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RESEARCH PAPER

Synthesis, Characterization and Biological Evaluation of $NiFe_2O_4$ Nanocomposite Against Bacteria and Breast Cancer Cells

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ABSTRACT

In this study, some spinel nanocomposites ($NiFe_2O_4$) were prepared by co-precipitation method ($NiFe_2O_4$) by green chemistry method. The spinel nanocomposites were characterized using FT-IR spectroscopy, ATR low total reflectance, XRD, EDX, SEM and AFM. FTIR spectroscopy of $NiFe_2O_4$ showed a sharp and medium band at frequency (1420 cm) because of the ʲ (Ni-O) bond's stretching and another acute and long band at frequency (1567 cm) due to the stretching of the ʊ (Fe-O) bond. ATR showed similar values to FTIR. XRD results showed that The produced nano-nickel ferrite has an average grain size of 30.70 nm. The average grain size in the scanning electron microscope (SEM) of $NiFe_2O_4$ was 94.795 nm, and in the AFM device the average grain size was 71.9 nm. The diagnosis was confirmed using the 2ᵉ VIT device, and the toxicity was studied. Spinel nanocomposite prepared on human umbilical vein endothelial cell (HUVEC) cell line and MTT and MCF-7 assay using ELISA device in Iranian laboratories. The results by using GraphPad Prism 8.0 software. The results showed that the effectiveness and toxicity of $NiFe_2O_4$ were as follows: The highest inhibition rate was 61.72% after 24 hours and 80.6% after 48 hours at a concentration of 400 µg/ml, and the lowest inhibition rate was 8.35% after 24 hours and 14.72% after 48 hours at a concentration of 25 µg/ml.MCF-7 in 24 h 95.17% and in 48 h 97.87% and the lowest inhibition rate was in 24h 26.58% and in 48h 32.62% The inhibition of the $NiFe_2O_4$ nano spinel composite was also measured using five serial concentrations (500, 600, 700, 800, 900, and 1000) µg/ml.

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INTRODUCTION

Nanotechnology is a area of study and invention that focusses on creating objects (materials and gadgets) at the atomic and molecular scale [1]. One billionth of a meter is called a nanometre. Human hair typically has a diameter of 80,000 nanometres. The standard laws of physics and chemistry do not apply at such scales. [2,3]. They are particles with at least two dimensions ranging from 1 to 100 nanometers on the nanoscale [4]. Nickel ferrite nanoparticles are considered an adsorbent because to their high adsorption capacity, low toxicity, robust superparamagnetic characteristics, ease of synthesis, and outstanding biocompatibility. $NiFe_2O_4$NPs exhibit the antiparallel spin magnetic moment of spinel,

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which arises from the antiparallel spin magnetic moment between tetrahedral sites of Fe3+ ions and octahedral sites of Ni2+ ions. $NiFe_2O_4$NPs have a low mass transfer resistance and a large surface area. These nanoparticles' magnetic behaviour is primarily size-dependent. [5]. Because of their great resistance and thermal durability, spinel ferrite nanoparticles, like $NiFe_2O_4$, are extremely significant magnetic materials. They are applicable to many different fields. Because magnetic nanoparticles (MNPs) are easily recognised by amino acids, proteins, DNA, and carbohydrates in vivo, they have become appealing solid scaffolds for catalysis and are utilised in drug design [6]. Synthesized nanoparticles with soft ferromagnetic nature [7]. Nanomaterials have applications in water and wastewater treatment [8]. Poor drinking water and sanitation pose risks of waterborne diseases, especially among vulnerable groups such as children and women [9]. removing hazardous and poisonous substances from water and wastewater by using nanostructured materials as adsorbents [10,11]. Used as antibacterial agents during wastewater treatment [12]. To further explore the multifunctionality of this material, we studied its cytotoxicity against human breast cancer cells (MCF-7) and HUVECs. MCF-7 cells were considered a harmful cell and HUVECs were considered a healthy cell. The goal was to compare the rate of cell killing and the toxicity of nanomaterials to healthy cells. $NiFe_2O_4$ demonstrated promising anticancer activity, with selective toxic effects on cancer cells and low toxicity to normal human cells (HUVECs).

MATERIALS AND METHODS

The following materials were used: $Ni(NO_3)_2.6H_2O$, $Fe(NO_3)_3.9H2O$, Citric acid anhydrous, deionized water, and NH4OH. Muller Hinton broth, Nutrient agar, MacConkey agar , Eosin Methylene Blue agar MCF-7 breast cancer cell dishes and umbilical cord cell dishes HUVECs.

Synthesis and characterization

Preparation of $NiFe_2O_4$ nanoparticles

1 g of $Ni(NO^3)_2.6H2O$, 1 g of Fe $(NO_3)_3.9H_2O$, and 1 g of Citric acid Anhydrous were mixed in 150 ml of deionized water and placed on a magnetic stirrer at 25°C for 20 minutes. The pH was adjusted to 7.5 by adding NH_4OH. The mixture was heated at 135°C until a gel was formed and a precipitate formed [13].

Inhibition test compounds spinel nanoparticles for bacteria

Escherichia coli, or Escherichia coli, was chosen for the antagonism test because it is one of the most important causes of waterborne diseases, especially severe diarrhoea in children, in addition to other members of the Enterobacteriaceae family, to which this bacterium belongs.

Removal and Identification of Bacteria from River Water

1. Water was collected from the Maqsudiya River (Diyala River and various locations) at a depth of 30 cm using plastic bottles with a top. These bottles were sealed immediately after signing the contract before being transported to the Microbiology Laboratory (Biotechnology Department/College of Science/University of Diyala) on foot, following the following steps:

2. 100 µL of thawed water was cultured on MacConkey agar medium, with three replicates, and incubated at 37°C for 24 hours in aerobic conditions.

3. After the incubation period, the expected colonies were identified (subcultured) on fresh MacConkey agar medium and incubated for twenty-four hours at 37°C. Until the bacteria were identified, the plates were kept in the refrigerator at 4°C.

