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Antioxidant Activity of Aluminum Oxide Nanoparticles Prepared by Laser Ablation Technique and Evaluating the Toxicity on Blood Human Components (*in vitro*)

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ABSTRACT

DPPH is one of the most widely used tests with scavenged-free radical effect, in which the color turns to yellow when tested onto a free stable dark-purple tulip, where natural antioxidants are returned by an electron and a proton. The optical, structural properties of Al_2O NPs prepared by the pulsed laser ablation (PLA) Nd: YAG laser method at various energies (500,800,1000 mJ) were studied using color absorbance, UV–VIS spectroscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM). The results showed that the average particle size is less than 41 nm. Spectroscopic analyses were used to study the antioxidant activity of Al_2O_3 nanoparticles by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The DPPH displacement was shown to be directly proportional to the high energies according to the results. The increased energy of (500, 800 and 1000 mJ) for free radicals are at (64.53 ± 5.487)%, (74.00 ± 2.887)% and (84.67 ± 4.372)% respectively, as compared to ascorbic acid. Furthermore, the toxicity of these nanoparticles on human blood parameters was studied using the complete blood count (CBC) (in vitro) by hematological parameters (PLT), (HGB–Hb), (RBCs), (WBCs), and count type white blood cells, and are compared to control groups. Our results demonstrate no significant changes in levels of (PLT), (HGB–Hb), (RBCs), (WBCs), and count type white blood cells between test and control groups. This data suggests that aluminum oxide nanoparticles have no harmful effect on hematological parameters (in vitro).

Keywords: Aluminum oxide nanoparticles; Antioxidants; Complete Blood Count (CBC)

1. INTRODUCTION

Biomedical nanoscience has a lot in common with getting benefits in the analysis, treatment, and identification of various diseases, with fewer side effects and a better quality of life for the patients [1]. Aluminium oxide nanoparticles (Al_2O_3 NPs) are a type of porous nanomaterial that belongs to the metal oxide nanomaterials category. With six oxygen atoms to one aluminum atom, they possess a corundum-like composition. Al_2O_3 NPs, like other metal oxide nanoparticles (NPs), are simple to manipulate and access. These low-cost nanoparticles also have a large surface area and mechanical properties, as well as outstanding chemical stability in high temperatures and severe environments like abrasive settings. They have little electrical properties as well [2]. Because nanoparticles have several advantages over bigger particles, such as better surface-to-volume ratios and magnetic characteristics, they are employed in biological applications [3]. The growth in medication resistance

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among dangerous bacteria, as well as the introduction of novel infectious diseases, has made the quest for new antimicrobials unavoidable. The unique phytochemical

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features of nanoparticles, together with their capacity to hinder microbe development, have prompted more research into nanoparticles and their potential as antimicrobials [4]. Depending on the nanoparticle's composition, structural features, and administration route, immunotoxin effects range from acute inflammation to lung, liver, and systemic damage [5]. Unwanted interactions with any of these blood components endanger the biocompatibility, biodistribution and efficacy of a cancer nanomedicine [6],which created the nanofluid model, in Gentile et al. [7]. Nadeem et al. studied the hemodynamic effects of stenosis by utilizing NPs analysis of blood movement across tapering arteries [8]. At the canter of the artery, the transmission of axial velocity curves for (Al_2O_3) nanoparticles is higher than that of both (CU) and (TiO_2) NPs, and the confrontation resistance remains higher for both titanium (TiO_2) and copper (Cu) NPs in the case of high aluminum concentration (Al_2O_3) in the blood [9]. There have been even fewer studies on the effects of size on cytotoxicity in Al_2O_3, and they have not yielded conclusive results [10]. The study's goal is to determine the impact of aluminum oxide nanoparticles on human blood components by using complete blood count (CBC) and their study of antioxidant activity of nanoparticles.

2. MATERIAL AND METHODS

2.1 Preparation of Al_2O_3 nanoparticles

Al_2O_3 nanoparticles were synthesized via laser ablation of an Al metal pellet in deionized water. As shown in Fig. 1, the aluminum target was placed in a glass vessel. The water level was roughly 3 mm above the target and contained 1 ml of deionized water. The nanoparticles were created using a pulsed Nd: YAG laser with the following parameters: wavelength = 1064 nm, frequency, f = 1 Hz, and pulse width = 9 ns, at various laser energy (500, 800, 1000 mJ/pulse). The ablation time was 30 min.

