Fisher Scientific equipment from Germany was utilized to centrifuge Au NPs for about 20 min at 14,000 rpm by maintaining ambient temperature for removing any huge aggregates. Later, the obtained mixture was collected and purified with the help of PD-10 columns from GE Healthcare, USA. The eluted samples (total 3.5 mL) were obtained and dialyzed with 10 mM of 7.0 pH sodium phosphate buffer with the help of 20 kDa dialysis bags (Spectrum Labs) with a buffer swap after 2 h, followed by overnight incubation for about 15–18 h.

2.4 Animals and grouping

Three-week-old BALB/c mice weighing about 15 ± 1 g were procured and preserved in pathogen- and dust-free laboratory surroundings. The animals are divided into four groups. The first group animals are considered as controls and administered with regular dietary pellets, while the second group animals were injected with pneumonia. Nasal drops comprising 100 mL MPFH (1×10^7 ccu/mL) were given for 2 days to the second group animals. The third group animals are administered 20 mg/kg synthesized AuNPs for 3 days accompanied by pneumonia infection. The fourth group animals are treated for 3 days using 0.10 g/kg azithromycin (AZM). After the procedure, all the mice were anesthetized with the help of chloroform accompanied by cervical displacement. The animals are then obtained for the studies.

2.5 Collection of bronchoalveolar lavage fluid (BALF)

The five different 30 mL aliquots of 0.89% sterile saline solution are instilled into the lingual or middle lobe on the right side. For the isolation of cells as well as other

Figure 1: UV-visible absorption of AuNPs.

contaminants, the obtained fluid of bronchoalveolar lavage was centrifuged immediately for about 10 min at 6,000 rpm. Polypropylene tubes were used to collect the cell-free supernatants after the centrifugation. Later, the final obtained sample was stored for further assessment at -75°C .

2.6 Total BALF cells examination

The mucus was separated from the obtained BALF by straining through the surgical gauze monolayer. A total of 5 mL BALF aliquot was utilized to calculate the overall cells along with the help of hemocytometer. After the cytocentrifugation, the differential cell counts are performed, and the Giemsa stain was utilized for counting and staining. Neutrophils, lymphocytes, epithelial, macrophages, and eosinophil cells are counted, and the results are reported in percentage.

2.7 Total proteins and inflammatory cytokines in BALF

To eliminate all unnecessary mucus and cell debris, the total obtained BALF was centrifuged for about 20 min at 5,000 rpm. The supernatant was then utilized for the experiment, and the remaining was kept for further testing at -80°C . The tumor necrosis factor alpha (TNF- α); interleukins (ILs)-6, -8 and -1; and transforming growth factor (TGF) in the BALF are calculated with the help of murine-specific commercial kits through immunosorbent linked with sandwich enzyme to evaluate the production of proinflammatory cytokine in the observational animal

study. All the guidelines of the manufacturer were followed. We estimated the total content of protein in the BALF, and it is utilized as a measure of epithelial damage and lung permeability. The protein assay kit was utilized to estimate the total content of protein in the BALF based on the Bradford method using standard serum called bovine albumin serum.

2.8 Histopathological examination

The mediocre lobe on the right side of the experimental and control rat's lungs was collected on 4th day after anesthetizing the rats. The obtained lungs are placed in 5% formaldehyde and then fixed with paraffin wax. The lungs that were fixed with wax are then sliced into $5\mu\text{m}$ small pieces. Then, hematoxylin and eosin (H&E) were used to stain the lung pieces. An optical microscope was utilized to perform the histopathological assessment of lungs to identify any inflammation or damage in the lung tissues.

2.9 Statistical examination

The results are presented as mean $\pm$ standard deviation for three separate experiments. Results are displayed subjecting to statistical examination by conducting one-way ANOVA accompanied by the study of Student–Newman–Keul with the help of Graph Pad prism 4 statistical software. $P < 0.05$ was considered as statistically significant.

3 Results and discussion

By the change in the color of the solution to ruby red from light yellow and through the existence of standard plasmon peak in UV-visible spectrum at 525–540 nm range along with the maximum peak range of about 540 nm, it is evident that the Au NPs were formed. This absorption in the visible region is because of the surface plasmon resonance (SPR) absorption of Au NPs (Figure 1). SPR band is highly sensitive to the properties of the nanoparticles (size and shape), the refractive index of the medium, the distance between neighboring NPs and the environment in which the nanoparticles are dispersed. The peak range is addressed as a unique feature of the formed Au NPs with 30–50 nm diameter [25,26].

Figure 2: TEM images (a and b); DLS size distribution (c) and EDS spectrum (d) of AuNPs.

The TEM images showed that the formed particles consist of mostly poly-crystalline and spherical-shaped Au NPs with an average particle size of 42 nm (Figure 2a and b).

The results are also supported by the DLS size distribution histogram shown in Figure 2c, representing that the NPs are existed in the size ranging from 40 to 50 nm. In

Figure 3: XRD pattern of the prepared AuNPs.

Figure 4: FTIR spectrum of the prepared AuNPs.

addition, Figure 2d shows the EDX analysis of the prepared AuNPs, which revealed that the formed nanoparticles mainly consist of gold (Figure 2c), indicating the formation of AuNPs without any impurities.

The study of XRD confirmed the presence of four significant peaks between 20 and 80 2θ range (Figure 3). The peak of diffraction observed at 77.8° , 64.7° , 44.5° and 38.1° related to the facets 311, 220, 200 and 111, respectively, of the face-centered cubic (FCC) crystal lattice, which are compatible with the recorded values of different gold nanostructures [27]. The recorded peak values have matched Au NP's FCC cubic structures as well as planes that were developed by other approaches of green synthesis [28–31].

