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Assessing the Toxicity of Aluminum Oxide Nanoparticles (Al_2O_3 NPs) Prepared by Laser Ablation Technique on Blood Components

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A B S T R A C T

Along with its uses in a wide range of sectors, NP toxicity research was one of the fastest-growing areas of research, so the growing commercial applications brought aluminum oxide nanoparticles under the purview of toxicologists. This study shows the toxicity of Aluminum oxide Nanoparticles on blood components prepared using the pulsed laser ablation (PLA) Nd: YAG laser method. We confirmed the synthesis of aluminum Oxide nanoparticles by measuring color absorbance, UV-vis, scanning electron microscope techniques (SEM), and FTIR as characterization of Aluminum oxide Nanoparticles. The complete blood count (CBC) was used in the study of the toxicity effect of these nanoparticles on human blood parameters (in vitro). The results of hematology parameter platelet (PLT); hemoglobin (HGB–Hb); red blood cell (RBCs); white blood cell (WBCs); Count type white blood cells) are compared with the control groups, our results show no significant differences in levels of platelet (PLT); hemoglobin (HGB – Hb); red blood cell (RBCs); white blood cell (WBCs); Count type white blood cells) between the test groups when compared with control groups. This result that there indicates no toxic effect of Aluminum oxide nanoparticles in the hematology parameter (in vitro). This work is done for the first time to investigate the non-toxicity effect of these Al_2O_3 NPs on human blood parameters.

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1. Introduction

Biomedical nanoscience has a lot in common with getting profits in the analysis, treatment, and identification of various diseases, with fewer side effects and a better quality of life for the patients [1]. Aluminum oxide nanoparticles (Al_2O_3 NPs) are a type of porous nanomaterial that belongs to the metal oxide nanomaterial category. They have a corundum-like structure with six oxygen atoms encircling one aluminum atom. Al_2O_3 NPs, like other metal oxide nanoparticles (NPs), are simple to manipulate and access. These low-cost NPs 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 NPs 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 among dangerous bacteria, as well as the introduction of novel infectious diseases, has made the quest for new antimicrobials unavoidable. NPs are

currently one of the most promising and new medicinal agents. The unique phytochemical features of nanoparticles, together with their capacity to hinder microbe development, have prompted more research into NPs 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, bio distribution and efficacy of a cancer nanomedicine [6], created the Nanofluid model, which *Gentile et al.* [7] later highlighted who discussed the longitudinal transport of NPs in blood vessels by treating blood as Casson fluid. *Nadeem et al.* studied the hemodynamic causes of stenosis by using NPs analysis of blood flow through tapered arteries [8]. The transmission of axial velocity curves for (Al_2O_3) NPs is higher than that of both (TiO_2) and (CU) NPs at the canter of the artery and the confrontation resistance in the case of aluminum concentration (Al_2O_3) in the blood remains higher for both copper (Cu) and titanium (TiO_2) NPs [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]. This study aims to detect the toxicity of Aluminium oxide NPs on human blood components by complete blood count (C.B.C).

2. Material and Method

2.1 Preparation of Al_2O_3 Nanoparticles

Aluminum pellet diameter of (15) × (15) mm and purity of (99.99%) was prepared via laser ablation of solids submerged in deionized distilled water [11], as shown in the experiment illustrated in Fig. 1. Then, the NPs were passed through a (0.22) m size filter for size uniformity by applying an Nd: YAG laser with a fundamental wavelength of (1064) nm., the target was irradiated for (15) min., using (150) pulses, a pulse length of (6) ns., a repetition rate of (2) Hz, spot size (10) mm, the focal length of the lens (8) cm, and the prepared at different laser energies (500, 800, and 1000) mJ.

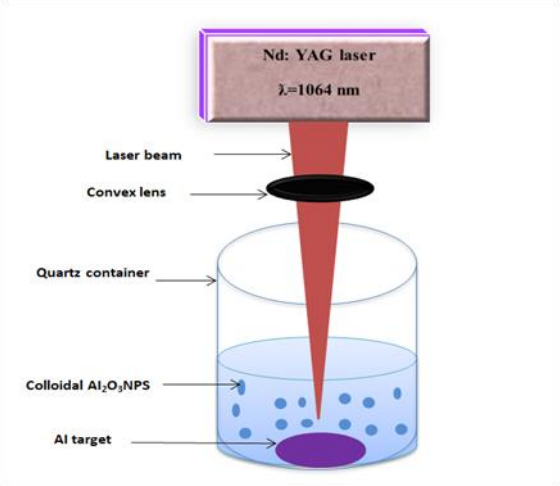

Figure 1: Setup for nanoparticle synthesis.