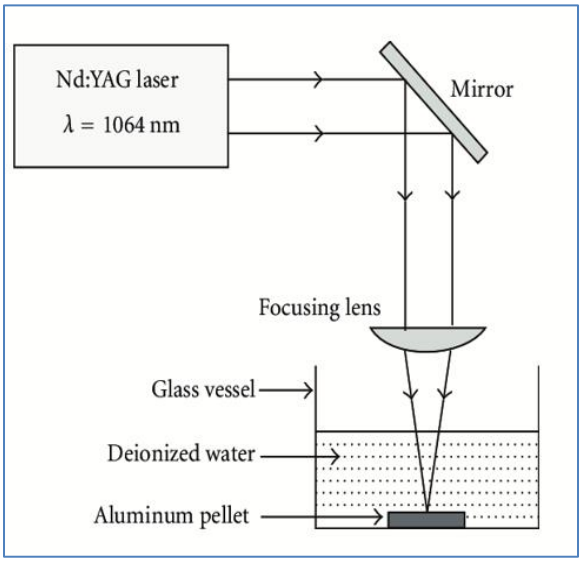


Figure 1. Photographs showing the laser ablation (Nd: YAG laser)

2.2 Characterization of Al_2O_3 NPs

UV-Vis. spectroscopy (UV-Vis, Shimadzu, Japan) was used to ration the absorption spectrum peak in the range between 250 and 1100 nm [11]. The study morphology properties of Al_2O_3 NPs using A Scanning Electron Microscope SEM (INSPECTS50-USA) [12], and atomic force microscope AFM [13]. These are done at room temperature. These tests (SEM, AFM) were conducted at the University of Tehran.

2.3 Antioxidant activity of DPPH assay

The antioxidant activity of produced NPs was determined according to Jawad et al. [1]. The DPPH scavenging capabilities of Al_2O_3 NPs were tested. After 750 µL of Al_2O_2NPs added at different energy (500, 800, 1000mJ) with 750 µl of DPPH solution, the samples were incubated in the dark at a fixed temperature of 37 °C for 30 min. The absorbances of samples was evaluated at a wavelength of 517nm. Using Equation (1), the scavenging activity of the produced NPs was calculated [3]:

$$\text{Antioxidant Activity} = OD^A - OD^B / OD^A * 100 \quad (1)$$

where OD are the absorbances of the control and the sample, respectively.

2.4 The examination on the effect of Al_2O_3 NPs on blood components by complete blood count test (C.B.C)

Blood samples were collected from ten healthy human (20 to 55) years old in EDTA tubes. Blood samples were treated with Al_2O_3 NPs at different energy (500, 800 and 1000 mJ) for 1hr compared with untreated samples,. followed by complete blood count test (CBC) and blood film. This experiment is done in Baghdad hospital.

2.5 Staining blood film sample [21]

Drop of bloods (control and test) were taken to slid glass and smear blood staining by Giemsa stain at 5 min, washed and dried for 10 min. They were examined microscopically (100X) to inspect the effected of Al_2O_3 NPs on blood cell. This experiment was done in blood laboratory in Baghdad hospital, Iraq.

2.6 Statistical analysis

All data were subjected to a Student's t-test, and the difference between them was deemed statistically significant when is 0.05.

3. RESULTS AND DISCUSSION

Figure 2 shows the sample's UV visible spectrum. The AI_2O_3 NPs absorb UV photons up to 410 nm at different energies (500 mJ, 800 mJ, and 1000 mJ) and they transmit practically all visible spectrum radiations with distilled water as a control. The AI_2O_3 NPs were placed in a quartz cuvette and monitored for wavelength scanning between 200 and 1000 nm.

The thresholds, ablation depths, and optical absorbance improvements in Al_2O_3 composites absorbance were found to be 0.64, 0.35, and 0.075 respectively, observed in 500 mJ the size of nanoparticles increases, their resonance absorption spectrum peak will be shifted toward a larger wavelength and vice-versa such that the absorption measurements provide a qualitative indication of the crystal size distribution. When the energy increased from 800 to 1000 mJ, leading to a reduction in the size of NPs is the instabilities that develop at the interface of molten NPs with the vapors of the surrounding liquid, so the absorbation peak is low.

Figure 3 depicts the colors of Al_2O_3 NPs colloidal nanoparticles created by laser using a Nd: YAG laser with a wavelength of 1064 nm and three energy levels (500, 800, and 1000) mJ for the goal of getting smaller sizes in double deionized water for three energies (DW). The degree of color is determined by the Al_2O_3 NPs laser energy. Because light absorption is saturated with energy, the colloid highly affects the efficiency of the fragmentation of NPs. Another interaction impacting the effectiveness of the fragmentation of NPs is a laser beam interaction with NPs in liquid, which results in a low-power laser on the sample surface. The color NPs are organized in a gradient from light yellow to light gray.

