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# EVALUATION OF EFFECT OF DOXORUBICIN CONJUGATED MAGNETITE NANOPARTICLES ON MOUSE LUNG CARCINOMA AND THEIR TOXICITY ON BIOLOGICAL SYSTEMS

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**ABSTRACT**

Cancer is a dreaded disease, which is the cause for a large number of deaths worldwide with a major part due to lung cancer, raising the head count every second. The chemotherapeutic agents used in the treatment of cancer are expensive and only a small fraction of the drug actually reaches the site of action, increasing a need for better drug targeting. Magnetite nanoparticles possess great potential as drug carriers. Due to their magnetic property they can be easily targeted to a specific site in the body using an external magnetic field. The Magnetite nanoparticles of approximately 20nm in size and varying shapes were prepared by solvo-thermal decomposition from hydrated Iron oxide in the presence of Fatty acids produced by saponification followed by acidification of vegetable oil. Solvent separation of nanoparticles followed by glucouronic acid mediated conjugation of doxorubicin, which were tested for conjugation by thin layer chromatography and fluorescence microscopy, proved to produce carriers with marked therapeutic value against mouse lung carcinoma cells. The particles also proved to be biocompatible in bacterial and zebra fish models.

**Keywords:** Cancer, magnetite, nanoparticles, Biocompatibility, doxorubicin and zebra fish

## INTRODUCTION

Cancer is the second largest killer diseases in developed countries. In the year 2008 it had been estimated that cancer had been the cause of 13% deaths worldwide, claiming more than 8 million lives, raising the head count every second. By the year 2020 the death rate is feared to increase up to 15 million cases a year. The major culprits, being rapidly changing lifestyle and pollution have created havoc in almost every human and animal leading to various other conditions which either directly or indirectly cause deterioration of health (Anand *et al.,* 2008 and Jemal *et al.,* 2011). There are several techniques for the diagnosis of cancer, which include blood tests, biopsy, X-rays, CT scans and endoscopy. These tests are used to identify abnormal levels of secretions in the blood or to physically locate abnormal tissue mass in comparison to a normal healthy human (Cleaver *et al.,* 2000 and Wilson and Jungner, 1968). Treatment of cancer is carried out by using individual or a combination of two or more techniques. The treatments used against cancer include surgery, radiation, chemotherapy, immunotherapy, hormone therapy and gene therapy.

Bionanotechnology can be used to design drug carriers due to their nanoscale size and targeting properties. Several carbon nanotubes are currently studied for their drug targeting properties since the drug of interest can be loaded into these nanotubes and targeted to the site of action without being affected by the external agents. Nanoparticles of gold can also be used for treatment for cancer without the use of a drug (Vickers, 2004). Magnetic nanoparticles are widely used in various applications such as lithography, biomedical sensors, bioelectrical sensors and several others. Doxorubicin is a multifunctional drug since it has both antimicrobial and anticancer properties, at present it also being used as anti malarial (Di Marco *et al.,* 1969). The zebrafish , *Danio reiro*, is a tropical freshwater fish belonging to the *minnow* family (*Cyprinidae*) of order *Cypriniformes*. It is a popular aquarium fish, frequently sold under the trade name *zebra danio*, and is a very important vertebrate model organism for scientific research. The zebrafish can grow up to 6.4 centimeters (2.5 inches), although it is uncommon for them to grow past 4cm in captivity. Lifespan in captivity is around 2-3 years, although in ideal conditions, may extend to 5 years. *Danio reiro* is one of the few species to have reached space. They may supplement higher vertebrate models, such as rats and mice. The major objectives of this study are, to reduce the concentration of drug used against cancer, to reduce side effects of the drug, to improve drug targeting to the site of tumor and to reduce cost of drug.

## METHODOLOGY

***Cell culturing:*** The glycerin stock of the mouse lung carcinoma cells was thawed to room

temperature about 1ml of the cell stock was aseptically transferred to 10ml of antimicrobial treated Alpha MEM which was taken in culture flasks and incubated for 48 hours. The cell growth was identified by a change of color in the medium and by staining for contamination (Doyle *et al.,* 1993).

**Synthesis of magnetite nanoparticles**

***Isolation of fatty acids***: The sesame seed oil was taken and 150ml of it was transferred to a beaker this was mixed with 30ml of 50% sodium hydroxide solution and stored at 35ºC for 24 hours. The hardened soap was then grated (Yavuz *et al*., 2010 ).