2.2 Characterization of Al_2O_3 NPs

2.2.1 Ultraviolet-visible Spectroscopy (UV)

UV-Vis. spectroscopy (UV-Vis, Shimadzu, Japan) was working to ration the absorption spectrum peak in the range between 200 and 1000 nm [11]. The optical absorption of colloidal Al_2O_3 NPs was measured using UV–Vis spectrophotometer.

2.2.2 Scanning Electron Microscope (SEM)

The obtained Al_2O_3 NPs were surface characterized via A Scanning Electron Microscope SEM (INSPECTS50-USA).

2.2.3 Atomic Force Microscope (AFM)

Atomic force microscope AFM was done by [12, 13].

2.2.4 Effect of Al_2O_3 NPs on Blood Components

Blood samples were collected in EDTA tubes from Ten healthy men only (20 to 55 years). Blood samples were treated with ten different energies microliter of Al_2O_3 NPs (500,800, and 1000 mJ) for an hour and compared to untreated samples. Following that, a complete blood count (CBC) and a blood film are performed [20]. This experiment is taking place in a hospital in Baghdad using a CBC device.

2.2.5 Staining Blood Film Sample

1. Take a drop of blood (control &test) into a slid glass and smear blood, 2. Giemsa stains for 5 min, 3. Washing & drying for 10 and 4 min. Examined microscopically (100X) to inspect the affected Al_2O_3 NPs on blood cells. This experiment was done in the blood laboratory in Baghdad hospital- Iraq.

2.2.6 Statistical Analysis

The student's t-test was applied to all data and the difference between them was accepted to be statistically significant when $P \leq 0.05$.

3. Results and Discussion

The UV-visible spectrum of the sample is shown in Fig. 2. The sample absorbs the radiations in the UV range up to 410 nm at different energies (500 mJ, 800 mJ, and 1000 mJ) and almost all the visible spectrum radiations are transmitted by the Al_2O_3 NPs. The Al_2O_3 NPs was put in a quartz cuvette and monitored for wavelength scanning between 200 and 1000 nm with distilled water as a reference.

