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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*)

Tuqa Sabah[1], *Kareem H. Jawad[2], Nebras E.Attar[1]

Department of Laser &Optoelectronics Engineering, University of Technology – Iraq1
Department of Medical Engineering, University of Technology – Iraq2
*Corresponding Author: Kareem H. Jawad @uotechnology.edu.iq

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ABSTRACT

DPPH is one of the most widely used tests is the scavenged free radical effect, in *which the color turns to yellow the scavenged effect tests a free stable dark-purple tulip, when 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 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); Count type white blood cells) are compared to control groups, our results demonstrate no significant changes in levels (PLT); (HGB–Hb); (RBCs); (WBCs);(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 profits 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 about one aluminum atom, they possess a corundum-like composition. Al2O3 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 among dangerous bacteria, as well as the introduction of novel infectious diseases, has made the quest for new antimicrobials unavoidable. The unique phytochemical 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,

which 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 (Al2O3) nanoparticles is higher than that of both (CU) and (TiO2) NPS, and the confrontation resistance remains higher for both titanium (TiO2) and copper (Cu)NPS in the case of high aluminum concentration (Al2O3) 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 (C.B.C) and their study the antioxidant activity of nanoparticles.

*Email of corresponding Author: Kareem H. Jawad @uotechnology.edu.iq

2. MATERIAL AND METHODS

2.1Preparation 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 above the target was roughly 3 mm 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.

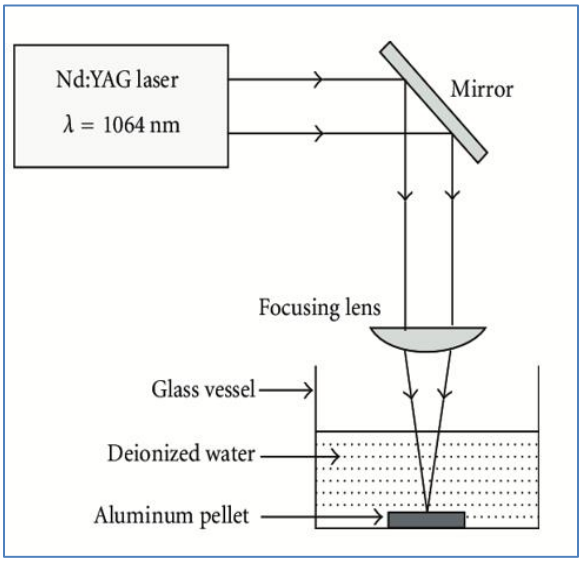


Figure 1: Photographs showing the laser ablation (Nd: YAG Laser).

2.2 Characterization of Al_2O_3NPs

UV-Vis. spectroscopy (UV-Vis, Shimadzu, Japan) was working to ration the absorption spectrum peak in the range between 250 and 1100 nm [11]. The study morphology properties of Al2o3NPs by 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 achieved at the University of Tehran.

2.3Antioxidant activity DPPH assay

The antioxidant activity of produced NPs was determined by Jawad et al. [1]. The DPPH scavenging capabilities of Al_2O_3NPs were tested. After added 750 µL of Al_2O_2NPs at different energy (500,800,1000mJ) with 750 µl of DPPH solution, The samples were incubated in the dark at a fixed temp. at 37 °C for 30 min. The absorbances of samples was evaluated at a wavelength of 517nm. Using formula, the scavenging activity of the produced NPs was calculated [3]:

Antioxidant Activity = $OD^A - OD^B / OD^A * 100$ (1)

Where: OD are the absorbances of the control and the sample, respectively

2.4 The examination 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_3NPs at different energy (500,800,and 1000 mJ) for 1hr compared with untreated samples. Followed by complete blood count test (C.B.C) and blood film. This experiment is done in Baghdad hospital.

2.5 Staining Blood Film Sample[21]

Take drop of blood (control &test) in to slid glass and smear blood. Staining by Giemsa stain at 5 mints. Washing and dried for (10 mints). Examining microscopically (100X) to inspect the effected Al2O3NPs on blood cell. This experiment done at 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 P 0.05.

