

# Evaluation the antibacterial action of polymer blends filled with ZnONPs for industrial applications

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## ABSTRACT

Composite materials with potential applications were formed by reinforcing polymeric blends containing 70% unsaturated polyester (UP) and 30% natural rubber (NR) with zinc oxide nanoparticles (ZnONPs) at weight fractions of (0, 1, 1.5, 2, 2.5, and 3%). The morphology and crystalline structure of ZnONPs were examined by using scanning electron microscopy (SEM) and powder X-ray diffraction (XRD) techniques. In addition, the ZnONPs sample exhibits a remarkably crystalline and wurtzite crystal structure, as indicated by the X-ray diffraction results. The antibacterial activity of the synthesized nanocomposites were evaluated against two types of bacterial growth, namely *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, with the aim of assessing their Potential applications. The experimental findings demonstrated that ZnONPs nanocomposites showed antibacterial activity against *E. coli* and *S. aureus* bacteria. The cytotoxic assessment of ZnONPs on healthy white blood cells (WBCs) was tested. The results indicated that ZnONPs do not show any toxic effects on normal white blood cells. Finally, cytological criteria, such as the blastogenic index (BI), mitotic index (MI), and total chromosomal abnormalities (TCA) were used to evaluate the genotoxic potential of ZnONPs against peripheral blood lymphocytes (PBLs). In addition, the toxicity of metal nanoparticles on various mammalian cells has been assessed.

## 1. Introduction

Composites may have superior thermal, optical, mechanical, and other properties compared to pure polymers [1]. Many researchers have interested study of composites due to their potential wide-ranging implications than polymers [2-4]. Composites are two or more different kinds of materials (like polymer, ceramic, metal, etc.) taken together to produce a new material with qualities combining constituents. Composites are materials composed of strong load-bearing material (known as

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reinforcement) embedded in weaker material (known as matrix). Reinforcement provides strength and stiffness, which helps to support structural loads. [5]. The matrix guarantees the unity and alignment of the load. Additionally, it is feasible to transfer the stresses experienced by the composite material to the load. The materials created in this manner exhibit significant heterogeneity and frequently display anisotropic properties [6].

Homopolymers and copolymers may not satisfy all practical specifications in certain domains.

Polymer blending, which combines two or more polymers or copolymers without strong chemical interactions, has become a viable approach for producing desired polymeric features. The blending process of polymers or copolymers is a more cost-effective method for the development of novel polymeric materials than chemical processes, and it also provides the desired features. Polymer blends are widely used in various industries such as automotive, electronics, packaging, and construction [7]. The physical properties of the blend differ from those of the parent polymers, depending on the ratios of the constituent components. There are two sorts of blends including miscible (homogeneous) and immiscible (heterogeneous). Miscible mixes are single-phase with a single glass transition temperature determined by the corresponding units. The most prevalent blends are immiscible blends. These blends have separation phases because of high interfacial tension and two or more unique glass transition temperatures [8]. Zinc oxide (ZnO) with a semiconductor photocatalyst, is commonly employed as a diagnostic disinfectant. The basic features of this substance are influenced by its specific surface area and the number of sites on its surface where interactions with absorbed molecules occur [9]. In addition to the ability of these nanoparticles to penetrate and damage bacterial membranes, they also include the release of  $Zn^{+2}$  ions. The growing concerns surrounding bacterial growth [3] have significant implications in various sectors of modern society, including the textile, water purification, food packaging, and biomedical industries. [10]. The search for novel anti-infective drugs is intensifying in response to the escalating threat posed by antibiotic-resistant microbes. In healthcare facilities, patient infections frequently arise from bacteria that exhibit resistance to multiple antibiotics, commonly referred to as multidrug-resistant (MDR) pathogens. There are two types of bacteria: Gram-positive and Gram-negative. There is a urgent demand for innovative, cost-effective, and highly effective antimicrobial therapies to effectively control bacterial activity [11-15].

Some studies have found that some nano oxides like CaO, MgO, and ZnO have significant antibacterial activity due to the formation of reactive oxygen species (ROS) on their surfaces. An advantage of utilizing these inorganic oxides as antibacterial agents is that they consist of environmentally favorable mineral components that are essential for human health and exhibit potent action even at low doses. The activity is quantified by examining the growth medium caused by bacterial metabolism [16]. Many studies have investigated the effect of nanoparticles on antibacterial activity for industrial applications. Douglas and colleagues have examined acceleration ageing affected the chemical, mechanical, and bactericidal properties of zinc oxide reinforced styrene-ethylene/butylene-styrene. They found that ZnO-loaded TPE composites retained their mechanical properties during thermal ageing [17].