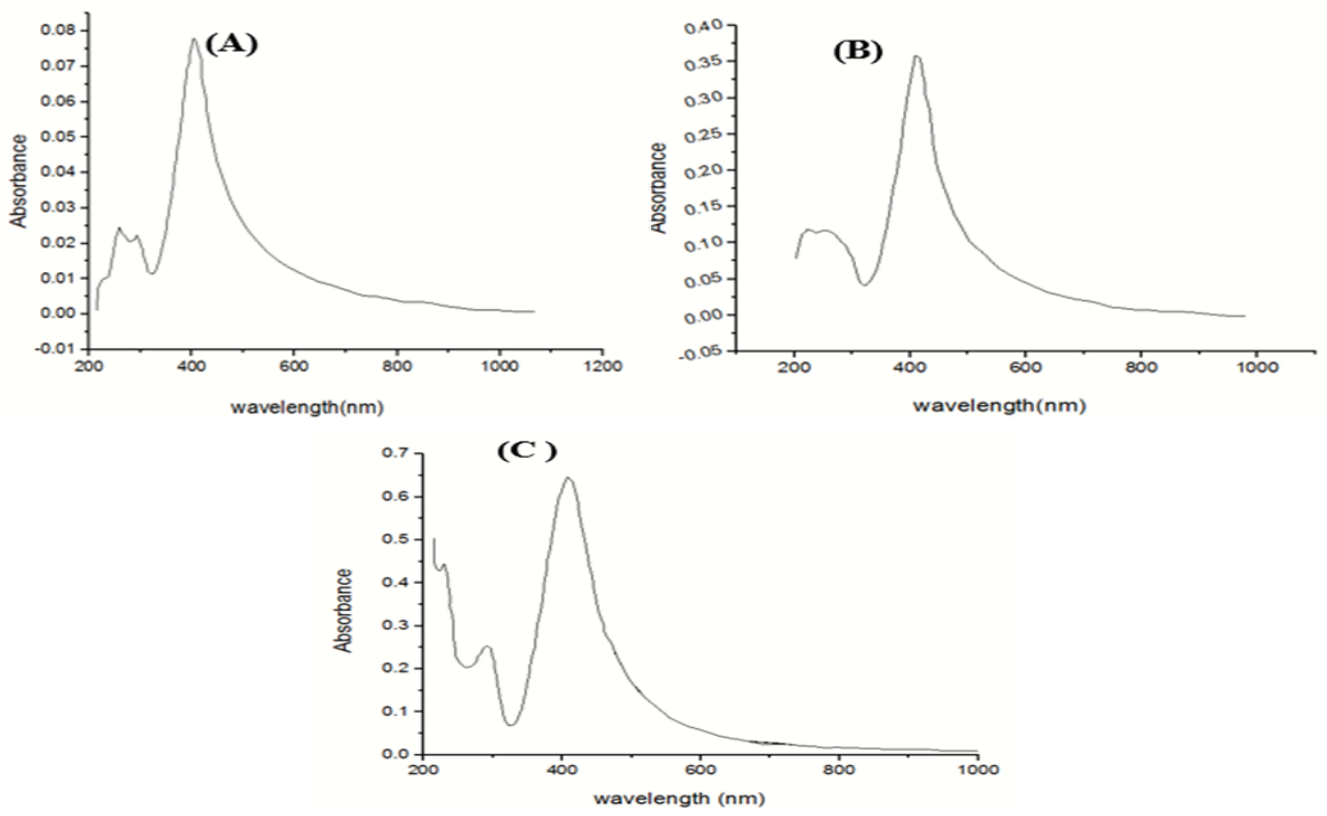

Figure 2: UV-Visible of Al_2O_3 NPs at (A) 500 mJ, (B) 800 mJ, and (C) 1000 mJ energy.

Fig. 3 displays the colors of Al_2O_3 NPs colloidal NPs produced by an Nd: YAG laser at three energies (500,800, and 1000) mJ. The degree of color depends on the Laser energy of Al_2O_3 NPs, As the laser energies increase, the number of NPs increases and the size decreases because light absorption is saturated with the energy of the colloid highly addition to this there is another interaction affecting the efficiency of the fragmentation of NPs is a laser beam interaction with NPs in liquid and this leads to a low-power laser on the sample surface. The color NPs are arranged from yellow light to light gray.

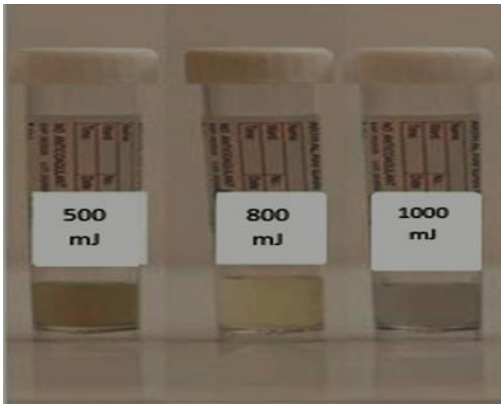

Figure 3: Al_2O_3 NPs colloidal prepared by laser ablation at different energy (500, 800, and 1000) mJ.

The AFM images for the nanostructures deposited utilizing 500,800.1000mJ energy are shown in Fig. 4. The topography of the surface of Al_2O_3 NPs particles was studied and measurements of surface roughness, particle size NPs rate, and particle size distribution were taken. The SEM image of the sample as shown in Fig. 5 (A, B, C) reveals that the particles are spherical and have granular nature. In the higher resolution SEM image, the agglomeration of particles is observed. Aging may have caused the agglomeration. The size distribution of the Al_2O_3 NPs sample was evaluated using ImageJ software. the size average value of particles is arranged from 40 to 60 nm.