4. The bacteria were identified by rapid colony formation, microscopic examination, biochemical tests, and growth on methylene blue eosin agar in the Advanced Microbiology Laboratory at the Department of Biotechnology, College of Science, University of Diyala. Then, a certification certificate was issued using the VITEK® 2 Compact system.

5. The isolate was stored on three plates of nutrient agar in the laboratory for viruses.

Bacterial suspension preparation

Prepare the bacterial suspension in Mueller Hinton Broth medium by raising 4-5 single colonies of activated bacteria and mixing them in the liquid medium using a Vortex mixer. The tubes are then compared with standard McFarland tubes to achieve a cell concentration of approximately 0.5% in the suspension)1.5 x 10^8 cfu/ml(.

Preparation of nano-nickel ferrite $NiFe_2O_4$ compound solution

Each nanocomposite solution was prepared at five multiple concentrations (200, 400, 600, 800,

1000 µg/ml) in deionized distilled water and mixed using an ultrasonic water bath and used directly in the antagonism test.

Primary antibacterial test compounds oxides nanoparticles

To identify the antagonistic effect of this compound ($NiFe_2O_4$) against E. coli isolated from river water, the test was conducted using microdilution method in Eppendorf tubes containing 100 µl of each concentration of nano oxide compounds and 100 µl of bacterial suspension to a final concentration of (500, 400, 300, 200, 100) µg/ml. The tubes were incubated at 37°C for 24 hours. After the incubation period, 100 µl of each tube was taken and spread on MacConkey agar medium and the plates were re-incubated at 37°C for 24 hours. 100 µl of bacterial suspension only was spread on the same medium as negative control. The plates were examined and compared with the negative control plates after 24 hours.

Secondary testing of compounds oxides nanoparticles

Based on the results of the initial antagonism test above, a secondary antagonism test was conducted using higher final concentrations of the nano oxide compounds (500, 600, 700, 800, and 1000 µg/ml) using the microdilution method, but in microtiter plates. The test was conducted according to the following steps:

A solution of each compound was prepared at five multiple concentrations (1000, 1200, 1400, 1600, and 2000 µg/ml) in distilled deionized water. The mixtures were mixed using an ultrasonic water bath and used directly in the antagonism test.

100 µl of the nano oxide diluent was added to the holes of the microtiter plates, with three replicates for each concentration.

100 µL of the bacterial culture prepared with the initial solution was added to each well.

200 µL of the bacterial culture was added in three replicates as a negative control.

A microplate reader was used to measure the light absorbance at a wavelength of 630 nm after the plate had been incubated for 24 hours at 37°C.

The percentage of bacterial growth inhibition (%) by the nano-oxide compounds was calculated using the Eq. 1:

Cytotoxicity assay $NiFe_2O_4$ On cells (HUVECs)

The cytotoxicity of the nanooxides against human umbilical vein endothelial cells (HUVECs) was evaluated by MTT assay. Cell lines were grown in sterile plastic cell culture flasks containing IRPM medium to a concentration of 1 x 10^5 cells/ml. The cultured flasks were incubated in a 5% carbon dioxide incubator at 37°C for 5 days, with daily monitoring for microbial contamination using an inverted microscope. Double concentrations of nanooxides were prepared in distilled deionized water and distributed into the wells of microplates. MTT staining was prepared in phosphate buffer saline and filtered using Millipore filters (0.22 µm) and stored in the refrigerator until use.

Nickel ferrite test for killing breast cancer cells (MCF-7)

100 microliters of cancer cell culture at a concentration of 1 x10^4 cell/ml was put into the microplate's wells, and the plate was incubated for 24 hours at 37°C with 5% carbon dioxide present. Following the incubation phase, 100 microliters of secondary metal sulfide solutions were added to obtain final concentrations (25, 50, 100, 200, and 400 µg/ml), with each concentration having three duplicates. The plates were then incubated under the same conditions for 24 hours. To achieve a color change, 10 microliters of MTT Each treated well in the microplate received an addition of staining solution. After that, the plate was incubated for five hours at 37°C with 5% carbon dioxide present. Using a microplate reader, a colour shift was detected at 570 nm in wavelength. Two control treatments were used in the test: the first consisted of cell culture alone, and the second consisted of cell culture with DMSO added instead of the secondary compounds. The percentage inhibition of cell growth was calculated using the following equation.

The method of work

The cell suspension was distributed into the wells of the microplate at a rate of 100 µL and incubated for 24 hours at 37°C in the presence

$$\text{Inhibition Percentage (\%)} = \frac{(\text{Absorbance of Negative Control} - \text{Absorbance of Sample})}{\text{Absorbance of Negative Control}} \quad (1)$$

of 5% carbon dioxide gas. Three replicates of 100 µL of each concentration of the nanocomposite solutions were added to each well, and the plate was re-incubated under the same conditions. After the incubation period, 10 µL of MTT staining solution was added to each well, and the plate was re-incubated under the same conditions for 5 hours. The color change of each well was read using a microplate reader at a wavelength of 570 nm. The first control treatment consisted of the cell suspension only, while the second control treatment consisted of the cell suspension supplemented with deionized distilled water. The percentage of cell growth inhibition was calculated using the following equation:

Inhibition percentage (%) = Read the control transaction - Read the nano transaction/ Read the control transaction x 100%

Characterization techniques

$NiFe_2O_4$ nanoparticles were characterized using several techniques, including X-ray diffraction (XRD) using a Shimadzu X-ray diffractometer (Kyoto, Japan). FTIR spectra of the samples were obtained using a Shimadzu FTIR spectrometer (Tokyo, Japan), using sulfur bromide granules. Scanning electron microscopy (SEM) was performed using a Zeiss (Germany) at 200 kV. EDS, AFM, and ELISA were also used.

