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Evaluation Effects of Synergistic Iron Oxide Nanoparticles and Antibiotics as Cytotoxic Effects of Activated Lymphocytes and Antibacterial Activity.

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Abstract

At the present time, the phenomenon of antibiotic resistance has increased by different species of bacteria. In this way, particularly in the situations of metal nanoparticles (MNPs) fabrication and MNPs surface modification, the emergence of nanotechnology as a new weapon has drawn increased attention. Currently, the safe way to manufacture nanoscales is at the lowest possible cost and the least harm to the environment of IO NPs with novel shape through Ultrasound-Assisted. Ultraviolet−visible spectrophotometer (uv-Vis), energy dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), atomic force microscopic (AFM), X-ray diffraction (XRD). these techniques were applied for physical characterization. well diffusion assay and minimum inhibitory concentration (MIC), were evaluated against gram negative (P.aeruginosa , Klebsiella spp) and gram positive (S. aureus , S. pyogenes) .Where the activity of iron nanoparticles prepared by a physical method showed a distinct activity against selected cancer cells. IO NPs with an average diameter size of 30nm.

Keywords

Fe3O4 NPs, UBC-40 cells, Salmonella.

Nanotechnology is the creation of materials and devices by controlling of matter at the levels of atoms, molecules and supramolecular (nanoscale) structures, is the use of very small particles of materials to create new large-scale materials. better understand difference among various scales(1). Antibiotics used to treat infectious illnesses are manufactured worldwide in around 100,000 tons per year. Antibiotic overuse has caused pathogenic strains, particularly in bacteria, to become multidrug resistant. [2] Iron oxide nanoparticles (NPs) have drawn a lot of interest because of their distinctive characteristics. Greater surface area, superparamagnetism, surface-to-volume ratio, and simple separation techniques. Were determined various physical, chemical, and biological methods have been adopted to synthesize magnetic NPs with suitable surface chemistry. The processes for producing iron oxide nanoparticles (NPs), size and shape regulation, and magnetic characteristics with contemporary bioengineering, commercial, and industrial applications. Iron oxides exhibit great potential in the fields of life sciences such as biomedicine, agriculture, and environment. Applications of magnetic NPs can be made more biocompatible and nontoxic by covering a specific surface with organic or inorganic molecules, such as surfactants, medications, proteins, starches, enzymes, antibodies, nucleotides, nonionic detergents, and polyelectrolytes [3] The worldwide increase in mortality and morbidity rates due to multi-drug resistant pathogenic bacterial strains poses a severe challenge in the field of medicines[4]. The compared to non-resistant bacterial infected

patients World Health Organization (WHO) reported that more than 64% of patients die due to methicillin-resistant Staphylococcus aureus (MRSA) infections. Novelty now the synergism different metallic nanoparticles of antibiotics worked on using different non-toxic and using eco-friendly techniques the synergistic antibacterial activities of different antibiotics after combinations (5). To increase the bioavailability and improve antibacterial activity of drugs found nanoparticles have been used, enhance efficient drug delivery (6).

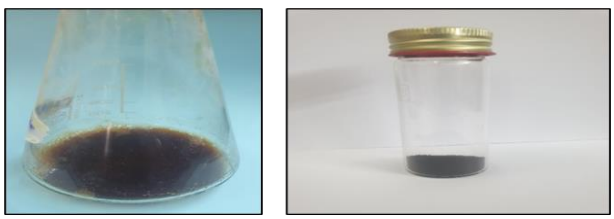
The use of nanoparticles as novel biomaterials to fully achieve this feat is currently gaining global attention. Nanoparticles could become an indispensable viable therapeutic option for treating drug-resistant infections. Of all the nanoparticles, the metals and metal oxide nanoparticles appear to offer the most promise and have attracted tremendous interest from many researchers (7).

