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RESEARCH PAPER

Evaluating the Hematological Impact of Zinc Oxide Nanoparticles in NMRI Mice: An in Vivo Study

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ABSTRACT

The burgeoning field of nanotechnology has led to the widespread use of zinc oxide (ZnO) nanoparticles due to their multifunctional properties. However, the hematological impact of these nanoparticles on biological systems is not fully understood. This in vivo study aimed to evaluate the effects of ZnO nanoparticles on hematological parameters in adult male Albino-NMRI mice. Over a 28-day period, mice were administered oral doses of ZnO nanoparticles at concentrations of 25, 50, 75, and 100 mg/kg body weight. Hematological parameters such as white blood cell (WBC), red blood cell (RBC) counts, hemoglobin (Hb) concentration, and platelet (PLT) count were measured, alongside coagulation tests. The study utilized a Sysmex XN-1000 hematology analyzer for blood cell counts and observed coagulation time and clot strength using standard laboratory techniques. Results indicated that most hematological parameters remained unchanged across the groups. Nevertheless, a significant increase in neutrophil count was observed in the 4th group (9.35 K/µL in Group 4 vs. 8.93 K/µL in the control group), suggesting a dose-dependent response. Additionally, a notable prolongation in blood coagulation time was observed in the same group (9.18 min in the 4th Group vs. 6.70 min in the control group), indicating potential alterations in the hemostatic function. These findings suggest that while ZnO nanoparticles do not significantly alter most blood parameters, they may affect neutrophil count and coagulation time at higher doses. This study underscores the importance of understanding the biological interactions of nanoparticles to ensure their safe application in various industries. The results contribute to the establishment of safety guidelines for the handling and disposal of ZnO nanoparticles, reflecting a commitment to public health and environmental stewardship in the advancement of nanotechnology.

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INTRODUCTION

The advent of nanotechnology has brought about a paradigm shift in material science, leading to the development of materials with unprecedented properties and functionalities. The transition from microscale particles to nanoparticles has been particularly transformative, as it has resulted in the manifestation of unique physical and chemical characteristics [1,2]. These changes are primarily attributed to the increased surface area-to-volume ratio and the quantum effects that become prominent at the nanoscale. The implications of these shifts are profound, influencing a multitude of industries and paving the way for innovative applications [3].

Nanoparticles, by definition, are particles that measure between 1 and 100 nm in at least one dimension. At this scale, the properties of materials can differ significantly from their bulk counterparts due to the increased influence of surface atoms and quantum mechanical phenomena. This has led to the exploration and exploitation of nanoparticles in various fields, including medicine, electronics, energy, and environmental science [4,5].

Among the various nanoparticles being studied, zinc oxide (ZnO) nanoparticles have garnered considerable attention due to their versatile properties. These include strong UV absorption, significant antimicrobial activity, high catalytic efficiency, and the ability to enhance the mechanical properties of composites [6,7]. Zinc oxide nanoparticles are also recognized for their safety profile, as they are generally regarded as safe by regulatory agencies such as the FDA [8].

The utility of ZnO nanoparticles spans across several domains. In the realm of textiles, they are employed to create antimicrobial and odor-resistant fabrics [9]. In the domain of personal care, they are a key ingredient in sunscreens, providing effective protection against UV radiation without the use of chemical absorbers [10]. In healthcare, ZnO nanoparticles are integrated into wound dressings to expedite healing and prevent infection [11]. Furthermore, their photocatalytic properties make them valuable in environmental applications, such as water treatment and air purification, where they aid in the degradation of pollutants [12–14].

Despite the promising applications of ZnO nanoparticles, their potential impact on human health and the environment remains a subject of intense scrutiny [15,16]. The field of nanomaterial toxicology is essential in this regard, as it seeks to understand the interactions between nanoparticles and biological systems. This research is vital for the responsible development and use of nanotechnology, ensuring that the benefits are not overshadowed by unforeseen risks.

Research has indicated that nanoparticles can enter the human body through inhalation, ingestion, or dermal absorption [17]. Once internalized, they may be distributed to various organs and tissues, potentially crossing cellular barriers and interacting with biological macromolecules. This raises concerns about the possibility of cellular toxicity, oxidative stress, and inflammatory responses [18–20].

