

Plant-Based Fabrication of Zinc oxide and Copper Nanoparticles Using Shorea robusta Resin: Dual Evaluation of Safety and Antimicrobial Efficacy

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Abstract

In this study, a green, sustainable, and cost-effective approach was employed for the synthesis of zinc oxide (ZnO) and copper (Cu) nanoparticles using *Shorea robusta* resin extract as a natural agent for reducing, capping, and stabilizing. The synthesis of metal nanoparticles utilizing *S. robusta* resin is being reported for the first time. The synthesized nanoparticles were characterized using Ultraviolet–Visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FT–IR), and Nanoparticle Tracking Analysis (NTA). The phyto-synthesized nanoparticles exhibited significant antibacterial and antifungal activity, particularly against *Pseudomonas stutzeri* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and the pathogenic fungi *Candida albicans* and *Aspergillus flavus*. Additionally, both ZnO and Cu nanoparticles demonstrated excellent hemocompatibility and cytocompatibility, highlighting their potential for pharmacological and therapeutic applications.

1. Introduction

Nanoscience and nanotechnology work toward enhancing the optical, electrical, magnetic, and catalytic functionalities of bulk materials to improve human lives[1]. Due to the damage caused by global warming, scientists worldwide are working hard to create new materials with unique features and minimal environmental hazard[2]. Nanomaterials are broadly classified into organic and inorganic nanoparticles, with metallic nanoparticles most commonly synthesized through chemical and physical methods[3]. Growing awareness of environmental degradation has prompted scientists to adopt greener, cost-effective, and non-toxic methods for synthesizing and fabricating nanomaterials[1]. Nanoparticle synthesis using plants is often favoured over microbial methods due to its simplicity, ease of scaling up, the vast diversity of plant species, and the abundance of phytochemicals they contain, such as alkaloids, steroids, terpenoids, tannins, saponins, polyphenols, and phenolic acids[4, 5]. They function as capping or stabilizing agents in addition to reducing agents[6–8].

Typically, metal nanoparticles possess distinct physical, chemical, and biological properties compared to their bulk forms, largely due to their exceptionally high surface-to-volume ratio[9, 10]. It has been proposed that the phyto-synthesis of nanoparticles is attributed to phenolic chemicals[11, 12]. Due to their antioxidant properties, phenolic compounds can be used as stabilizing and reducing agents while creating nanomaterials[13, 14]. Bioactive substances, viz., alkaloids, glycosides, phenols, tannins, steroids, and terpenoids that contribute to strong antibacterial properties, were found in very high concentrations during phytochemical screening of the oleoresin of *Shorea robusta*[15].

Zeghoud et al. claim that the plant species used for green synthesis has a minimal influence on the yield and morphology of ZnO nanoparticles, except when the extract lacks sufficient bioactive compounds. [16]. ZnO is the most widely used of them at the nanoscale due to its remarkable scientific characteristics, including high excitonic binding energy and band gap[17]. ZnO NPs are well-known among nanometal oxides for their antimicrobial, anti-inflammatory, and anticancer properties[18]. Moreover, ZnO is also classified as a "GRAS" (Generally Recognized as Safe) chemical by the US Food and Drug Administration (FDA)[19]. Copper is an abundant metal and serves as an essential trace element in most living organisms[20]. It has been acknowledged by the U.S. Environmental Protection Agency (EPA) as the first and sole metal officially

registered for its antimicrobial activity. [21]. When reduced to the nanoscale, copper exhibits unique physicochemical properties that make it highly versatile, with applications across various industries such as solar cells, wood preservatives, gas sensors, and high-temperature superconductors. Similarly, the catalytic, high electrical conductivity, optical, antifungal, and antibacterial properties of CuNPs (Copper nanoparticles) make them the most sought-after of the NPs [22].

This paper describes a green method for synthesising CuNPs and ZnO NPs using resin extract from *Shorea robusta*. All characterization results provided clear evidence of nanoparticle formation. Its antifungal and antibacterial properties against the pathogenic fungus *Aspergillus flavus* and *Candida albicans*, as well as *Staphylococcus aureus* and *Pseudomonas stutzeri* were evaluated. The Cu and ZnO nanoparticles were further evaluated for their biocompatibility, specifically focusing on hemocompatibility and cytocompatibility.

2. Materials and Methods

2.1. Materials

Shorea robusta (SR) resin was procured from DKC Agrotech Pvt. Ltd., India, and used for nanoparticle synthesis. Ethanol, zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$; QUALIGENS), and copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; CAS No. 7758-99-8; Sigma-Aldrich) were employed as precursor materials. Nutrient Agar, Nutrient Broth, and Potato Dextrose Agar (Himedia) were used for microbial culture and antimicrobial assays. The microbial strains utilized in this study included *Staphylococcus aureus* (MTCC 3160), *Pseudomonas stutzeri* (MTCC 863), *Candida albicans* (MTCC 183), and *Aspergillus flavus* (MTCC 277). All strains were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India.

The reagents and consumables used for the assays comprised sterile petriplates (TARSONS), 96-well microtiter plates, phosphate-buffered saline (PBS, pH 7.4), Triton X-100 (Sigma), Amphotericin B (100 units/mL), and Streptomycin (10 $\mu\text{g}/\text{mL}$). For cytotoxicity and bioactivity assessments, MTT dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Himedia), RPMI-1640 culture medium (Himedia), fetal bovine serum (FBS; Sigma), and red cell lysis buffer (RCLB; Sigma) were used. The ELISA readings were recorded using a BioTek 800 TS Absorbance Reader. DMSO (dimethyl sulfoxide; Sigma) was used as a solvent, and Histopaque-1077 (Sigma) was used for cell isolation and density gradient separation.

2.2. Characterization Techniques:

The UV–Visible absorption spectra were recorded using a Shimadzu UV-1900i double-beam spectrophotometer. FTIR spectra were obtained using a Nicolet iS50 FTIR spectrometer, from Thermo Scientific. The particle size distribution of the samples was determined through nanoparticle tracking analysis (NTA) using a NanoSight NS300 instrument (Malvern Panalytical, Netherlands) equipped with a 488 nm laser and a 500 nm long-pass filter. For NTA measurements, all samples were diluted to a concentration of 10^6 – 10^7 particles/mL. Each sample was analyzed in five replicates, and 30–60 s videos were recorded for each run, capturing at least 200 valid particle tracks per analysis. During all measurements, the light scatter mode (LSM) camera level was maintained between 12 and 14. Data acquisition and analysis were performed using Nanosight software (version 3.0) with standard parameters. The mean $\pm$ standard deviation values were calculated from three independent video recordings.