FTIR spectra of $NiFe_2O_4$ NPs

Nickel ferrite ($NiFe_2O_4$) differs from the FT-IR spectrum of Ni(NO3)2.6H2O and $Fe(NO3)_3$.9H2O salts, as clearly shown in (1) [6]. The appearance of a fast and medium band at a frequency of (1420 cm) is due to the clock stretch U(Ni-O) and another sharp and long band at a frequency of (1567 cm) is due to the clock stretch U(Fe-O) [14–16] . as shown in Fig. 1a. A direct and long band at a frequency of (11384 cm) is due to the squeeze stretch U(C-O) and a medium inspection band at a frequency of (11591 cm) is due to the victory stretch U(C = O). A broad and distinct band appears at a frequency of (13408 cm) especially for the compression stretch U(O-H) [17].

The crystal structure of the samples was assigned to the prepared nano-nickel ferrite ($NiFe_2O_4$) by X-ray diffraction as shown in Fig. 1b. The X-ray diffraction spectrum of the prepared nickel ferrite was matched with the standard spectrum ($NiFe_2O_4$) according to the data of the International Centre for X-ray Diffraction (ICDD), Card No.: 742081 [18]. The differential nickel ferrite ($NiFe_2O_4$) was detected by the diffraction peaks (18), (26), (31), (36), (38), (43), (54), (57), (63), (72), and (77), respectively. The average crystalline zonal nickel ferrite was 30.70 using the

Table 1. Shows the calculation of the average grain size of nano nickel ferrite.

K	λ (Å)	Peak position 2θ (°)	FWHM B_{size} (°)	Dp (nm)	Dp Average (nm)
0.94	1.54178	18.5544	0.3936	21.38	30.70
		25.6619	0.1476	57.70	
		30.442	0.2952	29.15	
		35.8338	0.246	35.48	
		37.423	0.1968	44.55	
		43.4679	0.2952	30.28	
		53.9703	0.3936	23.67	
		57.5005	0.3936	24.06	
		63.1015	0.2952	33.01	
		71.6516	0.5904	17.35	
		74.7739	0.492	21.24	
		75.6695	0.3444	30.53	

Debye-Scherer equation [19,20], as shown in the Debye equation (Eq. 2).

$$D_N = \frac{K\lambda}{\beta COS\theta} \quad (2)$$

Where, D = Average crystallite size (nm), K = A constant that depends on the crystal form (0.94 -0.89), λ = X-ray wavelength (1.54059 Å) Most λ for copper, (FWHM = B midpoint of highest peak, θ = Bragg angle.

The elemental content of the nano-sized nickel ferrite ($NiFe_2O_4$) was characterized by energy dispersive X-ray diffraction (EDX), as shown in Fig. 1f. The results showed the presence of nickel (38.4%) and iron (34.4%). There were also very small percentages of carbon, oxygen, calcium, and the prepared spinel material had good purity [21,22]. The morphological and structural compositions of the nano-nickel ferrite ($NiFe_2O_4$) were studied using scanning electron microscopy (SEM). Fig. 1c shows that the prepared nano-nickel ferrite particles are within the nanometer range. SEM images showed that While the majority of the nanoparticles were present in an agglomerated form, others were well separated from one

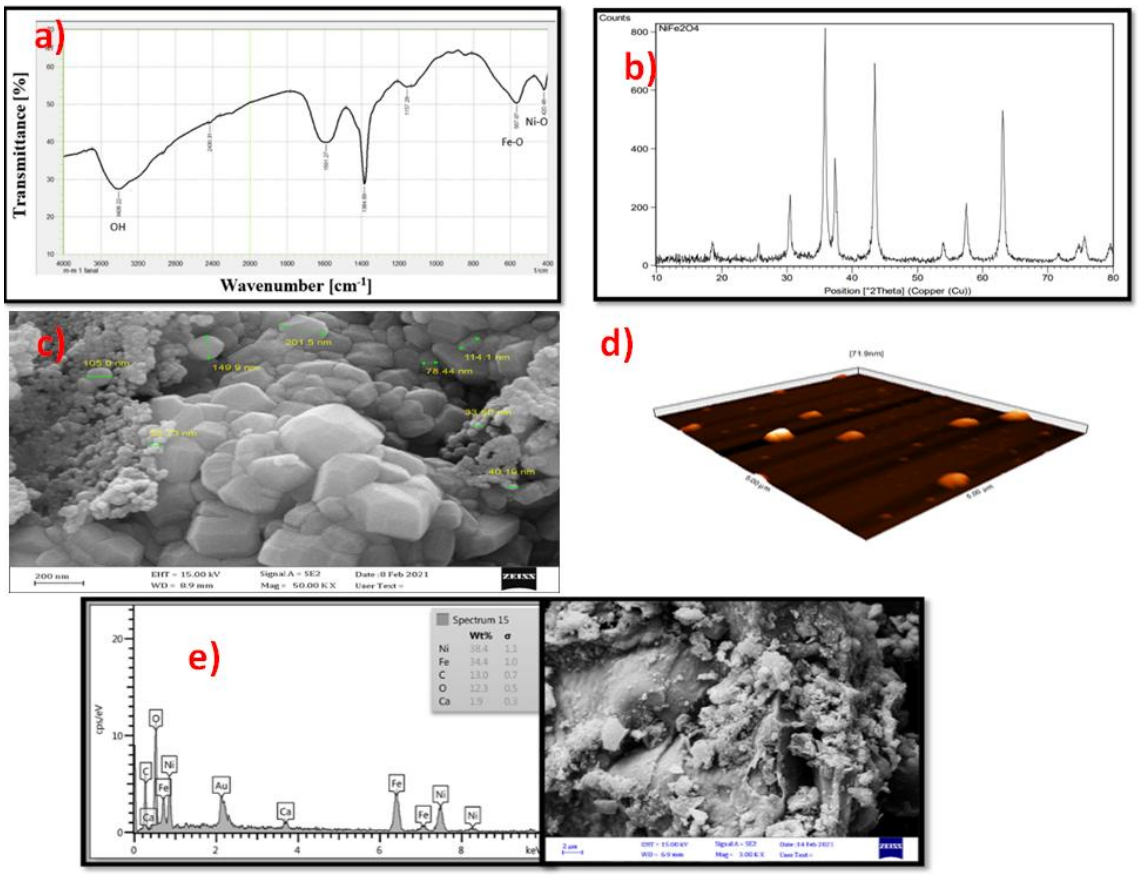

Fig. 1. a) FT_IR, b) XRD, c) SEM; d) EDX; and e) AFM for nanocomposite

Table 2. Shows the effect of nickel ferrite on bacteria.