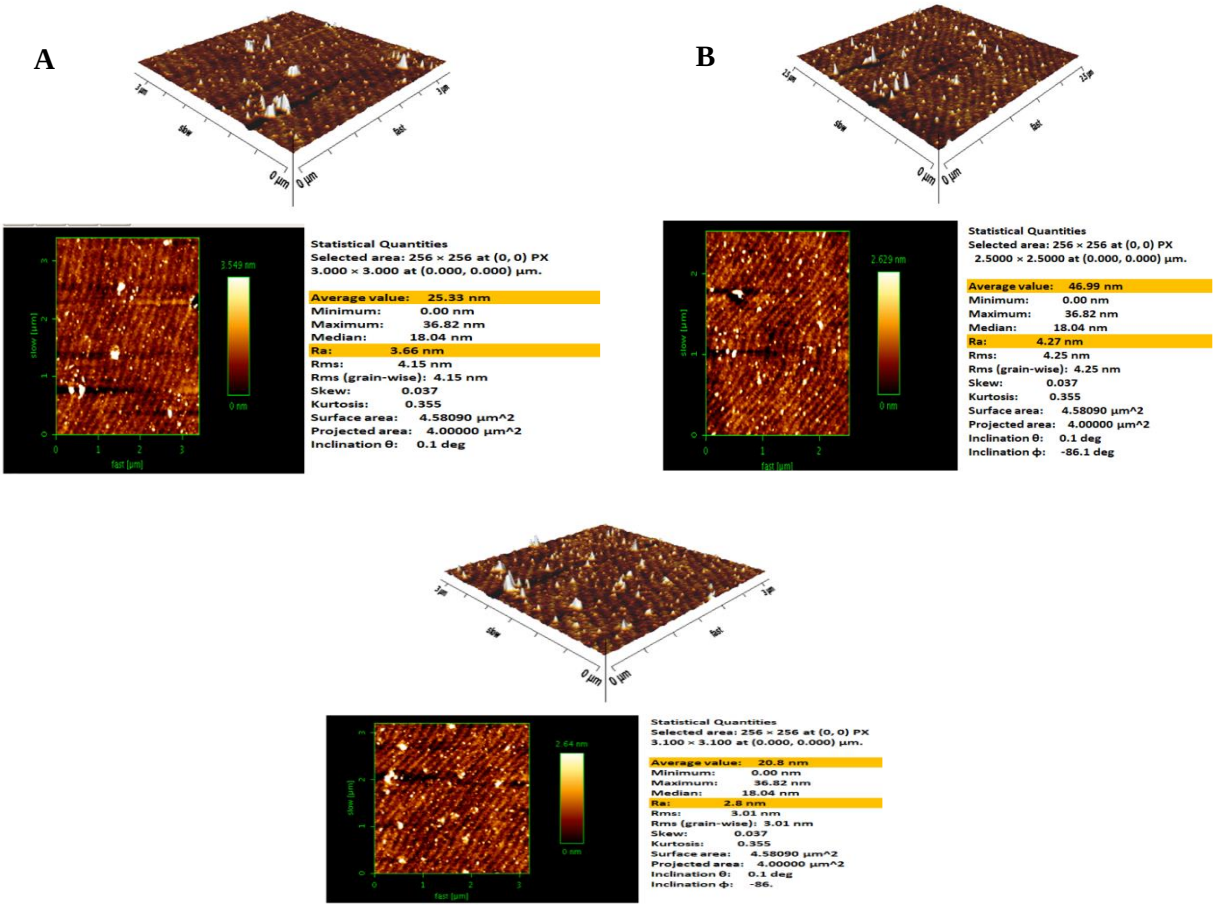

Figure 4: AFM image of Al_2O_3 NPs at different energy (A) 500, (B) 800, and (C) 1000) mJ.