2.3. Preparation of ethanolic *Shorea robusta* resin extract:

An ethanolic extract of *Shorea robusta* resin was made by measuring 10 g and thoroughly crushing it with a mortar and pestle. Pure ethanol was used to dissolve the crushed resin, which was then filtered through Whatman 40 filter paper and utilized as the biogenic source to produce the nanoparticles.

2.4. Green synthesis of ZnO NPs:

In brief, 25 mL (0.5 M) zinc acetate dihydrate was mixed with 2.5 mL of *Shorea robusta* resin extract. The pH of the mixture was 6.13, and to maintain it at 8, 2 M NaOH was added drop by drop. It was then stirred and heated at 70°C for 30 minutes, resulting in complete reduction and the appearance of a white precipitate. The resultant material was then collected by decantation, cleaned of residues using distilled water, and oven-dried for an entire night at 70°C to produce powdered ZnO nanoparticles. For subsequent characterization and application, the material was stored at ambient temperature in an airtight container. [23].

2.5. Green Synthesis of CuNPs:

50 mL (5 mM) of copper sulphate solution was combined with 5 mL of *Shorea robusta* resin extract to create plant extract resin copper nanoparticles. A solution of NaOH (1N) was added to bring the pH down to 7. Furthermore, a green colour mixture solution was produced. After centrifugation, the pellet collected was dried overnight at 60 degrees in a hot air oven to produce powder. Powder was collected and stored at 4 degrees temperature for further use [22].

2.6 Agar well diffusion assay:

Nutrient agar media was poured into the plates in sterile conditions. In the appropriate conical flasks, nutrient broth was made for the cultures of *Pseudomonas stutzeri* (MTCC 3160) and *Staphylococcus aureus* (MTCC 863), which were then maintained in the shaker for a whole day. The cultures were spread throughout the nutrient agar medium using a glass spreader. For the controls and test compounds, Positive control: 10 mg antibiotic streptomycin, Negative control: pure ethanol, Test compounds: ZnO and Cu NPs with various stock concentrations 50, 25, 12.5, 6.25 mg/mL, were used, and the wells were punched using a gel punch. 50 µL of different concentrations of ZnO and CuNPs were loaded into the wells, and the plates were kept in a Biochemical Oxygen Demand (BOD) incubator for 24 hours. Using a vernier calliper, the diameter of the zone (in millimetres) was recorded. The inhibitory zone was calculated using the formula:

Zone of inhibition (mm) = Diameter of zone (mm) - Diameter of well (mm).

2.7. Anti-fungal agar well diffusion Assay:

We procured *Aspergillus flavus* (MTCC 277) and *Candida albicans* (MTCC 183) from the IMTECH Microbial Type Culture Collection in Chandigarh. One colony was inoculated in Potato Dextrose Broth (PDB), and was cultured for overnight at 37°C with continuous shaking at 150 rpm. After transferring 60 µL of the overnight culture into 3 mL of PDB, it was cultivated at 37°C (150 rpm) until the optical density at 600 nm reached the 0.5 McFarland standard. The 100 µL of *Candida albicans* culture from the previous stage was distributed onto Potato Dextrose Agar (PDA) using a sterile cotton swab. Following this, the PDA plates were incubated at 37°C for 48 hours. ZnO and Cu NPs doses ranging from 6.25 to 50 mg/mL were added to the wells in 50 µL

increments. Amphotericin B (100 units/disc) acted as a positive control, and the solvent as a negative control. The inhibition zone, or clear area around the disc, was measured with a vernier calliper to determine its diameter.

2.8. Hemocompatibility test:

Hemolysis assay was used to examine the cytotoxic effects of ZnO and Cu NPs [24]. The experiment was carried out under the applicable institutional policies and procedures, and informed consent was acquired. The Sharda University School of Medical Sciences and Research (SMS&R) Institutional Ethics Committee examined and accepted the study protocol, assigning approval reference number SU/SMS&R/76-A/2024/125. A healthy individual's freshly obtained blood was collected in an anticoagulant-containing tube, and centrifuged for five minutes at 3000 rpm after adding phosphate-buffered saline (PBS) (v/v; pH 7.2). The pellet was further washed with PBS at 3000 rpm. The RBCs obtained were further diluted with PBS. ZnO and Cu NPs were added to diluted erythrocyte suspensions at concentrations of 50, 75, 100, 250, 500, 750, and 1000 µg/mL, gently mixed, and then incubated for 4 hours at 37°C. Triton X-100 (10%) was used as the positive control, and PBS (normal) as the negative control. The samples were incubated and then centrifuged. The supernatant was then collected, and absorbance at 540 nm was measured using an ELISA reader (BioTek 800 TS). To calculate the percentage hemolysis, the formula was as follows:

Hemolysis (%) = [(Mean OD of sample - Mean OD of negative control) / (Mean OD of positive control - Mean OD of negative control) × 100].

2.9. Cytocompatibility test

In a heparinized vacutainer, human whole blood was drawn. 10 mL of whole blood was diluted with 10 mL of RPMI-1640 before being stacked on top of 12.5 mL Histopaque-1077 gradient in centrifuge tubes. Following centrifugation at 2000 rpm for 20 minutes, the peripheral blood mononuclear cells layer was re-suspended in RPMI-1640 with 10% FBS after being washed with PBS. Different doses of ZnO and Cu NPs were used in the MTT test on peripheral blood mononuclear cells (PBMCs). Cells were seeded at 0.5×10^5 cells/mL density in a 96-well plate. After being treated for 24 hours with varying concentrations of (50, 100, 200, 300 µg/mL) of ZnO and Cu NPs, the cells were incubated for 3–4 hours at 37°C in media containing 5 mg/mL of MTT. After dissolving the resultant formazan crystals in DMSO, the absorbances were measured at 570 nm.

Cell Viability% = [(Absorbance of treated sample - Absorbance of blank) / (Absorbance of untreated - Absorbance of blank)] × 100.

