Toxicity Assessment of Cobalt Ferrite Nanoparticle on Cancer Cell Lines

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Abstract
Cobalt ferrite Nanoparticles (NPs) considered as promising biomedical tools in cancer treatment. Our purpose was to investigate the cytotoxicity of cobalt ferrite on mammary carcinoma cell lines CAL-51, AMN-3 and REF as normal cell lines by MTT (Methyl Thiazolyl Tetrazolium) and crystal violet technique. The reduction in cellular growth was concentration dependent. The inhibitory concentration (IC50%) after 72 hrs of incubation with a concentration 25 µg/mL CoFe2O4nanoparticles were 50%, 50.5%, on CAL-51, AMN-3 cancer cell lines respectively by MTT assay. The anti-tumor potentials of biogenic CoFe2O4nanoparticles which confirmed by using crystal violet method was significantly decreased in concentration 25 µg/mL CoFe2O4nanoparticles when reviewed microscopically after 72 hrs of exposure as compared to (REF) normal cell line. However, the morphological changes of CoFe2O4nanoparticles - treated cells exhibited apoptotic-like characteristics such as shrinkage and loss of normal fusiform appearance Furthermore, cells got separated from surfaces and from one another.
Keyword: nanoparticles CoFe2O4 sample Concentration,toxicity MTT crystal violet,REF,CAL51 AMN3,cell line.

1. Introduction

Cancer is the second major cause of death globally, and it is also connected with a significantly reduced quality of life. Presently, promising novel strategies for the treatment of malignant neoplasms are indeed being studied, with a special emphasis on nanotherapy. (Albarqi et al.,2019).
Magnetic nanoparticles (NPs) are widely used among various kinds of nanomaterials; in particular, cobalt ferrite NPs are a prospective contender for medical application due to their increased magnetic anisotropy (Srinivasan et al., 2018). Environmental restoration (Srivastava et al., 2016), better genetic and medication delivery (Zhang et al., 2020), MRI magnetic resonance imaging (Hankiewicz et al., 2019), biosensing (Krishna et al., 2012), intracellular therapies (Lee and Kim 2012), and magnetic hyperthermia are all applications for these Nanoparticles (Al Lehyani et al., 2017).
Cobalt ferrite nanoparticle is a member of a mineral's family known as spinel ferrites, that is widely used in applications for nanomedicin due to its strong coercive fields, moderate magnetic saturation, enhanced chemical stability, increased permeability, and electromagnetic properties. (Ahmad and Zhou 2017).
Although these characteristics make cobalt ferrites notable and remarkable representatives for a wide range of medical applications, difficulties such as increased release of cobalt and iron ions, diminished surfaces functioning as a result of coating material (macrobiomolecules), high toxic effects, and a greater tendency to aggregate must be adequately explained. (Marmorato et al., 2011). Cobalt ferrites have been shown to be hazardous to multiple species, including the mouse model (Abudayyak et al., 2017) as well as human, due to their genotoxicity and cytotoxicity. (Colognato et al., 2007).
Due to the existence of residual chemicals, physiochemical characteristics, and cationic distributions, synthesis techniques play a vital role in determining cytotoxicity. Cobalt ferrites nanoparticles (Nanoparticles) can be produced by a variety of ways, including sol–gel (Gul and Maqsood 2008), traditional hydrothermal (Millot et al., 2007), co - precipitation (Nikumbh et al., 2014), and microemulsion techniques (Vestal and Zhang 2002).
After intrcellular uptake by the innate immune cells, NPs are sequestered in intracellular vesicles known as lysosomes, where they are subjected to the action of hydrolytic enzymes, an acidic environment with a pH lower than 4.7, and a broad range of cellular proteins that are collectively responsible for the NPs' metabolic transformation. Crystal fragmentation, the liberation of metallic ions, and the subsequent formation of metal complexes are all controlled by the removal of the protective layer on the oxide or metal's surface, as well as by chemical etching. Therefore, the cellular recycling of nanoparticles (Singh and Ramarao 2012), iron oxide nanoparticles (Mosaiab et al., 2013), quantum dots (Liu et al., 2013), and zinc oxide nanoparticles (Cho et al., 2011) has significant implications: first and foremost, the production of highly reactive metal ions; furthermore, a change in the capabilities and physical characteristics of nanomaterials as a result of chemical reactions due to microenvironmental exposure. (Lartigue et al., 2013). Because of the intracellular breakdown of iron oxide nanoparticles (NPs), charged iron species generated by ferritins are often toxic (Chasteen and Harrison 1999). Ferritin

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possesses cellular protective characteristics as well as controlling iron excess, which can be detrimental and exacerbate lysosomal stress. (Volatron et al., 2017b). The current research focuses on the in vitro evaluation of cobalt ferrite NPs and their potential toxicity at different concentrations.