Materials and methods

Iron (III) chloride ($FeCl_3$.) anhydrous, Iron sulfate heptahydrate ($FeSO_4.7H_2O$), Ethanol and Sodium hydroxide (NaOH) pelts. (2−3) beakers glass (100−200) mL, deionized water and ultrasonocator(30 KHz, 20 W).

Synthesis Of Iron Oxide Nanoparticles (Ionps)

To maintain the molar ratio 2:1, 0.05 mmol of $FeSO_4.7H_2O$ were made by dissolving in 250ml of deionized water. however 0.1 mmol of $FeCl_3$ anhydrous. Ultrasonocator was placed to both salts aqueous solution in 100ml round bottom flask with heating at 60-70 c^0 (resulting from hotplate stirrer and by sonocater) .4M of aqueous NaOH solution was prepared which constantly added to the mixture drop by drop until the pH reached 11−13. The flask was covered with a cork, and the reaction was allowed to run at the specified settings for one hour. After the reaction the mixture was centrifuged at 6000 rpm for 5 minutes after being allowed to cool at room temperature. However, the supernatant was throwed and kept the black precipitate, the precipitate was dried overnight at 40°C in an oven to get the dry powder. figure (1).



Fig(1): Synthezied IONPs by Ultrasonoator method

Characterization of IONPs

Characterization and understanding nanoparticles. Methods were used to determine NPs characteristics: FESEM and TEM to characterize the shapes and sizes, atomic force microscopy (AFM) was used. UV-visible absorption spectrophotometer (UV-vis) then used to investigate the optical properties of colored samples. (XRD) was a method used in materials research to identify material's crystallographic structure [8].

Determination Of Minimum Inhibitory Concentration (Mic)

IONPs activities of bacteriostatic were measured by MIC assays [9]. An appropriate volume of bacteria (2 L) in Mueller Hinton broth MHB. The MHB of two fold dilution serials were added to MHB (64,32,16,8,4) µg/ml by using 6 tubes of MHB respectively. the Tubes were incubated for 24 hours at $37C^0$. These amounts of IONPs at 0.1M concentration was demonstrated an against stronger antibacterial impact in an agar diffusion experiment. Turbidity was observed in growth and non-turbidity as no growth after. The medium had been incubated for 24 hours at 37°C. The lowest concentration was shown by the MIC values, and showed clear fluid with no turbidity formation.as shown in Figure (2).

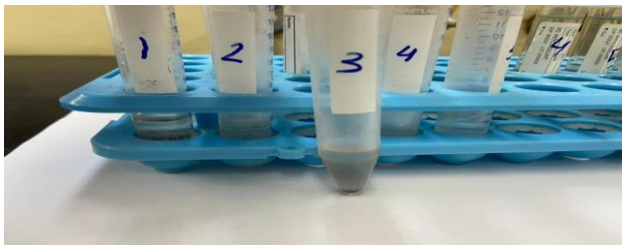


Fig (2): Prepreation MIC concentration of IONPs

Multiple Drug Resistance Bacteria (Mdr Bacteria)

The gram negative and gram positive bacteria isolation from patient suffer were collected from burn and wound infection shown table (1), all sample identified by biochemical method and preservation in 20% glycerol were obtained from Department of Biology, College of Science, Baghdad University, and confirmation Identification by vitek 2 system to chosen one strain multidrug resistant antibiotic have be done

Table -1- Bacteria isolation burn and wound infection

Pseudomonas aeruginosa	Lactobacillus
Escherichia coli	Staphylococcus epidermidis
Salmonella spp	Staphylococcus aureus

Proteus mirabilis	
Klebsiella spp	

Antibacterial Susceptibility Test

The antimicrobials tested of antibiotic activity included Pseudomonas aeruginosa anti- (Gentamycin) and anti- S. aureus (Vancomycin) Standard suspension of bacterial pathogens (5 Ч 10^8 cell/ml) was added to microdilution wells containing 100µL MHB and different concentrations between (4, 8, 16, 32, 64 µg/ml) for both antibiotic ampule and broth served as negative control and positive control, respectively. After 24 h incubation at 37C°, the tubes were examined for growth. The lowest concentration that showed no growth was expressed as the MIC of antibiotics. [10].