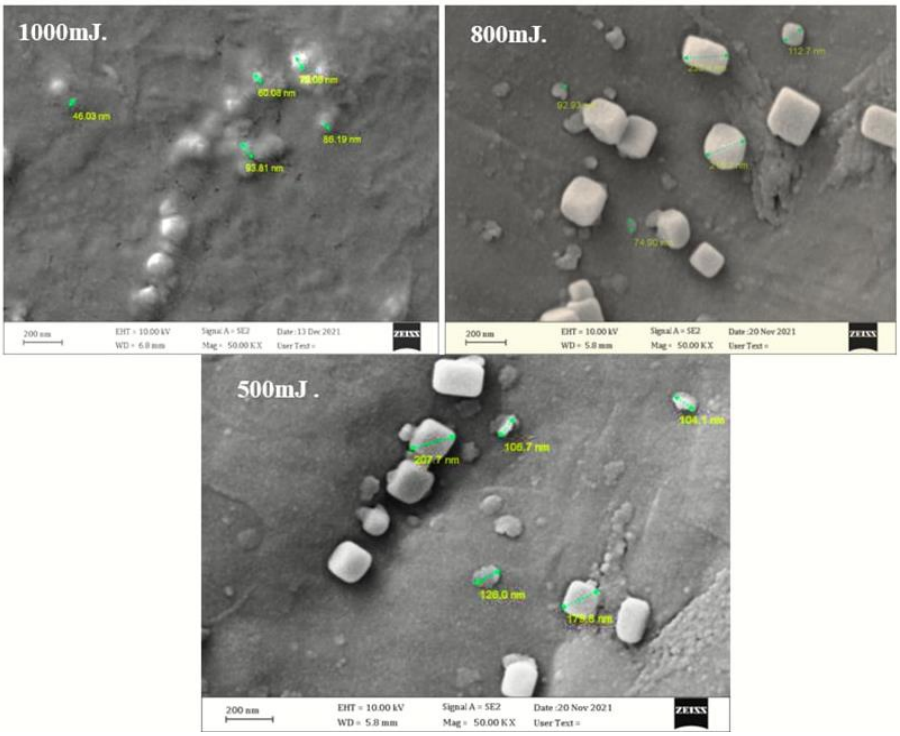

Figure 5: SEM image of $Al_2O_{3\ NPs}$ at (A) 500mJ, (B) 800mJ, (C) 1000mJ energy.

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 37ºC. These tests showed no differences within or between groups at different energy (500, 800, and 1000) mJ. Blood parameters in Complete Blood Count (C.B.C.) from Al_2O_3 NPs at different energy show no changes in hematological 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; HGB; and Count Type White Blood Cells (WBC) show no differences between treated and non-treated blood samples. Fig. 6, 7, 8, 9, 10, and 11.

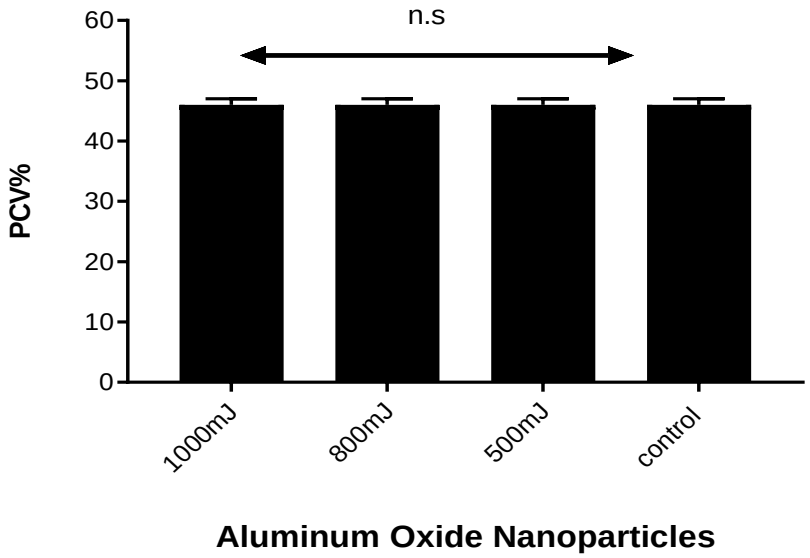

Figure 6: PCV level in blood samples in the presence and absence of Al_2O_3 NPs at different energy (500, 800, and 1000) mJ.