3. Results and Discussion

3.1 ZnO and Cu NP Nanoparticle Characterizations

The UV–Vis absorption spectrum of the prepared ZnO nanoparticles (Fig. 1a) exhibits a pronounced absorption edge/peak centred at ~ 366 nm. This absorption corresponds to a direct band-to-band transition; using $E_g \text{ (eV)} = 1240 / \lambda \text{ (nm)}$ gives an optical band gap of ~ 3.39 eV. This value is in very good agreement with the expected band gap of wurtzite ZnO (~ 3.3–3.4 eV) and indicates that the material is phase-pure ZnO with no large amounts of other semiconducting impurities [25]. The absorption peak at 366 nm is slightly

blue-shifted (shorter wavelength) compared with bulk ZnO values reported near $\sim 368\text{--}380\text{ nm}$, which can be ascribed to quantum-confinement effects for small nanoparticle sizes. The broad decrease in absorbance toward longer wavelengths and the weak tail extending into the visible region could be attributed to sub-band-gap absorption from defect states (e.g., oxygen vacancies) and to light scattering by nanoparticles in the suspension[26]. Formore accurate determination of the optical band gap, a Tauc plot analysis (Fig. 1b) for a direct band transition $(\alpha h\nu)^2$ vs $h\nu$ was performed and the linear extrapolation of the low-energy edge yielded $E_g \approx 3.39\text{ eV}$, consistent with the value calculated directly from the absorption edge. These observations corroborate the formation of nanoscale ZnO with electronic properties appropriate for UV-active photocatalytic and optoelectronic applications. The UV–Vis absorption spectrum of Cu-NPs is depicted in Fig. 1c. The spectrum features a band at $\approx 252\text{ nm}$, which confirms the successful synthesis of Cu NPs. These results corroborate with previously reported observations[22].

The FTIR spectrum of both the green-synthesized ZnO and Cu nanoparticles were found to be similar and (Fig. 1d) showed distinct absorption bands at 3295 cm^{-1} , 2117 cm^{-1} , and 1636 cm^{-1} . The broad and intense absorption band around 3295 cm^{-1} corresponds to the O–H stretching vibrations of hydroxyl groups and adsorbed water molecules present on the nanoparticle surface. This is a common feature in ZnO/Cu nanoparticles synthesized via aqueous or plant-extract-mediated routes, indicating the presence of surface –OH groups and hydrogen-bonded moisture[27]. The weak absorption observed near 2117 cm^{-1} can be attributed to $\text{C} \equiv \text{C}$ stretching or $\text{C} = \text{O}$ overtone/ C–N–C vibrations that arise from organic residues originating from plant phytochemicals [28]. Such features suggest partial retention of organic molecules that may act as capping or stabilizing agents during the green synthesis process. The peak appearing at 1636 cm^{-1} is assigned to the H–O–H bending vibration of molecular water and may also overlap with $\text{C} = \text{O}$ stretching vibrations of amide groups or carboxylate ions from biomolecules in the plant extract, further confirming the interaction between phytochemicals and metallic ions during Cu/ZnO nanoparticle formation [29]. The particle size distribution of the synthesized ZnO nanoparticles was analyzed using Nanoparticle Tracking Analysis (NTA), as shown in Fig. 2a. The NTA profile exhibits a prominent peak centred around $\sim 120\text{ nm}$, indicating that majority of the particles fall within this size range. A smaller shoulder observed near $70\text{--}80\text{ nm}$ suggests the presence of a minor population of smaller nanoparticles or aggregates. The concentration of nanoparticles at the main peak reaches approximately $3.2 \times 10^8\text{ particles/mL}$, reflecting a well-dispersed colloidal suspension with good stability. The relatively narrow distribution implies that the synthesis process produced uniformly sized ZnO nanoparticles with minimal aggregation, which is a desirable feature for optoelectronic and photocatalytic applications. Minor variations in particle size can be attributed to the presence of naturally formed agglomerates or residual capping molecules from the green synthesis process. The particle size distribution of the synthesized Cu nanoparticles was also analysed using NTA, as presented in Fig. 2b. The NTA profile reveals a bimodal distribution, indicating the presence of two predominant nanoparticle populations. The first major peak appears around $95\text{--}110\text{ nm}$, while a secondary peak is observed near $180\text{--}200\text{ nm}$. The total particle concentration reaches approximately $4 \times 10^8\text{ particles/mL}$, suggesting a high colloidal density of dispersed Cu nanoparticles. The presence of a bimodal distribution may arise from partial aggregation or variation in nucleation and growth rates during synthesis. The smaller size fraction corresponds to well-dispersed primary Cu nanoparticles, while the larger fraction likely represents agglomerated or fused particles formed due to the high surface energy of nanosized Cu. Overall, the observed size range confirms the nanoscale nature of the synthesized Cu nanoparticles, typically below

200 nm. The relatively sharp and well-defined peaks indicate that the colloidal suspension is reasonably monodisperse with moderate stability, suitable for optical and catalytic applications.

Representative screenshots from the Nanoparticle Tracking Analysis (NTA) video are shown in Fig. 2(c) and 2(d), displaying the Brownian motion of ZnO and Cu nanoparticles dispersed in aqueous medium, respectively. Each bright spot corresponds to an individual ZnO/Cu nanoparticle scattering laser light as it moves randomly due to collisions with solvent molecules. The intensity of scattered light varies with particle size—larger particles appear as brighter spots, while smaller ones exhibit weaker scattering. The observed random trajectories confirm that the nanoparticles are well-dispersed and remain colloidally stable without significant sedimentation or aggregation during measurement. This dynamic visualization, recorded by NTA, was used to calculate the hydrodynamic diameter distribution shown in Fig. 2(a) and 2 (b). The clear and distinct scattering points further validate the nanoscale dimensions and optical activity of the synthesized ZnO and Cu NPs.

3.2. Anti-bacterial activity:

The antibacterial activity of synthesized ZnO and Cu nanoparticles was evaluated against selected pathogens, namely *Staphylococcus aureus* and *Pseudomonas stutzeri*, using the disc diffusion method. The diameters of the zones of inhibition were measured in millimeters and are presented in Table 1 and Figs. 2a–d and 3a–d. In the disc diffusion assay, the synthesized ZnO nanoparticles exhibited significant antibacterial activity against all tested bacterial strains. The results clearly demonstrated that antibacterial activity, expressed as the zone of inhibition, increased with rising concentrations of ZnO nanoparticles (6.25, 12.5, 25, and 50 mg/mL). This effect may be attributed to the higher production of H_2O_2 and the generation of reactive oxygen species (ROS) from the nanoparticle surface at high concentrations.