Well Diffusion Method

Antibacterial activity was determined by using well diffusion method [11]. For each test, overnight MHB cultures of a specific strain of bacteria were freshly made, and include applied using swabs to the surface of the solidified medium MHA. IONPs were placed on well at various concentrations (IONPs, Gentamycin and Vancomycin) between (64,32,16,8,4) µg/ml after the medium had dried for 10 minutes [12].

Evaluation Of The Synergistic Ionps And Antibiotics Against Clinical Pathogens.

As previously to determine the fractional inhibitory concentration indexes of each antibacterial compound according to the work Masoumi, S [13]. When dividing the MIC for a drug in combination by the MIC for drugs acting alone. including were tested at MIC levels with prepared IONPs against the selected MDR. Stock solutions of IONPs and antibiotics with sub-MIC values were prepared using this method. The MICs of the antibiotics and IONPs in combination were determined after 24 h of incubation at 37 °C.

Results and discussions

Identification of bacterial strain by Vitic 2-System all slected strain have been classified as MDR as shown in Figure (3).

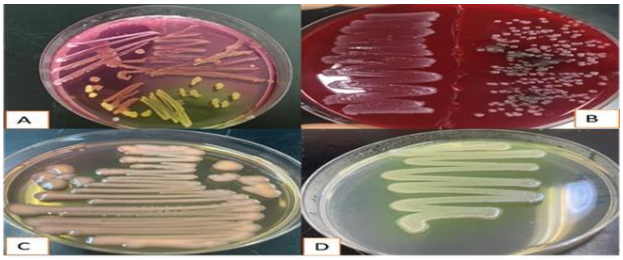


Figure (3) Bacteria isolation A) S. aureus B) Streptococcus pyogenes C) Klebsiella spp

Pseudomonas aeruginosa

Synthesized Of Iron Oxide Nanoparticles:

Iron Oxide nanoparticles were preparing by sonochemical method by mixing component. The realization the formation of nanoparticles by get black Precipitate was shown in figure (4). The result in agreement with El-Sigeny (14). Composition of Iron Oxide was confirmed by changing the color to black with ultrasound.

Figur (4): IONPs prepared by sonochemical method

Physicochemical properties of NPs

Ultraviolet-Visible (Uv-Vis) Spectroscopy

UV-visible absorption spectrophotometer is widely used as a technique to examine the optical properties of certain nanoscale particles. The optical properties of the synthesized Iron Oxide Nanoparticles by UV-vis spectrophotometer at room temperature is demonstrated in Figure (5). Broad peak has been found in 345 nm, which is a characteristic standard peak of spherical phase Iron Oxide, demonstrating that the synthesized products are Iron Oxid. This sharp peak shows that the particles are in nanosize. The result agrees with (15).

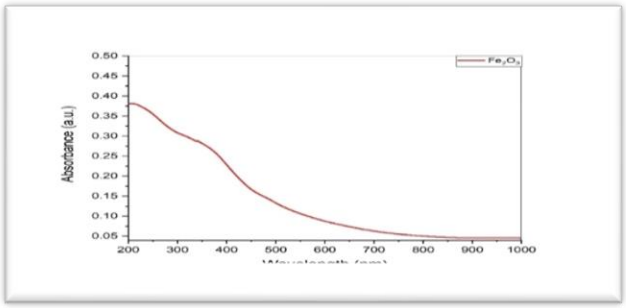


Fig (5) UV–vis spectrophotometry of IONPs.