To increase ZnONPs importance toward antibacterial properties, Khalfa et al. have precipitated zinc oxide nanoparticles (ZnO) from  $Zn(NO_3)_2 \cdot 4H_2O$  and studied chloramphenicol adsorption on ZnONPs. Zinc oxide nanoparticles coated with chloramphenicol (100 mg/mL) inhibited *S. aureus* and *Acinetobacter* better. The most resistant bacteria in this test was *K. pneumonia* which did not react with zinc oxide nanoparticles at any concentration [18].

Kindnew and colleagues created ZnO-filled polyvinyl alcohol (ZnO/PVA) nanocomposites from oleifera leaf extract and studied their electrochemical and antibacterial properties against gram negative *E. coli* and gram positive *S. aureus* bacteria. They demonstrated that loading ZnO enhanced the anti-bacterial efficacy of ZnO/PVA NCs against the above types of bacteria [19]. Rafael et al. synthesized (ZnO) nanoparticles using plant species as main resource. *Dysphania ambrosioides* extract was employed to synthesize hexagonal prism-shaped ZnO-NPs with average

diameters of 7nm to 130nm at 200, 400, 600, and 800°C. They examined how particle size affects minimum bactericidal concentrations, antibacterial testing, and disk diffusion. They found that smaller of ZnONPs (4–10 nm) eliminated strain better [20]. The mechanical properties and antimicrobial activity of a composite material of polymethyl methacrylate (PMMA) reinforced with ZnONPs were investigated by Khlifi et al. in relation to *Escherichia coli* (ATCC 25,922) and *Staphylococcus aureus* ATCC 29,213. They confirmed that this composite exhibited superior mechanical properties and demonstrated exceptional resistance to antimicrobial activity against these two categories of bacteria [21]. Zhao et al. made synergistic antibacterial composites by melt blending low-density polyethylene (LDPE) reinforced zinc oxide nanoparticles (ZnONPs) with quaternary ammonium compound 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride (QAS) with siloxane group. Antibacterial activity of the PE/ZnO-QAS composites was 99.9% against *Escherichia coli* (*E. coli*) and 99.75% against *Staphylococcus aureus* (*S. aureus*). [22].

The essential aim of this study is to examine the effects of ZnONPs on the antibacterial activities of blends including unsaturated polyester and natural rubber at various weight fractions. Many researchers studied the activity of bacterial growth using polymeric composites with zinc oxide, while in this study, polyester and rubber were employed as a base material in an 70:30 ratio to create a high shock resistance that can be used in the containers.

## 2. Materials and methods

Saudi Industrial Resins Limited Company (SIR) <sup>TM</sup> supplies unsaturated polyester (62143090) and natural rubber (40129049). Zinc oxide nanoparticles (Z713) with particle size 20–30 nm were offered by Hong International Group Ltd., China. (density: 5.06 g/cm<sup>3</sup>, purity: 99.8% and melting point: 1975 °C).

The two standard isolates of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were obtained from the laboratories of Environment Research Center/University of Technology/Baghdad/Iraq for studying the effect of ZnONPs on their viability and activity *In vitro*. These two isolates were grown on Nutrient Agar medium.

### 2.1 Preparation of nanocomposites

To create a polymer blend, UP and NR were blended in a 70:30 ratio. Using a glass rod, ZnONPs were mixed with the blend in weight fractions of 1, 1.5, 2, 2.5, and 3%. A magnetic stirrer was also utilized for 30 seconds to improve the mixing homogeneity of nanomaterial blends. The hardener was added into the composite for 2 minutes before being cast on a galvanized metal plate. Finally, all castings were allowed for two days for primary solidification before being removed from the mold and placed in a dryer for curing and stress reduction.

### 2.2 Antibacterial activity assay

The disc diffusion method (DDM) has been utilized in order to study the effect of ZnONPs on the survival of Gram-negative *E. coli* (ATCC 25922) and Gram-positive *S. aureus* (ATCC 25923), through the steps shown below [23].

- Activation of bacteria cells by culturing them in Brain Infusion Broth for 24 hours at a temperature of 37 °C with shaking at 300 RPM.
- Cultivation of activated bacteria cells on Muller Hinton medium intended to investigate the antibacterial properties of these cells. Subsequently, the discs measured with 6 mm in diameter were positioned, and accommodated with the necessary concentrations of zinc nanoparticles at equal distances between them. After being kept at room temperature, the dishes were subsequently transferred to an incubator set at a temperature of 37°C for duration of 24 hours. Subsequently, the

inhibition zone surrounding the discs was measured using a ruler. The inhibition rate was then calculated using the following equation [13].

$$\text{Inhibition rate \%} = (1 - x - \text{min}) / ((\text{max} - \text{min})) \times 100\% \quad (1)$$