Table 1

Anti-microbial activity of Zinc oxide and Copper nanoparticles synthesized from *Shorea robusta* resin extracts

Micro organisms	Zinc Oxide Nanoparticles				Copper Nanoparticles				Reference Standards	
	50	25	12.5	6.25	50	25	12.5	6.25	Streptomycin 10 mcg	Amphotericin-B
Concentration (mg/mL)										
Gram-positive bacteria	Zone (in mm)									
<i>Staphylococcus aureus</i>	13	11	11	9	13	12	11	11	29	-
Gram-negative bacteria										
<i>Pseudomonas stutzeri</i>	20	15	16	15	17	16	12	11	27	-
Yeast										
<i>Candida albicans</i>	19	18	17	16	13	13	11	11	-	23
Fungi										
<i>Aspergillus flavus</i>	29	27	20	19	13	12	11	11	-	4

Among the tested pathogens, *Pseudomonas stutzeri* showed the largest zone of inhibition (20 mm), whereas *Staphylococcus aureus* exhibited a comparatively smaller zone (12 mm), as shown in Table 1 and Figs. 2a–d. These findings are consistent with previous studies. For instance, Chinnammal Janaki et al. (2015) reported antibacterial activity of ZnO nanoparticles with inhibition zones of 10 mm against *Staphylococcus aureus*, 10 mm against *Candida albicans*, and 12 mm against *Penicillium notatum*[30].

Cu nanoparticles also exhibited notable antibacterial activity, with a stronger effect against *Pseudomonas stutzeri* (17 mm) compared to *Staphylococcus aureus* (13 mm), as shown in Table 1 and Figs. 3a–d. The antibacterial action of Cu NPs is primarily attributed to electrostatic interactions with the cell walls of gram-negative bacteria. Their strong affinity for carboxyl and amine groups which are key components of bacterial cell walls further enhances their antimicrobial potential [31].

At the nanoscale, Cu particles exert antibacterial effects through multiple mechanisms. These include adhesion of NPs to gram-negative bacterial cell walls due to electrostatic attraction, disruption of cell membrane proteins, denaturation of intracellular proteins, and interactions with phosphorus- and sulphur-containing biomolecules such as DNA[32]. In a comprehensive study, Chatterjee et al. used *E. coli* as a model system to investigate these mechanisms. They reported that treatment with the minimum bactericidal concentration (MBC) dose of CuNPs induced a 2.5-fold increase in intracellular reactive oxygen species (ROS). This ROS overproduction triggered lipid peroxidation, protein oxidation, and DNA degradation, ultimately leading to bacterial cell death[33].

3.3. Anti-fungal activity:

The antifungal activity of synthesized ZnO nanoparticles was evaluated against the selected fungal pathogens *Aspergillus flavus* and *Candida albicans* using the disc diffusion method. The diameters of the zones of inhibition (in millimeters) are presented in Table 1 and Figs. 3, 4 (e–h). The synthesized ZnO nanoparticles exhibited significant antifungal activity against both fungal strains. Similar to the antibacterial results, the antifungal activity increased with increasing concentrations of ZnO nanoparticles (6.25, 12.5, 25, and 50 mg/mL). Notably, *A. flavus* was more sensitive, showing the highest zone of inhibition (29 mm), while *C. albicans* exhibited a smaller zone (12 mm). By contrast, Cu nanoparticles displayed only moderate antifungal action, with inhibition zones of 13 mm for both fungal species.

3.4. Bio-compatibility:

In the present study, ZnO and Cu nanoparticles synthesized using *Shorea robusta* resin extract were employed for hemolysis testing. Hemolysis results from either direct or indirect damage to the red blood cell (RBC) membrane and is widely accepted as a reliable marker of biological incompatibility. One of the standard methods to evaluate whether a biomaterial is safe for blood-contacting applications is to measure its hemolytic activity. RBCs undergo lysis when their membranes are disrupted, releasing hemoglobin into the medium[34]. Among the various cell types that can be utilized for assessing nanoparticle-induced toxicity[35], RBCs remain the most suitable, as nanoparticles, regardless of origin, intended use, or route of administration, eventually reach the blood circulation and react with them, which are the predominant cellular constituent of blood circulation. This interaction often compromises RBC functionality. Since RBCs are structurally well studied, readily available, and simple to handle, they serve as excellent model cells for nanotoxicity studies. Several investigations have examined the impact of nanoparticles on RBCs, particularly their hemolytic activity, highlighting its importance as a key test for nanoparticle safety evaluation. However, comparison across different studies is often challenging due to variations in nanoparticle characterization methods and hemolysis testing protocols [36]. To address this, the American Society for Testing and Materials (ASTM) issued in 2008 a standardized procedure for evaluating hemolytic properties of nanoparticles [37], which measures the hemoglobin content released following interaction between nanoparticles and RBCs.

In our experiments, hemolysis assays were carried out at nanoparticle concentrations of 50, 75, 100, 250, 500, 750, and 1000 µg/mL. The results demonstrated minimal hemolysis, with the maximum observed at less than 1% for ZnO NPs and 2% for Cu NPs at the highest concentration tested (1000 µg/mL) (Figure. 5). These findings fall well below the 5% threshold defined by ASTM for hemocompatibility, thereby confirming that the synthesized nanoparticles are non-hemolytic. Overall, the results indicate that green-synthesized ZnO and Cu nanoparticles from *Shorea robusta* resin extract exhibit excellent blood compatibility, supporting their potential for future in vivo applications in drug delivery and other biomedical uses.

