Original Article

Laser Fragmentation of Green Tea-synthesized Silver Nanoparticles and Their Blood Toxicity: Effect of Laser Wavelength on Particle Diameters

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Abstract

Background: The efficacy of fractionation is significantly impacted by the colloidal particles' spontaneous absorption of laser beam radiation. The classification of silver nanoparticles during fragmentation processing is regulated through the collection of a combination of laser pulses with wavelengths of 1064 nm and 532 nm. **Aims and Objectives:** This study presents an investigation of the efficacy of a plant extract in conjunction with the incorporation of supplementary silver nanoparticles, as well as the generation of smaller-sized silver nanoparticles using laser fragmentation.and then measure thier toxity on the blood. **Results:** Ag nanoparticles were synthesized using pulsed laser fragmentation on green tea AgNPs. The synthesis process involved the utilization of a Q-switch Nd:YAG laser with wavelengths of 1064 nm and 532 nm, with energy ranging from 200 to 1000 mJ. Initially, a silver nano colloid was synthesized through the process of fragmented of the Ag target using the second harmonic generation of 532 nm at various energy levels. The optimal energy within the selected wavelengths was determined in order to facilitate the ultimate comparison. Transmission electron microscopy (TEM) was used to determine surface morphology and average particle size, while a spectrophotometer was used to analyses UV light's spectrum characteristics. The measurements focused on the surface plasmon resonance (SPR) phenomenon. The absorption spectra of silver nanoparticles exhibit distinct and prominent peaks at wavelengths of 405 nm and 415 nm. The mean diameter of the silver nanoparticles was found to be 16 nm and 20 nm, corresponding to wavelengths of 1064 nm and 532 nm, respectively. **Conclusion:** As a consequence, there is a decrease in the range of particle sizes and a decrease in the mean size to lower magnitudes, resulting in a very stable colloid. This particular methodology has demonstrated considerable efficacy in the production of colloidal suspensions with the intended particle dimensions. Moreover, by the analysis of nanoparticles in human blood, no discernible alterations in the blood constituents were seen, indicating their non-toxic nature.

Keywords: Fragment, laser pulse, nanoparticles, toxicity blood

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Introduction

Nanoparticles are the foundation of nanotechnology, used in biosensors and electronic nanodevices.[1,2] Due to their size-dependent physical and chemical characteristics, metal nanoparticles are fascinating. The utilization of laser ablation of solids to generate metal nanoparticles inside a liquid medium presents an alternate approach to the conventional chemical procedure. The simplicity of the laboratory configuration distinguishes this procedure. Laser fragmentation produces metal nanoparticles in a manner comparable to chemical methods but without the presence of residual ions originating from the reactants.[3] Surface plasmon resonance (SPR), as described in reference,[4] occurs when mineral nanoparticles undergo a transition toward longer wavelengths in the optical absorption spectrum due to an increase in their size. Nevertheless, the presence of noble metallic nanoparticles will be discernible within this particular location.

The phenomenon of reciprocal oscillation exhibited by electrons plays a significant role in determining the power associated with their plasmon resonance. According to

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previous research,[5] it is commonly believed that these organisms possess adaptability in relation to their size, shape, and surrounding environment.[6] Hence, by subsequent ultraviolet-visible (UV-VIS) absorption, the plasmon resonance would facilitate the unambiguous transmission of both shape and size through this particular absorption mechanism. The transfer frequency between these ranges is significantly distant from the SPR energy of Ag. It is worth mentioning that these silver nanoparticles (AgNPs) possess a distinct edge over the majority of other metallic nanoparticles, including gold and copper. The plasmon resonance of AgNPs is a widely recognized phenomenon wherein the wavelength of this resonance is contingent upon the size and form of the nanoparticles, typically occurring at a wavelength of 400 nm.[7] While it is of great importance to regulate the distribution of particles in terms of size, the mechanism by which colloids are formed through laser ablation is a subject of considerable interest. The investigation was not conducted comprehensively. Several researchers have conducted studies to examine the impact of status ablation on the efficiency of ablation and particle size.[8,9] Our research is centered on the fabrication of AgNPs through the utilization of laser ablation to remove mineral components from pure water, without the inclusion of any chemical additions. In this study, Q-switched Nd:YAG pulses with wavelengths of 1064 nm and 532 nm were employed to synthesize AgNPs in an aqueous medium. In addition, distinct laser energy was utilized to irradiate silver (Ag), hence facilitating the production of these nanoparticles. The resulting AgNPs hold promising potential for various biomedical applications, thereby opening up new avenues for their utilization in this field.