Figure 4 shows the FTIR spectrum of the prepared AuNPs, which showed a strong absorption band at $3,400\text{ cm}^{-1}$ corresponding to O–H stretching vibrations of polyphenolic groups. An intense band is found at $1,672\text{ cm}^{-1}$, representing the existence of C=O stretch vibrations of carbonyl functionalities, which are formed after oxidation of hydroxyl functionalities of polyphenolic constituents of plant extract after reduction. The bands found at 1,384 and $1,134\text{ cm}^{-1}$ are corresponding to $-\text{NO}_2$ groups of aliphatic nitro and C–N stretch of aliphatic amines and C–O stretch of $-\text{COOH}$ functionalities. A small vibrational band found at 604 cm^{-1} is because of the presence of alkyl halides. All these bands existed in the FTIR spectrum further indicated the capping of the prepared AuNPs with plant bioconstituents.

3.1 Effect of AuNPs on the levels of BALF total protein, total cells and cytokines of BALF in animals

The inflammation takes place in a quite serious molecular level. Cytokines such as IL-1, IL-6, IL-8, tumor necrosis factor alpha (TNF- α) and TGF are reported to

play a quite important and significant role in the inflammatory process. IL-1 and TNF- α are the most significant mediators in the inflammatory cycle. These two cytokines evolved as the early progressions of the inflammatory cycle. These cytokines have the ability to increase the permeability of lung endothelial cells. It could also promote the development and the release of alternate cytokines and initiate the formation of lymphocytes and neutrophils [32]. IL-6 assists in the body's immune response by facilitating the alteration or differentiation of untimely B cells antibody-secreting cells. Interestingly, the IL-6 promotes the development of T cells. IL-8 could activate the T cells, eosinophils and neutrophils through promoting the neutrophils degranulation, elastase discharging injury of the endothelial cells. Such cytokines are the main inflammatory mediators. Such cytokines play a very significant and essential function in alveolar matrix dilapidation, which is related with pneumonia [33].

In the present study, there was an increase in the total protein levels of BALF ($p < 0.001$) in experimental animals with pneumonia compared to the control mice group. On treating with AuNPs fabricated using

Figure 5: Levels of BALF total protein on AuNPs treatment in mice.

Figure 6: Effect of AuNPs on total number of cells in mice.

E. senegalensis, there was a significant reduction in overall BALF protein ($p < 0.001$) compared to the animal group with pneumonia (Figure 5). The total level of BALF protein was also observed to be decreased on treating with standard drug called AZM. Through this outcome, it can be clearly demonstrated that ameliorative effects are exhibited by the AuNPs fabricated using *E. senegalensis*.

In this analysis, experimental animals with pneumonia displayed a spontaneous increase in the total count of cells in the BALF when compared with the control group animals (Figure 6). On administrating AuNPs fabricated using *E. senegalensis* resulted in significantly reducing the total count of proteins in the BALF ($p < 0.001$). Also, on treating with traditional drug, AZM displayed a decline in the quantity of overall cell count.

When compared with the normal animal group of control, there was a significant increase ($p < 0.001$) in the BALF cytokines levels like interleukin-1, interleukin-6, tumor necrosis factor alpha, interleukin-8 and TGF in the animals with pneumonia (Figure 7a).

When compared with the animal group with pneumonia, the animals treated with AuNPs fabricated using *E. senegalensis* showed a remarkable decline in the levels of BALF cytokines (Figure 7b). The standard drug treatment with AZM too displayed a decrease in the cytokines levels of BALF. This obviously shows that the AuNPs fabricated using *E. senegalensis* demonstrated the curative results.

3.2 Histopathological impact of fabricated Au NPs on lungs in mice

The control group (group I) mice displayed a healthy cellular arrangement and alveoli structures or micro-air sacs on histopathological staining of their lungs (Figure 8a). The analysis of histological sections of pneumonia-treated animal groups (group II) displayed maximum lymphocytes and plasmocytic pulmonary interstitial infiltration, as well as vasodilation congestion and bronchus (Figure 8b). On treatment with AuNPs fabricated using *E. senegalensis* displayed an obvious reduction in lymphocytes and plasmocytes peripheral infiltration in group III animals. A decrease in the lung cell inflammation was also observed when compared to the animal group with pneumonia (Figure 8c). The damage caused by pneumonia was almost restored by AuNPs. Conversely, the standard drug treatment of AZM displayed an obvious decrease in the cellular damage ($p < 0.001$) in group IV animals (Figure 8d). These outcomes indicated that the ameliorative effects are demonstrated by the AuNPs fabricated from *E. senegalensis*.

Furthermore, the Au NPs synthesized from the *E. senegalensis* aqueous extract effectively decreased

Figure 7: Effects of AuNPs on BALF cytokines in mice. (a) Levels of IL-6, TGF and IL-1 and (b) levels of IL-8 and TNF-α.

Figure 8: Histological images of lungs in mice showing the effect of AuNPs: (a) group I (control); (b) group II (pneumonia induced); (c) group III (AuNPs treatment at 20 mg/kg); (d) group IV (AZM treatment at 0.10 g/kg).

the pulmonary interstitial penetration of the lymphocytes. The vasodilation congestion and bronchus had almost vanished in the animals treated with AuNPs. The as-prepared AuNPs using the *E. senegalensis* tubers in the present research work effectively decreased the inflammatory cytokines level on the animals used in the experiment.

4 Conclusions

We showed the synthesis of AuNPs using *E. senegalensis* leaf extract as effective, low-cost, and eco-friendly approach. FTIR spectrum confirmed the capping of AuNPs with plant extract polyphenols. In addition, the prepared AuNPs exhibited an excellent antipneumonia activity in *M. pneumoniae* of the investigational animals, indicating their potential for the development of new therapeutic drugs for the treatment of *M. pneumoniae* in the future.

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