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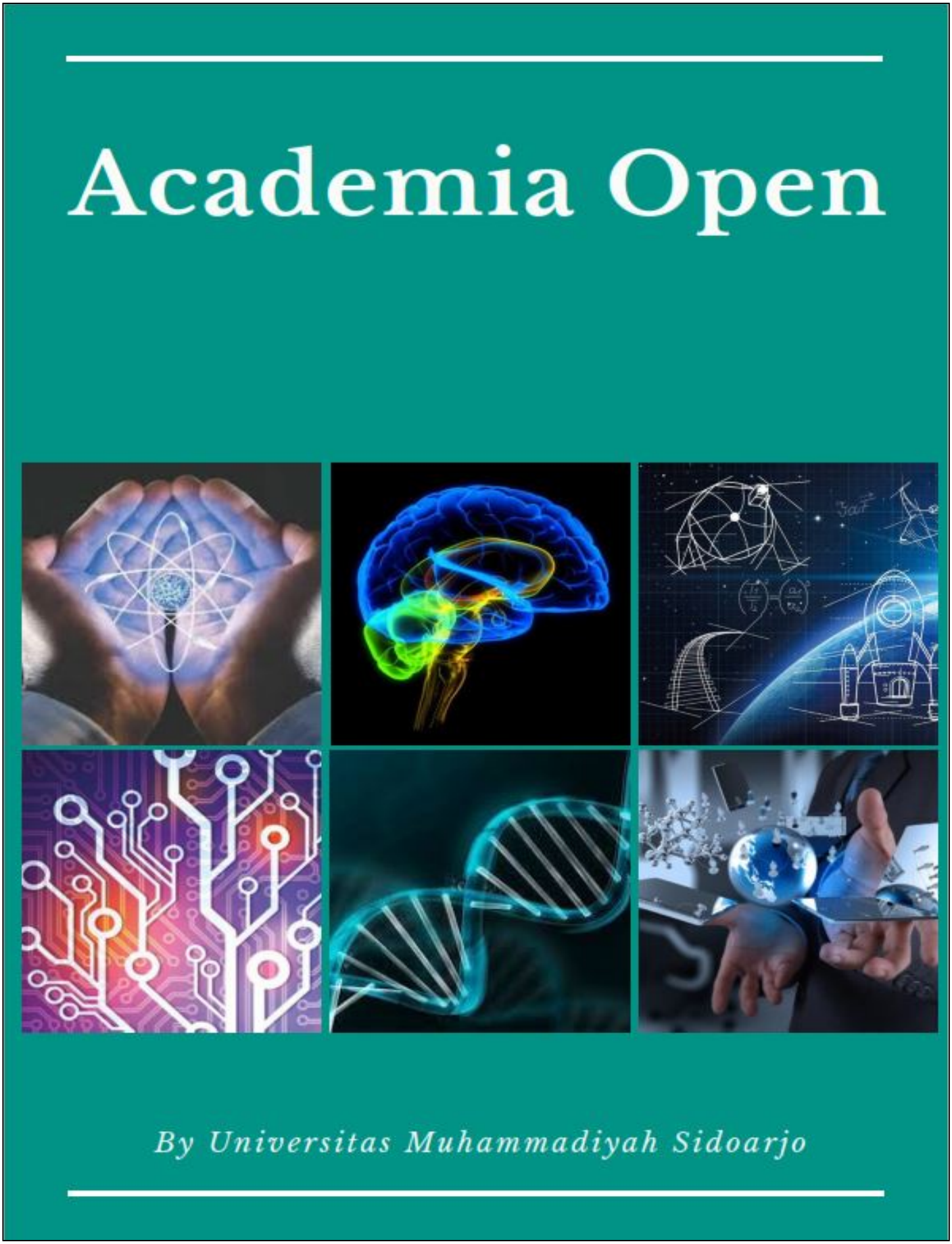

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Development of Tetracycline by AgO Nanoparticles and Studying its Activity on Antibiotic-Resistant Bacteria

