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Growth inhibition of mice mammary carcinoma cell line with green synthesized Magnetic Iron Oxide Nanoparticles

Amer T. Tawfeeq[1], Ghassan M. Sulaiman[2], Amal Naji[2]

1 Department of molecular biolog, Iraqi center for cancer and medical genetics research, University of Mustansiriyah.
2 Division of Biotechnology, Department of Applied Science, University of Technology.

Abstract:

Mammary adenocarcinoma represents one of the most cancers that effect woman in Iraq and worldwide. Finding a definite cure for this type of cancer is a target of considerable research in many laboratories over the globe. In this study, a green synthesized magnetic iron oxide nanoparticles (MNP) was used to specify their ability to inhibit the growth of mice mammary carcinoma cell line (AMN3), it comes as a preliminary study of wider ongoing research. After successfully synthesizing magnetic iron nanoparticles, it employed in different concentrations to expose AMN3 cells for 24 and 48 hours. The viability of the exposed cells was determined using standard MTT assay. The inhibition concentration 50 was determined afterward and used to test the possibility of inducing apoptosis in the carcinoma cells using a mixture of fluorescent stains acridine orange and propidium iodide. Results indicated that green synthesized MNP can inhibit the growth of mammary adenocarcinoma cell line up to 79% and 89% after 24 and 48 hr of incubation with 1000µg/ml. The IC50 of MNP was capable of inducing apoptosis in this cell line as shown by fluorescence microscope. The standard hemolysis assay used to test the reaction between the green synthesized magnetic oxide nanoparticles and normal cells. The IC50 of AMN3 cells growth inhibition only induce 14% of RBC hemolysis, while the percentage induced by negative control was 10%. In conclusion, green MNP have an effect upon cancer cells greater than normal cells. This material must tested against other types of cancer and normal cells to verify its action and study mechanisms of apoptosis induction in detail.

Key words: *Magnetic iron oxide nanoparticles, AMN3, green synthesis, nanotherapy.*

Introduction:

Mammary adenocarcinoma is one of breast cancer types represents a major type of cancer that affect the life of many women in Iraq. According to the Iraqi cancer registry of 2011, breast cancer was the first type of cancer on the top of cancer types that effect Iraqi people (1). The current types of cancer treatments employ different types of cytotoxic drugs as well as some targeted therapies. Cytotoxic therapies relays on the differences between the rate of proliferation in cancer cells and normal cells, so they target most likely the DNA synthesis machinery. In recent decades concept of targeted therapies emerged to reduce to some extent the side effect occurred when cytotoxic drugs are used. These therapies designed to focus more on the mutated genes and proteins, which are, not exist in normal cells therefore sparing them from any effect of those therapies (2-4).

Corresponding address:
Amer T. Tawfeeq
Department of molecular biolog, Iraqi center for cancer and medical genetics research, University of Mustansiriyah.
Email: amer.tawfeeq@iccmgr.com

In recent years nanoparticles interred on the line for making these therapies, more specific and more targeted to cancer cells and less effective on normal cells. Nanoparticles of different engineering methods employed to achieve this vision (5).

Methods to synthesis such nanoparticles varied substantially between chemical or physical procedures, each possesses it own beneficial and defects. Some drawbacks of these methods rise from the use of health and environmentally hazardous compound. These concerns lead the way to think of employing more health and environmental friendly compound and techniques and thinking directed toward the use of extracts of different parts (fruits, leafs, stem, and roots) and different types of plant. The phytochemicals present in these extracts play the major role in reducing and stabilizing the metallic synthesized nanoparticles (6-8). Magnetic iron nanoparticles (MNPs) is a type of particles that show a durable characteristics such as capability to be attracted by magnets, as well as surface properties like surface charge that plays an important role in their physical stability and influence its interaction with the biological system and their safety (9). This study presents preliminary

results of ongoing research taking place in the department of molecular biology of Iraqi center for cancer and medical genetics research (ICCMGR) and department of applied science of the University of Technology. The purpose was to address the question if the green synthesized MNPs can be used as targeted nanotherapy of cancer per ipsum. Therefore, experiments were designed to compare between the capabilities of green synthesized magnetic iron oxide nanoparticles (MNPs) to reduce the growth of mice mammary gland carcinoma cell line (AMN3) of a papillary type and its toxicity toward blood normal cells.