3. RESULTS AND DISCUSSION

Figure 2 showed 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 vise-versa such that the absorption measurements provide a qualitative indication of the crystal size distribution. and when increasing the energy 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_3NPs 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_3NPs' 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 500,800.1000mJ 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.

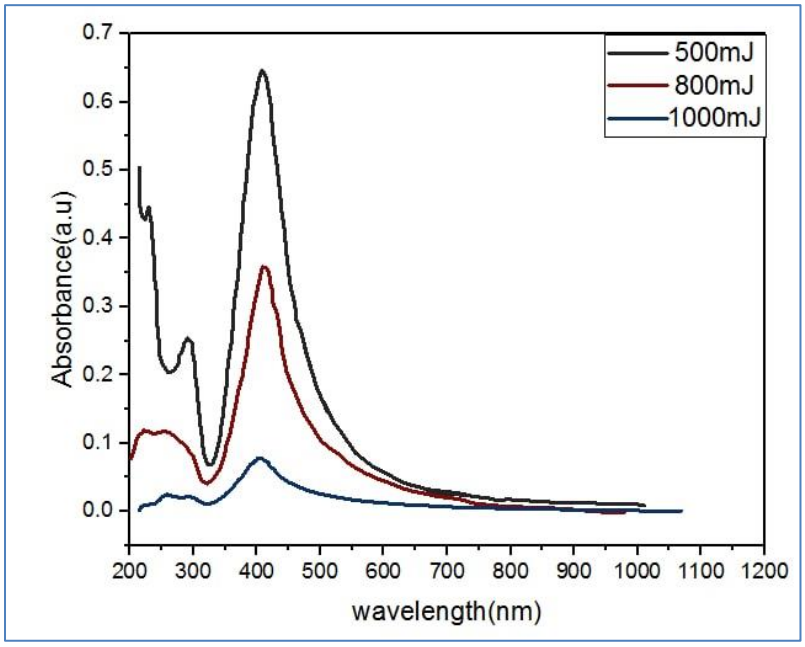


Figure2: UV-Visible of Al_2O_3 NPs at 500mJ, 800mJ, 1000mJ energy.