2. Materials and Methods

Tissue

Culture Medium

Rosswell Park Memorial Institute" (RPMI)-1640 Medium (HiMedia India) was done in the following manner:
RPMI-1640 medium" powder (with Hydroxyethyl piperazineethane sulfonic acid (HEPES) buffer pH (7.4), L-glutamine (8.2g) was dissolved in 400 ml of triple distilled water (TDW) and then, Sodium bicarbonate powder (1.1g), (0.5ml) Ampicillin 100 µg/ ml, (0.25 ml) Streptomycin 100 IU/ ml, (5 ml) AmphotercinB and fetal Calf Serum (FCS) 100 ml were added.
Trible distilled water was added to bring the volume to one liter, and the medium was sterilized by running through a Nalgene filter (nalgenunc, USA) with a 0.2-micron filter unit. (Freshney, 2000).

Minimum Essential Medium'' (MEM) (HiMedia,India)

It was prepared as follows

Minimum Essential Medium (with L-glutamine) 8.3g powder was dissolved in 400 ml of (TDW) after that the additional elements were incorporated:
Sodium bicarbonate powder (1.1g); HEPES, (1 M) 10 ml; (0.5 ml) Ampicillin100 µg/ ml; (0.25 ml) Streptomycin 100 IU/ ml; (5 ml) amphotericin B and Fetal Calf Serum (FCS) 100 ml.
Trible distilled water was added to bring the volume to one liter, and the medium was sterilized by running through a Nalgene filter (nalgennunc, USA) fitted with a 0.2-micron filter unit.

Serum Free Medium" (SFM)

It is either RPMI-1640 or MEM prepared without (fetal calf serum) FCS. (Freshney, 2000).

Tissue culture reagents

Phosphate buffer saline" pH7.2

In order to make the PBS, the following components were dissolved
Sodium chloride (NaCl) 8.0 g, potassium chloride (KCL) 0.2 g, disodium hydrogen phosphate (Na2HPO4) 1.15 g, potassium dihydrogen phosphate (KH2PO4) 0.2 g, and triple distilled water (T.D.W) up to 1L.
After dissolving the components in triple-distilled water and the pH was corrected to 7.2, the solution was autoclaved at 121 C° for fifteen minutes, and then it was stored at four C° until it was required. PBS was brought up to 37 degrees Celsius before it was used (Freshen, 2000)

Sodium Bicarbonate

A solution of sodium bicarbonate was obtained by dissolving (2.2g) in one liter of culture media. (Freshney, 2000)

Trypsin –versene solution

In order to make a trypsin-versene solution, first 10.1 grams of Trypsin –versene were dissolved in 900 milliliters of triple-distilled water (T.D.W). After that, 1 gram of sodium bicarbonate was added while the mixture was being stirred continuously on a magnetic stirrer at room temperature. Finally, the volume of the mixture was brought up to 1 L. After that, the solution was filtered using a 0.22-micron Nalgene filter, and it was then kept at a temperature of 4 C°. (Freshney, 2000)

Methyl Thiazolyl Tetrazolium (MTT) Solution

In order to produce a dye with a concentration of 2 mg/ml, 0.2 grams of methylthiazolyl tetrazolium were dissolved in 100 ml of phosphate-buffered saline (Betancur-Galvis, et al.,2002). After following the instructions provided by (Denizot & Lang, 1986), In order to get rid of any blue formazan bodies, the solution was filtered using a syringe filter with a 0.2 m. It was then placed in sterile, darkened bottles with screw-capped closures and chilled to 4 C°. Following its preparation, the solution was put to use no more than two weeks later. (Freshney, 2005)

Crystal Violate Stain

To make crystal violate stain, 5 g. of crystal violate powder were dissolved in 200 ml of methanol, and then the resulting solution was filtered using Whatman No. 1 filter paper. The volume of the liquid was increased to 1000 ml by addition of distilled water followed by addition of 50 ml of formaldehyde solution at a 37 percent concentration. The excess solid residue was filtered out with Whatman No. 1 filter paper, and then it was stored in a blank container at room temperature and away from any sources of light. (Freshney, 2000).