Materials and Methods:

Synthesis and characterization of green magnetic iron oxide nanoparticles

This was described in details by Sulaiman et. al, (10) .Typically, 1.11 g of Ferric chloride hexahydrate (FeCl3-6H2O) and 0.53g of ferrous chloride tetra hydrate (FeCl2-4H2O) (1/2 molar ratio) were dissolved in 100 ml of sterile deionized distilled water in a 250 ml beaker and heated at 80 ^{o}C using magnetic stirrer and under atmospheric pressure. Five ml of aqueous solution of A. adianthifolia leaf extract added after 10 min, to the above solution drop wise. Color of the mixture changed to reddish brown color and after 5 min, 20 ml of 1 M NaOH (0.8 gm) dissolved in sterile deionized distilled water in a beaker 50 ml and added to the mixture with rate (3 ml/min) for allowing the magnetite precipitations uniformly. From the first addition of NaOH the reddish brown color of mixture changed to brownish black suspended particles. The mixture allowed to cool down to room temperature and the iron oxide nanoparticles obtained by decantation after that dilution with sterile distilled water and centrifugation to remove heavy biomaterials of plant leaf extract. The iron oxide nanoparticles purified by dispersing in sterile distilled water and centrifuged three times.

Cells cultivation and cytotoxicity assay

The mammary carcinoma cell line (AMN3) used in the study obtained from cell bank unit of the department of experimental therapy in ICCMGR (11). Cell line cultivation and standard MTT cytotoxicity assay was carried out as described by Freshney (12). Different concentration of green synthesized MNPs was used (1000, 500, 250, and 125 µg/ml). The concentrations were tested for 24 and 48 hour of incubation. All concentration tested in quadruplicate and the assay repeated three times.

Blood cells hemolysis assay

This assay carried out as described by Evans et al (13) on human blood sample from a volunteer. The pH used was 7.4 and absorbance of micro-titer plate reader was set on 550 nm. Phosphate buffer saline was used as negative control and 1% SDS was used as positive control. The IC50 concentration of MNPs that determined against AMN3 cells tested on human red blood cells in quadruplicate and the experiment repeated three times.

Staining with mixture of fluorescent dyes

The IC50 of the green synthesized MNPs that determined against AMN3 cells was used to detect the late event of apoptosis in AMN3 cells after exposure to MNPs for 24 hr. The procedure carried as described by Twafeeq 2014 (14). Images was recorded using CCD camera mounted over inverted fluoresces microscope (Lieca, swetzerland).

Statistical analysis

One-way analysis of variance (ANOVA) was used and the significant differences were calculated on probability level of 0.05 and 0.01(15).

Results:

The green synthesized MMPs were spherical in shape with rough surface and its size ranged between 32-100 nm (10). When AMN3 cells exposed to these nanoparticles its growth reduced substantially according to MNPs concentration used (Fig.1). Growth inhibition was 79, 58, 16, and 19 percent of control untreated cells for 1000, 500, 250, 125 µg/ml respectively for 24 hr of incubation. When incubation extended for 48hr the growth inhibition increased with all concentrations used up to 87, 77, 73, 82 percent of control untreated cells respectively (Fig. 2). The IC50 of growth inhibition determined using logarithmic scale of 24 hr incubation and it was 445 µg/ml (Fig. 3). This concentration used to detect the late apoptosis event by florescence staining with acridine orange and propidium iodide. Observations of both nuclear DNA condensation and fragmentation noticed almost in 100% of the exposed cells (Fig. 4). To test the reaction between the green synthesized magnetic oxide nanoparticles and normal cells such as red blood cells, we used the standard hemolysis assay. The IC50 of AMN3 cell growth inhibition was used and 1% SDS and PBS as positive and negative control respectively. The green synthesized MNPs only induce 14% of RBC hemolysis that was almost the same as the percentage induced by PBS (negative control) of 10% (Fig. 5).

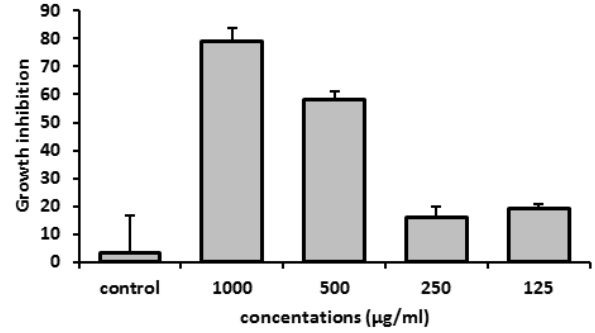

Figure 1: Growth inhibition percentages of AMN3 cells after exposure to different concentrations of magnetic iron oxide nanoparticles (MNPs) after 24 hr of incubation ±SD.* p<0.01, ** P<0.01