The AFM images for the nanostructures deposited utilizing 500, 800 and 1000mJ energy are shown in Figure 4. The topography of the surface of Al_2O_3NPs particles was studied and measurements of surface roughness, particle size nanoparticles rate and particle size distribution were taken.

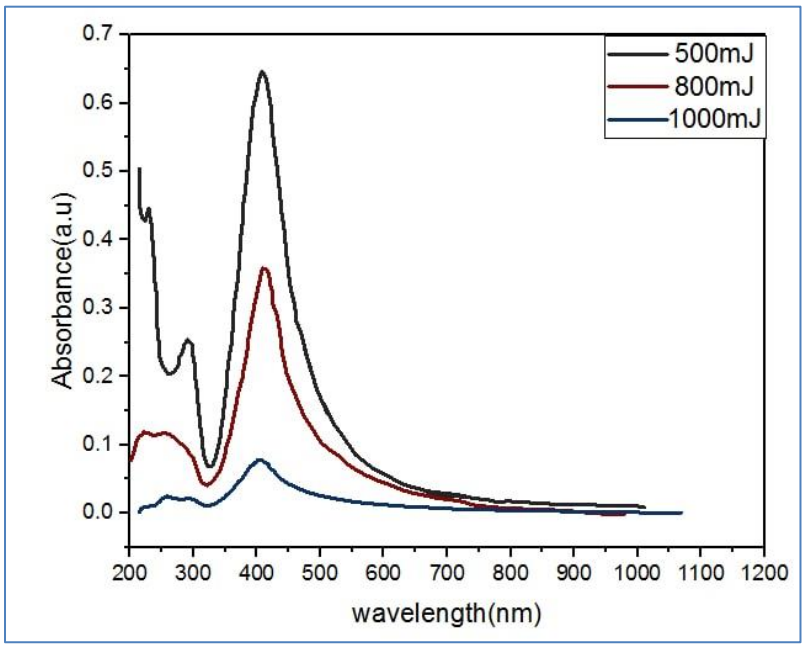


Figure 2. UV-Visible of Al_2O_3 NPs at 500mJ, 800mJ, 1000mJ energy

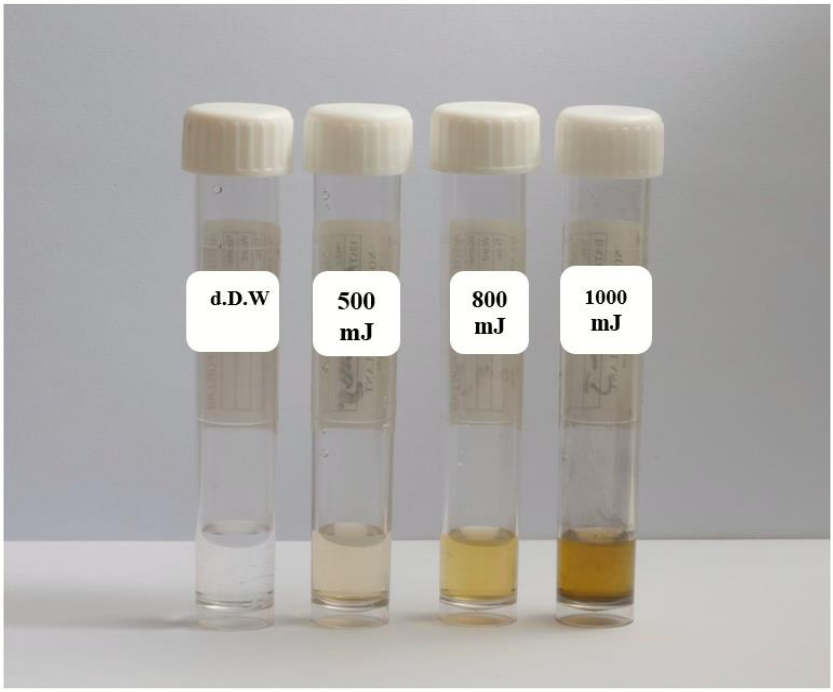
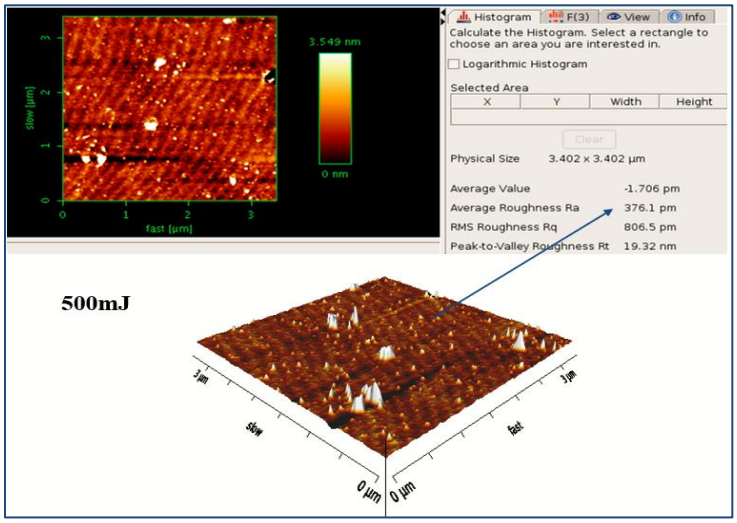