Trypan Blue Dye

To prepare trypan blue, 1g of powder was dissolving in 100 mL of PBS to prepare concentration 1%, then filtered by using Whitman No.1 filter paper, stored at 4°C until use. Later, 10% dilution in PBS was made from the stock solution for immediate use (Freshney, 2000)

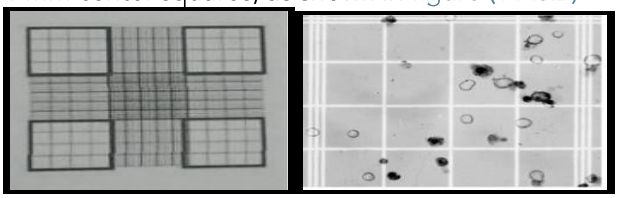
Antibiotics" (Freshney, 2000)

One gram of streptomycin was dissolved in five ml of triple-distilled water, and then half a ml of this solution was added to one liter of culture medium.
One gram of ampicillin was dissolved in five milliliters of triple-distilled water, and one milliliter of that solution was afterwards added to one liter of culture medium.

Viable Cell Count

Trypan blue exclusion was used to estimate a viable cell count. A second after the dye (stain) was applied, dead cells were clearly differentiated from living cells. Dead cells appear blue color under a light microscope if their membranes are undamaged.as fig (1):
According to Freshney, R. I.et al., (2005). The cell

suspension was prepared at a high concentration by trypsinization and resuspension in known media volume as following:
The coverslip was securely fastened in position atop a freshly cleaned haemocytometer.
One portion (0.2 ml) of cell suspension, one portion (0.2 ml) of trypan blue, and eight portions (1.6 ml) PBS were mixed, and a sample of cells counted using an Improved Double Naubauer Ruling Counting Chamber (Assistant, Germany). Magnification powers of 40X were used to count the cells. As (20µl) of samples were transferred to the edge of the coverslip, along running into the counting chamber. After 1-2 min, counting the viable cells It was possible to get this result by employing a light microscope with a 40 X objective. Beginning with the first chamber of the haemocytometer, surviving cells were counted in the 1 mm center square and the four 1 mm center squares, as shown in figure (1 A&B):



A B

Figure (1): (A) four corners of haemocytometer. (B) High-power (40×objective) microphotograph of one of the smaller, bounded by three parallel lines and containing 16 of the smallest. Live cells (viable) are unstained and clear, with a refractive ring around them; nonviable cells are dark and have no refractive ring.
The concentration of the cells (cells/ml) was determined using the following formula:
" c = n X d X 104 "
Where: c is the average cell concentration (cells per milliliter) n is the average number of viable cells detected,
and d is the mean dilution factor, which equals 10.
The value 104 indicates an inversion of the volume index.

Cell lines

The cell Bank Unit, Experimental Therapy Department, in Iraqi center for cancer and medical genetic research (ICCMGR), University of Al-Mustansiriyah, Baghdad, Iraq supplied all the cell lines used in this study.

Ahmed-Mohammed-Nahi-2003 (AMN3) Cell Line

This murine exocrine gland carcinoma or mammary adenocarcinoma cell was primarily obtained from in vivo passage for a spontaneous mammary cancer of female BALB/c mice. Afterwards, the cell was cultured in vitro (Al-Shammari, *et al.*, 2008). During the whole of this investigations, a cell line with passages (120-126 of AMN3) was used, and the cells' viability was maintained by culture in RPMI-1640 medium that was supplemented with 10% FCS.

Rat Embryo Fibroblast (REF) Cell Line

This normal cell line was derived from rat embryos that were 14 days old when they were cultured. The cells of this murine cell line were a mixture of fibroblastic and endothelial cells, and their chromosomes were normal. Throughout the entirety of this work, a cell line including passages (120-129 of REF) was utilized, and the cells were maintained with RPMI-1640 containing 10% FCS.