The utilization of botanical extracts in the synthesis of metallic nanoparticles is a burgeoning field of study that has garnered significant attention in recent years. This research endeavor aims to supplant hazardous and finite chemical substances with more sustainable alternatives. The topic of environmentally sustainable synthesis of nanoparticles has garnered significant interest in recent years.[10] The core notion underlying synthesis involves the ability to reduce metal ion precursors through the use of flavonoids and alkaloids. Several types of metal and metal oxide nanoparticles, such as AgNPs,[11] gold nanoparticles,[12,13] zinc oxide nanoparticles,[14] and platinum nanoparticles, are synthesized using plant extract as a reducing agent.[15] The development of green-synthesized AgNP is a topic of interest in academic study, as numerous studies have demonstrated the influence of plant extract type, content, and synthesis methods on the characteristics of the synthesized nanoparticles. The purported occurrence of antibacterial, antifungal, anticancer, and antioxidant activities has been attributed to the green synthesis of AgNPs. Shape and form, which depend on plant extract, composition, and synthesis method, affect AgNPs' activity, and physical and chemical properties.[16] Several studies have indicated a strong correlation between the biological and chemical activity of AgNPs and their physicochemical features. The current study investigates the utilization of green tea leaf extract as a reducing agent during the production of AgNPs. The primary objective of this study is to investigate the extraction, optimization, synthesis, and evaluation of the antibacterial characteristics of the generated AgNPs. In addition, the use of plasma-liquid interactions (pulsed laser fragmentation in liquid) is employed to mitigate the formation of nanoparticles that occur during the extraction and chemical reduction processes involving green tea plants.

Experimental Setup

Materials

All reactants used in this inquiry are of analytical quality and have not undergone any further purification. The Ag nitrate ($AgNO_3$) was procured from Avonchem Limited, a company based in the United Kingdom. The collection of green tea leaf extract took place in Baghdad, Iraq, procured from a local market. The technique of immersion was employed in the production of green tea leaf extract (GTE). A total of 50 g of dehydrated green spinach leaves were immersed in approximately 100 ml of water-based solvent for an extended period. The GSE was isolated from the mixture using the solution filtration method. The soaking extract was, thereafter, subjected to drying at a temperature of 50°C within an oven. Following the drying process, a quantity of 1 g from the resulting powder was carefully measured and subsequently subjected to analysis. This analysis involved diluting the extract with 50 ml of distilled water and subsequently combining it with diluted $AgNO_3$ in a molar ratio that was specifically determined for the purpose of research.

The process of synthesizing silver nanoparticles

The synthesis of AgNPs involved the combination of $AgNO_3$-10-2 M with GSE at a fixed volume ratio of 20%. The mixture was subjected to a 5-h treatment at ambient temperature to ensure a reduction reaction between Ag+ and Ag0.[8] The decline investigation was validated using the utilization of UV-VIS spectroscopy. The investigation of AgNPs was further conducted using an analyzer of particle size, an infrared microscope, and a Transmission -Electron Microscope (TEM) Fourier transform. The Particle Size Analyzer HORIBA and the JEOL TEM apparatus were employed in conducting the tests, utilizing a dynamic light dispersion system running at 120 kV. To assure the acquisition of a single phase of Ag during synthesis, the Perkin-Elmer spectrometer equipment was employed. Next, X-ray diffractometry was conducted using the Shimadzu ×6000 equipment, emitting CuKα wavelength filtered by nickel.