Where X= diameter of the colony at any concentration

Min = zone of smallest inhibitions

Max= zone of biggest inhibitions

### 2.3 MTT Cytotoxic Assay

The effect of cytotoxicity of ZnONPs on healthy white blood cells (WBCs) was assessed. A volume of 10 milliliters of venous whole blood was obtained from a 35-year-old male who is in good health, utilizing a sterile heparinized syringe. Next, the blood sample was conducted to centrifugation at a speed of 5000 (RPM) for 10 minutes. After the removal of plasma, the buffy coat of white blood cells was meticulously collected using a sterile glass Pasteur pipette. The acquired white blood cells were transferred into a 5 ml test tube containing sterilized RPMI-1640 medium and 15% FCS. The cytotoxic effects of synthesized nanoparticles on white blood cells were assessed by preparing a final concentration of  $10^4$  cells/mL. The study employed RPMI-1640 culture media to investigate the cytotoxicity of white blood cells. For the experiment, a 96-well flat-bottom microliter plate was utilized.

A volume of 100  $\mu$ L of a white blood cell (WBC) solution with a density of  $10^4$  cells/mL was seeded to each well. Various concentrations of ZnONPs were added to each well. The culture plate was incubated at 37°C for 24 hours. Following a period of approximately 3-5 minutes of gentle circular agitation, any surplus medium was carefully removed from the plate. The first line served as the negative control. The wells were washed three times with phosphate-buffered saline (PBS) to eliminate any remaining nanoparticles and non-adherent cells after discarding the growth media and unattached cells. Subsequently, 10 microliters of MTT solution were introduced into each well, yielding a final concentration of 0.5 milligrams per milliliter. The process of PBS washing was repeated until all excess dye will be eliminated from the cells. The plate underwent a drying process. The absorbance was measured at 500 nm using an ELISA plate spectrophotometer.

### 2.4 Clonogenic evaluation

To investigate the genotoxic effects of ZnONPs at different concentrations, pe-stimulated peripheral blood lymphocytes (PBLs) were incubated with 10 g/mL phytohemagglutinine (PHA) for 72 hours at 37 oC and 5% CO<sub>2</sub> ambient concentration. Using a sterile syringe covered in heparin, whole blood was drawn from a healthy 25-year-old man. 0.5 mL of blood was added to 4.5 mL of finished RPMI-1640 culture medium, which also contained 10% fetal bovine serum and a penicillin-and-streptomycin antibiotic cocktail. The cells were incubated for 20 minutes with 10 g/mL of colchicine before being given another 20 minutes of treatment with hypotonic KCl 0.075M. The pellet were recieved three washings with a fixative solution (3:1 methanol to glacial acetic acid) after a five-minute centrifugation at 3000 rpm. The extra fluid was removed. After the translucent cell suspension was left to air dry on pristine, cold slides for an entire night, Giemsa stain was used. At various dosages of the three botanical extracts, chromosomal abnormalities, the mitotic index, and blastogenic index were assessed in exposed and non-exposed cells [24].

### 2.5 Statistical analysis

The data groups were analyzed statistically by (SPSS, Version 22 software). One-way analysis of variance was utilized to calculate the valuable differences at ( $P < 0.05$ ) by the use of the least significant differences (LSD). All data was expressed as (Mean  $\pm$  SD).

## 2.6 Characterization of ZnONPs

The X-ray diffraction patterns of ZnONPs were acquired using the Miniflex XRD instrument (B-XRD1127 ) manufactured by Rigaku, a company Tokyo\ Japan.

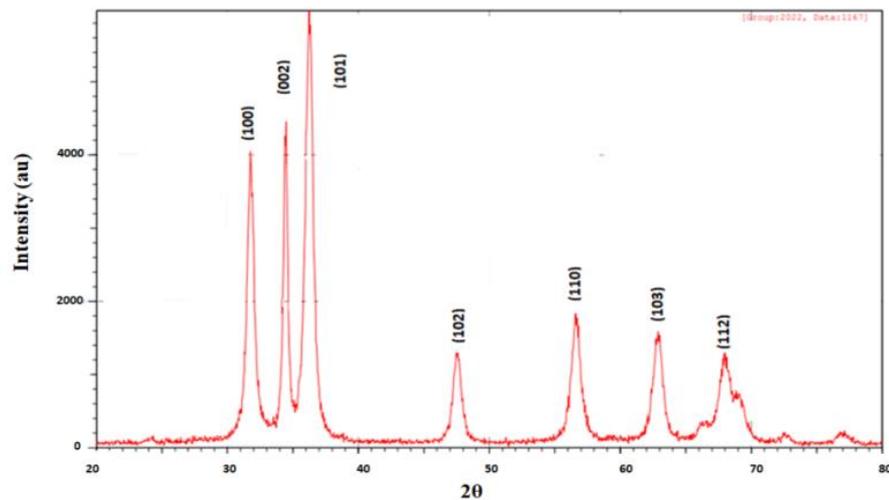
The morphology of ZnONPs was used utilizing a scanning electron microscope (SEM) with 5 kV accelerating voltage S4800 field emission SEM (FE-SEM, Japan, Hitachi).