Figure 3. Al_2O_3 NPs colloidal prepared by laser ablation at different energy (500, 800 and 1000) mJ



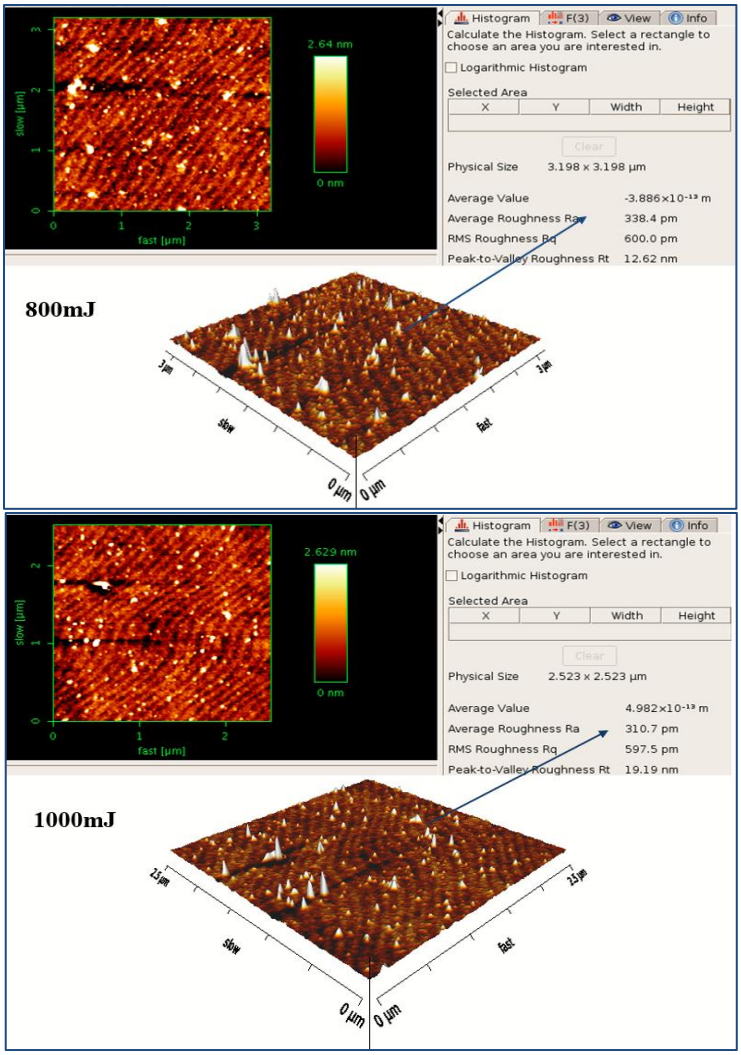


Figure 4. AFM image of Al_2O_3NPs at different energy (500, 800 and 1000) mJ

Figure 5 shows the SEM image of the sample. The nanoparticles are round and granular, as shown in Figure 5 (A), (B) and (C). The aggregation of particles may be seen in the higher resolution SEM image. Agglomeration could have been produced by aging. ImageJ software was used to assess the size distribution of the Al_2O_3 NPs. The average particle size ranges from 40 to 60 nm.