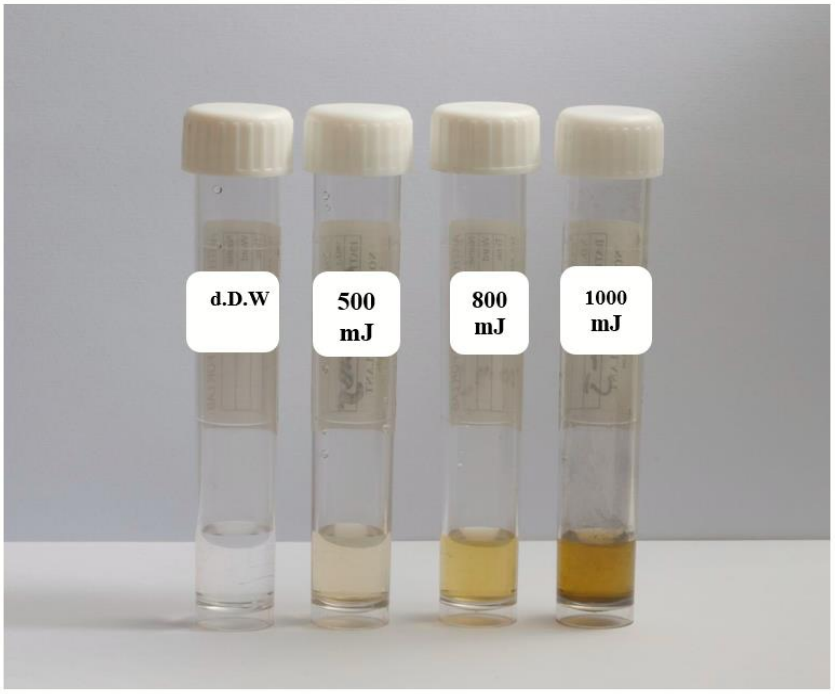
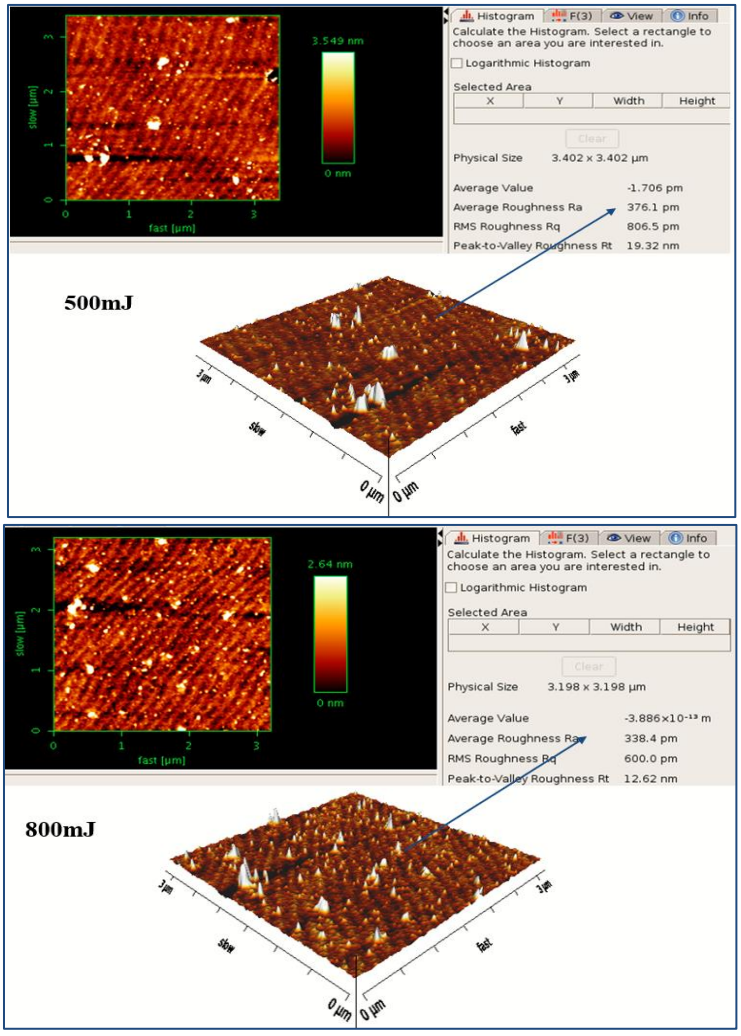


Figure3: Al_2O_3NPs colloidal prepared by laser ablation at different energy (500, 800 and 1000) mJ.



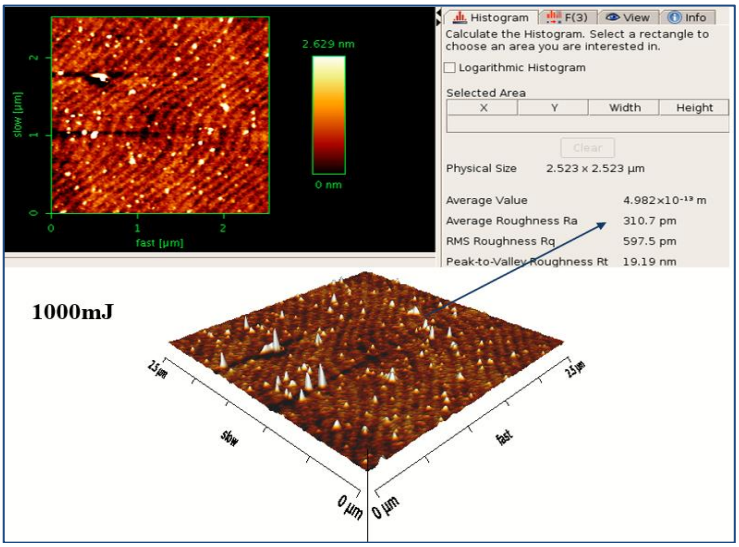


Figure4 : AFM image of Al_2O_3NPs at different energy (500, 800 and 1000) mJ.