Pengembangan Tetrasiklin dengan Nanopartikel AgO dan Mempelajari Aktivitasnya pada Bakteri yang Resisten terhadap Antibiotik

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Abstract

General background: Antibiotic resistance in bacteria has become a critical global health issue, necessitating the development of new strategies to enhance antibiotic efficacy. **Specific background:** Nanoparticles, particularly silver nanoparticles (AgNPs), have emerged as potential enhancers of antibiotics due to their unique properties and interactions with bacterial cells. **Knowledge gap:** However, the combination of nanoparticles with existing antibiotics, such as tetracycline, and their impact on bacterial inhibition and safety has not been fully explored. **Aims:** This study aims to investigate the antibacterial activity of silver oxide nanoparticles (AgO NPs) combined with tetracycline (TCS) and evaluate their effectiveness against resistant bacterial strains. **Results:** AgO NPs were synthesized using a photodeposition method, yielding nanoparticles with an average diameter of 2.24 nm. The AgO NPs + TCS combination demonstrated superior antibacterial activity, with a minimum inhibitory concentration (MIC) of 16 µg/mL against Staphylococci and 32 µg/mL against Pseudomonasaeruginosa,significantlyoutperformingstandardtetracycline.Hemolysisassays confirmed the safety of the synthesized compound at all concentrations. **Novelty:** Silver oxide nanoparticles and tetracycline exhibit a unique synergistic interaction, enhancing antimicrobial effects by increasing bacterial membrane permeability, facilitating greater antibiotic infiltration. **Implications:** These findings suggest that AgO NPs combined with tetracycline offer a promising solution to overcome bacterial resistance, providing a potent and safe alternative to conventional antibiotic treatments.

Highlights:

1. AgO NPs and tetracycline show enhanced antibacterial effects.
2. More effective than standard tetracycline against resistant bacteria.
3. Safe with no toxicity observed in hemolysis tests.

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Keywords: Antibiotic resistance, silver nanoparticles, tetracycline, photodeposition, antibacterial activity

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Introduction

Tetracycline (TC) has been widely used as an effective antibiotic compound for the treatment of various infectious diseases [1,3,2]. Antibiotics can inhibit cell wall production, cell membrane function, and DNA and protein synthesis [4]. However, bacteria cause the spread of antibiotic-resistant genes. Multidrug-resistant pathogens continue to grow; Therefore, the development of effective antimicrobial antibiotics is important. Recent developments in the field of nanotechnology especially in the fabrication of metal nanoparticles of specific size and shape have attracted microbiologists to this field. Nanoparticles have multidisciplinary uses in industry, health, food, feed, aerospace, chemicals, and cosmetics industries [6,7,8,9,10]. The conjugation of nanoparticles (NPs) with antimicrobial agents such as antibiotics, peptides or molecules One of the successful techniques in targeting antibiotic resistance is various biochemicals. Nanoparticles play a range of roles, for example. As a carrier, synergistic agent and therapeutic agent, it henceforth facilitates the effectiveness of treatment. Nanoparticles also have special properties due to their dissolution of metal ions, nanoscale size, large surface area and antimicrobial effects [5]. Among various metal oxide nanoparticles, silver nanoparticles (AgNPs) are receiving great attention not only in research laboratories but also in human and veterinary field due to their unique optical, catalytic and biological properties. In the field of biological activity, these nanoparticles exhibit high antibacterial activity dependent on size, morphology and surface modification 14-11]]. .Application of AgNPs with different antimicrobial agents increases their antimicrobial effect. Many scientific groups have addressed the possibility of chelating antibiotics with nanoparticles through hydroxyl and amide groups, which are present in antibiotic molecules [15-17]. AgNPs-based products have been used for treatment in human medicine for wound dressings and even catheters in surgery. Most AgNPs-based drugs for animals are designed for use as drops, ointments, sprays, or gels to treat bacterial infections. Some researchers claim that silver ions in AgNPs interact with bacterial cell membranes and cell death ensues [17,18]. The effect of silver can be generated by chelation of silver (I), which prevents DNA unwinding [18] . AgNPs can also be used as drug carriers. The bacterial cell membrane is composed of phospholipids and glycoproteins (hydrophobic groups), therefore nanoparticles target the bacterial cell membrane because ATBs like amoxicillin are hydrophilic and nanoparticles are not, which is why the antimicrobial groups simplify the transport of amoxicillin to the bacterial surface. [17]. Compounds can be linked together by strong covalent bonds (amide). Homogeneous conjugation was observed, due to the very small size of AgNPs and tetracycline, which in turn provides a synergistic effect of increased antimicrobial activity. Show the most effective effect by Tetracycline AgNPs- conjugation against Gram-positive bacteria S. aureus by effectively damaging the bacterial membrane. Due to the binding of nanoparticles to proteins in the bacterial cell membrane, the permeability increases and the infiltration of antibiotics into the bacterial cell increases. Thus, nanoparticles bound to antibiotics via chelation can increase the concentration of antimicrobials at specific sites of the cell membrane. [19].