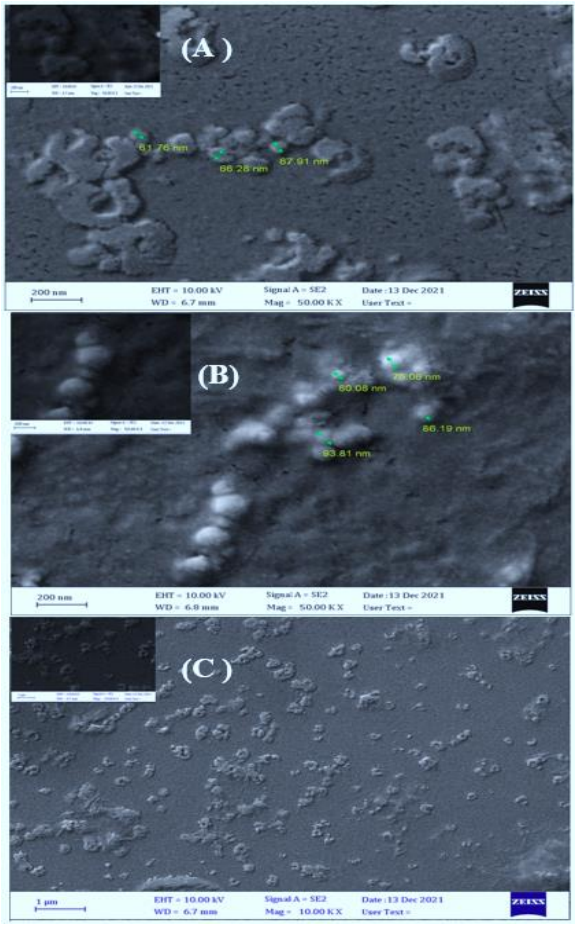


Figure 5. SEM image of Al_2O_3 NPs at: (A) 500mJ, (B) 800mJ, (C)1000mJ energy

3.1 DPPH assay

DPPH is a class of stable free radicals that attach to replacement electrons [12]. When DPPH is combined with a hydrogen-producing substrate, a hydrogen atom is produced, with the hue shift from purple to yellow [13]. Figure 6 shows the DPPH radical scavenging activity (%) of Al_2O_3 NPs.The cytotoxic reaction, instead of ROS generation, could be the major mechanism of Al_2O_3 NPs cytotoxicity. Although the interaction of Al_2O_3 with the cell produces ROS, the main cause of ROS production may be the lethal response of Al-dependent protein activity disequilibrium, such as the permeabilization of organelles, that releases large amounts of ROS in the tissue [12]. Al_2O_3 NPs had an antioxidant activity of (64.53 ± 5.487)% at 500 mJ and (74.00 ± 2.887)% at 800 mJ. While the proportion for 1000 mJ was (84.67 ± 4.372)%. The results showed that Al_2O_3 NP_S can release hydrogen atoms and removes the unstable electron from DPPH at more than 1000 mJ 500 mJ.

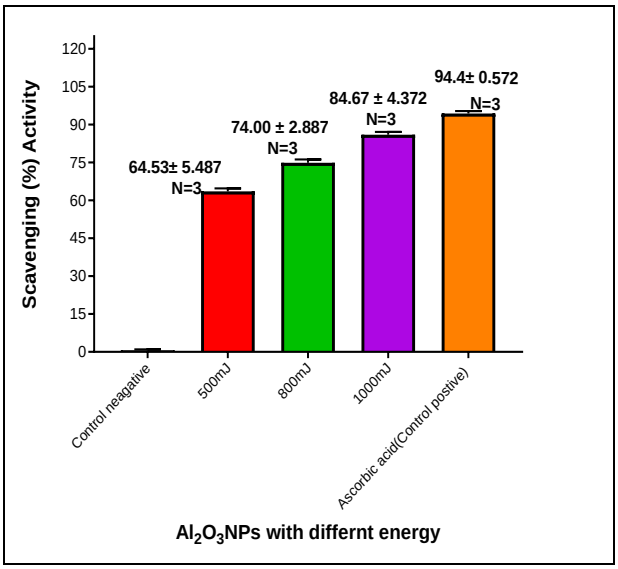


Figure 6. DPPH scavenging activity of Al_2O_3 NPs

We investigated the effect of Al_2O_3 NPs at different energy 500, 800 and 1000 mJ on major human blood components *in vitro* after incubating blood samples for 1 hour at 37C ◦ . These tests showed no differences within or between groups at different energy (500, 800 and 1000) mJ. Blood parameters in complete blood count (CBC) from Al_2O_3 NPs at different energy show no changes in haematological examination in the blood of humans treated with Al_2O_3 NPs at different energy. The results show the mean and standard deviation of PCV, platelets, red blood cells, white blood cells, count type white blood cells (WBC) and HGB show no differences between treated and non-treated blood samples, as can be seen in Figures 7, 8, 9, 10, 11 and 12.