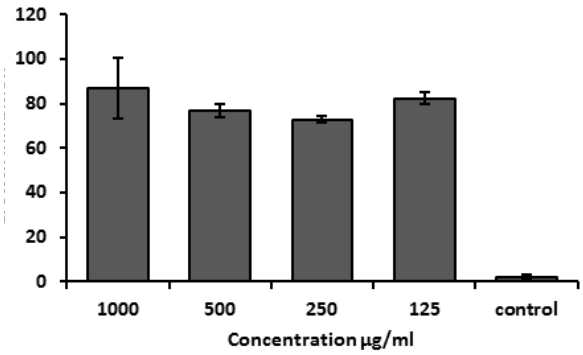

Figure 2: Growth inhibition percentages of AMN3 cells after exposure to different concentrations of magnetic iron oxide nanoparticles (MNPs) after 48 hr of incubation ±SD. ** P<0.01

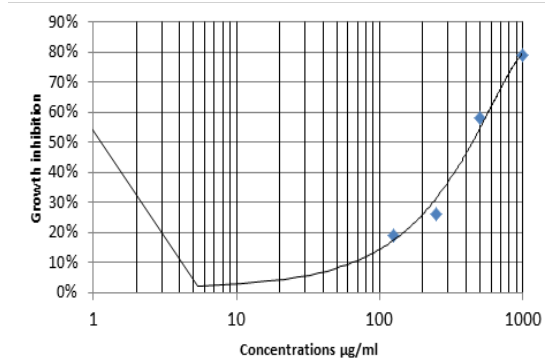

Figure 3: Logarithmic scale for determination of inhibitor concentration 50 for AMN3 cell line exposed to the iron magnetic nanoparticles for 24 hour of incubation.

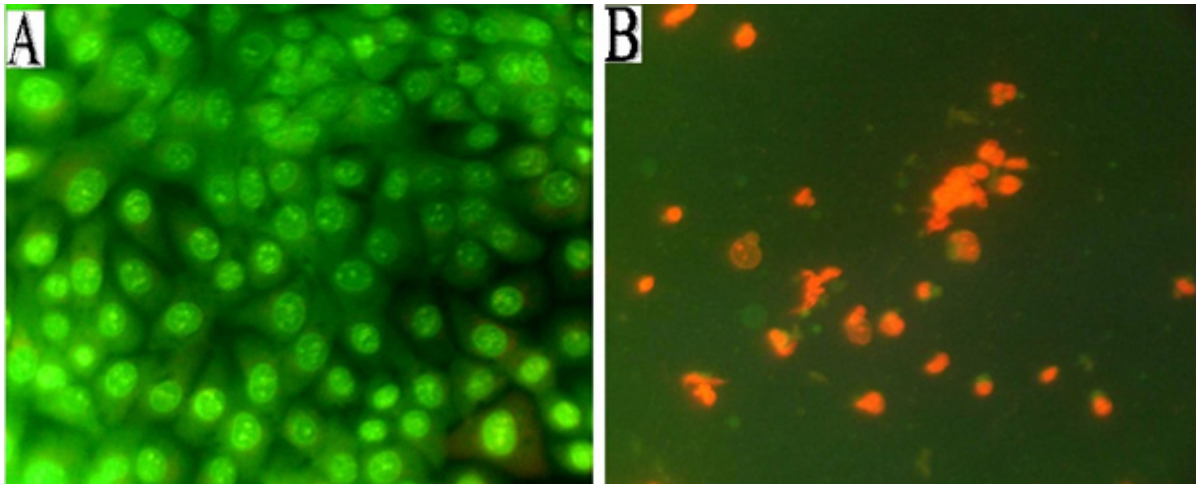

Figure 4: Mammary carcinoma cells AMN3; A, control untreated cells; B, cells exposed to IC50 of magnetic iron oxide nanoparticles (445µg/ml) and incubated for 24 hr, white arrows indicate nuclear DNA condensation and fragmentation, black arrows indicate an obvious apoptotic bodies.