The morphology of the sample was analyzed utilizing a scanning electron microscope (SEM) with code (90129000) manufactured by FEI Company, United States.

## 3. Results and Discussion

### 3.1 XRD of ZnONPs

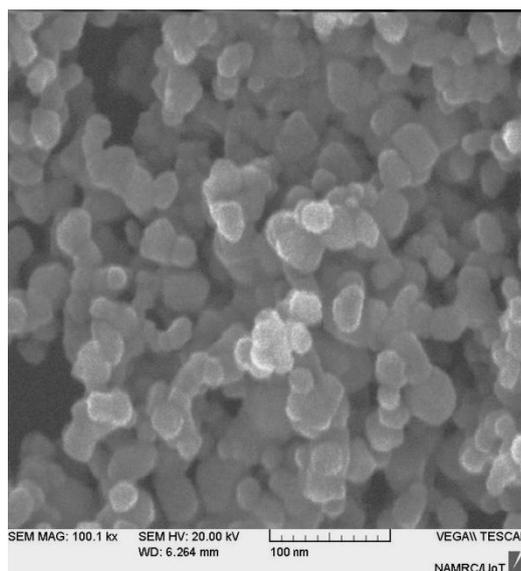
Figure 1 depicts an XRD pattern of ZnONPs, the strong peak shows that the nanoparticles were crystalline in form. Additionally, the peak was amplified, suggesting a very small particle size. Most of the peak broadening seen can be attributed to the ZnONPs effect [25]. A typical XRD pattern of ZnONPs can be seen in the region of  $20^{\circ}$ - $80^{\circ}$  with a scanning step of 0.01. This includes Bragg reflections with values of  $31.79^{\circ}$ ,  $34.45^{\circ}$ ,  $36.26^{\circ}$ , and  $47.53$ , which correspond to (100), (002), (101), and (102) planes, respectively. These values are similar to the results of researchers Govinda and Monika [26, 27].The product's crystallinity was shown by the strong and narrow diffraction peaks. The results were consistent with Asmaa [28].



**Fig.1.** The XRD pattern of the ZnO nano-particles.

### 3.2 SEM of ZnONPs

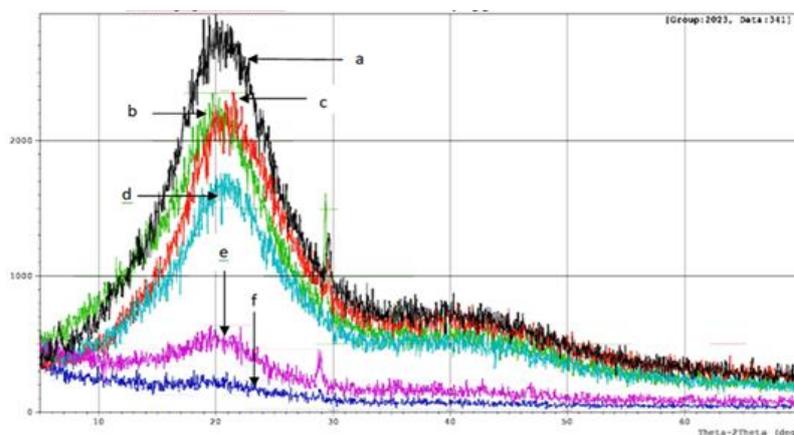
The scanning electron micrograph of ZnONPs is shown in Figure 2. The image shows spherical zinc oxide (ZnO) particles with a shiny surface. These results are consistent with the findings of Hamrayev's study [29].



**Fig. 2.** Shows SEM of ZnONPs.

### 3.3 XRD of nano composites

The X-ray patterns of the prepared samples are depicted in Fig.3. The study pointed out the intensity of the pure semi-crystalline blend exhibited the highest value, while the intensity of the composites were either equivalent or in close proximity to one another. The results of this study provided a similar perspective with previous research conducted by Albalawi [30]. The X-ray diffraction pattern of the pure matrix exhibits peaks at  $2\theta$  values of 18.9, 22.77, and 29.32, which closely compatible with the findings reported in the literature by Al-Harbi [31]. The effect of rubber is minimal due to its low percentage (20%). Due to the amorphous nature of natural rubber, it did not show any peak, as confirmed by Althubiti [32]. An alteration in the theta values of the composites was seen when the content of zinc oxide was increased. Specifically, the composite with a concentration of 1% exhibited the lowest values of  $2\theta$  in comparison to the other composites. The observed phenomenon pertains to the reaction of ZnO with the matrix. It is noteworthy that a slight increase in values was observed with increasing concentration of ZnONPs. This can be attributed to the occurrence of particle agglomeration or de-intercalation at high levels of filler loading. Similar findings have also been published [33].