Given the widespread application and the increasing human exposure to ZnO nanoparticles, it is critical to investigate their biokinetics and potential health effects. The primary objective of this study is to examine the impacts of ZnO nanoparticles on blood cells in adult male Albino-NMRI mice. Blood cells serve as a vital indicator of physiological and immunological health, and any alterations in their function or viability could have significant implications for overall health.

This study will focus on evaluating hematological parameters, including platelet aggregation, hemoglobin concentration, white blood cell count, and red blood cell count. These parameters will provide insight into the effects of ZnO nanoparticles on the circulatory and immune systems. As the use of ZnO nanoparticles continues to expand, it is of paramount importance to understand their biological interactions and to establish safety guidelines for their handling and disposal. This study represents a step towards achieving a comprehensive understanding of the biocompatibility and potential risks associated with ZnO nanoparticles, ensuring that the advancements in nanotechnology are accompanied by a commitment to public health and environmental stewardship.

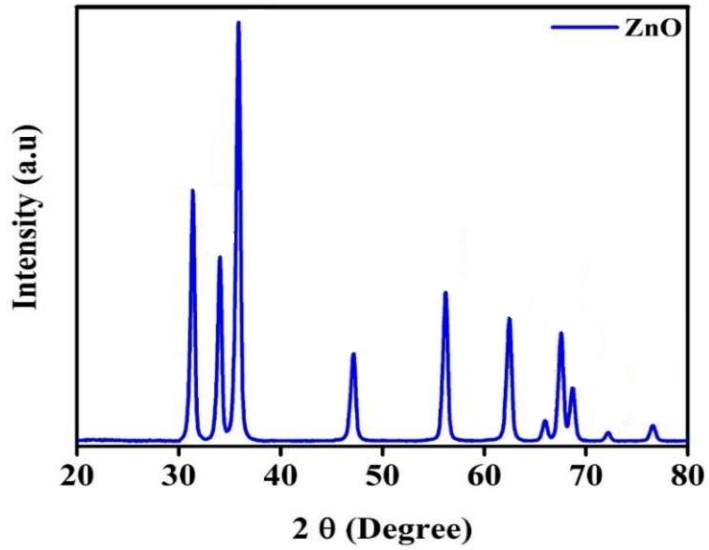
MATERIALS AND METHODS

In this experimental study, blood cell counts were performed using a Sysmex XN-1000 hematology analyzer (Sysmex Co., Japan), and the characterization of manufactured nanoparticles was conducted with an atomic force microscope (AFM) (Multimode, Nanoscope 8, Bruker), which provides very high-resolution imaging.

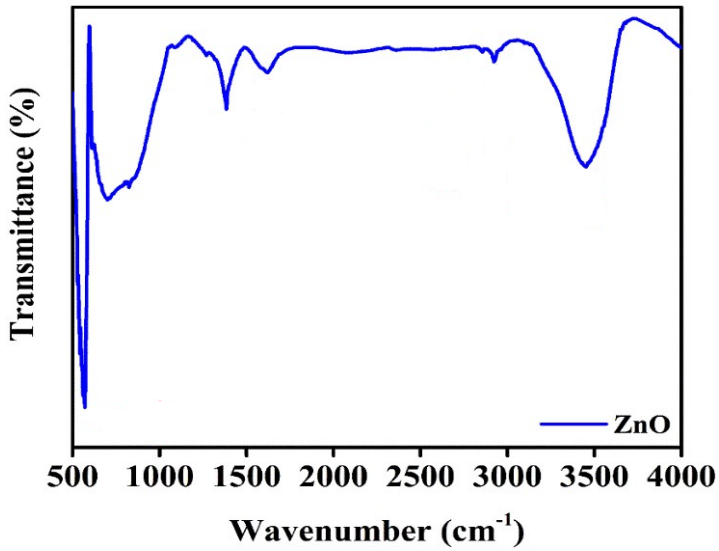
Zinc oxide nanoparticles with sizes ranging

from 50 to 60 nm were synthesized by the Nanotechnology and Advanced Materials Research Center at the University of Baghdad. The XRD pattern and FTIR spectrum of the ZnO nanoparticles employed in this investigation are all displayed in Fig. 1. The surface area of these nanoparticles was analyzed using the AFM microscope.