Figure 5 showed a SEM image of the sample. The nanoparticles are round and granular, as shown in (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.

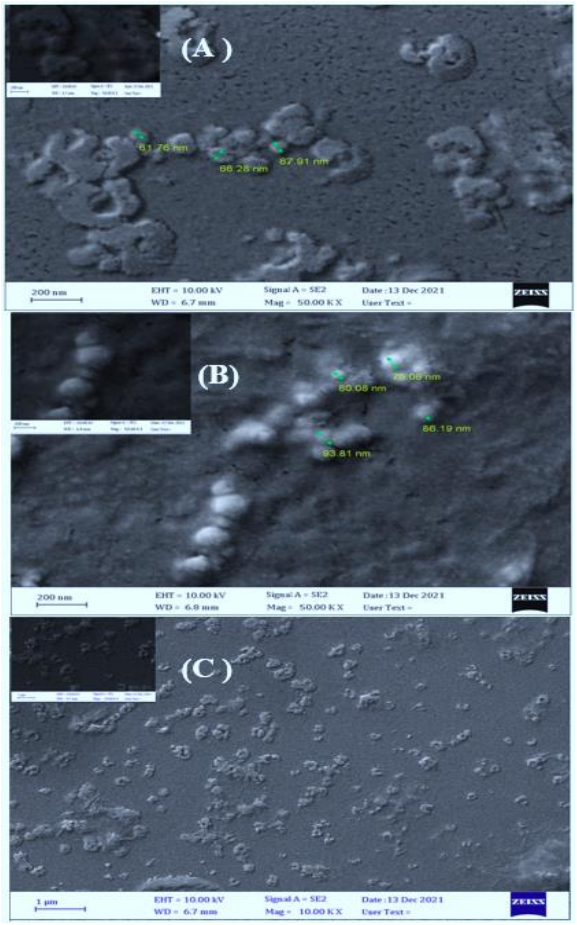


Figure5: SEM image of Al_2O_3 NPs at (A) 500mJ, (B)800mJ, (C)1000mJ energy.

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_3NPs .The cytotoxic reaction, instead of ROS generation, could be the major mechanism of Al_2O_3 NP 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 Al2O3NPS can release hydrogen atoms and removes the unstable electron from DPPH at more of than 1000 mJ 500 mJ.

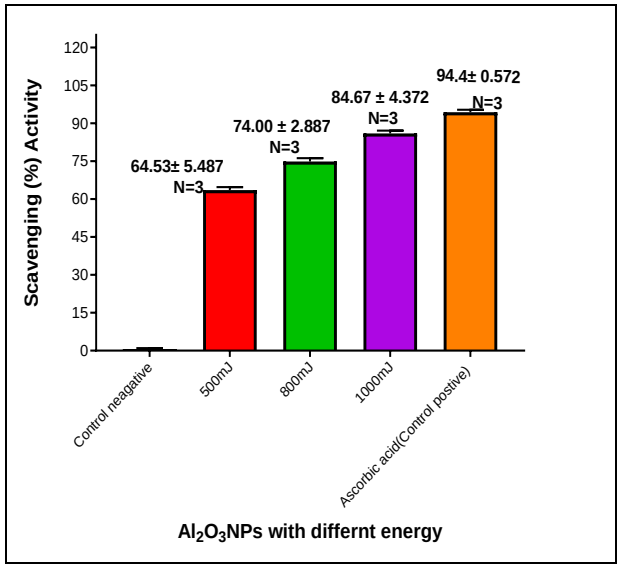


Figure 6: DPPH scavenging activity of Al_2O_3 NPs.

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 (C.B.C.) 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. Figures (7,8,9,10,11 and 12).