Method work

Figure 1 depicts the laboratory experimental setup employed for the generation of Ag colloids using laser fractionation, as well as its impact on the extract solution containing $AgNO_3$. The liquid solution of the nano extract that had been created was transferred into the baker using a glass container. During

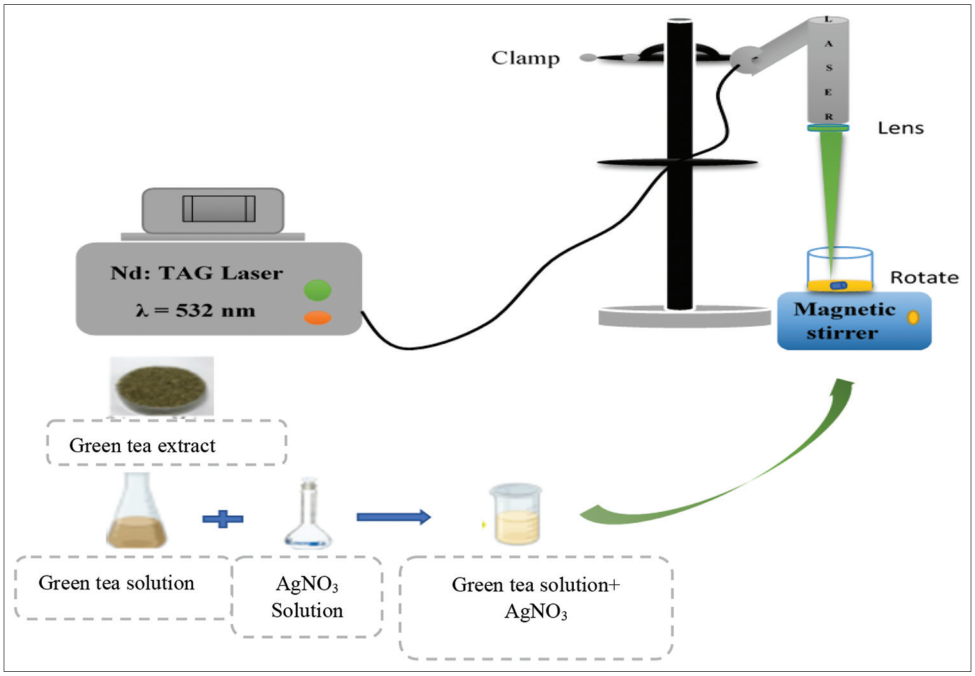

Figure 1: The present study outlines the experimental methodology employed for the synthesis of silver colloids by the process of laser ablation. $AgNO_3$: Silver nitrate

the fractionation process, the solvent is subjected to a steady stirring motion, facilitating the dissolution of the colloidal particles. Preparations were conducted under aerobic conditions in an open-air environment. The experimental setup involved the utilization of a Q-Switched Nd:YAG laser system, which operated at a pulse energy range of 200–1000 mJ/pulse. The system emitted pulses with a duration of 10 ns and a repetition rate of 1 Hz. In this experiment, a convex lens with a focal length of 12 cm was employed to concentrate laser beams with diameters of 2.1 mm (532 nm) and 2.4 mm (1064 nm). The intensity of the laser beam was measured by selecting the center portion.

Colloidal Silver Characterization

The colloid solution was collected in increments of 3 ml after each pulse or fragmentation. A 1 cm wide quartz cell was used to characterize the UV-VIS optical spectrum. The Japanese-made Hitachi U-3410 spectrophotometer was used to record the colloid absorption spectrum. For the purpose of taking micrographs of the samples prepared.

The electrons of the resulting nanoparticles were studied using a transmission electron microscope (JEM-200CX) with an acceleration voltage of 200 kV. A 2-mL blood sample was collected using two different tubes. Whole blood was in the second tube, whereas AgNPs were in the first. For 1 h, blood samples were incubated at 37°C. The researchers used a hematological auto analyzer, namely an Orphee Mythic 22 Hematological Analyzer made by Diamond Diagnostic in the USA, to check the numbers of red blood cells (RBCs), white blood cells (WBCs), and hemoglobin (HB) in the participants' blood.

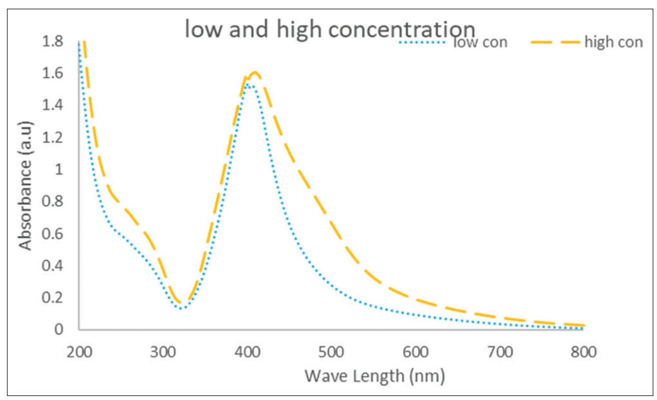

Figure 2: Laser excitation at 532 nm and 1064 nm is used to examine the ultraviolet-visible absorption spectrum of silver-water colloidal particles

Results and Discussion

The absorption spectra of the colloidal solution created by the process of fractionation and targeting of Ag in the extract using $AgNO_3$ and focused laser radiation are depicted in Figure 2. The dimensions of the laser beam in relation to the focal point.