The ZnO nanoparticle suspensions were subjected to ultrasonication for 10 min using the equipment available at the research institute. Following this, the suspensions were agitated for 2 min. Subsequently, various doses were administered to the 40 adult male Albino-NMRI mice (25-35 g) via oral gavage. These mice were divided into five groups of eight, including one control group and four experimental groups. The mice were accommodated in cages made of



(a)



(b)

Fig. 1. Analysis results of ZnO nanoparticles: (a) XRD pattern, (b) FTIR spectra.

propylene, situated in the biology department. The cages were lined with sawdust, and the mice had unrestricted access to water. The environmental conditions were regulated, with temperatures kept at 23±2°C, humidity at 20%, and a light/dark cycle that lasted 12 hours each. A full diet was provided to the mice, which included commercial chow and water, both of which were accessible to them without any limitations.

Prior to the initiation of the experiments, the mice underwent a two-week acclimatization period under the specified environmental conditions. All procedures involving animals were approved by the ethics committee. The experimental groups received oral doses of ZnO nanoparticles at concentrations of 25, 50, 75, and 100 mg/kg body weight via gavage for a duration of 28 days [21–23].

For the preparation of ZnO nanoparticle solutions, the nanoparticles were procured from University of Baghdad and suspended in deionized water (EC>1) at the aforementioned concentrations. Each mouse received 1 ml of the solution at 24-hour intervals.

Environmental temperature and humidity were monitored daily to ensure optimal conditions, maintaining a temperature of 23±2°C and a humidity level of 20%. Additionally, the body weight of the mice was recorded regularly throughout the treatment period.

Blood samples for biochemical analysis were collected after 21 days using the Orbital Sinus method [24], which involves drawing venous blood from the orbital sinus at the inner canthus of the mice eyes using a hematocrit tube. The tubes had a length of 75 mm and an internal diameter of 1.2 mm. The blood samples were then placed in specialized centrifuge tubes for serum separation.

A Sysmex XN-1000 hematology analyzer, utilizing isotonic solutions, hemoglobin lysis buffer, white blood cell lysis buffer, and control blood, was employed for blood cell counting. To assess coagulation time and clot strength, blood samples were collected in capillary tubes and transferred to simple tubes.

Blood specimens were gathered into tubes designed for a complete blood count (CBC), which included Ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. These samples were then placed on a hematology mixer for 10 min to ensure thorough mixing and homogenization before being processed by the cell counter. The cell counter was used to enumerate various blood cell types, including white blood cells, red blood cells, platelets, neutrophils, and lymphocytes. Additionally, blood smears were prepared to examine potential morphological changes in the blood cells.

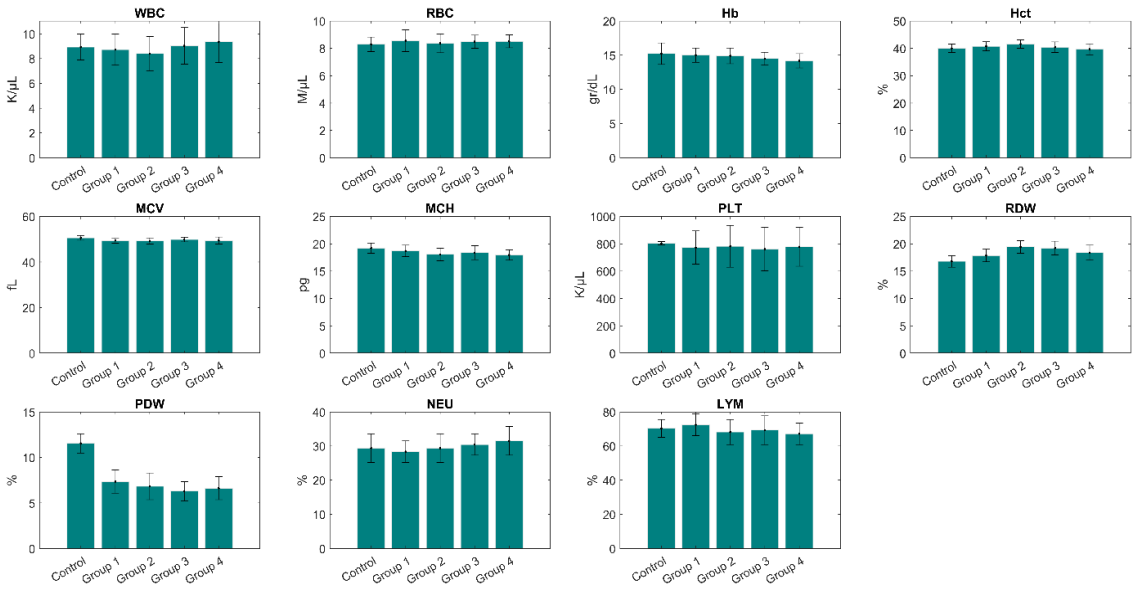


Fig. 2. The results of hematological tests in mice after 28 days of oral intake of ZnO nanoparticles.