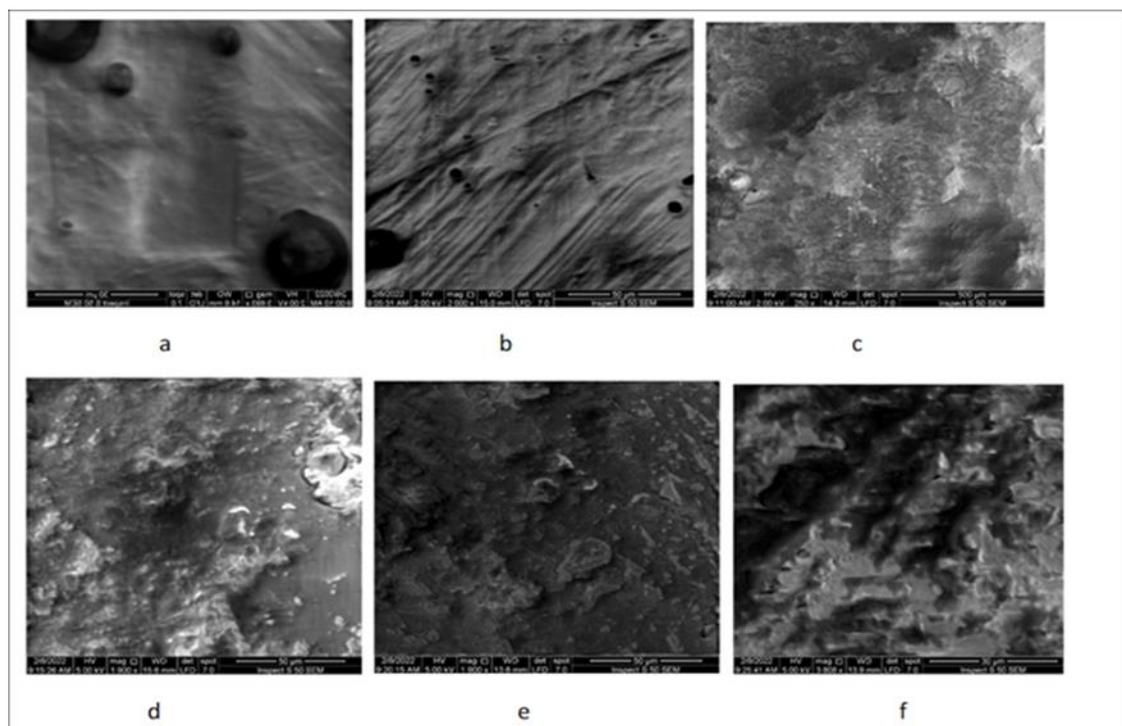
**Fig. 3.** X-Ray Diffraction patterns of (a) Pure blend, (b) blend+ ZnONPs (1wt%), (c) blend + ZnONPs (1.5 wt%) ,(d) blend+ ZnONPs (2wt%), (e)blend+ ZnONPs (2.5wt%), and (f) blend+ ZnONPs ( 3wt%).

### 3.4 SEM of nano composites

Scanning electron microscopy (SEM) images in Fig. 4 depict the surface morphology of the prepared samples. SEM image of a pure blend is shown in Figure 4(a). The sample contains empty spaces, and there is a lack of clear boundaries between the two components of the blend (unsaturated polyester and natural rubber). Additionally, there is evident shrinkage of the sample.

Figure 4(b) shows that the blend, reinforced with 1% ZnONPs, exhibited a more uniform distribution due to decreased agglomeration and aggregation. The primary reason for the straightforward interaction with the matrix is the homogeneous dispersion.

Figures 4(c) and 4(d) show a blend reinforced by 1.5% and 2% ZnONPs, with considerable similarities between the two samples. These specimens exhibit clusters of ZnONPs dispersed throughout the matrix, creating open space between them. These agglomerates demonstrate the general nature of nanoadditions [34]. Figure 4(e) displays the composite reinforced with 2.5% ZnONPs, revealing the presence of several randomly produced agglomerates. This is due to the fact that ZnO and the matrix have poor interfacial contacts and weak interactions of van der Waals forces [35]. The blend reinforced by 3% ZnONPs has agglomerations of ZnONPs and some free space in the matrix, but it is denser and has less free space than the blend reinforced by 2.5% ZnONPs. This is illustrated in Fig. 4(f).