Center Antoine Lacassagne-51(CAL-51)

This human breast cancer cell lineCAL-51 was derived from a lady with metastatic breast cancer's malignant pleural effusion. These cells proliferate in continuous culture and have the phenotypic, ultrastructural, and immunohistochemical characteristics of mammary epithelial cells. Passages (120-126 of the CAL-51) cell line were used in this work, and cells were cultured in MEM media with 10% FCS. (Passage No.20-22),

Handling and Maintenance of Cell Lines

this study was conducted in the laboratories of the Iraqi center for cancer and medical genetic research (ICCMGR). All the cell culture procedures were carried out inside of a vertical laminar flow hood that had been sterilized by being exposed to UV radiation and then being wiped off with ethanol that was 70 percent strong. Every piece of equipment and reagent was either sterile or manufactured to be sterile, every glass, pipette, and piece of material was cleaned with ethanol that was 70% strength, and every step of the process was carried out in a laminar flow hood that was either autoclaved or filtered with a 0.22 m Nalgene syringe filter. The in vitro approach was used to study the effect of CoFe2O4 in varying doses using one normal cell line (REF), two distinct kinds of carcinoma cells (AMN-3, and CAL-51), and after 72 hours of exposure durations using the MTT test and crystal violet staining.
The cell lines used were sub-cultivated when the cells in the flask formed a confluent monolayer, using the techniques outlined below (Freshney, 2005)
The growing media (RPMI&MEM) was discarded, and the cell sheet was rinsed twice with sterile PBS. Two to three milliliters of trypsin/versine solutions were put to the reverse sid surface of the flask as the cells. After inverting the flask so that the liquid completely covers the monolayer, the flask is heated to 37 degrees Celsius and left for a few minutes until the cells rise to the surface, at which point they are gently shaken apart into individual cells.The growth media (RPMI&MEM) was added (0.1-0.2ml/cm2) and the cells were dispersed by pipetting growth medium across the monolayer surface.
Redistribution of the cells at the specified concentration into culture flasks, followed by re-incubation at 37 C°.
Initially, the medium was orange, but as the cells grew, it turned yellow, necessitating a change in the reducing medium. The presence of turbidity indicates that the culture is polluted and should be discarded.

Cell lines Preparation for Cytotoxicity Study

After the cells underwent incubation and formed a monolayer, the confluent monolayer was handled as mentioned above. Twenty milliliters of growth medium were added after trypsinization, and cells

were pipetted into the growth media.

Evaluation of cytotoxicity using the MTT stain

The MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay was used to assess whether CoFe2O4 nanoparticles possessed any anticancer properties when tested on CAL-51 AMN3 and REF cells.

3. Method

The colorimetric MTT method using to determine the proliferation of cancer cells CAL-51 AMN3 and REF normal cell line. Cell suspension (100µl) with a density of 1x104 cell/well was seeded in 96 – well plate that have flat bottom. Then incubated at 37°C for 24 hours to generate a monolayer (inverted microscope is used to confirm monolayer generation), RPMI-1460 growth media with serum 10% was used for seeding. By using maintenance media two-fold serial dilution was prepared for CoFe2O4 NPs. After the end of seeding the media decanted off and the cells had been treated with 100µl of different concentrations of CoFe2O4 nanoparticles, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, and 12.5 µg/ml /72 hrs).100 µl of maintenance media was added to well of control group, at the end of the exposure time the media was removed and the plate washed with PBS, then 100 µL of tetrazolium MTT solution which have yellow color was added to all wells, and incubated at 37°C for 4 hrs. At the end of incubation period the dye was removed, and the plate was washed with PBS. Subsequently,intracellular purple MTT formazan crystals that remaining in the wells were solubilized by the addition of 50µL DMSO for 30 min at 37°C to.The optical density of each well was read by micro-ELISA reader at wavelength 584 nm (Nikalje et al., 2015).

The inhibitory rate was calculated using (untreated cells) a control, so the percentage of cytotoxicity was determined according to (Nikalje,2015; Betancur-Galvis, et al., 1999; Alexander, 2001) as follow:

Growth Inhibition (G.I) rate % = (A - B/ A) X 100

Were A = optical density of control.
B = optical density of treated sample.
Then use Computer softwere for synergism determination. (Freshney *et al.*,2005).