For coagulation studies, mice were anesthetized, and blood was drawn from the internal veins of the eye using capillary action. The collected blood, approximately 1 ml, was immediately placed in a bain-marie at 37°C. The coagulation time was periodically checked by removing the tube every minute, and the duration of clot formation was measured with a chronometer.

In another procedure, after anesthetizing the mouse, a small incision of about 3 mm was made at the tip of the mouse's tail. Blood droplets were absorbed using filter paper to observe the cessation of bleeding, and the bleeding time was recorded with a stopwatch.

The examination of blood smears and the counting of blood cells were conducted using a Sysmex XN-1000 hematology analyzer at the Baghdad teaching hospital. Coagulation time, as well as the strength and quality of the clots, were assessed at University of Baghdad.

Upon completion of data collection, statistical analyses were carried out using Statistical Analysis Software (SAS). The Tukey-Kramer and t-tests were applied to determine the significance of the results. In the conducted analysis, results yielding a p-value lower than 0.05 were recognized as statistically significant. The findings are reported as the mean value with the corresponding standard deviation (SD).

RESULTS AND DISCUSSION

The impact of ZnO nanoparticles on various hematological parameters in mice was assessed, including white and red blood cell counts, hemoglobin concentration, neutrophil count, lymphocyte count, platelet count, and blood coagulation time. The results indicated no significant changes across most parameters, except for neutrophils, where an increase was observed with higher concentrations of ZnO nanoparticles in the diet (Figs. 2 and 3).

In Fig. 2, the parameters of a complete blood count are presented. WBC (K/µL), or white blood cell count, measures the number of white blood cells, which are crucial for the body's immune response. RBC (M/µL), or red blood cell count, indicates the number of red blood cells that carry oxygen throughout the body. Hb (gr/dL) stands for hemoglobin, the protein in red blood cells that binds to oxygen. Hct (%), or hematocrit, represents the proportion of blood volume occupied by red blood cells.

Additionally, MCV (fL), or mean corpuscular volume, quantifies the average size of red blood cells. MCH (pg), or mean corpuscular hemoglobin, measures the average amount of hemoglobin per red blood cell. PLT (K/µL) refers to the platelet count, which is essential for blood clotting. RDW (%), or red cell distribution width, assesses the variation in red blood cell size. PDW (%), or platelet distribution width, evaluates the variability in platelet size. Lastly, NEU (%) and LYM (%) represent the percentages of neutrophils and lymphocytes, two types of white blood cells that play key roles in the body's defense mechanisms. Neutrophils are the first responders to infection, while lymphocytes are involved in the adaptive immune response.

Additionally, the study revealed a notable alteration in blood coagulation time in the 4th group of mice, with a dose-dependent prolongation when compared to the control group ($p<0.05$) (Fig. 4).

The present study aimed to evaluate the hematological effects of zinc oxide (ZnO) nanoparticles in adult male Albino-NMRI mice. Our findings demonstrate that the administration of ZnO nanoparticles at varying concentrations

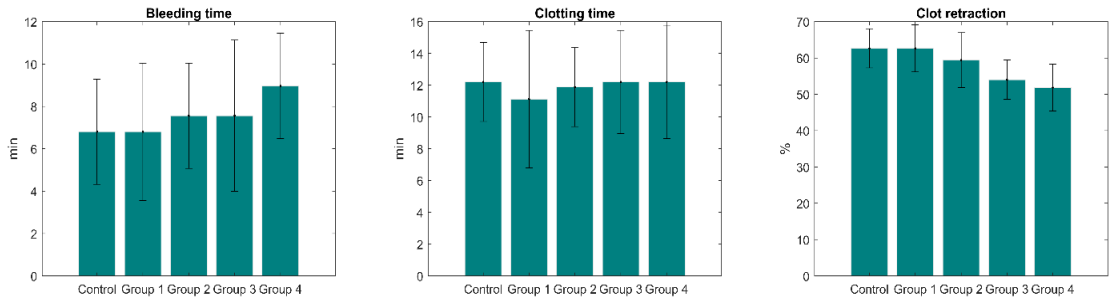


Fig. 3. The results of coagulation tests in mice after 28 days of oral intake of ZnO nanoparticles.