Compound	Inhibition (%) Mean±SD					
	Concentration (µg/ml)					
	500	600	700	800	900	1000
$NiFe_2O_4$	17.6±1.51	29.5±0.47	37.6±0.72	42.4±1.94	55.7±0.26	60.4±0.46

another. Electrostatic forces are the cause of this aggregation, which is in line with comparable nanoparticle agglomeration behaviour observed in earlier research. The average diameter of these particles is about 94.795 nm as shown in Fig. 1f [23]. The prepared nano-nickel ferrite (NiFe2O4) was analyzed using Atomic Force Microscope (AFM) and the average grain size (Ave. Diameter)

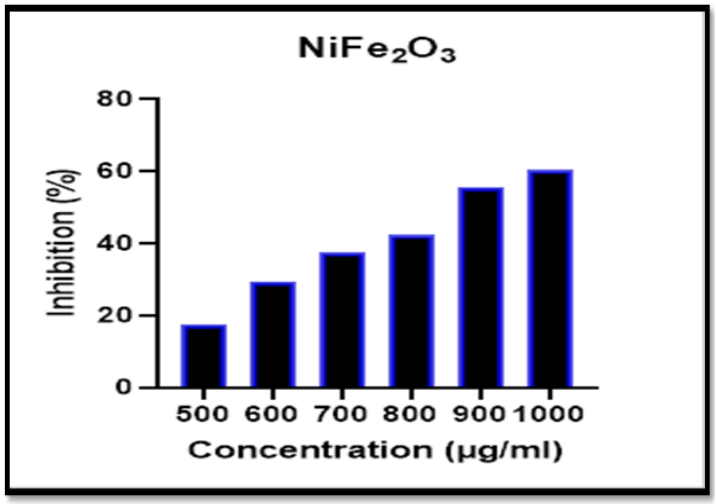

Fig. 2. Shows the effect of nano nickel ferrite concentration on the growth of E. coli.

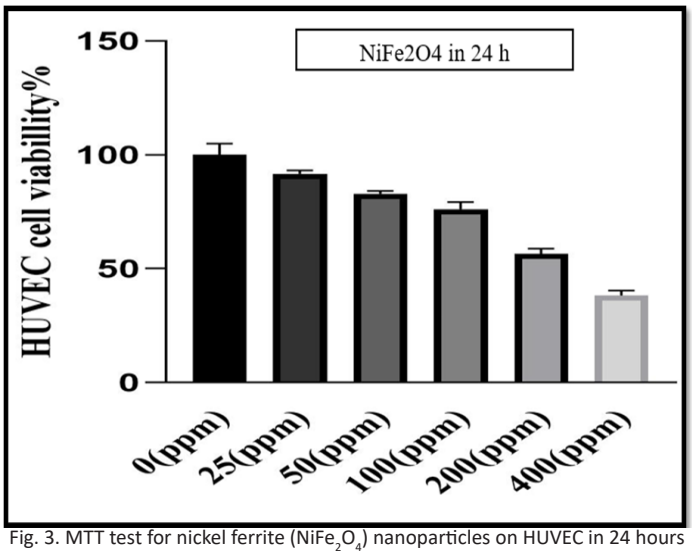

Fig. 3. MTT test for nickel ferrite ($NiFe_2O_4$) nanoparticles on HUVEC in 24 hours

Table 3. Shows the cytotoxicity of nano-nickel ferrite in 24 hours for HUVEC cells.

Concentration	R1%	R2%	R3%	Mean%	SD
0	95.61338	99.06313	105.3238	100.0001	4.92254
25	93.44131	91.14148	90.37487	91.65256	1.595827
50	82.83654	84.24199	81.55885	82.87913	1.342075
100	77.08696	78.74795	72.61506	76.14999	3.171987
200	54.08864	58.6883	56.64401	56.47365	2.304559
400	40.28965	35.94552	38.62866	38.28795	2.192014

was (71.9 nm), the average roughness (Sa. Roughness average) was (207 pm) picometers and the root mean square (Sq. Root mean square) was (351 pm) picometers[24,25].

Antibacterial activity of nano-nickel ferrite against E. coli

The effect of adding different concentrations of nano-nickel ferrite ($NiFe_2O_4$) on inhibiting E. coli was studied as shown in Table 2 and Fig. 2, where it is noted that there is a direct relationship between the concentration of ($NiFe_2O_4$) and the percentage of bacterial growth inhibition, as The greatest percentage of inhibition attained 60.4% at a concentration of (1000µg/ml). While the lowest inhibition percentage reached 17.6% at a concentration of (µg/ml500). The results of the statistical analysis showed that nickel ferrite (NiFe2O4) inhibited the growth of E. coli bacteria using the program (Graph Pad Prism 8.0) and (correlation) analysis was used to infer the significance, reaching a value of (P-value <0.0001) and there is a significant significant difference between the concentration and the percentage of cell survival Significant diff. Among means ($P < 0.05$) yes and the value of R square was equal to (0.9918) which indicates the presence of a strong correlation between inhibition of E. coli growth and increasing concentration ($NiFe_2O_4$).

Nickel ferrite ($NiFe_2O_4$) nanostructures have demonstrated antibacterial activity against Escherichia coli. These results are important for understanding their effects in toxicological studies [26], indicating that nano-nickel ferrite ($NiFe_2O_4$) has the ability to kill a variety of bacterial types. Potential uses for the nanocomposite could include disinfecting water. Because of its low rate of corrosion, nickel ferrite ($NiFe_2O_4$) is a more cost-effective and efficient structure for industrial applications than Fe_3O_4 [27]. It has shown important applications for inactivating common water-associated bacteria, as well as viruses and other pathogens [28].