Primary lymphocytes serve as an effective model for evaluating genotoxic effects, as they exhibit normal cellular responses unlike cancer or transformed cells, and are more likely to encounter nanoparticles in daily life[38]. The biosynthesized ZnO NPs and Cu NPs were tested for their cytotoxic effects on lymphocytes derived from PBMCs using the MTT assay. The findings in Fig. 6 demonstrate that ZnO NPs and Cu NPs had cytotoxic effects on blood cells that were dose-dependent. Overall, ZnO NPs showed less cytotoxicity

compared to CuNPs at the highest concentrations (300 µg/mL). Existing reports on the effects of ZnO NPs on human cells remain inconsistent; while some studies highlight their potential as strong anticancer agents, others describe them as non-toxic, supporting their application in consumer products[38]. Some findings suggest that ZnO nanoparticles exert low toxicity toward normal immune cells while maintaining significant anticancer activity, supporting their potential as a promising nanotherapeutic agent. ZnO nanoparticles demonstrate selective biological effects, exhibiting minimal cytotoxicity in normal peripheral blood mononuclear cells (PBMCs) even at concentrations as high as 300 µg/mL, thereby underscoring their relative biocompatibility with non-transformed cells[39, 40]. Zivari Fard et al. reported concentration-dependent cytotoxicity of CuO nanoparticles in human PBMCs assessed by MTT after exposure to 1–200 µg/mL, with viability decreasing progressively at higher concentrations [41].

4. Conclusion

The present study demonstrated that ZnO and Cu nanoparticles synthesized using *Shorea robusta* resin extract possess remarkable antimicrobial properties and excellent biocompatibility. Both types of nanoparticles exhibited concentration-dependent antibacterial and antifungal activity, with ZnO NPs showing larger inhibition zones, particularly against *Pseudomonas stutzeri* and *Aspergillus flavus*. Cu NPs also displayed strong activity, mainly through electrostatic interactions and ROS-mediated mechanisms, though their effects were comparatively moderate against fungal pathogens. Importantly, hemolysis assays confirmed that the synthesized nanoparticles were non-hemolytic even at high concentrations, remaining well below the ASTM threshold of 5%. This highlights their blood compatibility and supports their safety for biomedical applications. Furthermore, MTT assays indicated that ZnO NPs were less cytotoxic to blood cells than Cu NPs, suggesting a more favourable safety profile. Overall, these findings indicate that green-synthesized ZnO and Cu nanoparticles not only exhibit potent antimicrobial and antifungal activity but also maintain good hemocompatibility, making them prospective candidates for future applications in the delivery of drugs, infection control, and other biomedical fields.

Future research could explore incorporating biogenic ZnO and Cu nanoparticles derived from *Shorea robusta* resin into advanced biomedical systems such as implant coatings, wound dressings, and controlled drug delivery platforms. Further studies on their long-term stability, in vivo performance, and molecular mechanisms of anti-microbial action would help strengthen their translational potential. Moreover, optimizing synthesis conditions and surface modification strategies could enhance their selectivity and overall performance, paving the way for the development of sustainable and therapeutically relevant nanomaterials.

Declarations

Conflicts of Interest

The authors have declared no conflict of interest.

Ethics Declaration

This study involving humans was approved by the Sharda University School of Medical Sciences and Research (SMS&R) Institutional Ethics Committee (SU/SMS&R/76-A/2024/125). All participants provided written informed consent to participate in this study.

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Author Contribution

Shuaib Burgee: Designed and performed the experiments, methodology, data collection, analysis, original draft, and writing.V. Barghavi: Designed the experiments, data collection, calculations, original draft, writing, and formal analysis.Swati Bhati: Designed the experiments and figures.S. Shankara Narayanan: Methodology, formal analysis, writing, review & editing.Soumi Sadhu: Conceptualization, Project Administration, Writing - Review & EditingAll authors have approved the final version of the manuscript.

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Data Availability

All data supporting the findings of this study have been included in the main text; further inquiries can be directed to the corresponding author.

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Figures

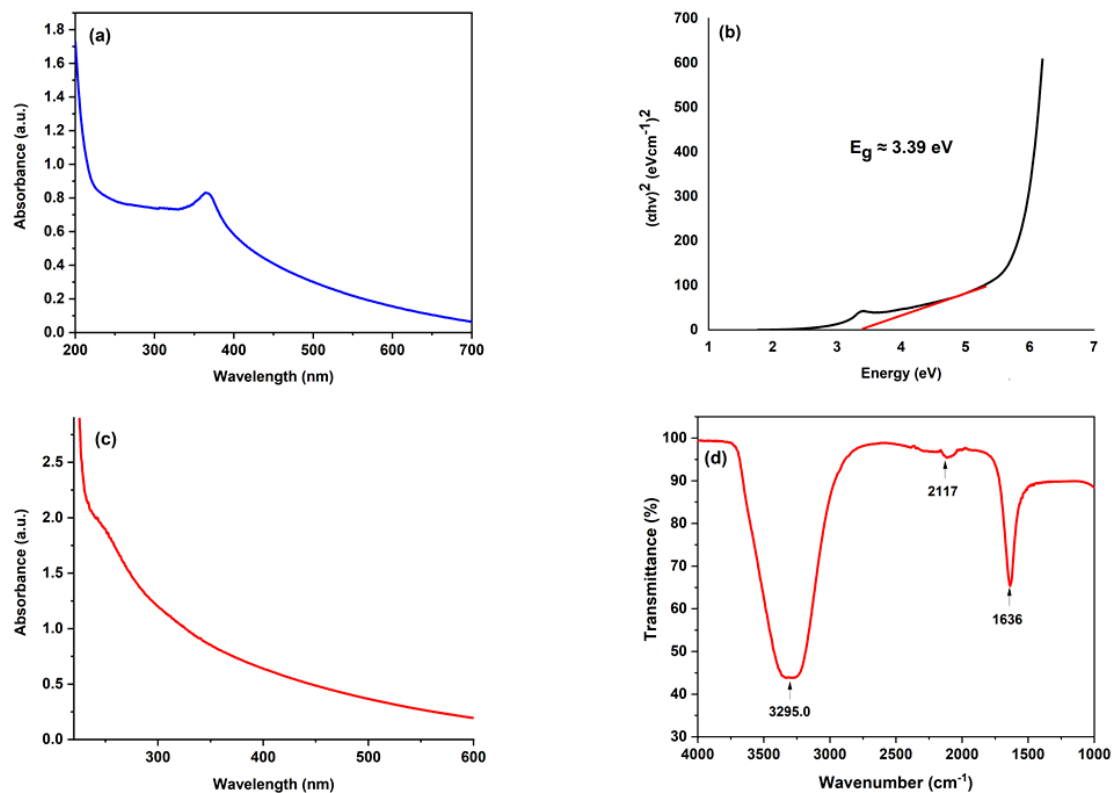


Figure 1

(a) UV–Vis absorption spectrum of green-synthesized ZnO NPs. (b) The corresponding Tauc Plot for calculating the optical direct band gap of ZnO NPs. (c) UV–Vis absorption spectrum of green-synthesized Cu NPs. (d) FTIR Spectrum of ZnO/Cu synthesized NPs.