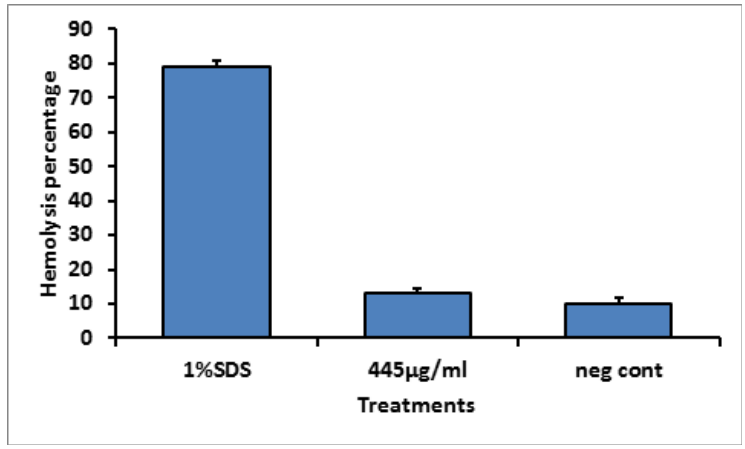

Figure 5: induction of hemolysis in human red blood cells using AMN3 inhibition concentration 50 of the green synthesized magnetic iron oxide nanoparticles.

Discussion:

In this study, which is a part of ongoing research, we have answered a question been asked about the extent of using green synthesized magnetic iron oxide nanoparticles as growth inhibition substances for mice mammary carcinoma cell line. The replacement of green synthesis of nanoparticles by other down-up procedures that depends on the use of toxic chemical compounds is an attractive method, especially when the final intended use of these nanoparticles in biological applications. For different reasons, the green synthesis is more favorable, since it only employed biocompatible substances such as plant extract or some other biocompatible compounds such as starch, gelatin, sugars, and proteins. The use of plant extracts are now documented to be able to produce several types of metallic nanoparticles such as silver, zinc, zinc oxide, iron, gold, cupper, and titanium. Enormous types of microorganisms, plants parts, and algae were invested. Most of them produce well-defined and highly functional metallic nanoparticles test to perform different types of biological functions such as antibacterial, Anti-inflammatory and anticancer (16-18). To expand this knowledge we have synthesized magnetic iron oxide nanoparticles using aqueous leaf extra of the plant A. adianthifolia it was characterized using the standard technique of nanoparticles characterization and found out that particles size was ranged between 32-100 nm (10). As preliminary study, we need to verify the ability of these nanoparticles to inhibit the growth on mammary carcinoma cells and in the same time to detect its toxicity toward normal cells. Therefore, we chose the mice mammary carcinoma cell line AMN3 (11) and human red blood cell lysis assay for that purpose. In these experiments, a large-scale concentration was employed as a beginning to reach some extant of understanding to what could possibly occurs for both type of cells. The MNPs successfully destroyed cancer cells with the all concentration ranged used. However, concentration lower than 250 µg/ml were unable to decrease cancer cell population lower than 50%. The calculated IC50 of MNPs against was 445 µg/ ml, this concentration was able to destroy AMN3 cell after 24 hr at 37°C by inducing the process of apoptosis as have been seen under fluorescent microscope. The distraction through apoptosis was massive, almost 100% of the exposed cells was showing late apoptosis event by nuclear condensation followed by nuclear fragmentation, and the apoptotic bodies were floating all around the cells as visualized under microscope (19). The concentration above the IC50 did not inhibit the growth by 100% after incubation for 24 hr or even after incubation to 48 hr. This may be due to what is known as the over saturation of the growth media with the effecter molecules that compete with each other over the effected receptors or spots on the cell surface. Therefore, the IC50 always used to conduct these assays and it may inhibit all the seeded cells or even less than the 50% of them (20). When it comes to toxicity different procedures suggested to assay the toxicity of nanoparticles, some of these assays use different types of cancer cell lines. Unfortunately this may not represent a convincing truth since there are a huge difference between normal and cancer cells in all aspects of biological parameters especially growth rate and metabolism. For that reasons an in vivo toxicity assessments of nanoparticles can be suggested to overcome this obstacles, once more these in vivo assays may annoy the animals care organizations and its demand a laborious work (21). Some researches lately suggest a 3D in vitro type of cultures of normal cells in order to bridging the gap between in vitro and in vivo outcomes in nanotoxicology (22). However, it's still needs a kind of sophisticated techniques, therefore and for rapid toxicity assessment we employed the red blood cell hemolysis assay to detect the extant of destroying normal cell phospholipids bi-layer membrane by the green synthesized MNPs. These nanoparticles did not exceed the normal negative control capacity to induce RBC lysis. For that reasons we believe that this type of nanoparticles may be a cancer cell targeted type of nanoparticles by itself. This believe in fact needs more investigation and in details to reach a convincing conclusion.

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