Toxicity test of nano nickel ferrite ($NiFe_2O_4$) on HUVEC cells

The cytotoxicity of nickel ferrite ($NiFe_2O_4$) nanoparticles on HUVEC cell lines was studied by MTT assay at different concentrations (25, 50, 100, 200, 400 µg/ml) for 24 and 48 hours compared to the control group. The results showed that the percentage of HUVEC cells survival after 24 hours of adding nano-nickel ferrite ($NiFe_2O_4$) at a concentration of (25µg/ml) was 91.65%, indicating that this low concentration had no effect on HUVEC cells. However, we find that the percentage of

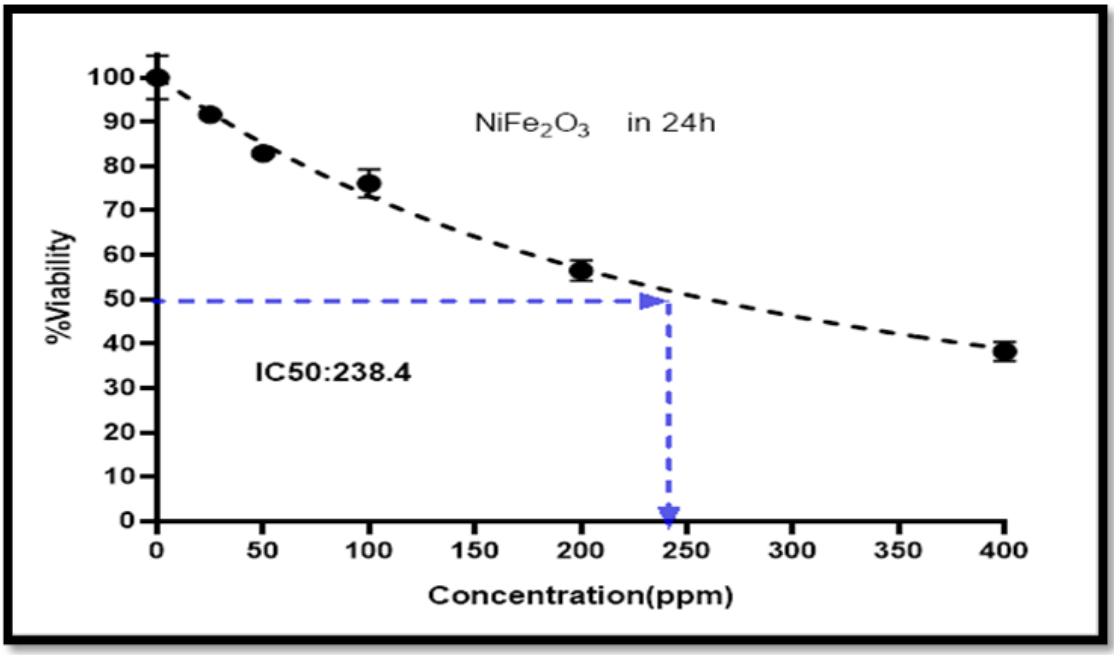

Fig. 4. IC50 for nickel ferrite nanoparticles ($NiFe_2O_4$) in 24 hours

HUVEC cells survival at a concentration of (50µg/ml) was 82.87%, as the percentage decreased, indicating that there is a relationship between increasing the concentration and the percentage of effectiveness or killing, while the percentage of cells survival at a concentration of (100µg/ml) was 76.14%, and at a concentration of (200µg/ml) the percentage of HUVEC cells survival was 56.47%, but at a concentration of (400µg/ml) the percentage of HUVEC cells survival was 38.28%, indicating a decrease in the number of living cells by more than half, as shown in Table 3 and Fig. 3.

The statistical analysis results showed that the nano-nickel ferrite ($NiFe_2O_4$) on HUVEC cells in 24 hours using the program (Graph Pad Prism 8.0) used one-way analysis of variance (One way ANOVA) to infer significance, where the value reached (P-value < 0.0005) and there is a significant significant difference between the concentration and the percentage of cell survival (Significant diff. Among means (P < 0.05) yes) and the value of R square was equal to (0.9498), which indicates the presence of a strong correlation between the variables. The half-maximal inhibitory concentration (IC50) of nano-nickel ferrite on HUVEC cells in 24 hours was measured using the program (Graph Pad Prism 8.0) using normalized response analysis and the value was (IC50= 238), as shown in Fig. 4.

While the results shown in Table 4 showed the cytotoxicity and were examined at different concentrations (from 25 to 400 micrograms/ml) compared to the control group, the results showed that the percentage of HUVEC cell survival after 48 hours of adding nickel ferrite ($NiFe_2O_4$) at a concentration of (25µg/ml) was 85.28%, which

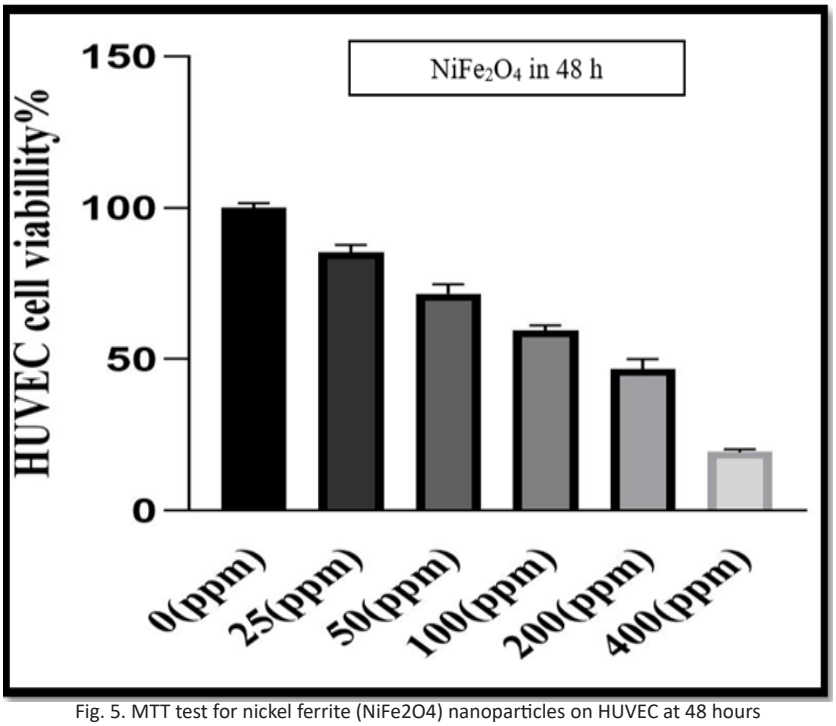

Fig. 5. MTT test for nickel ferrite (NiFe2O4) nanoparticles on HUVEC at 48 hours

Table 4. Shows the cytotoxicity of nano-nickel ferrite in 48 hours for HUVEC cells.