A. X-Ray Diffraction (Xrd)

The determination XRD of crystallinity analysis was applied of IO nanoparticles. comparing with XRD patterns (16). As shown in Fig (5) .The XRD spectra of nanoparticle powder was proven in the figure revealed distinct peaks that corresponded to the diffraction peaks, where the grains (nanoparticles) is 33.64 nm. The IONPs was formed. (31.95) ,(35.8), and (45.6) were shown in Figure (6) which used to estimate the size of the crystallite by Debye-Scherrer equation of IO NPs (17).

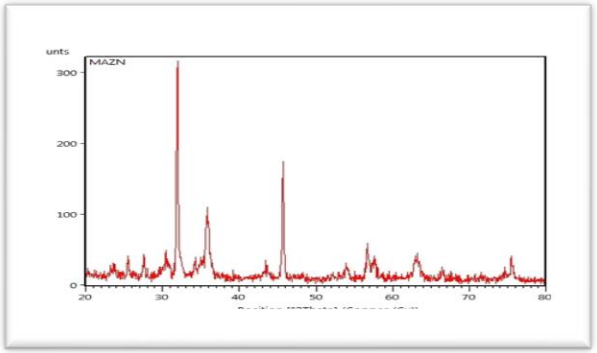


Figure (6): XRD analysis of synthesized IONPs

Afm Analysis

The confirmatory technique to characterize the synthesis of IONPs by AFM. The mean of their detecting average diameter were achieved from this study confirmed that the synthesized IONPs had average size of 25 nm as shown in the figure (7).with surface roughness 1.27 nm .The result agreed with the X-ray and TEM output. The topography Surface, size, and height of IONPs which surveyed by AFM analyses

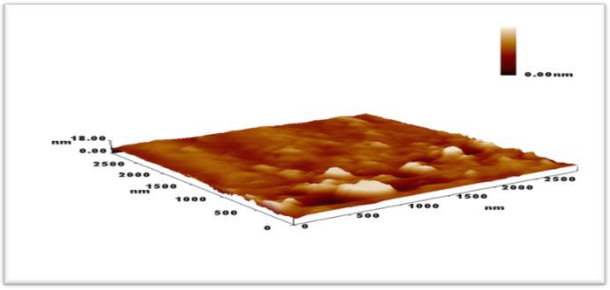


Figure (7): The synthesized IONPs 3 Dimension AFM for IONPs

D. Tem Analysis

. The morphology characterization was also carried out by the transmission electron microscopy (TEM). Figure (8) represents the TEM image of Ions. These images confirm that IONP is a scale of nanoparticles which the average size is in the range 25-30, grown in spherical shape ,which demonstrates the good quality of the IONPs and have good homogeneity. This is in close agreement with the results obtained from powder XRD measurement agreement with (18).

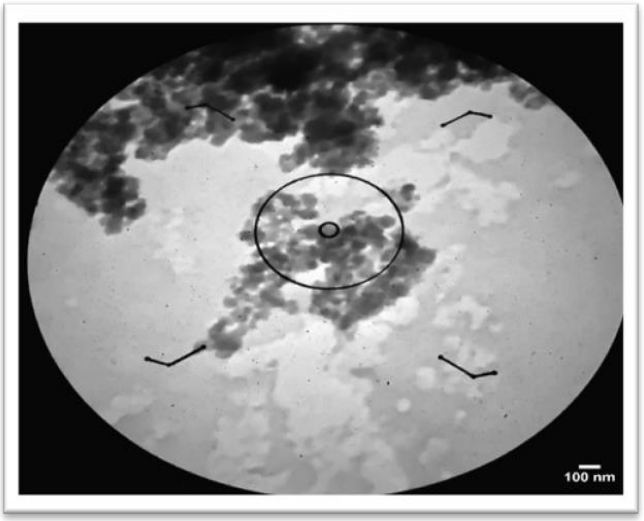


Figure (8): The Transmission electron microscopic (TEM) image of the IONPs

E. Energy Dispersive X-Ray Spectrometry (Edx)

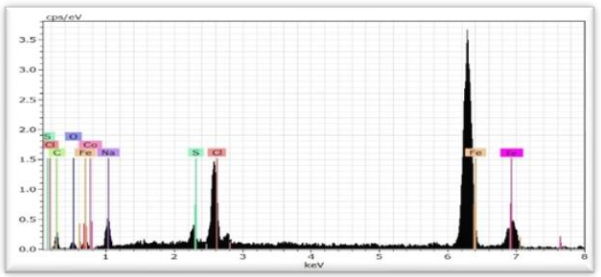


Figure 9 Energy dispersive X-Ray spectrometry (EDX)