**Fig. 4.** X250 SEM micrographs of unreinforced blend (a), X2000 SEM micrographs of blend reinforced with 1% ZnO (b), X250 SEM micrographs of blend reinforced with 1.5% ZnO (c), X1900 SEM micrographs of blend reinforced with 2% ZnO (d), X1900 SEM micrographs of blend reinforced with 2.5% ZnO (e) and X3808 SEM micrographs of blend reinforced with 3% ZnO (f).[34]

### 3.5 Antibacterial effects of blends reinforced by ZnONPs.

The bactericidal influence of blends reinforced by ZnONPs against *E. coli* and *S. aureus* was assessed by monitoring the inhibition zone values which were represented as ( $M \pm SD$ ). Table 1 and Figure 5 illustrate the decrease in cell growth observed following treatment, as well as the ratio of ZnONPs-enriched mixes to the inhibitory zone. The inhibition zone in *E. coli* ranged from 11.03 to

27.16, with significant variations at ( $p < 0.05$ ) for the ratio response mode, while for *S.aureus* the inhibited zone ranged from (9.83-24.03) and there is a significant differences at ( $p < 0.05$ ) ( $p$  value= 2.55).

The proliferation of bacterial cells is a complex and dynamic process that begins with the synthesis of the membrane protein that will drive all functions connected to cell division and cell wall synthesis [36]. The conventional tubulin saved in bacteria, which exerts its activities depending on the nucleotide guanosine triphosphate (GTP) [37-39]. Some antibacterial compounds work by stopping the GTP activity of cell wall proteins, which will inhibit cell proliferation and lead to cell death [9], stopping the cell division cycle also induces cell filamentation which can be easily assessed by microscope. In a previous study, the bacterial species were used, showed the cytoplasmic membrane affected within the first 15 min of exposure to ZnONPs and caused membrane damage in more than 70% of the bacterial cells because formed of ZnO, electron-hole pairs that lead to the production of hydroxide anions from oxidizing water molecules and generate strong oxidizing species [40].

This reaction leads to product of reactive oxygen species (ROS), which are generated by the radical's hydroxyl ( $\text{OH}^\cdot$ ), hydroperoxide ( $\text{H}_2\text{O}_2$ ), and superoxide radical anion ( $\text{O}^{2-}$ ), as the paths of bactericidal action [41,42].

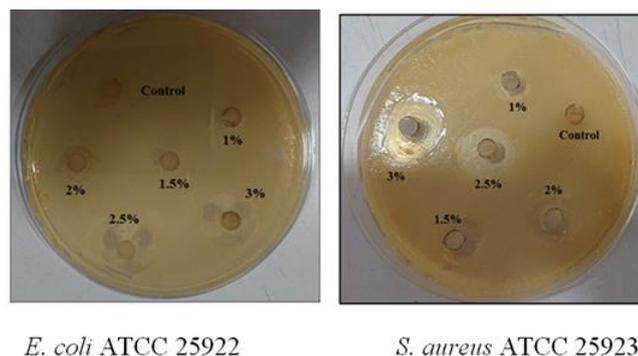
These ROS from ZnONPs along with oxidative stress can inhibit DNA replication and protein synthesis [43]. During this process, the conductivity of ZnONPs rises, leading to the instability and rupture of the charges in the cytoplasmic membrane. When ZnONPs are dissolved in water, they may harm cells by releasing  $\text{Zn}^{2+}$  ions from the cytoplasmic membrane, causing damage. The  $\text{Zn}^{2+}$  ion serves as the mechanism by which it inhibits the glycolytic enzyme [44].

The antibacterial effects of ZnONPs are attributed to their ability to cause membrane damage. This occurs when ZnONPs adhere to the surface of the cell and induce structural and functional changes in the membrane, leading to the creation of pores and leakage of cytoplasmic contents. This process is related to the gradual release of  $\text{Zn}^{+2}$  ions from the surface of the polymer blends, resulting in the generation of reactive oxygen species (ROS). These ROS have detrimental effects on essential components, including proteins, DNA, and structural lipids. In addition, the presence of  $\text{Zn}^{+2}$  ions on microbial outer surfaces serves to inhibit microbial cell adhesion and the formation of biofilms. Alternatively, the disruption of the microenvironment of these microorganisms by  $\text{Zn}^{+2}$  ions interfere with the functioning of the potassium-sodium pump and hinders the activation of crucial enzymes required for colonization [45].

**Table 1.** The inhibitory effects of reinforced blends with ZnONPs against Gram negative bacteria (*E. coli*) and Gram positive bacteria (*S. aureus*) using the disc diffusion method.