Concentration	R1%	R2%	R3%	Mean%	SD
0	101.8288	98.83628	99.33504	100	1.603267
25	87.24027	82.37743	86.24277	85.28682	2.568498
50	74.89613	68.53703	71.52955	71.65424	3.181384
100	60.43229	57.43978	60.43229	59.43479	1.727731
200	43.22531	49.83379	47.09065	46.71658	3.320082
400	18.66172	19.16047	20.40736	19.40985	0.89914

indicates that there is a relationship between the time factor and concentration on the percentage of HUVEC cell survival, but we find that the percentage of HUVEC cell survival at a concentration of (50µg/ml) was 71.65%, the percentage decreased, which indicates that there is a relationship between increasing the concentration and the percentage of effectiveness or killing, while the percentage of cell survival at a concentration of (100µg/ml) was 59.43%, as it appears that the percentage decreased, and at a concentration of (200µg/ml) the percentage of HUVEC cell survival was 46.71%, as it appears that the percentage decreased to half, but at a concentration of (400µg/ml) the percentage was HUVEC cell survival was 19.40%, indicating a decrease in the number of live cells by more than half, as shown in Table 4 and Fig. 5.

While the results of the statistical analysis showed that the nano-nickel ferrite (NiFe2O4) on HUVEC cells in 48 hours, where the value reached (0.0014 P-value < 0.05) and there is a significant difference between the concentration and the percentage of cell survival (Significant diff. Among means (P < 0.05) yes) and the value of R square was

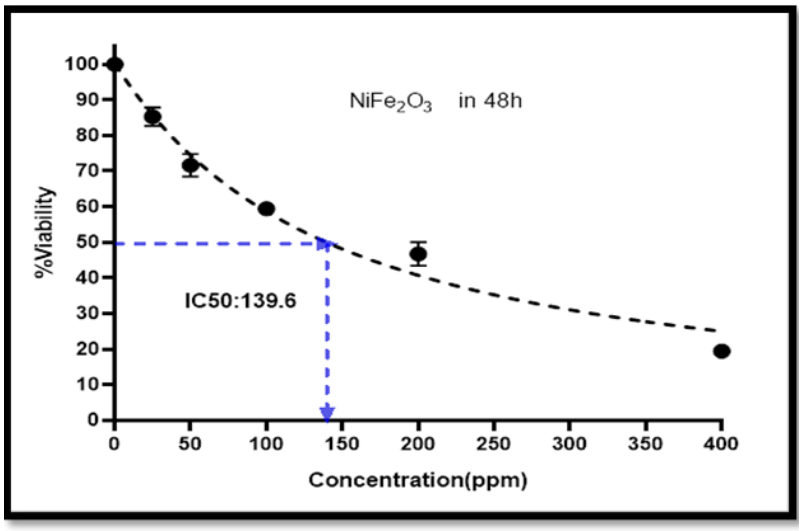

Fig. 6. IC50 for nickel ferrite nanoparticles ($NiFe_2O_4$) in 48 hours

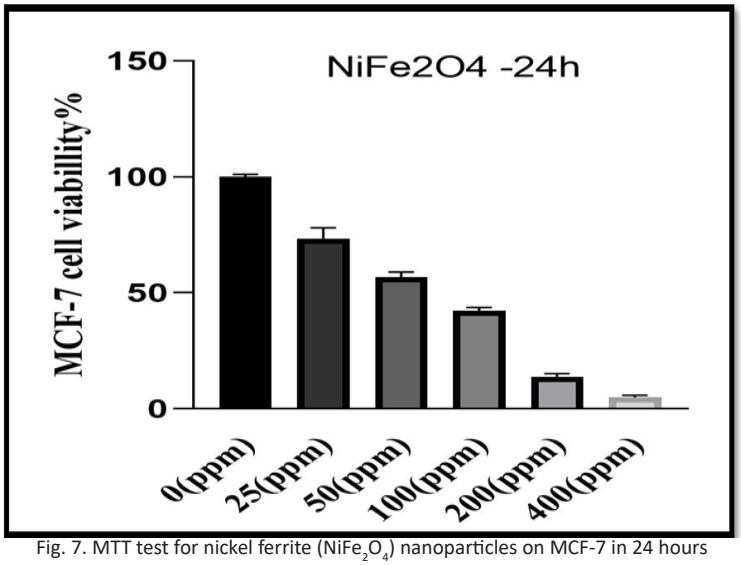

Fig. 7. MTT test for nickel ferrite ($NiFe_2O_4$) nanoparticles on MCF-7 in 24 hours

equal to (0.9160), which indicates the presence of a strong correlation between the variables. The half maximal inhibitory concentration (IC50) of nano-nickel ferrite on HUVEC cells in 48 hours was measured using the program (Graph Pad Prism 8.0) using normalized response analysis and the value was (IC50= 139), as shown in Fig. 6.