Items of Research

AgNO3 %99.99 Spain,Tetracycline %99.9 Pharmaceutical Company Samarra Iraq, Nutrient agar& Muller Bacterial isolates (Pseudomonas aeruginosa, Staphylococcus aureus) were acquired and diagnosed from Baquba Teaching Hospital in Iraq using Hinton broth from HIMEDLA

Methods

Synergistic TCS with AgNO3 Nanoparticles

Silver was deposited on tetracycline by the photodeposition method when a mixture consisting of dissolving 2 grams of tetracycline in 100 ml of hexane with the required amount of silver nitrate AgNO3 0.2 grams was exposed to irradiation with a strength of 1.3 mW of Uv light for 6 hours at a temperature of 40 C using a mercury lamp. At a power of 200 watts, the precipitate resulting from irradiation was collected after drying at 45 degrees C.

Minimal - Inhibitory Concentration

Multiple sequence concentrations of antagonist (TCs loaded with AgO NPs) ranging from (32-64-128-256-512-1024µg/ml) were formed by adding varying amounts of this antagonist from their prepared safe solutions and transferring (100 µL) of deionized water to the plate pits. Each bacterial isolate was divided into three replicates using a polystyrene plate with 96 holes, with (100 µL) of the antibody dispersed in the holes with the highest concentration of 1024, except for the holes containing the control, which were water and cultured bacteria. Shifts were accomplished by shifting (100 µL) of the 1024-concentrated counter-pit to the next hole. The transition from one hole to the other went well as well. When we reached the last hole, we removed (100µL), and the bacteria were distributed in (100µL) for each hole in the plate. The plate was covered and incubated at 37 degrees Celsius [20] before being monitored with an ELISA reader at 630 nanometers [21].

Hemolysis assay

The hemolysis assay was used to screen for TCs with AgO NPs at various concentrations (64, 128,256 ,500 µg/ml)

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to identify dangerous or non-toxic compounds. The blood sample was retrieved from the lab and deposited in an (EDTA) tube, then tested on a slide and inspected under a microscope at a magnification of (100), confirming that the person's blood was healthy and free of illnesses, with no platelet breakage. The method was used to determine the cytotoxicity of the chemical substances employed [12]. Placed an (EDTA) tube in the (Universal Centrifuge) for 10 minutes to separate the blood cells from the plasma, then removed the plasma layer from the cells and washed the cells numerous times with PBS, each time adding (1ML) of PBS and centrifuged for 10 minutes. The cells were removed from the PBS after two minutes. The blood cell suspension was made by mixing (1ML) with (9ML) PBS after the cells had been washed numerous times. In each tube with a volume of (1200 µL), the antagonist is added at different concentrations, and (300 µL) of the cell suspension is added to the final volume (1.5 ml) and incubated in the incubator for two hours, then separated by a centrifuge device at a rate of 1000 cycles/min for five minutes. The difference in hemolysis was then measured using the Heh control settings (test tube containing blood and deionized water only, test tube containing blood and PBS). If the blood components are combined after centrifugation, the (+) option shows the compound's toxicity. The (-) option shows that following centrifugation, the blood components were not mixed, indicating that the substance was not hazardous.