Crystal violet staining assay

This assay was performed in accordance with Liu et al., 2015. The cancer cells including CAL-51 and AMN3 and REF as normal cell line were seeded on the 96- plate well with density 1x104 cell/well. After 24 hrs. when cell reached to monolayer confluence. The cell exposed to IC50% concentration"25µg/ml " of CoFe2O4 nanoparticles, after 24hrs. of the treatment step, the medium that had been consumed was thrown away, and the cells were washed with PBS. After 30 minutes, 50µ L of crystal violet was added to the tissue culture plate. After that, the cells were washed carefully with tab water for three times, and then they were left until they became dry. Under the 20X magnification of an inverted microscope, the morphological characteristics of the cells were investigated.

4. Results and Discussion

In vitro assays for CAL-51, AMN3 and REF cells

Evaluation of cytotoxicity and cell viability (Mtt).
The ability to reduce MTT to formazan crystals was used to determine cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cellular metabolic activity can be assessed with this colorimetric cytotoxicity test. Several laboratories throughout the world employ this test because it is well-characterized, simple to use, and widely accepted. as a result, are utilized in the research that investigates how susceptible cultured cells are to potentially toxic substances. Utilizing these methods, one is able to ascertain both the survival of cells and their metabolic processes. (Laia et al., 2014).

The viability examined by MTT assay for CAL-51, AMN3 cancer cell lines and REF (control) cells after 72 hrs of exposure to different concentrations of CoFe2O4, show inhibition of the cell's growth significantly (P<0.05) and the effect was more potent as compared to control cell culture, however, the reduction was concentration dependent manner. the inhibition caused by CoFe2O4was seen after 72 hrs of incubation, as normal cell line, Rat Embryo Fibroblast (REF) Cell Lines were used and exposed to the effect of cobalt ferrite NPs (200-12.5) µg/ml after 72 hrs the results showed minimum G.I% for five concentrations in comparison with cancer cell lines. Therefor normal cells exhibited the highest resistance. In table (1) and figure (2) The result of G.I% for REF normal cell line at concentration (200) µg/ml revealed higher significant inhibition (17%) in comparison with the fifth concentration (12.5) µg/ml which showed lower inhibition rate (2.2%), also we showed significant variation between them.

Table (1) Cytotoxicity of CoFe2O4nanoparticles on normal REF Cell line.

Concentration of CoFe2O4 NPs (µg/ml)	G.I% Mean± SD
200	A17.00±2.00
100	AB14.20±2.00
50	BC9.20±0.20
25	CD4.70±0.30
12.5	D2.20±0.29
* (P<0.05)	
Means with a different capital letter in the same column are significantly different (P<0.05).	

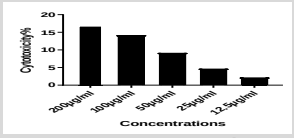


Figure (2) MTT assay for REF cell line.

Table (2) and figure (3) show the effect of CoFe2O4 on G.I% of AMN3 cell line, after 72hrs. The result of

G.I% for AMN3 cancer cell line at concentration (200) µg/ml exhibited significant inhibition (61%) compared with the fifth concentration (12.5) µg/ml which revealed lower inhibition rate (32), as well as significant variance across concentrations.

Table (2)Cytotoxicity of CoFe2O4nanoparticles on AMN3 Cell line.	
Concentration of CoFe2O4 NPs (µg/ml)	G.I Mean± SD
200	A61.00±2.50
100	AB57.00±1.00
50	BC53.00±2.75
25	C50.50±1.25
12.5	D32.00±2.00
*(P<0.05)	
Means with a different capital letter in the same column are significantly different (P<0.05).	

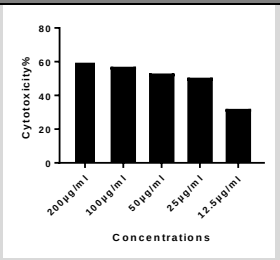


Figure (3) MTT assay for AMN3 cell line.

Cellosaurus cell line (CAL-51) cells were incubated with CoFe2O4 (200-12.5 µg/ml) for 72 h, after which the cells were analyzed by MTT assays Table (3) and Figure (4). The incubation with CoFe2O4 NPs for 72 hrs reduced cell viability significantly or increase growth inhibition significant (60%) at 200 µg/ml compared with (37%) at 12.5 µg/ml.