The energy levels were measured at 2.1 mM (for a wavelength of 532 nm) with a pulse energy of 1000 mJ, and at 2.4 mM (for a wavelength of 1064 nm) with a pulse energy of 1000 mJ, in a sequential manner. The ablation period lasted for 15 min. The observed spectrum has distinct features that are indicative of colloidal solutions containing Ag particles.[10] Specifically, it displays a symmetrical peak centered at 401 nm, accompanied by a significant tail that extends into the ultraviolet region of the electromagnetic spectrum. The absorption bandwidth of electronic devices is around 70 nm (full width at half maximum), which is significantly narrower compared to that of a colloid generated through chemical processes.[4] The molecules' peak and tail regions are extracted at a wavelength of 400 nm. Sequentially, the stimulation of plasmons and the transmission between interband states occur. The presence of a singular peak observed at a wavelength of 405 nm can be attributed to the reality that the solution contains nanoparticles that exhibit a spherical shape with a high level of certainty.

The UV-VIS spectrum of AgNPs at different wavelengths is illustrated in the accompanying photos. Figure 2 displays the absorption spectra of Ag colloids in the form of Ag foil, which underwent fragmentation due to continuous fluence. This observation showed an increase in the number of crumbs absorbed within the UV-VIS spectral area at a wavelength of 532 nm, accompanied by a slight decrease in their average size. The phenomenon under consideration can be elucidated by the utilization of the self-absorption technique, as a multitude of metallic particles are formed along the trajectory of the incident laser beam. The absorption bandwidth of the peak has been reduced to a wavelength of 462 nm. This statement can be interpreted as indicating a limited and restricted distribution of particles.[17,18]

The objective of this study was to examine the stability of the Ag nanoparticles by analyzing the energy absorption of the colloidal solution. As depicted in Figure 3, there is a discernible shift toward a higher wavelength in the vicinity of the surface plasmon (SP) peak, accompanied by a marginal reduction in absorption. Therefore, in the context of these energy levels, it can be observed that AgNPs exhibit a lack of coagulation and possess a notable degree of stability. The plasmon band of the Ag particles was significantly influenced by their size and form, as stated in the reference.[11] The measurement of the absorbance of the interband absorption at 250 nm was conducted to evaluate the performance of the fragmentation. The variability in the effectiveness of the ablation procedure is mostly contingent upon the impact of the laser. To establish an accurate correlation, it is important to conduct tests pertaining to the enduring spot size. When comparing between the fluency of the quality and the fragmentation of laser to get the required size.

The absorption spectra of Ag colloidal solutions, as depicted in Figure 4 demonstrate variations in response to different levels of laser fluence. The diameter of the spot measured 2.1 mm at a wavelength of 532 nm, and the ablation procedures were conducted for 15 min. The augmentation of laser fluence results in a corresponding increase in both ablation and absorption at the plasmon resonance peak. The observation of a minimal average particle size is indicative of a concurrent blue shift alteration. The energy per pulse is measured at 200, 400, 600, 800, and 1000 mJ.

Figure 5 illustrates the absorption spectra of Ag colloids. In this instance, the Ag foil undergoes ablation through