Figure (9) shown EDX (Energy dispersive of X-Ray spectrometry) analysis for the nanoparticles which emphasis that the product is IONps.

F. Zeta Potential

The procedure for measuring the electrostatic potential at the electrical double layer around a nanoparticle in solution is described in this test. this is referred to as the zeta potential. Nanoparticles with a zeta potential between -10 and +10 mv are considered approximately neutral ,while nanoparticles with zeta potential of greater than +30 mv or less than -30 mv are considered strongly cationic and strongly anionic, respectively since cellular membranes are negatively charged ,zeta potential can affect a nanoparticles tendency to permeate membranes ,with cationic particle generally displaying more toxicity associated with cell wall disruption. IONPs with a zeta potential test has -19.54 as shown in figure (10).According (19) to analysis was performed to detect the surface charges acquired by iron oxide nanoparticles (Fe_3O_4). This test was conducted to get an idea of the stability of the obtained IO nanoparticles.

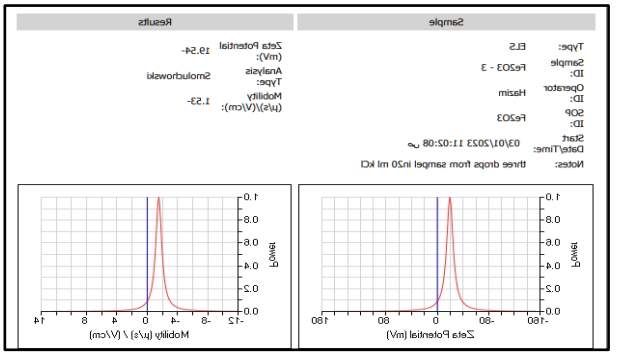


figure (10) showed the of IONPs nanoparticles

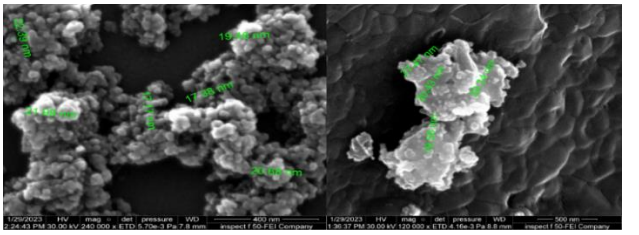


Figure (11): FESEM and size distribution of biosynthesized IONPs.

G. Field Emission Scanning Electron Microscopy Study (Fesem):

Results clearly exhibited that the majority of the synthesized nanoparticles were spherical in shape. The average size of chemically synthesized IONPs was 19-30 nm. Nanoparticles synthesized Ultrasound route were agglomerated in many areas which might be due to high surface energy (20). The sizes agglomeration of the IONPs and size of NPs are shown in Figure (11) for the FESEM images for different magnifications

Antibacterial Activities

The antibacterial capacity of novel preparation IONPs by sonicater physical method was evaluated at different concentration(64, 32,16, 8.4) µg/ml firstly against S. aureus by well diffusion method .The plates were incubated in (37c), the inhibition zone diameter was evaluated which proof the antibacterial capacity (Table 1). This experiment demonstrated that a higher concentration was (64 µg/ml) and the highest inhibition zone diameter value.as shown in figure (12).E. coli. In this case, a basic concentration of 16µg/ml was employed to measure the MIC assay.