Proportion (%)	<i>E. coli</i> (ATCC 25922)		<i>S. aureus</i> (ATCC 25923)	
	Inhibition zone(mm)	IR%	Inhibition zone(mm)	IR%
0.0	0.0	0.0	0.0	0.0
1.0	11.03±0.71*	40.6	9.83±0.35*	40.9
1.5	13.16±1.89*	48.45	11.8±0.2*	49.11
2.0	21.5±1.32*	79.16	18.5±0.45*	76.98
2.5	24.33±2.08*	89.58	21.0±0.2*	87.39
3.0	27.16±1.04*	100	24.03±0.25*	100
P value	2.55			

- Each number in table represent (Mean ±SD) for three replicates
- (\*) Significant differences at  $p \leq 0.05$ . IR; inhibition rate.



**Fig. 5.** The inhibitory effect of blends reinforced by ZnONPs on *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) at different proportion by disc diffusion method.

### 3.6 Cytotoxic MTT Assay

To investigate cytotoxic influence of ZnONPs an in vitro, WBCs from healthy persons were incubated with different proportions of ZnONPs. The anti-proliferative effect was detected utilizing the MTT assay, which seems more dependable [46]. The results indicated that ZnONPs had a slight inhibitory effect on the multiplication of white blood cells (WBCs) in a dose-dependent manner. However, this effect was not statistically significant ( $p < 0.05$ ) according to Table 2.

The ZnONPs did not reveal any toxic impact on normal WBCs Pertinent to the evaluation of the toxicity of metal nanoparticles against numerous mammalian cells; cancer cell lines have been noted. Akhtar et al. showed that three kinds of cancer cells were destroyed by the influence of ZnONPs, while regular rat astrocytes and hepatocytes were not influenced [47]. Based on the data presented in Table 2, it is evident that the optical density  $OD^{500nm}$  exhibits a negative correlation with powder concentration. Consequently, as the absorbance decreases, the bacterial growth also decreases.

**Table2.** Cytotoxic effect of ZnONPs on WBCs at 24 h of exposed was assessed by mitochondrial activity using MTT assay.

Proportion (%)	$OD^{500nm}$	IR (%)
0.0	1.92±0.18	0.0
1.0	1.86±0.21	3.125
1.5	1.79±0.11	6.77
2.0	1.76±0.12	8.33
2.5	1.72±0.26	8.86
3.0	1.71±0.14*	10.93
P value	0.0035	

- Each number in table represent (Mean ±SD) for three replicates

(\*) Significant differences at  $p \leq 0.05$ ; (IR): inhibition rate

### 3.7 Cytogenetic Assay

Cytological criteria such as the blastogenic index (BI), mitotic index (MI), and total chromosomal abnormalities (TCA), were used to evaluate the genotoxic potential of ZnONPs against peripheral blood lymphocytes (PBLs). Table 3 and Figure 6 show that the blastogenic index of cells was 63.41 before ZnONPs were added, but this value jumped dramatically afterward. The mitotic index increased from 1.88 to 2.87 at the concentration 3% of ZnONPs, while the value of the mitotic index was reduced in hazardous material (MMC) to reach  $(0.25 \pm 0.01)$ , and the number of chromosomal abnormalities increased to 1.88 from  $1.25 \pm 0.01$  in the control group. Cells treated with various

nanoparticle fractions revealed elevated levels of biomass, total soluble proteins, superoxide dismutase (SOD), and peroxidase (POX), suggesting that ZnONPs operate as a growth-promoting agent. This may have resulted from increased activity of antioxidant defense enzymes, which regulate isoenzyme expression patterns and reduce ROS. Therefore, ZnONPs incorporated into biomolecules are a practical choice for use in healthcare [48]. However, zinc nanoparticles may be hazardous in high doses. One hypothesis concerns the chemical toxicity based on the concentration and content of the chemical, while the other concerns the stress induced by the surface, size, and shape of the ZnONPs. Cell responses are profoundly impacted by both features [49].

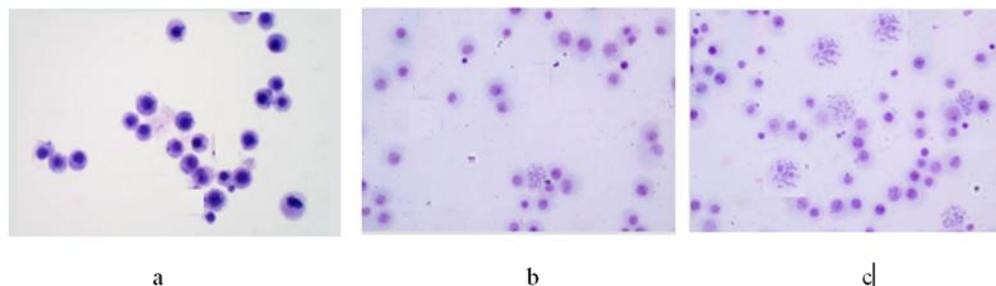
**Table 3.** Cytogenetic analysis of ZnONPs at different portions on peripheral blood lymphocytes (PBLs).