Table (3)Cytotoxicity of CoFe2O4nanoparticles on CAL-51 Cell line.	
Concentration of $CoFe_2O_4$ NPs (µg/ml)	G.I Mean± SD
200	A62.00±2.25
100	A59.00±3.75
50	B53.00±1.00
25	B50.00±1.75
12.5	C37.00±1.50
*(P<0.05)	
Means with a different capital letter in the same column are significantly different (P<0.05).	

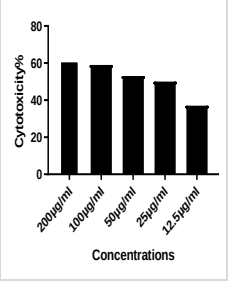


Figure (4) MTT assay for CAL-51 cell line.

Table (4): MTT assay for CAL-51, AMN3 and REF cell lines after exposed to $CoFe_2O_4$ NPs.					
	CoFe2O4 NPs concentrations				
Groups	12.5µg/ml	25µg/ml	50µg/ml	100µg/ml	200µg/ml
AMN3	D32.00±2.00a	C50.50±1.25a	BC53.00±2.75a	AB57.00±1.00a	A61.00±2.50a
CAL51	C37.00±1.50a	B50.00±1.75a	B53.00±1.00a	A59.00±3.75a	A62.00±2.25a
REF	D2.20±0.29b	CD4.70±0.30b	BC9.20±0.20b	AB14.20±2.00b	A17.00±2.00b
LSD	5.93				

Means with a different small letter in the same column are significantly different (P<0.05).
Means with a different capital letter in the same row are significantly different (P<0.05).

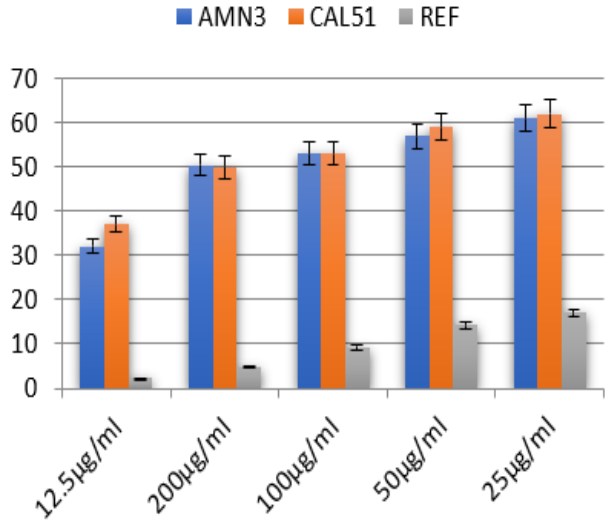


Figure (5): MTT assay for CAL-51, AMN3 and REF cell lines after exposed to $CoFe_2O_4$NPs.

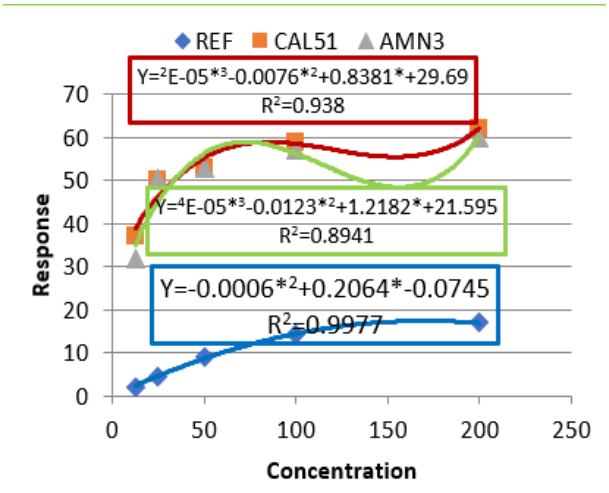


Figure (6): MTT assay for CAL-51, AMN3 and REF cell lines after exposed to CoFe2O4 NPs.

The results proved that CoFe2O4induced death of triple negative (ER-/PR-/HER2-) breast cancer cells and the CAL-51 cells were comparable insensitive to CoFe2O4 as compared with AMN3 cells in which the inhibition rate(IC50%) at the same concentration was

50% and 50.5% respectively ,while the REF cell line which represent as control cells show more resistance than the previous two cancer cell lines in which the cell viability rate was about 4.7% at similar concentration of CoFe2O4 .