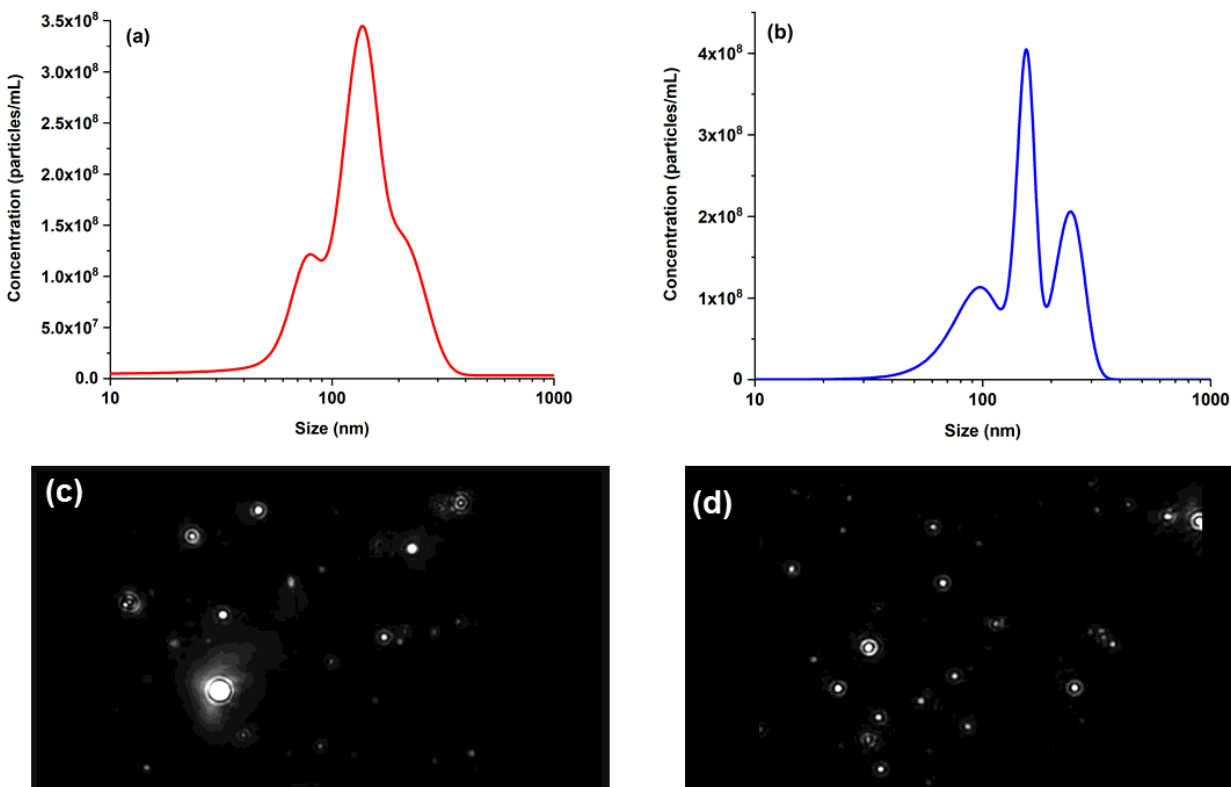


Figure 2

(a) Nanoparticle Tracking Analysis (NTA) profile of green-synthesized ZnO nanoparticles showing a dominant size distribution centered around ~ 120 nm with a particle concentration of approximately 3.2×10^8 particles/mL. (b) Nanoparticle Tracking Analysis (NTA) profile of Cu nanoparticles showing a bimodal size distribution with dominant peaks around 100 nm and 190 nm, and a total particle concentration of approximately 4×10^8 particles/mL. Screenshot from the Nanoparticle Tracking Analysis (NTA) video showing the Brownian motion of dispersed (c) ZnO nanoparticles and (d) Cu NPs in aqueous suspension. Each bright spot represents an individual nanoparticle scattering light as it moves randomly, confirming good dispersion and colloidal stability.