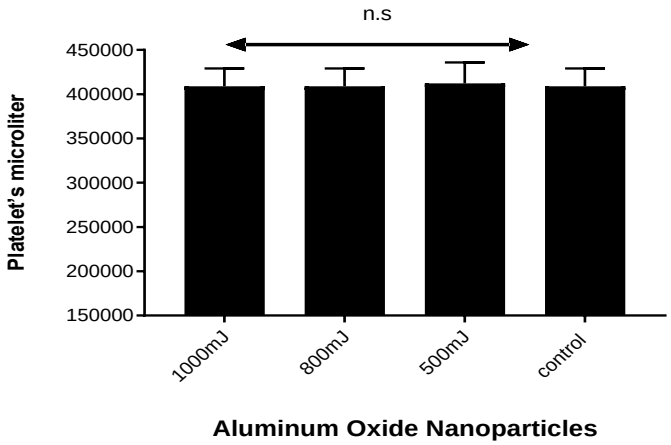

Figure 7: Platelet's count level in blood samples in the presence and absence of Al_2O_3 NPs at different energy (500, 800, and 1000) mJ.

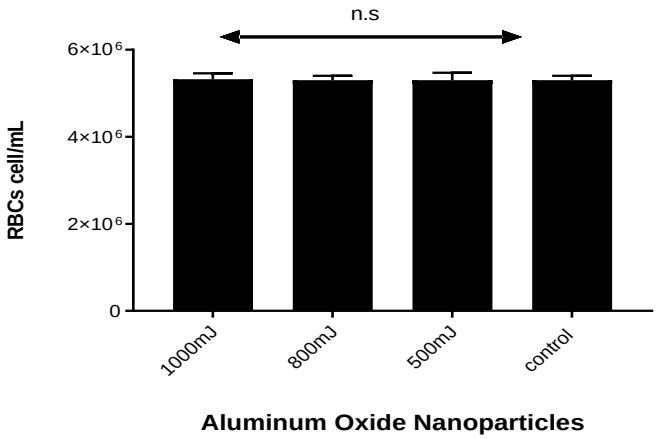

Figure 8: RBCs count in blood samples in the presence and absence of Al_2O_3 NPs at different energy (500, 800, and 1000) mJ.

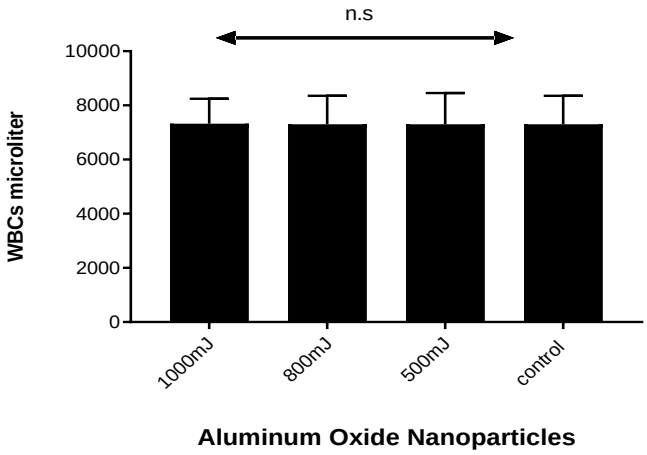

Figure 9: WBCs count in blood samples in the presence and absence of Al_2O_3 NPs at different energy (500, 800, and 1000) mJ.

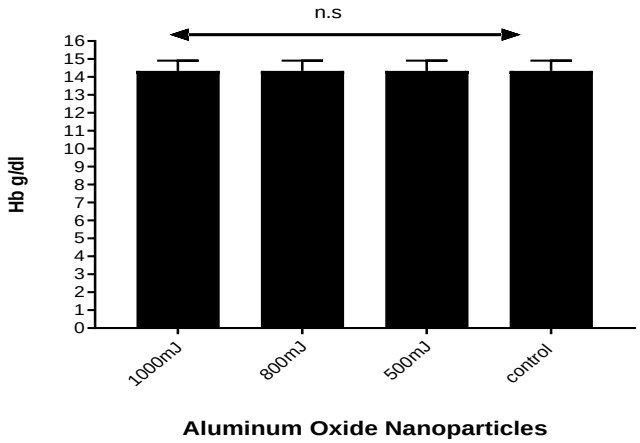

Figure 10: HGB level in blood samples in the presence and absence of Al_2O_3 NPs at different energy (500, 800, and 1000) mJ.