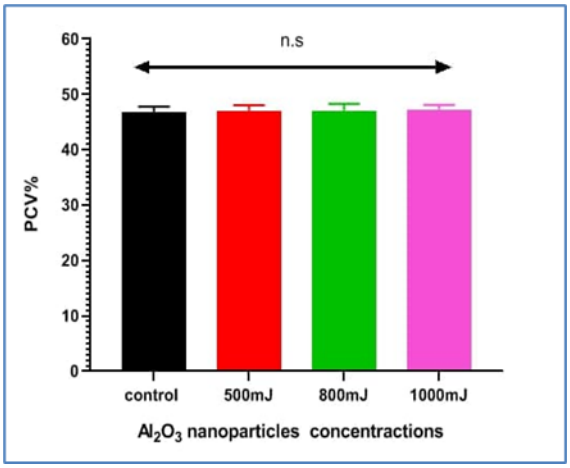


Figure7: PCV level in blood samples in presence and absence of Al_2O_3 NPs at different Energy(500,800,and 1000)mJ.

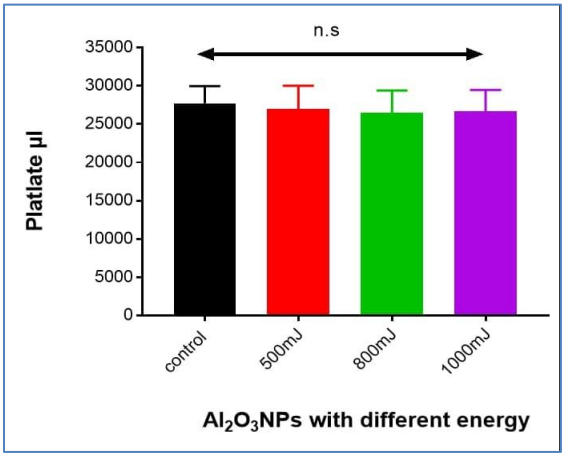


Figure 8: Platelet's count level in blood samples in presence and absence of Al_2O_3 NPs at different Energy(500,800,and 1000)mJ.

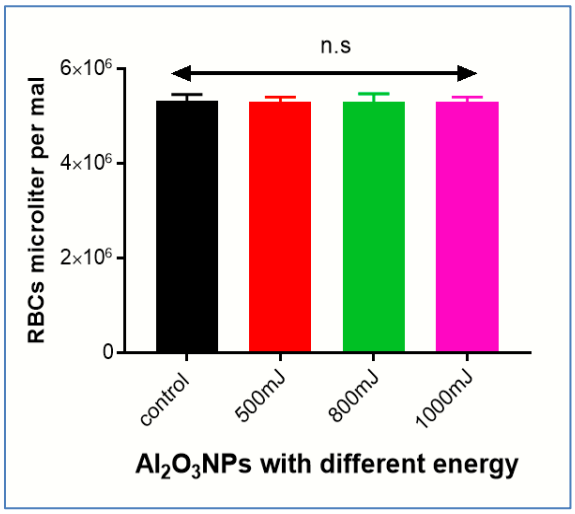


Figure 9: RBCs count in blood samples in presence and absence of Al_2O_3 NPs at different Energy(500,800,and 1000)mJ.

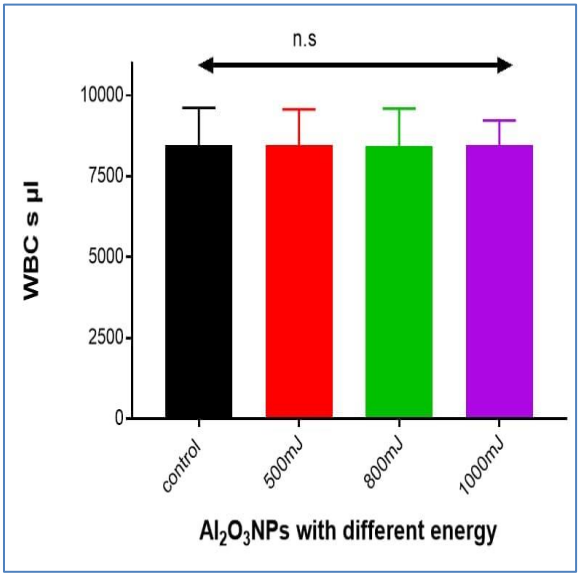


Figure 10: WBCs count in blood samples in presence and absence of Al_2O_3 NPs at different Energy(500,800,and 1000)mJ.