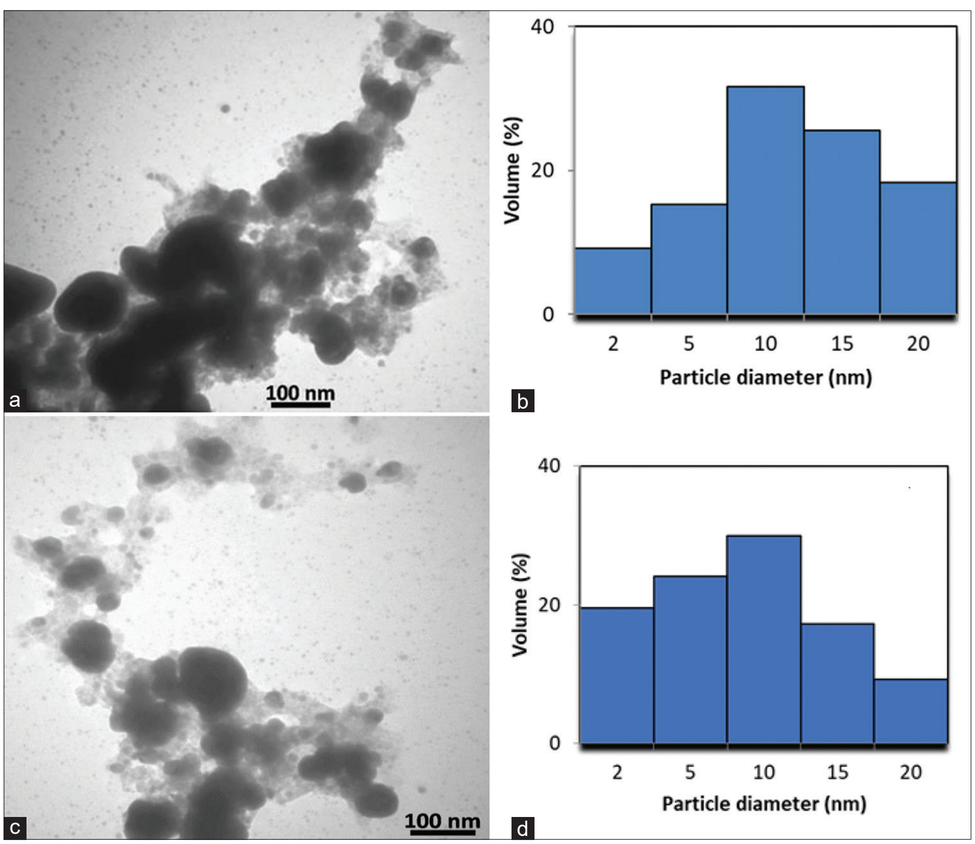

Figure 3: (a and c) Silver nanoparticles were synthesized using a plant extraction method involving green tea. The images were captured using two different wavelengths, specifically 532 nm and 1064 nm. The size distribution of these particle are in b & d , respectively

continuous fluence, leading to the generation of crystals with diverse energy levels. The spot size measured 2.4 mm (at a wavelength of 1064 nm), and the ablation procedure lasted for 15 min. Enhanced absorbance levels and improved ablation performance were the results of increasing the ablation energy per pulse from 200 to 1000 mJ, which enhanced the fragmentation process. This observation demonstrates an increase in the quantity of crumbs absorbed in the UV-VIS spectral region, accompanied by a slight reduction in their average size. The ablation adequacy and absorption levels exhibited a drop to 400 nm following a duration of 15 min of ablation. Continued enhancement of the ablation capabilities beyond 1000 mJ per pulse once again results in an escalation of absorption. The phenomenon under consideration can be elucidated through the lens of self-absorption, as numerous metal particles are generated along the trajectory of the laser beam's incidental light. Therefore, the phenomenon of blue transition is linked to the decay of macroscopic particles. The absorption bandwidth of the peak has been decreased to 69 nm. This term specifically denotes a densely packed distribution of particles. In earlier investigations,[8,12] it has been observed that the performance of colloid formation at a wavelength of 532 nm was superior to that achieved with 1064 nm lasers operating at an energy density below 1 J/cm^2.