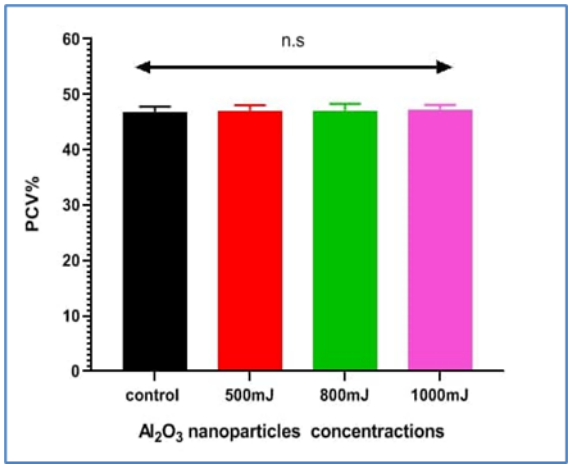


Figure 7. PCV level in blood samples in presence and absence of Al_2O_3 NPs at different energy (500, 800 and 1000) mJ

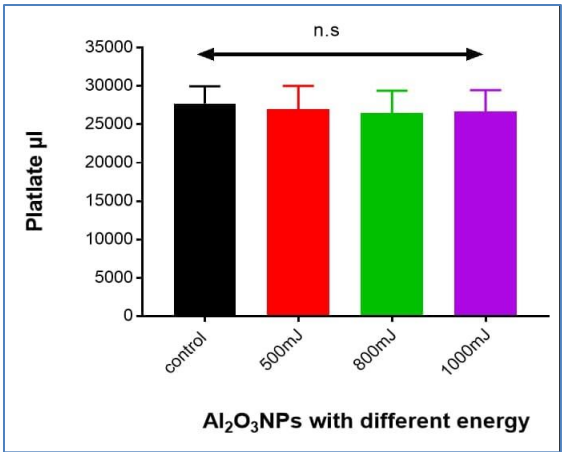


Figure 9. Platelet's count level in blood samples in presence and absence of Al_2O_3 NPs at different energy (500, 800 and 1000) mJ

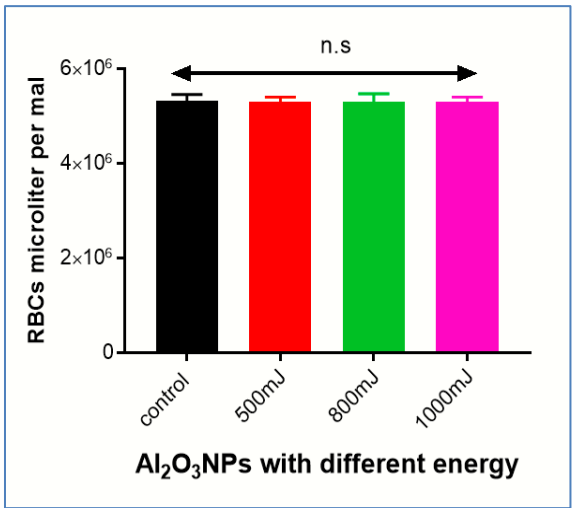


Figure 9. RBCs count in blood samples in presence and absence of Al_2O_3 NPs at different energy (500, 800 and 1000) mJ

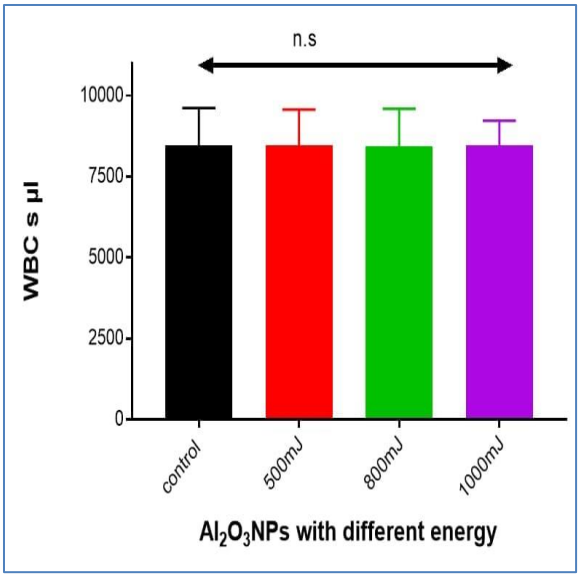


Figure 10. WBCs count in blood samples in presence and absence of Al_2O_3 NPs at different energy (500, 800 and 1000) mJ