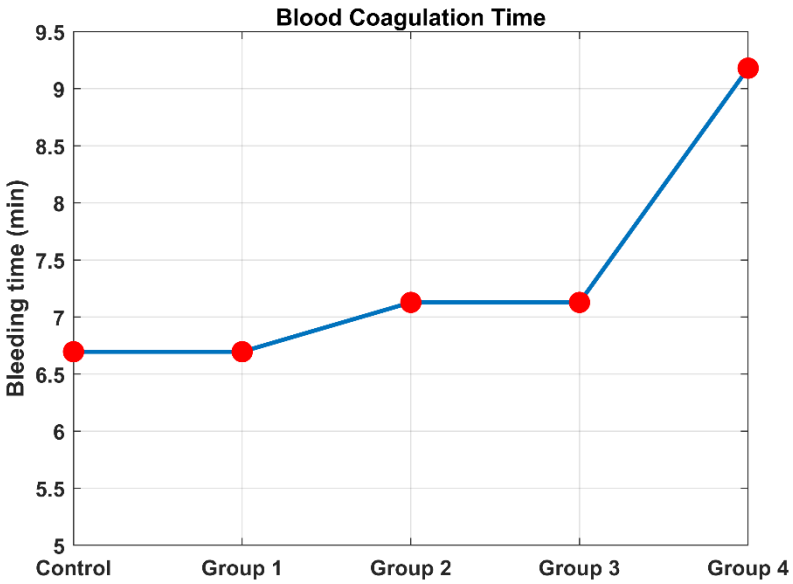


Fig. 4. The average blood coagulation time between the treatment and control groups.

did not significantly alter most hematological parameters, such as red blood cell count, hemoglobin concentration, and platelet count. However, a dose-dependent increase in neutrophil percentage (NEU %) was observed, which suggests a potential immunological response to the presence of ZnO nanoparticles.

The observed increase in NEU % aligns with the role of neutrophils as the first responders to infection and inflammation. Neutrophils are known to react to foreign bodies, including nanoparticles, which may be perceived as potential threats by the immune system [24]. The increase in neutrophils could indicate an acute inflammatory response, as has been suggested by previous studies that reported similar hematological changes upon exposure to various nanoparticles [22,23]. This response could be attributed to the recognition of ZnO nanoparticles as foreign particles, leading to an innate immune response characterized by neutrophil recruitment.

The lack of significant changes in other hematological parameters is consistent with some previous studies, suggesting that ZnO nanoparticles may not have a pronounced effect on red blood cells (RBCs), hemoglobin (Hb), and platelets (PLTs) at the tested concentrations [21]. However, it is essential to consider that the size, shape, surface charge, and coating of nanoparticles can influence their hematocompatibility, as highlighted by Modena et al. [4]. The ZnO nanoparticles used in our study had a size range of 50-60 nm, which may have contributed to the observed biological responses.

In contrast to our findings, some studies have reported cytotoxic effects, including hemolysis and alterations in RBC morphology, upon exposure to ZnO nanoparticles [19,20]. These discrepancies could be due to differences in nanoparticle characteristics, exposure duration, or animal models used. It is also possible that the systemic effects of ZnO nanoparticles may manifest over a more extended period or at higher doses than those employed in our study.

The notable alteration in blood coagulation time observed in the 4th group of mice, with a dose-dependent prolongation compared to the control group, is particularly significant ($p<0.05$). This finding is in agreement with previous research that has indicated the potential of nanoparticles to interfere with hemostasis, affecting both coagulation and anticoagulation pathways [7]. The prolonged coagulation time suggests that higher concentrations of ZnO nanoparticles may have anticoagulant effects, which could be due to direct interactions with blood components or indirect effects mediated by the immune system [8].