The result agrees with (21)-resistant Gram-positive Staphylococcus aureus was performed using different concentrations (125 µg mL−1 to 30 µg ml, and Functionalized nanoparticles act as an antibacterial agent by interacting with the peptidoglycan cell wall and plasma membrane

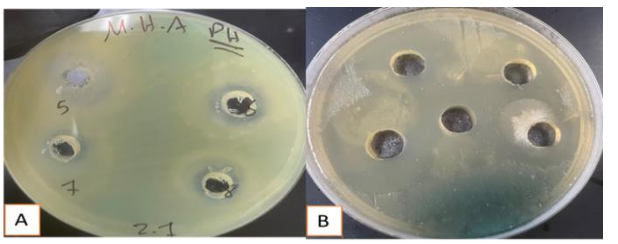


Figure (12): The inhibition zone values of IONPs different concentrations (64,32,16,8,4) µg/ml against two bacteria strains at 37Co for 24 hrs.

Table 1. Results for IONPs effects on bacterial strain

Different concentrations of IONPs dimeter of inhibition zone (mm)					
Bacterial strains	64 µg/ml	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml
S. aureus	15	13	10	8	5
P. aeruginosa	16	14	10	7	4

A) P aeruginosa B) S. aureus

Minimum inhibitory concentration of Vancomycin (VAN) against S. aureus by Well Diffusion Assay method (WDA):

Table (2): Inhibition zone (mm)of IO NPs combination Vancomycin (VAN)

IONPs+VAN	IONPs	Bacterial app
20	12	Staphylococcus aureus
21	18	Staphylococcus epidermidis
16	15	Pseudomonas aeruginosa
17	15	Salmonella spp
14	17	spp Klebsiella
13	12	Proteus mirabilis
19	19	E. coli

Figure (12) shows comparison of the inhibition zone diameter of each

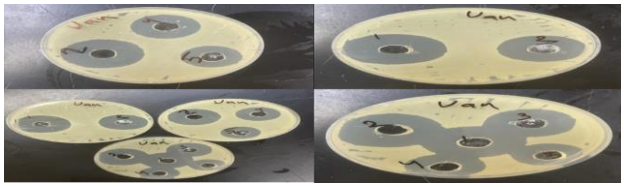


Figure (13): comparison of the inhibition zone diameter of each S. aureus bacteria after treatment with different concentration of Vancomycin (64, 32, 16, 8, and 4) µg/ml at 37C0 for 24 hrs on MHB.

S. aureus bacteria after treatment with different concentration of Vancomycin (64, 32, 16, 8, and 4) µg/ml. Where the lowest concentration showed significant inhibition against S.aureus bacteria, Where the lowest concentration 16 µg/ml showed significant inhibition against isolated. The bacteria as inhibition zone area 15mm is enlarged with increased concentration prepreation shown fig. [13], while at concentration 64µg/ml showed the highest inhibition against S.aureus reached 22 mm and lesser diameter reached 4 mm at 4µg/ml concentration . as shown in table (2). where the result showed the lowest effectiveness was in diameter of the inhibition reached 4mm at 8 µg/ml against negative bacteria. The result agreement with (22) Then, the antibiotic was loaded on synthesized nanoparticles and mixed with the IO NPs+Antibiotic and evaluated for antibacterial activities .

Synergistic IO NPs with Vancomycin (VAN) 16 µg/ml

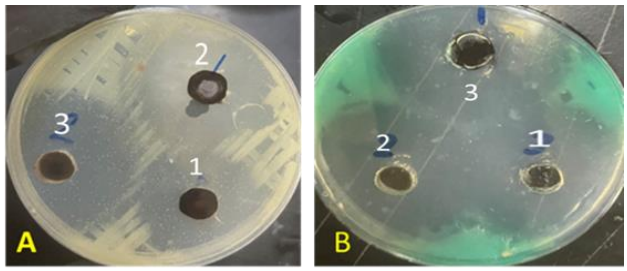
The MIC of Vancomycin and IO NPs for the Multidrug resistance bacteria isolates was determined as shown in table (2) Where we notice the synergistic effect of Vancomycin against positive bacteria of the S.aureus

more effective than negative bacteria, where the inhibition diameters (18)mm. Compared with IO NPs the work alone, it has less effect against Klebsiella spp , as it reached inhibition diameters (14) mm and less effects Synergistic nanoparticles against gram negative resistance bacteria Proteus mirabilis reached 13 mm inhibition diameters .