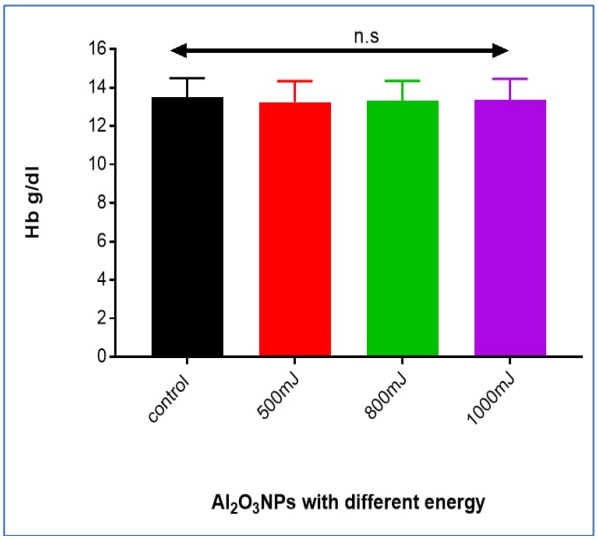


Figure 11. HGB level in blood samples in presence and absence of Al_2O_3 NPs at different energy (500, 800 and 1000) mJ

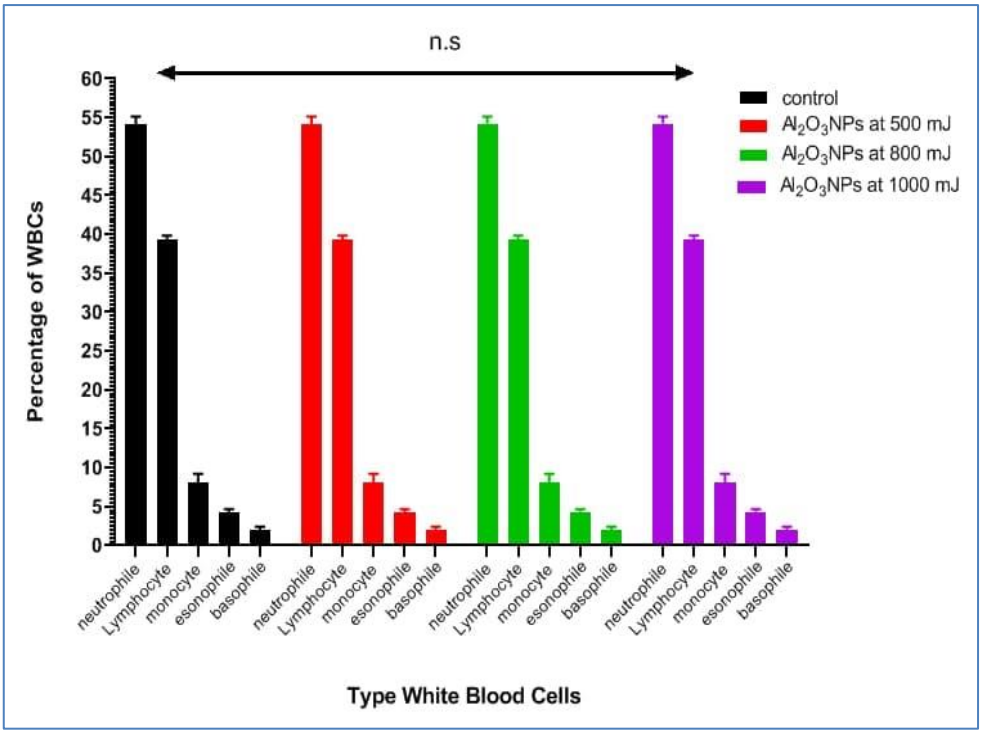


Figure 12. Percentage of WBCs in blood sample in presence and absence of Al_2O_3NPs at different energy (500, 800 and 1000) mJ

We studied the effect of Al_2O_3 NPs on blood cell components *in vitro*, after 1hr incubated blood samples (control and test) at 37ºC by staining blood film and examined microscopically (100X) to inspect the effected Al_2O_3NPs on blood cell. These examinations were not different either within or between groups at different energy (500, 800 and 1000) mJ, as shown in Figure 13.