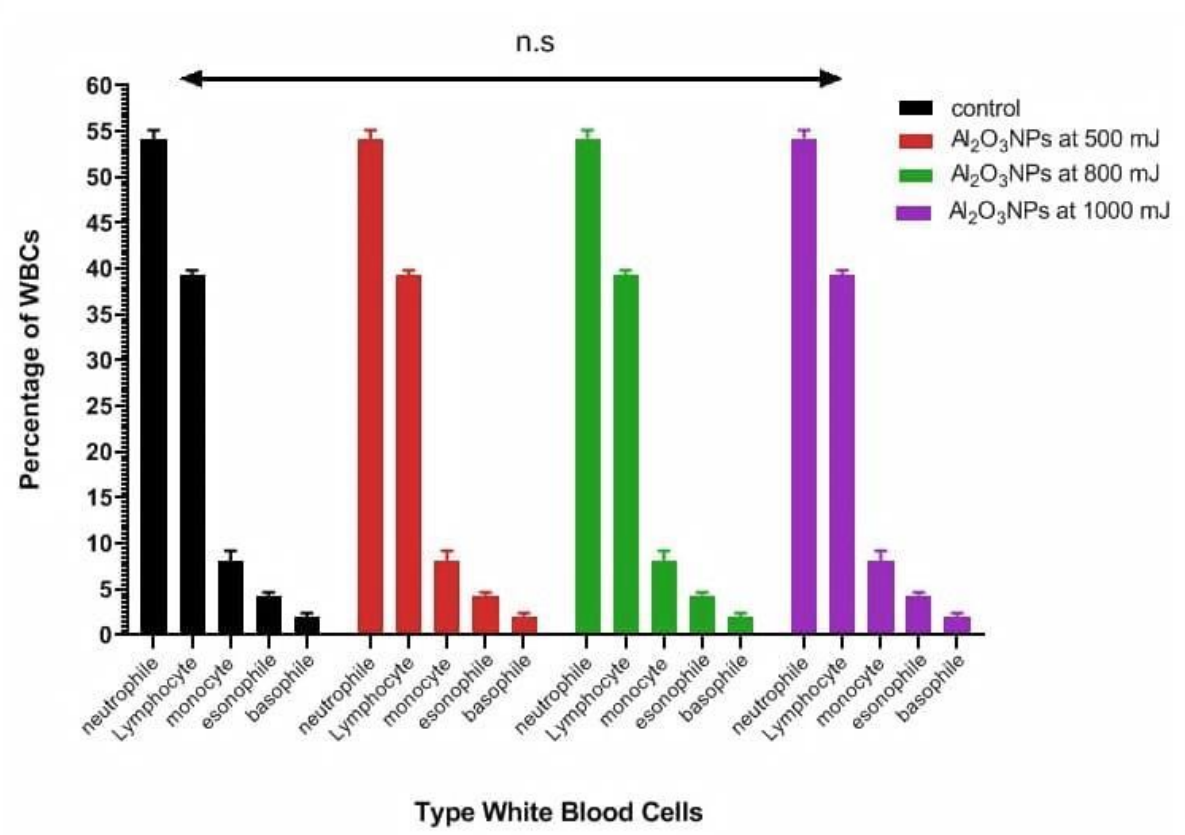

Figure 11: Total percentage of WBCs in a blood sample in the presence and absence of Al2O3 NPs at different energy (500, 800, and 1000) mJ.

We studied the effect of Al_2O_3 NPs on blood cell components *in vitro*, after 1 hr incubating blood samples (control &test) at 37 ºC by staining blood film and examined microscopically (100X) to inspect the affected Al_2O_3 NPs on the blood cell. Show in Fig. 12. These examinations were not different either within or between groups at different energy (500, 800, and 1000) mJ. Biomaterial-based appliances can improve therapeutic function by bio memicking naturally occurring structures. Because red blood cells (RBCs) allow for long-term blood distribution, we hypothesized that biologically inspired NPs that mimicked both the chemical and physical properties of RBCs could improve blood circulation as well. The cell membrane layer can be used for a variety of purposes. Mimicking NPs delivery properties, improving the functionality of various cell types, and extending the half-life of blood