An anti-cancer effect of CoFe2O4 on breast cancer cells, which able to decrease cell viability by inducing apoptosis in treated cells through activating the cyrochrome-c and caspase dependent apoptotic pathway ROS generation causing DNA damage then inducing cell death.(Debarati et al.,, 2021).

Moreover, Previous studies have reported that growth inhibitory effects of CoFe2O4 agglomeration and dissolution of ions induces severe mechanical damage to cells membranes and, oxidative stress. Severe apoptosis of cells with inhibition of catalase confirms ROS induced acute toxicity and this activity was concentration-dependent manner.(Farooq et al.,, 2015).

In terms of their antiproliferative nature against cancer cells, CoFe2O4 NPs have been shown to have a weak response, and the fact that they have little cytotoxicity reflects the fact that they are human-safe and friendly, making them appropriate for use in bioapplications..(Sumayya et al.,,2016).

Crystal violet staining

A crystal violet method used to evaluate the anti-tumor potentials of CoFe2O4nanoparticles which confirmed by using in regard to the cytotoxic effect toward breast carcinoma cell lines CAL-51 and AMN3. The viability of cancerous cells was significantly declines when reviewed microscopically after 72 hrs. of exposure to (IC50%) concentrations (25 µg/ mL) of CoFe2O4nanoparticles, in contrast to the absence of any cytotoxic effects against the normal cell line. (REF) Figure (7)

At 72 hours of exposure, although, the cells that had been exposed to CoFe2O4nanoparticles showed morphological alterations and apoptosis. These alterations included a reduction in size and a loss of the typical fusiform shape. In addition, cells began to separate from the surfaces they were attached to as well as from one another.

Staining cells that are adhered to cell culture plates is the basis for the Crystal Violet assay. During the process of cell death, it is necessary for the cells to detach themselves from the cell culture plates. This property can be utilized for the indirect assessment of cell death as well as the determination of changes in proliferation in response to stimulation with death-inducing substances. Crystal violet dye staining of connected cells is one easy approach for determining whether or not cells have retained their adhesion to one another (Maria et al.,2016).

Any dead or disconnected cells by washing them. After an initial step of washing, any remaining attached living cells are stained with crystal violet, which binds to DNA. The crystal violet is then solubilized, and the absorbance of the dye at 570 nm is used to determine the results. The results of this test were based on the affinity that existed between the dye and the outside surface of the DNA double helix. According to Liwka et al., (2016), the amount of dye that is absorbed is dependent on the total amount of DNA present in the culture (liwka at al., 2016).

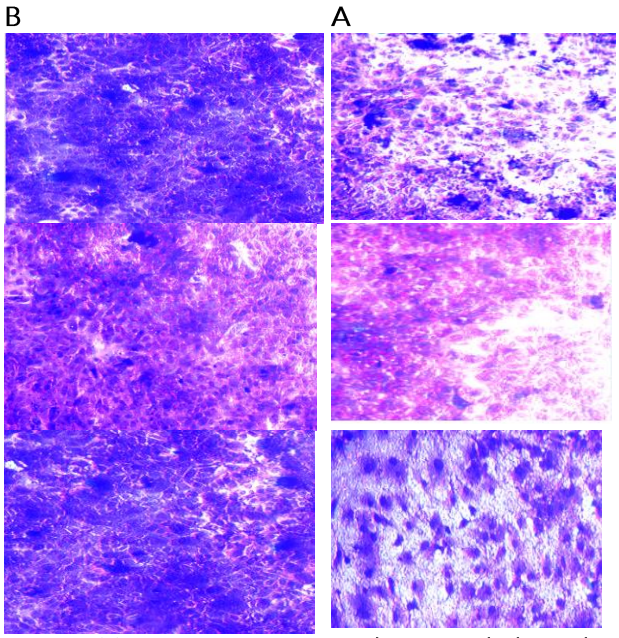


Figure (7): Microscopic images show morphological alterations of CAL-51, AMN3 and REF cell lines: A (Right line) cells treated with $CoFe_2O_4$NPs, and B (Left Lane) untreated cells. The cells stained with crystal violet (20X).

5. Conclusion

A significant reduction in growth of CAL-51, AMN-3 mammary carcinoma cell lines than normal REF cell line when exposed to IC50% if CoFe2O4 nanoparticles 25 µg /mL as measured by MTT and crystal violet.

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