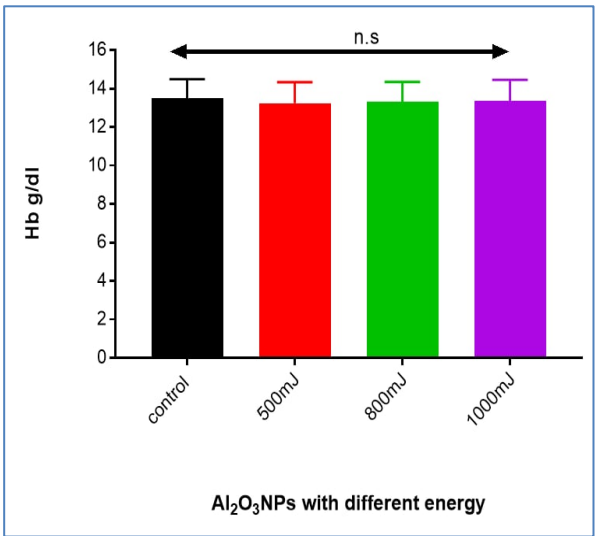


Figure 11:HGB level in blood samples in presence and absence of Al_2O_3 NPs at different Energy(500,800,and1000)mJ.

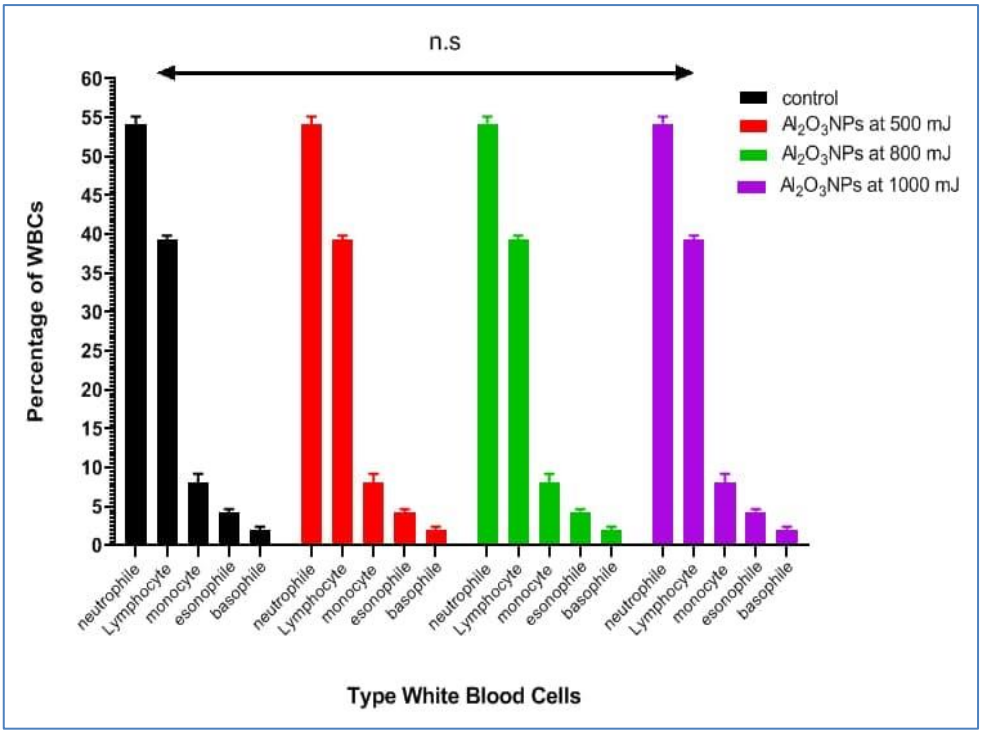


Figure12: Percentage of WBCs in blood sample in presence and absence of Al_2O_3NPs at different Energy(500,800,and 1000)mJ.

We studied the effect of Al_2O_3 NPs on blood cell components in *vitro*, after 1hr incubated blood samples (control &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.Show in Figure 13.