***Acidification and isolation of fatty acids:*** The soap was grated and weighed to 60gm this was then dissolved in 650ml of 9% acetic acid solution with continuous mixing on a hot plate at 90ºC. After approximately 30 minutes a brownish fatty acid layer separates out. This fatty acid mixture was separated and stored (Yavuz *et al*., 2010).

***Formation of iron oxide:*** For the formation of hydrated iron oxide 100 ml of 5% ferric chloride solution was mixed with 50ml of 3% sodium hydroxide solution and mixed thoroughly. After mixing the solution mixture was stored for the formation of a precipitate. The precipitate was the separated and dried in a hot air oven to remove moisture content (Yu *et al*., 2004). $FeCl_3 + 3NaOH \rightarrow Fe(OH)_3 + 3NaCl$

***Solvo-thermal decomposition:*** The iron oxide was grinded using a mortar and pestle to a fine powder, then 1gm of it was mixed with 5ml fatty acid mixture and heated at 350ºC in a hot air oven for 4 hours. This forms a black waxy mixture. The mixture was then partially dissolved in dodecane to suspend the magnetite nanoparticles (Yavuz *et al*., 2010). $2\ Fe(OH)_3 \rightarrow Fe_2O_3 + 3\ H_2O$.

***Conjugation of doxorubicin with magnetite nanoparticles:*** The particles were separated by dissolving the fatty acid in chloroform. 600mg of magnetite particles were suspended in 300ml of 5M sodium hydroxide solution and sonicated in an ultra sonicator for 10 minutes. Then 1800mg glucuronic acid was added and sonicated for 1.5 hours. Then the magnetite particles were magnetically separated and washed thrice in PBS solution. These particles were then separated and suspended in 600ml of distilled water. 300ml of the stock solution was mixed with 30 ml of 0.2M sodium bi carbonate solution and 3ml 0.088M Sodium per iodate, the solution mixture was incubated in dark conditions for 20 minutes at 20ºC. The particles were then centrifuged and separated in 30ml of 0.2M sodium bi carbonate solution and mixed with 60mg of doxorubicin dissolved in 6ml of sodium bi carbonate and mixed under the same conditions in a shaker for 4 hours. To the solution 360ul of 5M sodium cyanoborohydrate was added and incubated for 30 minutes under the same conditions. After incubation the 1.8ml of 2-amino ethanol was added to the particles and incubated again. The particles were then separated and washed 10 times using Phosphate buffered saline and resuspended in 300ml of distilled water (Tiefenauer *et al*., 1996) (Scarberry *et al*., 2008).

***Direct treatment of cells:*** A microfuge tube with 1ml of the cultured mouse lung carcinoma cells were taken, to it 0.9ml of alpha MEM and 0.1ml of Conjugated particles and doxorubicin of varying concentration was added aseptically. Then10µl of antibiotics was added to each of the tubes and sealed using paraffin film or aluminum foil. The treated cells were then incubated in an incubator for 24hours (Stacey *et al*., 1981 and Wenzel *et al*., 1983).

***Trypan blue absorbance:*** 100µl treated cells from each of the tubes were taken and centrifuged. The supernatant was discarded and to the pellet 10µl of Trypan blue and 10µl of 1% SDS solution was added and treated for 15minutes. Then the cells treated were centrifuged and the pellet was suspended in Phosphate buffered saline. The absorbance of the suspended cells was measured at 590 nm (Zu-hua Gao *et al*.,1998 and Tracy *et al*., 2008).

***Agar diffusion assays:*** The filter paper discs were soaked in the particle solution. LB agar was poured in the plates and allowed to solidify. The *lactobacillus* broth culture was the spread over the agar using an L-rod. The soaked disks were placed on the agar and allowed to diffuse for 10 minutes. The plates were then inverted and incubated in an incubator at 32ºC for 24 hours after which the plates were observed (Andrews *et al*., 2001) (Turnidge *et al*., 2003).

***Biocompatibility in zebrafish:*** The conjugated particles were serially diluted in phosphate buffered saline to obtain various concentrations. 10µl of these samples were then injected into the fishes using a 31 gauge needle and syringe. The fishes were then observed for 96 hours (Adrian *et al.,* 2005).