The biocompatibility and safety of ZnO nanoparticles are of paramount importance, given their widespread use in consumer products and

potential for human exposure. While our study provides valuable insights into the hematological impacts of ZnO nanoparticles, further research is needed to elucidate the underlying mechanisms of their interaction with the immune system and their long-term effects. Additionally, studies investigating the potential genotoxicity, oxidative stress, and organ-specific toxicity of ZnO nanoparticles would contribute to a more comprehensive understanding of their safety profile [13,18].

In conclusion, the present study contributes to the growing body of literature on the biological effects of nanoparticles and underscores the importance of thorough toxicological evaluations. As the field of nanotechnology continues to advance, it is crucial to balance the benefits of novel applications with a commitment to public health and environmental stewardship.

CONCLUSION

This in vivo study aimed to evaluate the hematological impact of ZnO nanoparticles on adult male Albino-NMRI mice, providing valuable insights into the biocompatibility and potential health risks associated with ZnO nanoparticles exposure. Our findings contribute to the growing body of knowledge in nanotoxicology, particularly concerning the physiological and immunological implications of engineered nanomaterials.

The thorough examination of blood-related indices, which includes the proportions of lymphocytes (LYM) and neutrophils (NEU), along with the measurements of platelet distribution width (PDW), red cell distribution width (RDW), platelet (PLT) count, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), hematocrit (Hct), hemoglobin (Hb) concentration, red blood cell (RBC) count, and white blood cell (WBC) count, indicated stability in most of the parameters with no significant alterations observed. However, a dose-dependent increase in neutrophil count was observed, which could indicate an immune response to the presence of ZnO nanoparticles.

Furthermore, the study identified a notable alteration in blood coagulation time in the highest dose group, suggesting that ZnO nanoparticles may affect the hemostatic system. This finding is particularly relevant given the crucial role of coagulation in maintaining physiological homeostasis and the potential implications for bleeding disorders.

The experimental approach, utilizing advanced hematology analyzers and rigorous statistical analysis, ensured the reliability of the results. The data obtained from this study underscore the importance of thorough toxicological assessments of nanomaterials, especially as their applications in consumer products and industrial processes continue to expand.

In conclusion, while ZnO nanoparticles are widely regarded as safe and are utilized in various applications, our study indicates that at certain doses, they may exert hematological effects in mice. These findings highlight the necessity for continued research into the long-term health effects of nanomaterials and the development of guidelines to manage exposure levels. It is imperative that the advancements in nanotechnology are paralleled by a commitment to safeguarding public health and the environment. Further studies are recommended to explore the mechanisms underlying the observed hematological changes and to assess the potential impact of chronic exposure to ZnO nanoparticles.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Chaikittisilp W, Yamauchi Y, Ariga K. Material Evolution with Nanotechnology, Nanoarchitectonics, and Materials Informatics: What will be the Next Paradigm Shift in Nanoporous Materials? Adv Mater. 2022;34(7).
2. Chafiq M, Chaouiki A, Ko YG. Recent Advances in Multifunctional Reticular Framework Nanoparticles: A Paradigm Shift in Materials Science Road to a Structured Future. Nano-Micro Letters. 2023;15(1).
3. Abdul Rani R, Zoolfakar AS, Mohamad Ryeeshyam MF, Ismail AS, Mamat MH, Alrokayan S, et al. High Surface Area to Volume Ratio 3D Nanoporous Nb2O5 for Enhanced Humidity Sensing. J Electron Mater. 2019;48(6):3805-3815.
4. Modena MM, Rühle B, Burg TP, Wuttke S. Nanoparticle Characterization: Nanoparticle Characterization: What to Measure? (Adv. Mater. 32/2019). Adv Mater. 2019;31(32).
5. The importance of silver nanoparticles in human life. Advances in Applied NanoBio-Technologies. 2020;1(1):5-9.
6. Vinitha V, Preeyanghaa M, Vinesh V, Dhanalakshmi R, Neppolian B, Sivamurugan V. Two is better than one: catalytic, sensing and optical applications of doped zinc oxide nanostructures. Emergent Materials. 2021;4(5):1093-1124.
7. Islam F, Shohag S, Uddin MJ, Islam MR, Nafady MH, Akter A, et al. Exploring the Journey of Zinc Oxide Nanoparticles (ZnO-NPs) toward Biomedical Applications. Materials.