circulation [14]. Biomedical applications are the most vital applications in all these areas. These applications aim to create lighter, more durable, more cost-effective, simpler, and more biocompatible materials and systems. Finally, NPs are fundamental structure cell blocks for the creation of materials that can contribute to the resolution of these issues. It is critical for human health to determine the toxicity of NPs that are widely used due to their unique properties such as surface area and charge, particle size, and chemical, and physical properties [15]. The bio-surface may undergo special reactions due to its small size, high surface curvature, large surface area, and abundance of surface reactive sites. When nanomaterial's come into contact with organisms, the most important biological surfaces with which they interact are cell membranes. As a result, the effects of nanomaterial's on cell membranes should be evaluated to determine nanomaterial applications and safety. Because there are concerns about the health effects of NPs, it is critical to understand how they interact with cells, particularly red blood cells (RBCs), which play an important role in blood functions, and how they compare to nano particle materials. Some studies demonstrated that Al_2O_3 they have a less cytotoxic effect than other NPs [16].

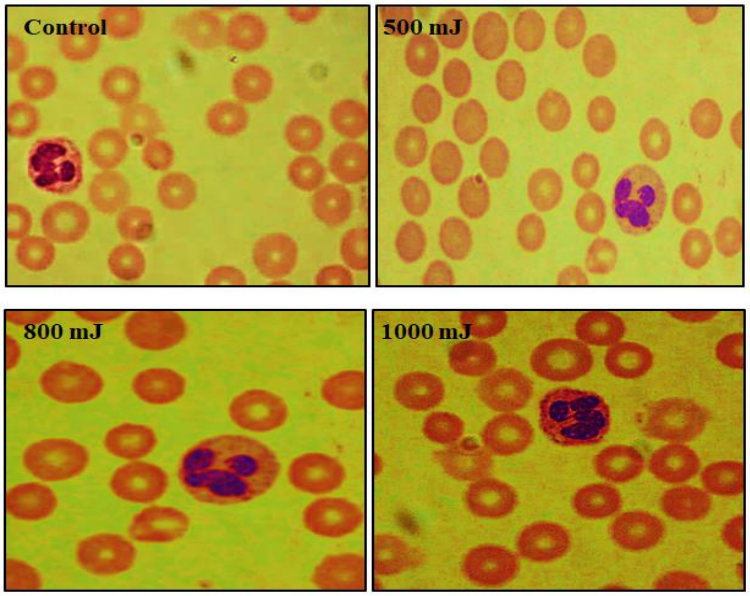

Figure 12: Morphology of a blood sample in the control group and treatment of $Al_2O_{3\ NPs}$ at different energy (500, 800, and 1000) mJ.

Through interactions with the cell surface, aluminum oxide NPs were shown to be toxic to microalgae [17]. NPs was found in almost every cell's cytoplasm. However, there have been few studies on its effects on membranes, which need to be investigated. As a reliable test for material biocompatibility, the hemolysis assay is recommended [18]. Another study on the effect of TiO_2 NPs on human blood components such as platelets, red blood cells (RBCs), hemoglobin, and whole-genome bisulfite levels was assessed in vitro by investigating their potential influence at different concentrations such as 20, 40, 60, and 80 µg/ml. There were no significant differences in the results between the control groups that were not treated and the test groups that were treated with TiO_2 NPs [19]. Research on another toxicity of colloidal PSNPs was assessed in mice; the effect of this nanomaterial toxicity on liver markers in laboratory animals is investigated utilizing four groups. Each group has three duplicates; this data demonstrates that porous silicon NPs have no damaging effect on renal functions [19-21]. While another study on the toxicity of colloidal Al_2O_3 NPs was assessed in mice [22].

4. Conclusions

Al_2O_3 NPs' preparation has a sizable impact on bio-compatibility. Al_2O_3 NPs are effectively used in medicine to prevent infectious and non-infectious diseases because of these qualities, and complete blood count was used in the study of these NPs toxicology effects on human blood in ex vivo (CBC). This finding shows that Al_2O_3 NPs had no toxic effects on the haematology parameter (*in vitro*). It was discovered that the size of the ablated Al_2O_3 particles was smaller with low laser energy than with high laser energy. On the other hand, higher levels of Al_2O_3 were produced by high laser energy than by low laser energy.

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Conflict of Interest
The authors declare that they have no conflict of interest.

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