**RESULTS AND DISCUSSION**

The transmission electron microscopy images of the magnetite nanoparticles suspended in dodecane showed that the particles were approximately 20nm in diameter consisting mostly spheres and cuboids of 20x10nm in a small amounts. The particles were tested for their magnetic properties using a magnet. The agglomerated particles suspended in distilled

water were found to be attracted to towards the magnet. To test the conjugation of doxorubicin to the particles, the conjugated and non conjugated particles after repeated washing with phosphate buffered saline solution were applied on a slide and viewed using a fluorescent microscope. No fluorescence was observed in the particles without conjugation due to the absence of doxorubicin (Figure A and B). The doxorubicin, conjugated to the magnetite particles, which is a fluorescent substance, was found to be fluorescing under UV radiation in a bright reddish orange fluorescence (Figure C and D)(Cafer *et al.,* 2010). The magnetite particles were tested for their biocompatibility in bacterial system using lactobacillus sp by using agar diffusion method. The bacterial colonies were found to be grown around the particle coated discs without any zone of inhibition. This proves the biocompatibility of the magnetite nanoparticles (Figure E). The cells treated with various concentrations of doxorubicin showed steady decrease in cell proliferation confirming the anticancer effect of doxorubicin on carcinoma cells. The cells treated with varying concentrations of the doxorubicin conjugated particles showed decrease in cell proliferation, but at lesser extent when compared to doxorubicin treated cells due to the small quantity of drug conjugated to the magnetite nanoparticles. The particles were found to be less toxic when injected in zebrafish model since the fishes injected with high concentration showed minimal death over a period of 96 hours.

The size of the magnetite nanoparticles, 20nm is an ideal size for drug targeting since it is not easily excreted, increasing its circulation time in blood. The magnetic property of the particles which was tested using a simple method proves the targeting and navigation of magnetite nanoparticles in a system. The biocompatibility of the magnetite particles in bacterial system shows that these particles can be used in wider applications such as gene transfer or magnetic separation of biomolecules without affecting the organisms.The minimal toxicity in zebrafish proves its possible applications in humans as drug targeting agents for various conditions such as cancer (Kirpotin, 2006).

**CONCLUSION**

The particles conjugated with doxorubicin an anticancer agent was found to exhibit anti cancer effect on mouse lung carcinoma cells, with magnetic properties which aid targeting of the drug conjugated particles using an external magnetic field. The particles were also found to be biocompatible in bacterial cells, which prove it's potent for various applications in bacterial cells. These conjugated particles were also found to exhibit minimal toxicity in zebrafish model.

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*Fig A:TEM image of magnetite nanoparticles*

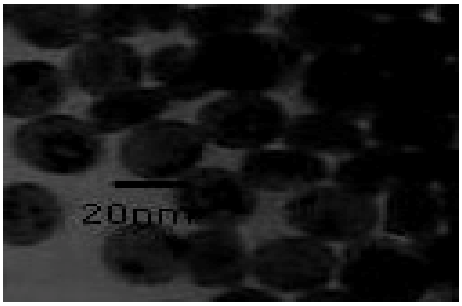



*Fig B:TEM image of doxorubicin conjugated magnetite nanoparticles*

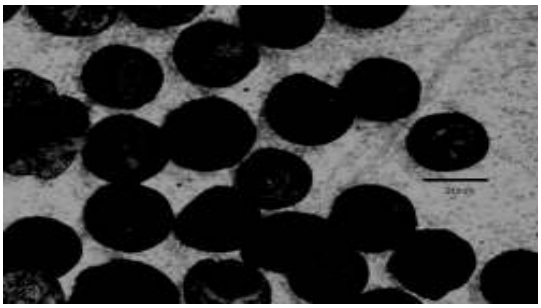


*Fig C: Fluorescence of Doxorubicin conjugated to magnetite nanoparticles*

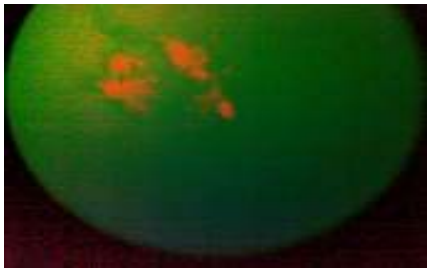
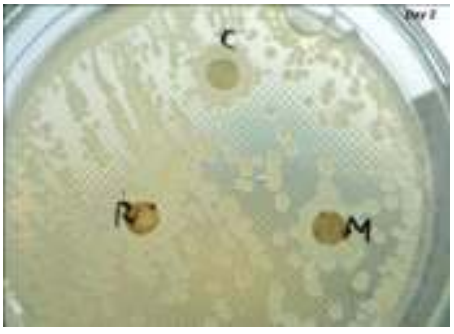


*Fig D: Magnetite nanoparticles without conjugation*

*Fig E: Biocompatibility of Magnetite nanoparticles in bacteria*

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