Inhibition assay of ($NiFe_2O_4$) nanoparticles on MCF-7 breast cancer cell lines

The cytotoxicity of nickel ferrite ($NiFe_2O_4$) nanoparticles on MCF-7 cell lines was studied by MTT assay at different concentrations (400, 200, 100, 50, 25 µg/ml) for 24 and 48 hours compared to the control group. The results showed that the percentage of survival of MCF-7 cells after 24 hours of adding nano-nickel ferrite ($NiFe_2O_4$) at a concentration of (25µg/ml) was 73.42%, indicating that this low concentration had no effect on MCF-7 cells. However, we find that the percentage of survival of MCF-7 cells at a concentration of (50µg/ml) was 56.74%, as the percentage decreased, indicating that there is a relationship between the increase in concentration and the percentage of effectiveness or killing, while the percentage of survival of cells at a concentration of (100µg/ml) was 42.19%. At a concentration of (200µg/ml), the percentage of survival of HUVEC cells was 13.80%, but at a concentration of (400µg/ml), the percentage of survival of MCF-7 cells was 4.93%, indicating a decrease in the number of living cells by more than half, as shown in Table 5 and Fig. 7.

The statistical analysis results of the nickel ferrite ($NiFe_2O_4$) nanoparticles on MCF-7 cells over 24 hours using GraphPad Prism 8.0 software showed that a one-way analysis of variance (ANOVA) was used to determine significance. The P-value was < 0.0001, and there was a significant difference between the concentration and the cell viability rate (significant diff. Among means ($P < 0.05$). The R square value was 0.949, indicating a strong correlation between the variables. The half-maximal inhibitory concentration (IC50) of the nickel ferrite nanoparticles on MCF-7 cells over

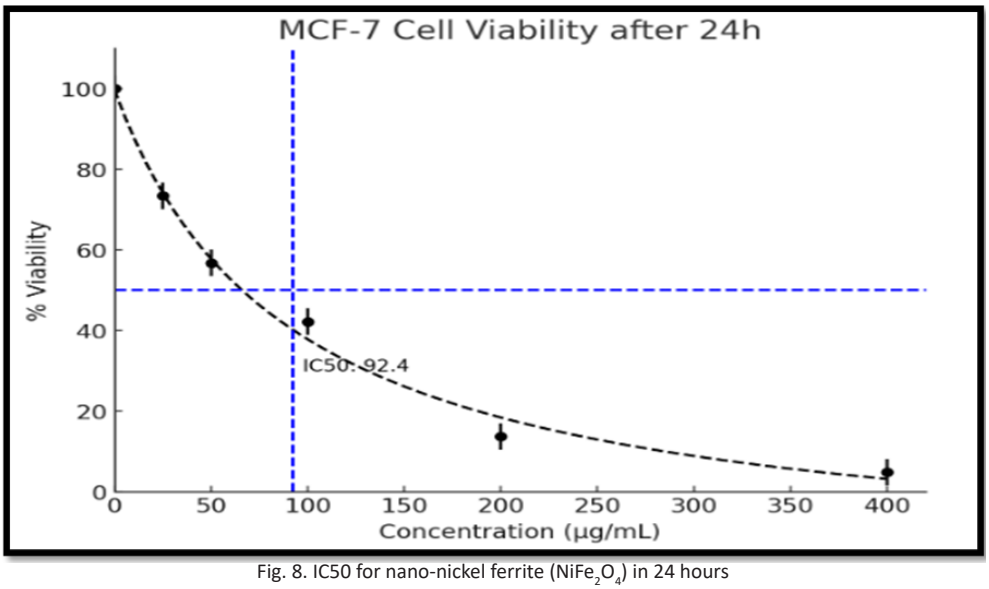

Fig. 8. IC50 for nano-nickel ferrite ($NiFe_2O_4$) in 24 hours

Table 5. Shows the cytotoxicity of nano-nickel ferrite in 24 hours for MCF-7 cells.

Concentration	R1%	R2%	R3%	Mean%	SD
0	100.0456	98.93628	101.0317	100.0045	1.048305
25	78.7218	69.97042	71.57278	73.42167	4.65945
50	58.87711	56.78171	54.56305	56.74062	2.157325
100	43.83952	41.99063	40.75804	42.19606	1.550975
200	15.3667	13.39455	12.655	13.80542	1.40176
400	5.136201	5.629237	4.02687	4.93077	0.820699

24 hours was measured using GraphPad Prism 8.0 software using normalized response analysis, and the IC50 value was = 92.45 µg/mL, as shown in Fig. 8.

While the results were listed in Table 6 for cell viability examination and different concentrations (from 25 to 400 µg/ml) compared with the set of clear results, the percentage of MCF-7 cells survival after 48 hours of adding nickel ferrite ($NiFe_2O_4$) at a concentration of (25 µg/ml) was 67.38%, which indicates that there is a relationship between the time factor and the percentage of MCF-7 survival,