Result and Discussion

Characterization by X-ray diffraction

The synergistic XRD and TCS spectra of AgO were matched, and the average crystal size was 1.59 nm, as shown in Fig 1.

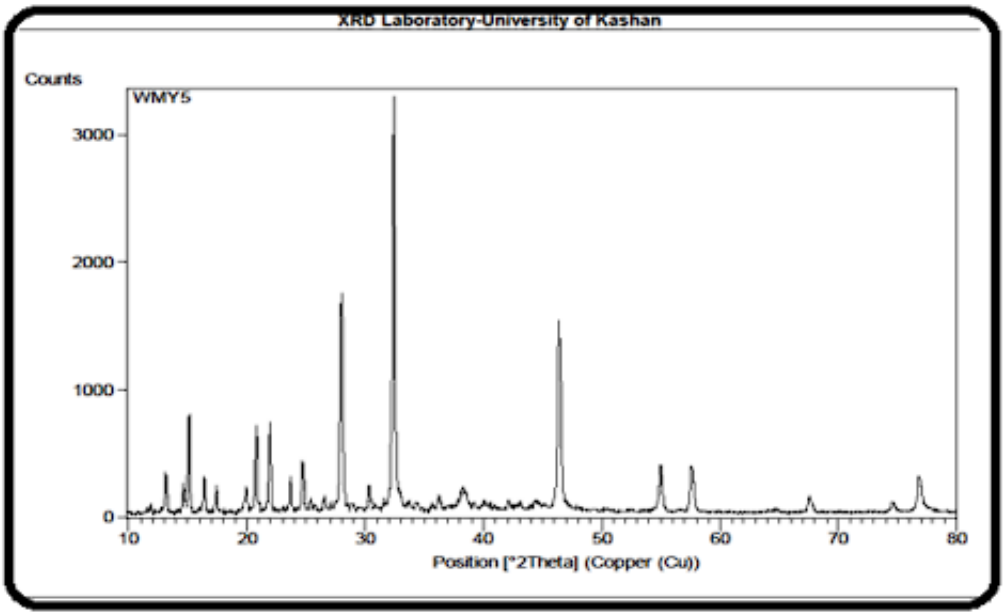

Figure 1. *shows the XRD spectrum of the TCs antibody with AgO nanoparticles*

Characterization by energy-dispersive X-rays

The percentage of elements present in the TCs with AgO NPs was determined using (EDX), as shown in the figure (2), and the results showed carbon (38.74%) and silver (61.26%), so the TCs with AgO NPs material appeared to be of high purity.

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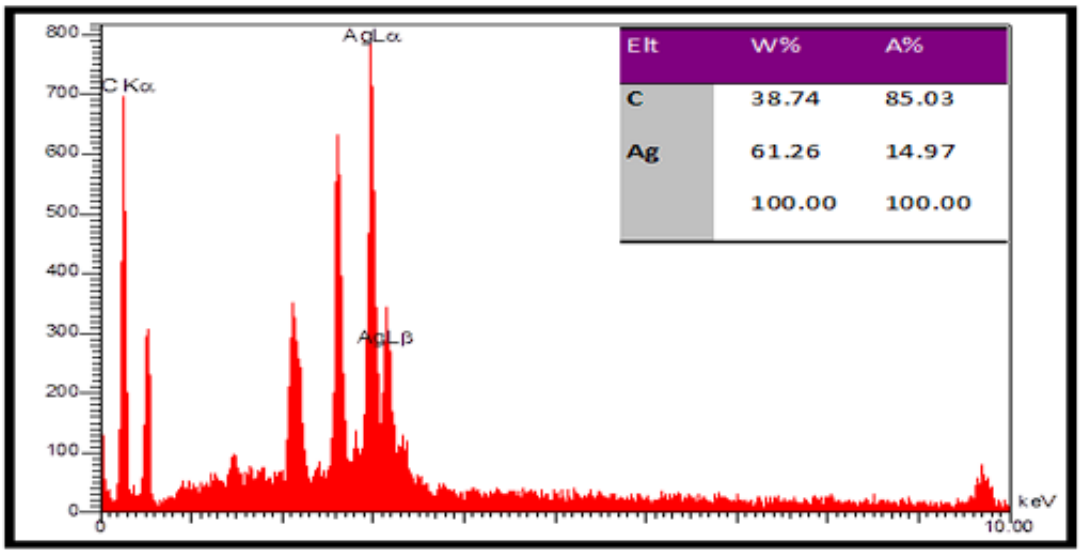