2022;15(6):2160.
8. Akbar N, Aslam Z, Siddiqui R, Shah MR, Khan NA. Zinc oxide nanoparticles conjugated with clinically-approved medicines as potential antibacterial molecules. AMB Express. 2021;11(1).
9. Sol-Gel Finishing for Protective Fabrics. Biointerface Research in Applied Chemistry. 2022;13(3):283.
10. Goyal N, Jerold F. Biocosmetics: technological advances and future outlook. Environmental Science and Pollution Research. 2021;30(10):25148-25169.
11. Manuja A, Raguvaran R, Kumar B, Kalia A, Tripathi BN. Accelerated healing of full thickness excised skin wound in rabbits using single application of alginate/acacia based nanocomposites of ZnO nanoparticles. Int J Biol Macromol. 2020;155:823-833.
12. Kusiak-Nejman E, Wojnarowicz J, Morawski AW, Narkiewicz U, Sobczak K, Gierlotka S, et al. Size-dependent effects of ZnO nanoparticles on the photocatalytic degradation of phenol in a water solution. Appl Surf Sci. 2021;541:148416.
13. Mustapha S, Ndamitso MM, Abdulkareem AS, Tijani JO, Shuaib DT, Ajala AO, et al. Application of TiO2 and ZnO nanoparticles immobilized on clay in wastewater treatment: a review. Applied Water Science. 2020;10(1).
14. Ji S-M, Tiwari AP, Oh HJ, Kim H-Y. ZnO/Ag nanoparticles incorporated multifunctional parallel side by side nanofibers for air filtration with enhanced removing organic contaminants and antibacterial properties. Colloids Surf Physicochem Eng Aspects. 2021;621:126564.
15. Karimi M, Mirkazemi SM, Vahidshad Y, Javadpour J. Preparation and characterization of zinc sulfide thin film by electrostatic spray deposition of nano-colloid. Thin Solid Films. 2021;737:138929.
16. V R, G S, R A, S B. Green synthesis of zinc oxide nanoparticles using Hyptis leaf extract and Activated carbon based Zinc oxide composite of Supercapacitor Applications. Indian Journal of Science and Technology. 2019;12(40):107-108.
17. Modi S, Fulekar MH. Synthesis and characterization of zinc oxide nanoparticles and zinc oxide/cellulose nanocrystals nanocomposite for photocatalytic degradation of Methylene blue dye under solar light irradiation. Nanotechnology for Environmental Engineering. 2020;5(2).
18. Subramaniam VD, Prasad SV, Banerjee A, Gopinath M, Murugesan R, Marotta F, et al. Health hazards of nanoparticles: understanding the toxicity mechanism of nanosized ZnO in cosmetic products. Drug and Chemical Toxicology. 2018;42(1):84-93.
19. Singh S. Zinc oxide nanoparticles impacts: cytotoxicity, genotoxicity, developmental toxicity, and neurotoxicity. Toxicology Mechanisms and Methods. 2019;29(4):300-311.
20. Czyżowska A, Barbasz A. A review: zinc oxide nanoparticles – friends or enemies? Int J Environ Health Res. 2020;32(4):885-901.
21. Fujihara J, Tongu M, Hashimoto H, Yamada T, Kimura-Kataoka K, Yasuda T, et al. Distribution and toxicity evaluation of ZnO dispersion nanoparticles in single intravenously exposed mice. The Journal of Medical Investigation. 2015;62(1.2):45-50.
22. de Souza JM, Mendes BdO, Guimarães ATB, Rodrigues ASdL, Chagas TQ, Rocha TL, et al. Zinc oxide nanoparticles in predicted environmentally relevant concentrations leading to behavioral impairments in male swiss mice. Sci Total Environ. 2018;613-614:653-662.
23. Esmaeillou M, Moharamnejad M, Hsankhani R, Tehrani AA, Maadi H. Toxicity of ZnO nanoparticles in healthy adult mice. Environmental Toxicology and Pharmacology. 2013;35(1):67-71.
24. Srivastav AK, Kumar M, Ansari NG, Jain AK, Shankar J, Arjaria N, et al. A comprehensive toxicity study of zinc oxide nanoparticles versus their bulk in Wistar rats. Human & Experimental Toxicology. 2016;35(12):1286-1304.