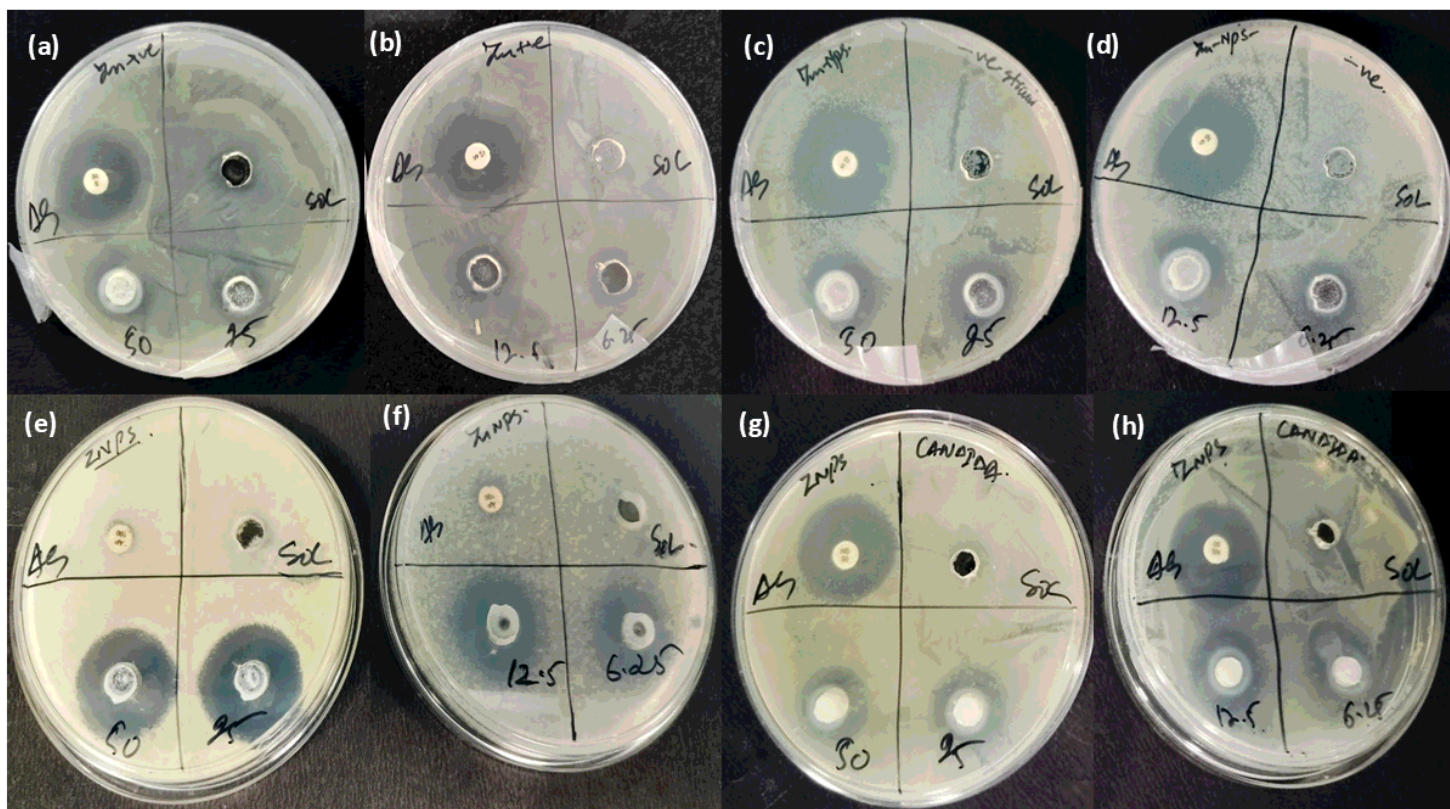


Figure 3

Antibacterial activity of ZnO nanoparticles against *Staphylococcus aureus* (a,b) and *Pseudomonas stutzeri*(c,d) at concentrations of 50, 25, 12.5, and 6.25 mg/mL, with streptomycin (10 µg) as control. Antifungal activity of ZnO nanoparticles against *Aspergillus flavus* (e,f) and *Candida albicans* (g,h) at the same concentrations, with amphotericin-B as control.

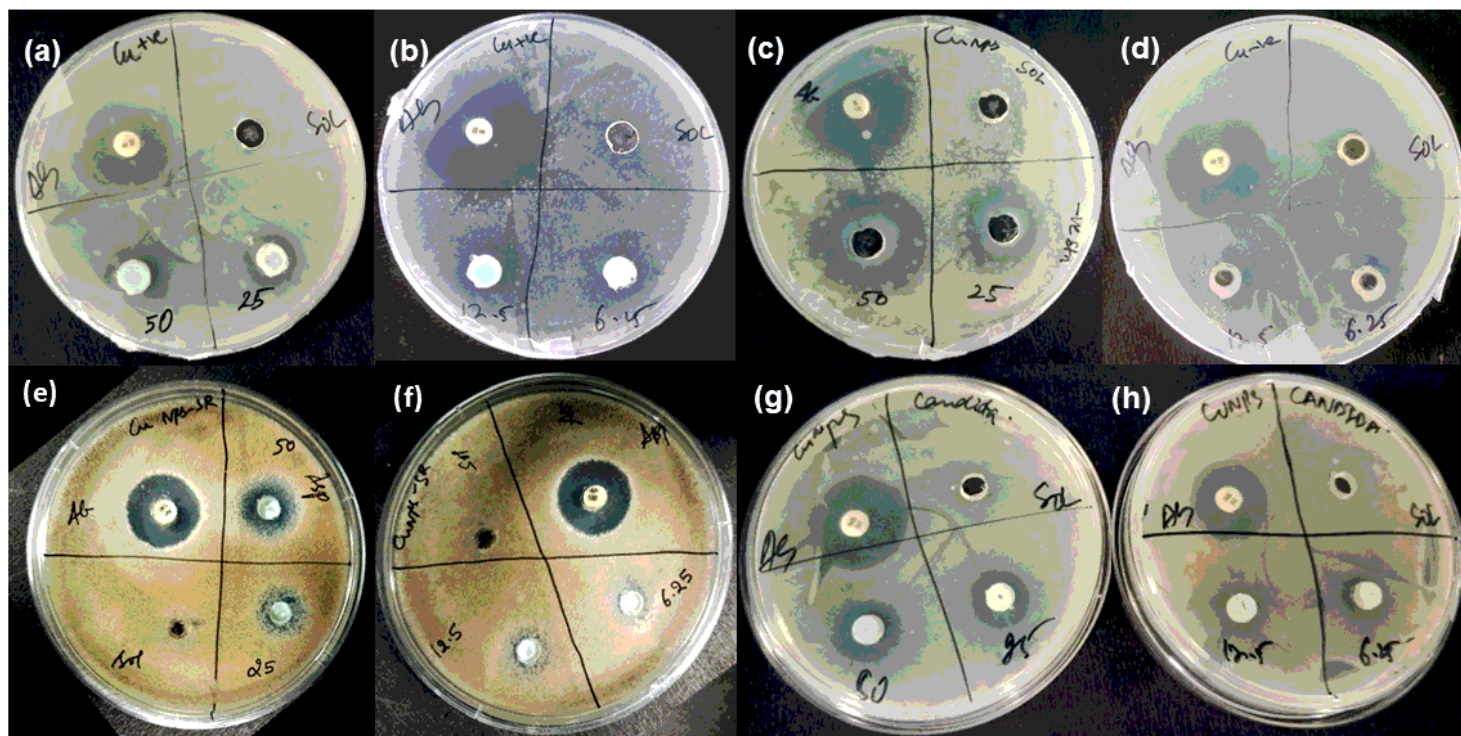


Figure 4

Antibacterial activity of Cu nanoparticles against *Staphylococcus aureus* (a,b) and *Pseudomonas stutzeri* (c,d) at concentrations of 50, 25, 12.5, and 6.25 mg/mL, with streptomycin (10 µg) as control. Antifungal activity of Cu nanoparticles against *Aspergillus flavus* (e,f) and *Candida albicans* (g,h) at the same concentrations, with amphotericin-B as control.