Figure 2. *shows the XRD spectrum of TCs with AgO NPs*

Characterization by scanning electron microscope

An SEM scanning electron microscope was used to investigate the morphological and structural compositions of TCs with AgO NPs. The nanoparticles were created in the nanoscale range, , and SEM scans revealed that some of the nanoparticles separated well from one another. Due to electrostatic influences, the majority of them were present in a lumpy form at the same time. This behavior is consistent with earlier investigations of nanoparticle agglomeration, and the average diameter of these nanoparticles is 2.24 nm. Figure 3

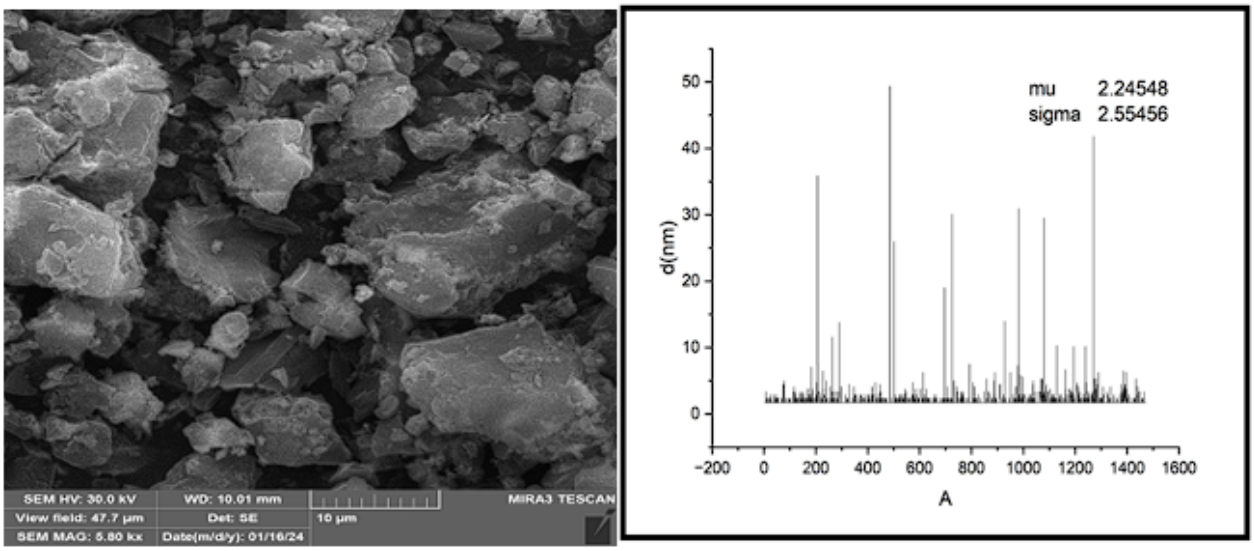

Figure 3. *shows TCs with AgO NPs using a scanning electron microscope*

Determination of Minimal Inhibitory Concentration

The lowest inhibitory concentration of (Tetracycline, Tetracycline with AgO NPs) compounds was determined using resistant bacterial isolates. The multiplicative serial concentration approach (32-64-128-256-512-1024µg/ml) was used to determine the MIC with Middle Mueller Hinton Broth. Table 1 shows the results. The minimum inhibitory concentration of of Tetracycline with AgO NPs against Pseudomonas aeruginosa was 32 µg/ml, whereas the standard base contributions were 128 µg/ml Tetracycline, showing that the aforesaid compound has a higher activity than the standard Tetracycline. The MIC of Tetracycline with AgO against Staphylococcus aureus was 16 µg/ml, while the MIC of standard Tetracycline was 32 µg/ml, showing that the aforesaid compounds were more effective than the standard components.