Colloidal particle absorption of laser beam light has a substantial impact on the efficacy of ablation, as demonstrated in the preceding section. There is an expectation that the capacity of Ag particles to absorb their light when exposed to a wavelength of 532 nm will exceed that which was observed at 1064 nm. This disparity can be mostly attributed to the existence of plasmon bands that are centered around 401 nm. According to Ruaa A. mohammed *et al*. (2021), the increase in plasmon bands can be ascribed to the clustering of Ag particles. This section investigates the improvements implemented in the laser ablation method for Downsizing Ag colloids to the nanoscale. The pulses emitted by the 1064 nm laser, each carrying an energy of 1000 mJ, demonstrate effective suppression for 15 min. The suppression of SPR is accomplished through the utilization of colloids, which demonstrate a peak extinction at 401 nm and a bandwidth of 84 nm. Furthermore, the colloids demonstrate a significant extension of their extinction tail into the near-infrared (NIR) spectral range. The laser pulse with a wavelength of 1064 nm caused the dispersion of particles, leading to the creation of a colloid. This colloid exhibited a SPR with a limited width of 74 nm, which was only observable for a short distance. However, the overall extinction of the SP band did not change. The utilization of laser pulses with a wavelength of 532 nm resulted in a decrease in the breadth of the extinction band. Furthermore, it was observed that the absorption was heightened in comparison to laser pulses with a wavelength of 1064 nm in this specific situation. In a broad sense, it can be deduced that the influence of the fragility of 1064 nm pulses is predominantly noticed in nanoparticles of considerable size that demonstrate substantial absorption in the NIR wavelength range. This phenomenon occurs due to the fact that the wavelength of 1064 nm exceeds the absorption range associated with the SPR during the process of preparing 532 nm. As a result, a significant proportion of these nanoparticles undergo redistribution and dispersion within the colloid. The absorption rate exhibits an increase, but the bandwidth experiences a relatively smaller decrease, when subjected to a preparation utilizing a wavelength of 532 nm as opposed to a subsequent treatment involving two

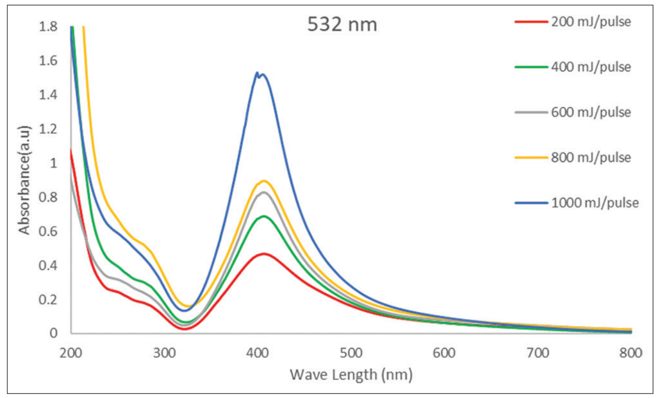

Figure 4: Illustrates the spectral absorption characteristics of silver colloids, which demonstrate varying responses to laser fluence at different wavelengths, specifically at 532 nm, as well as in crystals

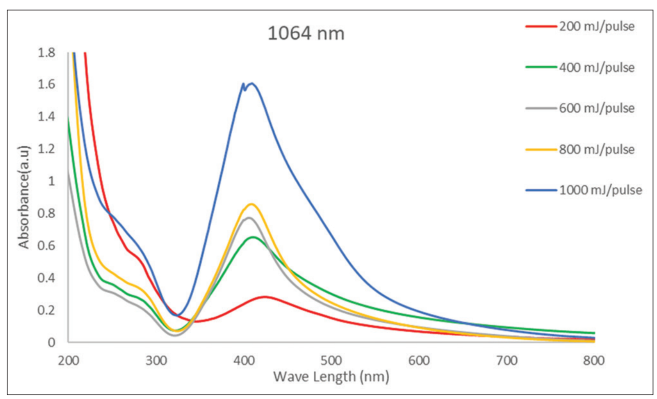

Figure 5: The absorption spectrum of silver colloid is excited at a wavelength of 1064 nm with the application of laser fluence at varying levels

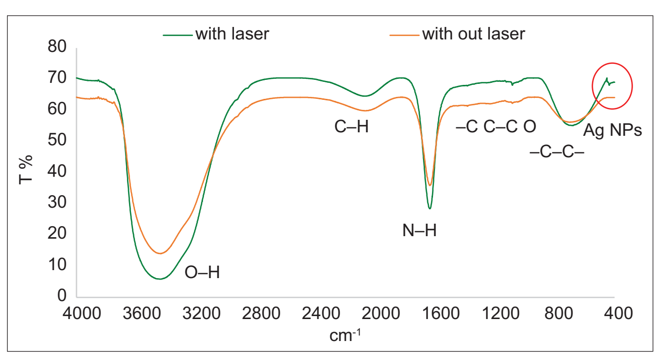

Figure 6: Fourier transform infrared spectra of silver nanoparticles synthesized using green tea, AgNPs: Silver nanoparticles