The IO NPs and VAN concentrations regarded as MIC are those where no detectable growth is observed. We note a synergistic effect of the VAN with iron nanoparticles against different species of bacteria, where the diameter of inhibition ranged (2) mm show Fig. (14).

The use action of IONPs of an alternating field allows additional increases in the bactericidal against S. epidermidis, P. mirabilis, and A. baumannii, causing cell death All the above-mentioned factors lead to the dissociation of bacteria from the bacterial cell wall, membrane rupture and death (23).

The bacteria inhibition mechanism is still under investigation; some theories state that the nanoparticles invade the cell remembrance to damage the enzymes of bacteria, which further induces cell death (24).



Fighter (14): Synergistic IO NPs with VAN by (WDA) method on MHA at 37C0 for 24hrs. Against (A) S. epidermidis (B) P. aeruginosa

1. IO NPs 2. IO NPs + GEN 3. IO NPs + VAN

The Cytotoxic Effect of Io Nps On Lymphocyte.

The results of the cytotoxicity assays revealed that no significant reduction in viability of human lymphocytes treated with the Iron Oxide Nanoparticles synthesized by sonochemical method These findings showed that these method could be

used as a good alternative to the current other physical methods associated with environmental toxicity.

The cytotoxicity was observed in human lymphocytes after 24 h of interaction of IONPs by MTT assays. MTT assays have been frequently used for determining the in vitro cytotoxicity of NPs in cell culture experiments, Results of MTT assay showed no significant reduction in cell viability compared with control in a dose-dependent manner. At (62.5, 125, 250, 500 and 1000 µg/ml). the Fe_2O_3 NPs have a similar significant decrease in cell viability and the lowest reduction in cellular viability. Table (3) and chart (1) shown the cellular viability in human lymphocytes by MTT assay when treated with IONPs. While the nanoparticles functionalized with show greater antioxidant activity, antilymphocyte and anticancer the potential use of nanoparticles as antibacterial agents, which can be a good alternative to marketed antibiotics.(25).

Table (3): Cellular viability in human lymphocytes by MTT assay when treated with IONPs

Viability %	Concentration µg/ml
103.1491003	1000 µg/ml
99.29305913	500 µg/ml
98.11482434	250 µg/ml
89.86718081	125 µg/ml
97.68637532	62.5 µg/ml

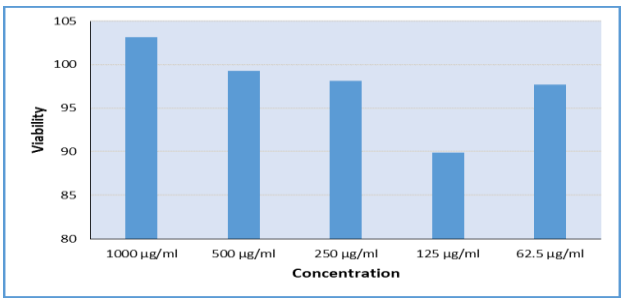


Chart (1): Cellular viability in human lymphocytes by MTT assay when treated with IONPs.

Conclusions

An alarming situation has been by multiseriate bacterial strains. IO NPs were successfully synthesized following an in, direct, eco- friendly, low cost, high-yield. And this study Iron nanoparticles showed exceptional antimicrobial activity against several bacterial strains. And this study demonstrates positive attributed of antibiotics- conjugated IONPs as promising antibacterial agent with low toxicity

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