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Compounds	Pseudomonas aeruginosa MIC µg/ml	Staphylococcus aureus MIC µg /ml
Tetracycline	128	32
Tetracycline with AgO NPs	16	32

Table 1. *Minimal Inhibitory Concentration of (Tetracycline, Tetracycline with AgO NPs)*

Determination Hemolysis assay

The cytotoxicity of the compound (Tetracycline with AgO NPs) was studied, and the results showed that the compound was safe (non-toxic) at all concentrations, as it did not show a toxic effect in the form of broken blood platelets, as shown in Table 2 and Figure 4.

Tetracycline with AgO NPs	64	-
	128	-
	256	-
	500	-

Table 2. *is used to evaluate the toxicity of a chemical (Tetracycline with AgO NPs) [18] .*

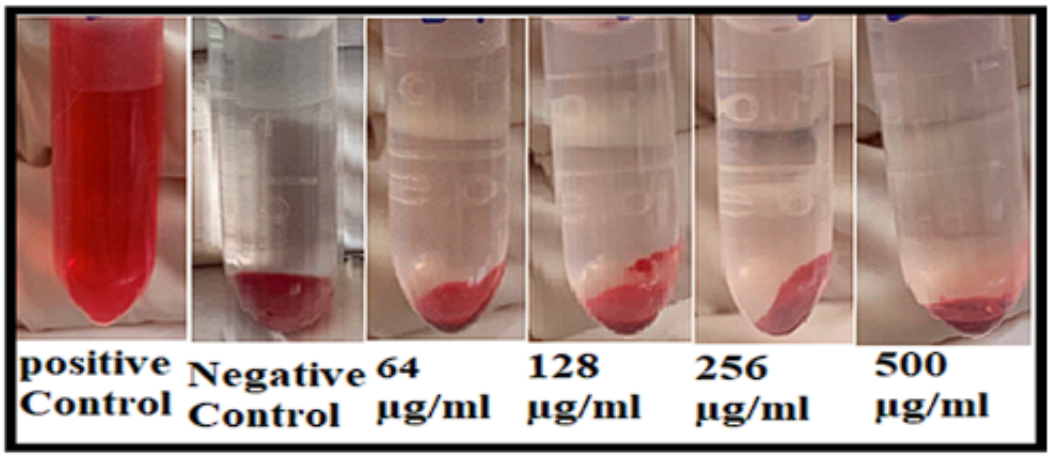

Figure 4. *Hemolysis test for Tetracycline with AgO NPs*

Conclusion

Tetracycline was developed by photodeposition using a 200-watt mercury lamp, where the tetracycline was linked to silver oxide nanoparticles. The results showed a consistent distribution of silver oxide nanoparticles in the tetracycline particle matrix. Through the results we reached by measuring the minimum inhibitory concentration for two types of bacteria and comparing the effectiveness of tetracycline bound to silver oxide nanoparticles with the effectiveness of standard tetracycline, which was obtained from the Samarra Pharmaceutical Laboratory, we found that the silver ions in AgNPs interact with bacterial cell membranes, resulting in cell death. Due to the very small size of AgNPs and tetracycline, which in turn provides a synergistic effect of increased antimicrobial activity. Show the most effective effect through conjugation of Tetracycline AgNPs- by effectively damaging the bacterial membrane. The binding of nanoparticles to proteins in the bacterial cell membrane causes increased permeability and increased infiltration of antibiotics into the bacterial cell. Moreover, these nanosilver oxide nanoparticles have no toxicity.

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