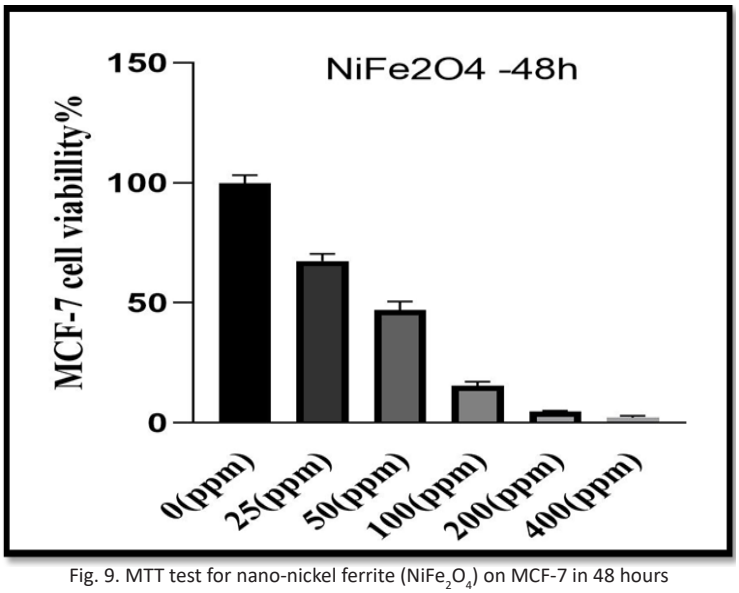

Fig. 9. MTT test for nano-nickel ferrite ($NiFe_2O_4$) on MCF-7 in 48 hours

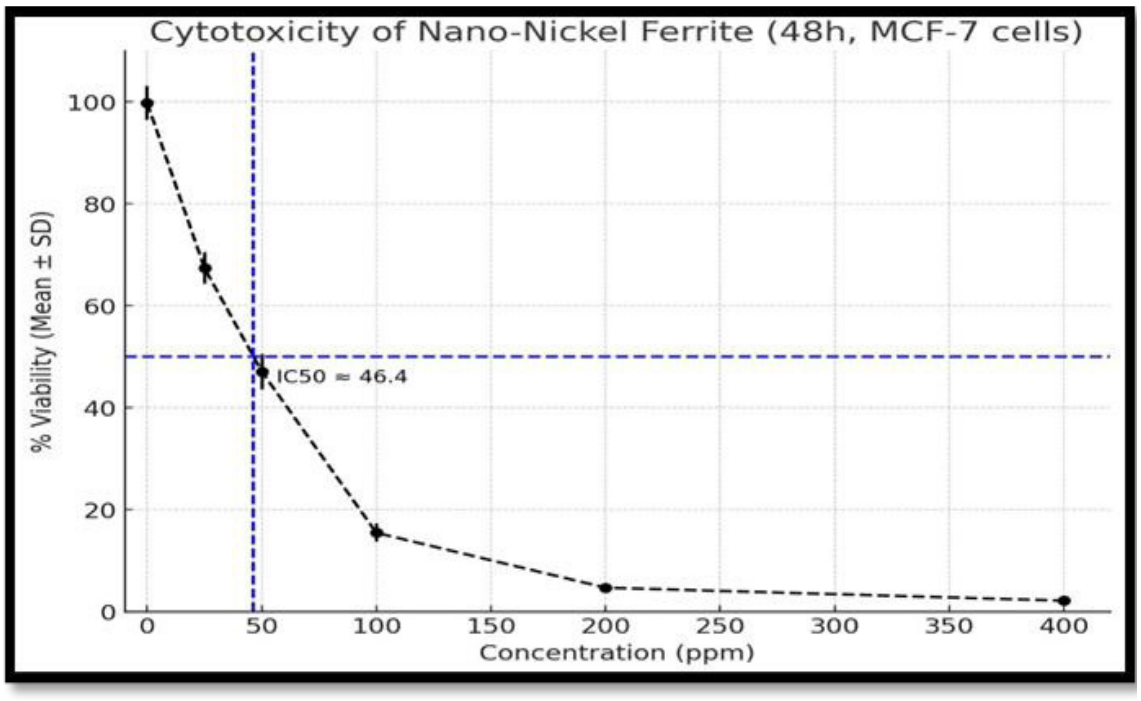

Fig. 10. IC50 for nano-nickel ferrite ($NiFe_2O_4$) in 48 hours.

Table 6. Shows the cytotoxicity of nano-nickel ferrite in 48 hours for MCF-7 cells.

Concentration	R1%	R2%	R3%	Mean%	SD
0	98.81302	103.4969	96.96413	99.758	3.367324
25	70.95649	65.40984	65.77961	67.38198	3.101133
50	47.41403	50.37224	43.46974	47.08534	3.46297
100	16.35277	13.39455	16.59929	15.44887	1.783355
200	4.766424	5.012942	4.273388	4.684252	0.376562
400	1.561691	3.040799	1.808209	2.1369	0.792444

but I noticed that the survival of MCF-7 cells at a concentration of (50 µg/ml) was 47.08%, which indicates that there is a relationship between the increase in concentration and the percentage of effectiveness or killing, while the percentage of cells survival at a concentration of (100 µg/ml) was 15.44%, as it was shown that the percentage of concentration of Vio is (200 µg/ml) the percentage of MCF-7 cells survival was 4.68%, as it was shown that the release calculation at a concentration of (400) µg/ml) the percentage of MCF-7 cells survival was 2.13%, which indicates The number of award-winning cells decreased by more than half, as in Table 6 and Fig. 9.

The statistical analysis of the effect of $NiFe_2O_4$ nanoparticles on MCF-7 cells after 48 h demonstrated a notable, dose-dependent decline in cell viability. One-way ANOVA analysis (GraphPad Prism 8.0) showed a highly significant difference among means ($P < 0.0001$). The regression model exhibited a strong correlation (R^2=0.98). The half maximal inhibitory concentration (IC_{50}) of $NiFe_2O_4$ nanoparticles on MCF-7 cells after 48 h was determined using normalised response analysis to be 42.25 µg/mL. As shown in Fig. 10.

CONCLUSION

In this work, we have described the synthesised $NiFe_2O_4$ nanoparticle by the coprecipitation method. The characterization of the structural properties of $NiFe_2O_4$ nanoparticles was carried out using FTIR, XRD, and SEM. According to the XRD and SEM analysis, the average size of NiFe2O4 nanoparticles sized 30.70 nm in XRD and 94.795 nm in SEM.in EDX nickel (38.4%) and iron (34.4%) analyzed using Atomic Force Microscope (AFM) and the average grain size (Ave. Diameter) was (71.9 nm), the average roughness (Sa. Roughness average) was (207 pm) picometers and the root mean square (Sq. Root mean square) was (351 pm) picometers The method used to evaluate the antibacterial activity revealed that the produced nanoparticles had outstanding activity against E. coli. $NiFe_2O_4$ nanoparticles showed low cytotoxicity on HUVEC cells and (MCF-7), so it represents one of the important compounds in various biomedical applications as antimicrobial and drug transporter. Used in wastewater treatment and filtration $NiFe_2O_4$ nanoparticles have a small size, large surface area, and super magnetic properties. Our study showed that this nano-complex has low toxicity to healthy cells and has a high killing rate for harmful cancer cells. The effects of $NiFe_2O_4$ nanoparticles are biocompatible and physiologically good for use, with no significant toxicity.

CONFLICT OF INTEREST

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

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