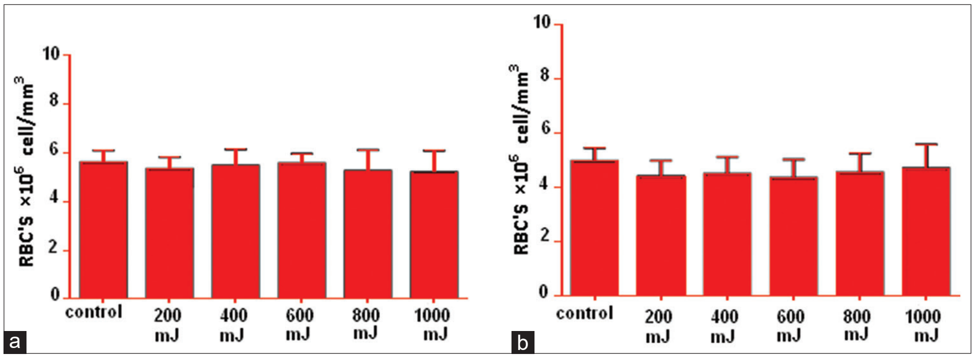

Figure 7: Investigates the impact of laser treatment at two specific wavelengths, namely 1046 nm (a) and 532 nm (b), on the count of red blood cells in human blood samples following the application of silver nanoparticles. RBCs: Red blood cells

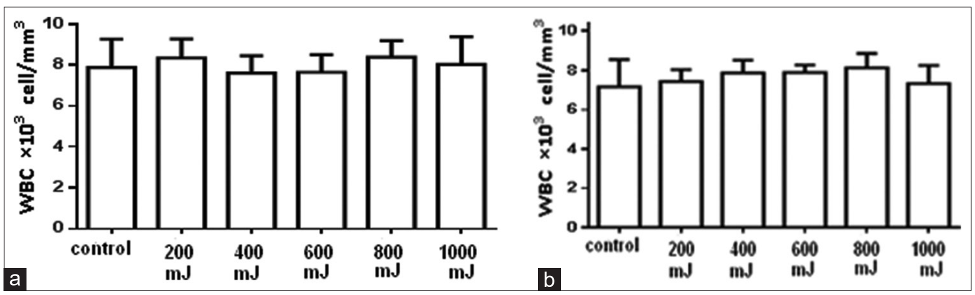

Figure 8: White blood cell count in human blood is depicted following the treatment of silver nanoparticles at laser wavelengths of 1064 nm for (a) and 532 nm for (b). WBC: White blood cell

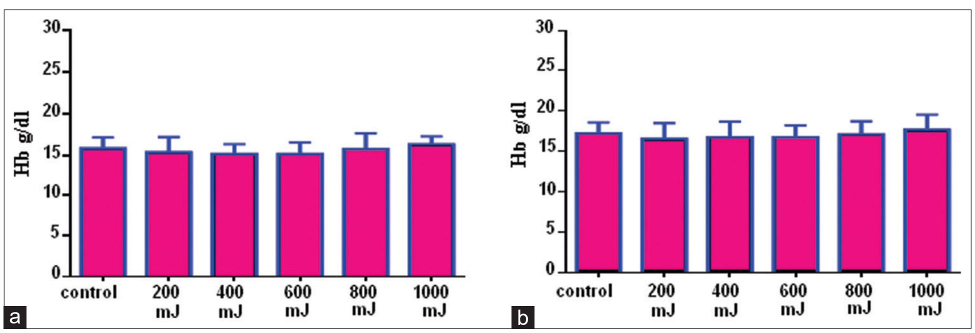

Figure 9: Illustrates the hemoglobin count in human blood following the application of laser wavelengths, namely at 1064 nm and 532 nm for a &b respectively , after treatment with silver nanoparticles, HB: Hemoglobin

wavelengths. Nevertheless, the stability of the Ag colloid generated under these specific conditions exhibits a greater degree of stability compared to other situations, as illustrated in Figures 4 and 5.

Fourier transform infrared (FTIR) spectroscopy was utilized in this study to identify the biophysical molecules that may be involved in stabilizing and reducing Ag + ions. In addition, the FTIR technique was employed to investigate the capping process of bio-reduced AgNPs generated using pulse laser extraction.