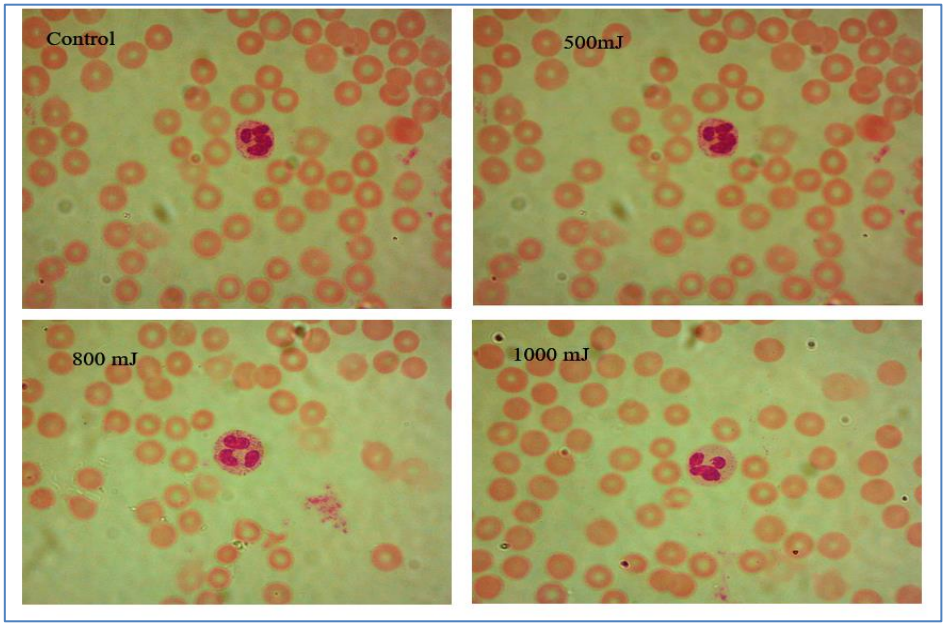


Figure13: Morphology of blood sample in control group and treated of Al_2O_3NPs at different energy (500,800, and 1000) mJ.

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's 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. Al2O3 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 TiO2 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 TiO2 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 Al_2O_3NPs have significant biocompatibility, biodegradability, and antioxidant effects. Due to these properties, Al_2O_3NPs 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). This result that there indicates no toxic effect of Al_2O_3NPS 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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REFERENCES