Portions%	BI	MI	TCA
0.0	63.41±0.56	1.88±0.02	1.25±0.01
1	68.81±0.77*	1.95±0.03*	0.21±0.01
1.5	71.25±0.39*	2.12±0.03*	0.13±0.01
2	77.12±0.45*	2.55±0.05*	0.0
2.5	82.55±0.89*	2.61±0.09*	0.0
3	87.18±0.66*	2.87±0.07*	0.0
MMC	21.11±0.60*	0.25±0.01*	1.88±0.01

0.25µg/mL

Each number represent (M±SD) four three replicates

\* Significant differences at  $p \leq 0.05$



**Fig.6.** The cytogenetic analyses of peripheral blood lymphocytes treated with a) Mitomycin C (positive control); b) Adding nothing (negative control); c) Treated with 3% of ZnONPs. Disappearance the mitotic cells in treatment (a) and their abundance in treatment (c).

#### 4. Conclusions

We fabricated composite materials composed of UP/NR reinforced with ZnONPs with varied weight fractions. The XRD pattern of ZnONPs revealed diffraction peaks, indicating the creation of zinc oxide's hexagonal wurtzite structure. SEM pictures revealed that the blend reinforced by 3 % ZnONPs has agglomerations of ZnONPs and some free space in the matrix, with a denser structure and less free space than the blend reinforced by 2.5 % ZnO specimen.

Our findings also revealed bactericidal effects of ZnONPs against two types of bacteria Gram Positive *S aureus* and Gram negative *E coli*. ZnONPs are multi-target chemicals that affect a variety of bacterial cell structures; nevertheless, their major action mechanism is in cytoplasmic membrane, with additional structure impacts operating as a consequence or secondary effect after the disruption of the membrane. For the cytogenetic assay, the results showed that the blastogenic index of cells and mitotic index are increased by 16.9% and 34.4% respectively compared to virgin blend, while total chromosomal abnormalities is

decreased. Finally, the results showed a slight dose-dependent decrease in white blood cell viability after exposure to ZnONPs.

Based on these findings, it can be inferred that the synthesized composites incorporating ZnONPs exhibit promising prospects as active water containers and in the pharmaceutical industry.

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## نموذج الملخص باللغة العربية لمجلة أبحاث البصرة (العلميات)

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المعلومات البحث	الملخص
الاستلام المراجعة القبول النشر	تم تصنيع مواد مركبة ذات خواص جيدة من خلال تدعيم الخلطات البوليمرية التي تحتوي على 70% بوليستر غير مشبع (UP) و 30% مطاط طبيعي (NR) مع جزيئات أكسيد الزنك النانوية (ZnONPs) بأجزاء وزنية (0، 1، 1.5، 2، 2.5، و 3%). تمت ملاحظة البنية المورفولوجية والبلورية لـ ZnONPs باستخدام المجهر الإلكتروني الماسح (SEM) وتقنيات حيود الأشعة السينية (XRD). تُظهر عينة ZnONPs بنية بلورية بشكل ملحوظ، كما هو موضح من خلال نتائج حيود الأشعة السينية. تم تقييم المركبات النانوية المُصنَّعة لنشاطها المضاد للبكتيريا ضد نوعين من النمو البكتيري، وهما <i>S. aureus</i> ATCC 25923 و <i>E. coli</i> ATCC 25922، بهدف تقييم مدى ملاءمتها للتطبيقات الصناعية. أظهرت النتائج التجريبية أن المركبات النانوية ZnONPs تظهر خصائص مضادة للجراثيم قوية ضد بكتيريا <i>E. coli</i> و <i>S. aureus</i> . تم إجراء تقييم السمية الخلوية لـ ZnONPs على خلايا الدم البيضاء السليمة (WBCs) تشير النتائج إلى أن ZnONPs لا تظهر أي آثار سامة على خلايا الدم البيضاء الطبيعية. أخيراً، تم استخدام المعايير الخلوية، مثل مؤشر الانفجار (BI)، ومؤشر الانقسام الفتيلي (MI)، وتشوهات الكروموسومات الكلية (TCA)، لتقييم إمكانات السمية الجينية لـ ZnONPs ضد الخلايا الليمفاوية في الدم المحيطية. الغرض من هذه الدراسة هو تقييم سمية الجسيمات النانوية المعدنية على خلايا الثدييات المختلفة.
الكلمات المفتاحية	مزيج البوليمر ، جزيئات أكسيد الزنك النانوية ، المركبات النانوية ، الخصائص ، نشاط مضاد للجراثيم ، السمية الخلوية.
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