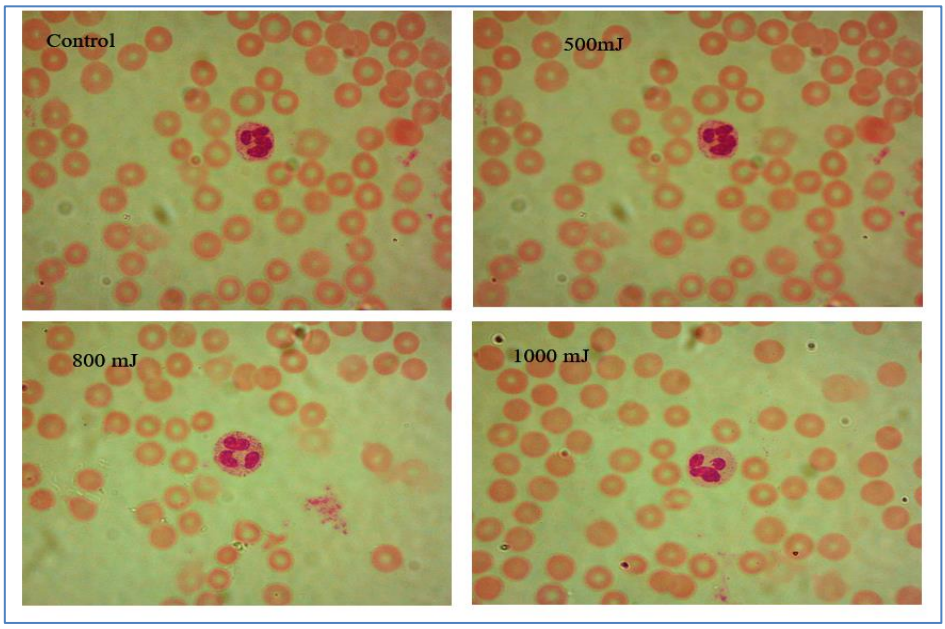


Figure 13. Morphology of blood sample in control group and treated of Al_2O_3NPs at different energy (500, 800 and 1000) mJ

Biomaterial-based appliances with increased therapeutic function can be created by biomimicry of naturally occurring structures. We anticipated that biologically inspired NPS that replicated both the chemical and physical features of RBCs could increase blood circulation, for example, since red blood cells (RBCs) allow for long-term blood dispersion. A cell membrane layer can be used for a variety of things. Enhancing the functionality of diverse cell types and increasing the half-life of blood circulation by mimicking the delivery properties of NPs [14]. In all these domains, biomedical applications are the most relevant and vital. These applications strive to make materials and systems that are lighter, more durable, more cost-effective, simpler and more biocompatible. Finally, NPs are essential building blocks for the development of materials that can aid in the resolution of these problems. The toxicity of nanoparticles (NPs), which are widely employed due to their unique features such as surface area and charge, particle size, chemical, and physical properties, is crucial for human health [15]. Because of its small size, high surface curvature, huge surface area and the number of surface reactive sites, the bio-surface may experience unique reactions. Cell walls are most significant biological surfaces with which nanomaterials interact when they come in touch with organisms. As a result, to identify nanomaterial uses and safety, the impacts of nanomaterials on cell membranes should be assessed. Because there are worries about nanoparticles' (NPS) health consequences, it is crucial to understand how they interact with cells, particularly red blood cells (RBCs), which play a major role in blood functions, and how they compare to non-nano particle materials. Al_2O_3 has been shown in certain research to be less harmful than other nanostructures [16]. Aluminum oxide nanoparticles have been found to be harmful to microalgae through interactions with the cell surface [17]. NPs was discovered in the cytoplasm of practically every cell. However, few investigations on its effects on membranes have been conducted, and more research is needed. The haemolysis assay is recommended as a credible test for material biocompatibility [18]. Another study looked at the impact of TiO_2 NPs on human blood components like platelets, red blood cells (RBCs), haemoglobin, and whole genome bisulfited levels *in vitro* at doses of 20, 40, 60 and 80 g/ml. The results of the control groups that were not treated and the test groups that were treated with TiO_2 NPs were not significantly different [19]. Research another toxicity of colloidal PSNPs was assessed in mice, The effect of these nanomaterials' toxicity on liver markers in laboratory animals is investigated utilizing four groups. Each group has three duplicates, which are then confirmed by a histological examination of a section of the liver. When comparing the test groups to the controls, the findings show no significant difference of opinion in levels (GOT, GPT, ALT). This data demonstrates that porous silicon nanoparticles have no damaging effect on renal functions [20,21,22].

4. CONCLUSION

Preparation of Al_2O_3 NPs have significant biocompatibility, biodegradability and antioxidant effects. Due to these properties, Al_2O_3 NPs are efficiently used in the field of medicine to prevent infectious and non-infectious diseases and the study of toxicity effects of these NPs on the blood of humans (*in vitro*) utilized complete blood count (CBC). Results indicate that there are no toxic effect of Al_2O_3 NPs nanoparticles in the haematology parameter (*in vitro*). The size of Al_2O_3 particles ablated with low laser energy was found to be smaller than that achieved with high laser energy. High laser energy, on the other hand, produced a higher concentration of Al_2O_3 than when the laser energy is low.

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