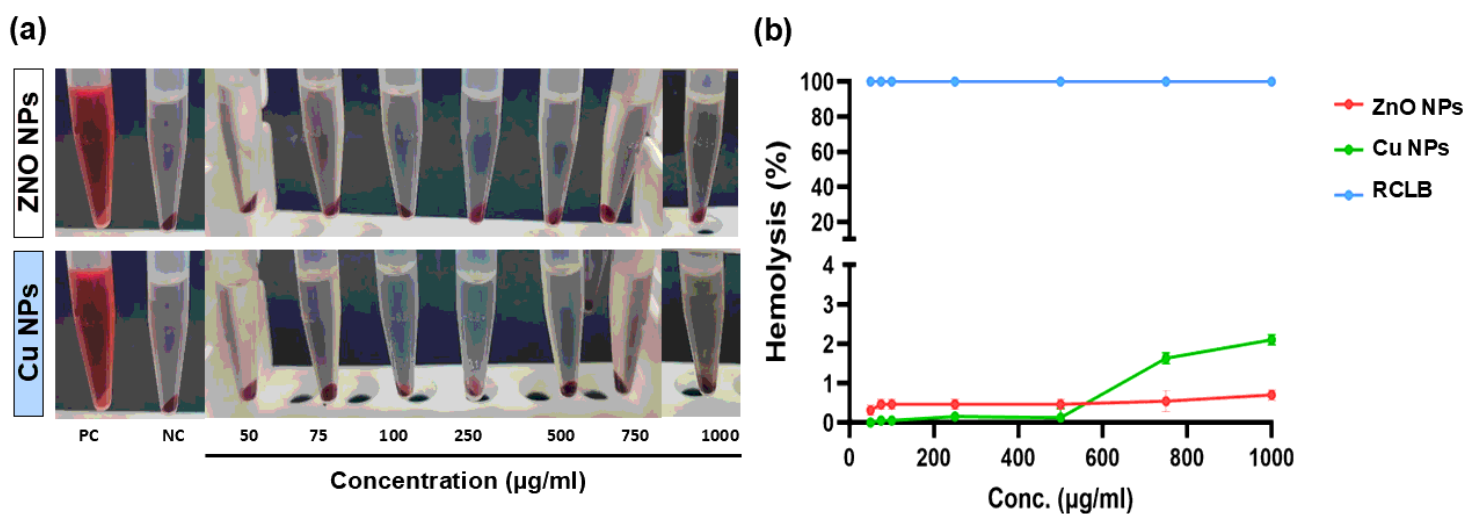


Figure 5

Hemolysis assay of ZnO and Cu nanoparticles showing minimal hemolysis. b. Cu NPs caused <2% hemolysis at 1000 $\mu\text{g/mL}$, while ZnO NPs showed only 0.5% hemolysis at the same concentration

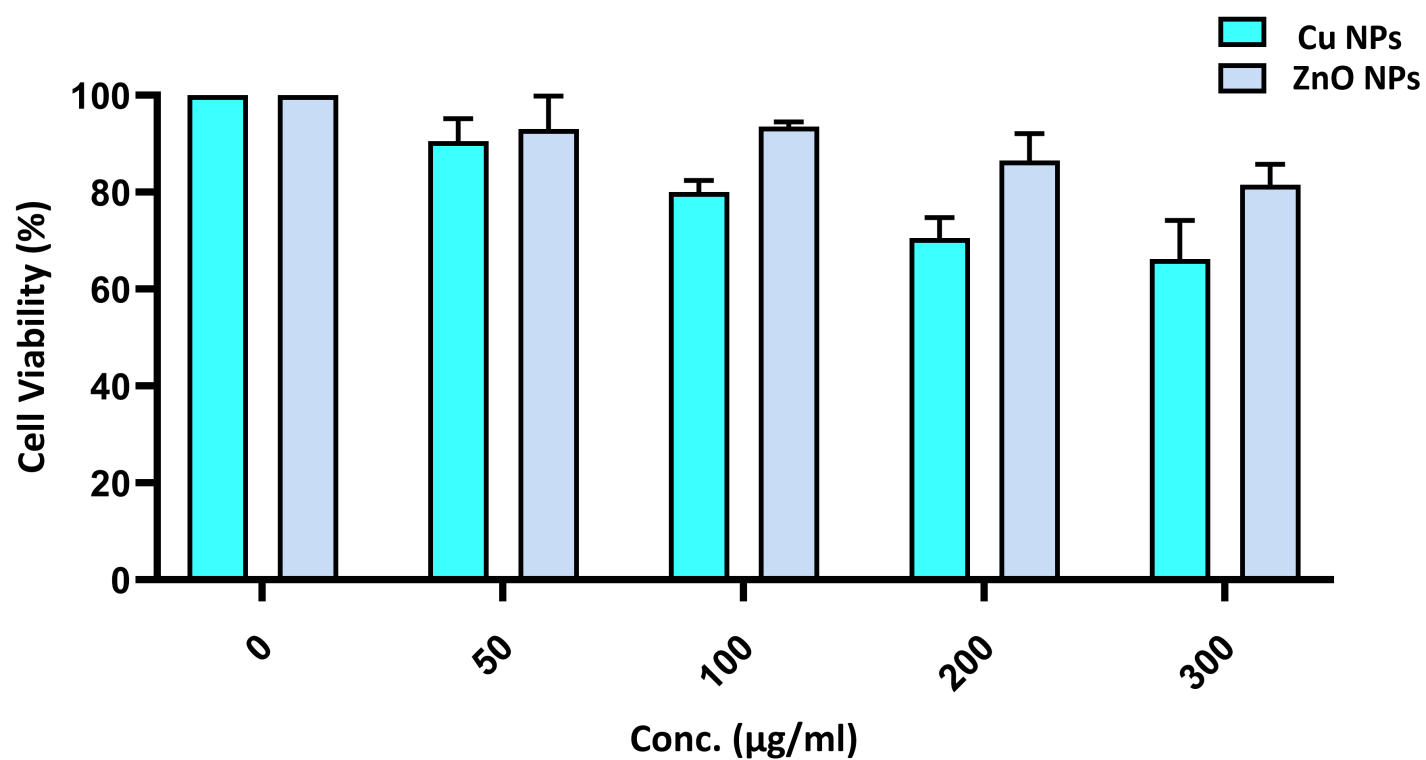


Figure 6

MTT assay showing lower cytotoxicity of ZnO nanoparticles compared to Cu nanoparticles in human white blood cells. At 300 µg/mL, ZnO NPs maintained ~80% cell viability, whereas Cu NPs reduced viability. Even at higher concentrations, ZnO NPs consistently exhibited higher cell viability than Cu NPs.

Supplementary Files

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