[1] Jawad K. H., Saleh T.H., and Hasoon B. A. "Preparation of Aluminum Oxide Nanoparticles by Laser Ablation and a Study of Their Applications as Antibacterial and Wounds Healing Agent", Nano Biomedicine and Engineering, 313-319.2019.
[2] Bala T., Armstrong G., Laffer F., and Thornton R., "Titania–silver and alumina–silver composite nanoparticles: novel, versatile synthesis, reaction mechanism and potential antimicrobial application," Journal Colloid Interface Sci., vol. 356, no. 2, pp. 395–403, 2011.
[3] Jawad K. H., Hasson B. A. "Developing Strategy for a Successful Antioxidant, Anticancer Activity via an Improved Method Prepared to Porous Silicon Nanoparticles" journal of Applied Sciences and Nanotechnology, 2021, Volume 1, Issue 4, Pages 1-11.DOI: 10.53293/jasn.2021.3890.1054
[4] Jawad A. S., Thewaini Q. N., Sh. Al-Musawi "Cytotoxicity Effect and Antibacterial Activity of Al2O3 Nanoparticles Activity against Streptococcus Pyogenes and Proteus Vulgaris" Journal of Applied Sciences and Nanotechnology, 2021, Volume 1, Issue 3, Pages 42-50.DOI: 10.53293/jasn.2021.3944.1061.
[5] Zolnik B. S., González-Fernández Á., Sadrieh N., and Dobrowolski M. A., "Minireview: nanoparticles and the immune system," Endocrinology, vol. 151, no. 2, pp. 458–465, 2010.
[6] Patton K. T. and Thibodeau G. A., "Anthony's Textbook of Anatomy & Physiology-E-Book". Elsevier Health Sciences, 2018.
[7] Gentile F., Ferrari M., and Decuzzi P., "The transport of nanoparticles in blood vessels: the effect of vessel permeability and blood rheology," Ann. Biomed. Eng., vol. 36, no. 2, pp. 254–261, 2008.
[8] Nadeem S., Ijaz S., and Akbar N. S. "Nano Particle analysis for the steady blood flow of Jeffrey fluid with stenosis with new analytical techniques," Journal Compute. Theory. Nanoscale., vol. 10, no. 11, pp. 2751–2765, 2013.
[9] Ahmed A. and Nadeem S., "The study of (Cu, TiO2, Al2O3) nanoparticles as antimicrobials of blood flow through diseased arteries," Journal. Mol. Liq., vol. 216, pp. 615–623, 2016.
[10] Lin W., Stayton I., Y., Huang X.-D. Zhou, and Y. Ma, "Cytotoxicity and cell membrane depolarization induced by aluminum oxide nanoparticles in human lung epithelial cells A549," Toxicol. Environ. Chem., vol. 90, no. 5, pp. 983–996, 2008.
[11] Maeh R. K., Jaafar A.I., Hasoon B.A., Hussein N. N. "Preparation and characterization of graphene oxide for biological application " Drug Invention Today,2019.
[12] Soud Sh. A., Hasoon B. A., Abdulwahab A. I., Hussein N.N., Maeh R. K. "Synthesis and characterization of plant extracts loaded PVA/PVP blend films and evaluate their biological activities" , Eurasian Journal of BioSciences,2020.
[13] Fadhil F.A., Hasoon B.A., Hussein N.N., and Khashan K. S. "Preparation and characterization of CuO NPs via laser ablation under electric field and study their antibacterial activity" AIP Conference Proceedings 2045, 020002 (2018); Doi: 10.1063/1.5080815.
[14] Parodi A. et al., "Synthetic nanoparticles functionalized with biomimetic leukocyte membranes possess cell-like functions," Nat. Nanotechnology., vol. 8, no. 1, pp. 61–68, 2013.
[15] Donaldson K., Stone V., Tran C. L., Kreyling W., and Brom P. J. A., "Nanotoxicology," Occupational and environmental medicine, vol. 61, no. 9. BMJ Publishing Group Ltd, pp. 727–728, 2004.
[16] Zhang X. Q., Yin L. H., Meng T., and Pu Y. P., "ZnO, TiO2, SiO2, and Al2O3 nanoparticles-induced toxic effects on human fatal lung fibroblasts," Biomed. Environ. Sci., vol. 24, no. 6, pp. 661–669, 2011.
[17] Sadiq I. M., Pakrashi S., Chandrasekaran N., and Mukherjee A. "Studies on toxicity of aluminum oxide (Al2O3) nanoparticles to microalgae species: Scenedesmus sp. and Chlorella sp.," J. Nanoparticle Res., vol. 13, no. 8, pp. 3287–3299, 2011.

[18] Jawad K.H., Hasoon B.A., Hussein. N.N. "Biological application of titanium dioxide nanoparticles prepared through laser ablation in liquid" Drug Invention Today, 12(10), 2019
[19] Lu S. et al., "Efficacy of simple short-term in vitro assays for predicting the potential of metal oxide nanoparticles to cause pulmonary inflammation," Environ. Health Perspect., vol. 117, no. 2, pp. 241–247, 2009.
[20] Jawad K. H., Jabir M. S., Nayef U. M. "Toxicity of Porous Silicon Nanoparticles on Liver of Mice", Al-Mustansiriyah Journal of Science, DOI: http://doi.org/10.23851/mjs.v28i3.552.
[21] Jawad K. H., Jabir M. S., Nayef U. M. "A Study of the effect of Porous Silicon Nanoparticles on Human Blood Components", Journal of College of Basic Education,2017.

[22] Jawad K. H., Jabir M. S., Nayef M. "study of kidney Parameters Induced by Porous Silicon Nanoparticles" Engineering and Technology Journal.2017.