The absorption peaks observed in the sample spectra of stabilized AgNPs, produced under both low and high conditions, are depicted in Figure 6. These peaks are located at approximately 3436.70, 2067.67, 1636.33, 1083.44, and 684.87. Conversely, the extraction leaf of laser fragmentation exhibited absorption peaks at 3436.72, 2067.64, 1636.44, 1083.53, and 696.49.

The laser pulse exhibits peaks at 3436.70 cm^{-1}, whereas the sample without laser pulse shows peaks at 3436.72 cm^{-1}, both of which can be attributed to the stretching of O–H bonds. In addition, the extracts display peaks at 2067.67 cm^{-1}, 2067.64 cm^{-1}, and 2068.04 cm^{-1}, which are assigned to the stretching of aldehydic C–H bonds. The observed absorbance bands in Spinacia oleracea, turmeric, and saffron at wavelengths of 1636.33, 1083.44, 684.87 cm^{-1}, 1636.44, 1083.53, 696.49 cm^{-1}, and 1653.46, 1636.92, 1630.24, 1083.47, 711.69 cm^{-1}, respectively, have been identified as being associated with specific stretching vibrations. These vibrations correspond to the following functional groups: −C C–C O, −C C− ([in-ring] aromatic), −C–C− ([in-ring] aromatic), C–O (esters, ethers), and C–O (polyols).[19]

The presence of carboxyl stretches and N–H deformation vibrations in extracts is responsible for the occurrence of a peak at 1636 cm^{-1}. This peak has been identified as being associated with the amide bonds seen in proteins.[20,21] Previous studies have demonstrated that carbonyl groups present in amino acid residues and proteins exhibit a heightened propensity for metal binding. This suggests that proteins could potentially envelop metal nanoparticles, such as AgNPs caps, to prevent aggregation and maintain medium stability.

Application

The objective of this experiment was to investigate the harmful effects of AgNPs by examining alterations in hematological parameters, including RBC count, WBC count, and HB level, following treatment with AgNPs. The assessment of nanoparticle toxicity is crucial in determining its suitability for various biological applications, such as antibacterial agents.

The potential adverse effects of silver nanoparticles on red blood cells

Figure 7 presents a comparative analysis of treatment groups involving the use of AgNPs and a control group with negative results in RBC samples. The findings indicate that the treatment groups did not exhibit any adverse effects on the count of RBCs. Furthermore, no statistically significant difference was observed between the treatment and negative control groups.

The potential adverse effects of silver nanoparticles on white blood cell

Figure 8 presents a comparative analysis of treatment groups involving the use of AgNPs and a negative control in terms of WBCs. The findings indicated that the treatment groups did not exhibit any adverse effects on WBC count. Furthermore, there was no statistically significant disparity observed between the treatment groups and the negative control group.

Effects of silver/gold nanoparticles on hemoglobin concentration

Figure 9 presents a comparative analysis of treatment groups involving the use of AgNPs and a negative control in the context of HB. The findings indicated that the treatment groups did not experience any adverse effects on their HB count. Moreover, no statistically significant disparity was identified between the treatment groups and the negative control group.

Conclusion

This study presents an investigation of the efficacy of a plant extract in conjunction with the incorporation of supplementary AgNPs, as well as the generation of smaller-sized AgNPs using laser fragmentation. The identification of nanoparticles was achieved through the application of transmission electron microscopy and UV-VIS spectroscopic techniques. The customary range for the measuring of diameters of AgNPs is approximately 16–20 nm. Conducted an investigation on the impact of laser flux and laser segmentation length. The efficacy of fractionation is significantly impacted by the colloidal particles' spontaneous absorption of laser beam radiation. The classification of AgNPs during fragmentation processing is regulated through the collection of a combination of laser pulses with wavelengths of 1064 nm and 532 nm. As a consequence, there is a decrease in the range of particle sizes and a decrease in the mean size to lower magnitudes, resulting in a very stable colloid. This particular methodology has demonstrated considerable efficacy in the production of colloidal suspensions with the intended particle dimensions. Moreover, by the analysis of nanoparticles in human blood, no discernible alterations in the blood constituents were seen, indicating their nontoxic nature.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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