



In vitro apoptosis and molecular response of engineered green iron oxide nanoparticles with L-arginine in MDA-MB-231 breast cancer cells

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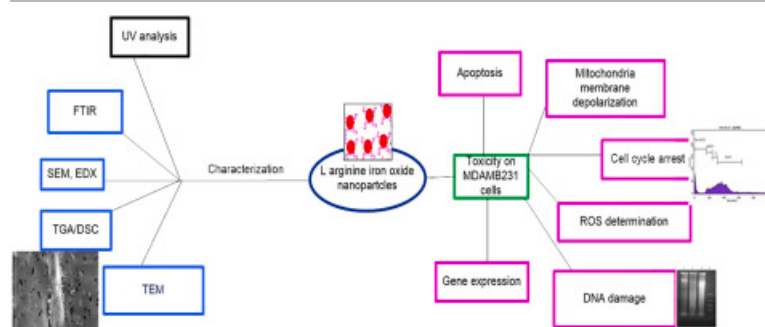
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Abstract

Iron oxide nanoparticles (IONPs) have immense applications in pharmaceutical industries, diagnostics, therapeutics, and biomedicine. In this study, the aqueous leaf extract of Mimosa pudica was utilized for the synthesis of IONPs. The color change of the solution after adding 3mM ferrous sulfate (FeSO₄) confirmed biosynthesis of IONPs. The spherical IONPs were coated with L-arginine (cIONPs) and were found to be in the size range of 20nm–48nm, and toxicity analysis revealed IC₅₀ value of these cIONPs were 67.69 µg/ml against MDA-MB-231 breast cancer cells and 156.28 µg/ml against normal 3T3 L1 cells. The apoptosis study revealed membrane blebbing, chromatin condensation and change in cell morphology and mitochondrial membrane potential assay confirmed mitochondrial membrane depolarization ($\Delta\psi_m$) with 38.19% membrane damage. Dichlorodihydrofluorescein diacetate (H2DCFDA) analysis confirmed the production of reactive oxygen species (ROS) (57.95%) in the cIONPs treated breast cancer cells. Flow cytometry results confirmed that cell cycle got arrested at S (16.52%) stage, even better in G2/M stage (52.82%). Extensive DNA fragmentation in treated cells was

detected via agarose gel electrophoresis. Quantitative reverse transcription PCR (RTqPCR) confirms the upregulation of endoplasmic reticulum (ER) stress XBP_s, PERK, CHOP, ATF-6 genes, and downregulation of IRE1 alpha gene. The study showed cIONPs could be used as potential anticancer agent in biomedical and pharmaceutical applications.

Graphical abstract



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Introduction

Nanoparticles in healthcare systems are mainly used in diagnostics and drug delivery [1]. Nanobiotechnology has an edge due to its small particle size (1 nm–100 nm) and its improved surface-to-volume ratio [2]. Gold, silver, and iron oxide nanoparticles are the most researched inorganic materials because of their nanoscale, optical, and magnetic properties. A fine example is the superparamagnetic characteristics of iron oxide nanoparticles, employed as a multipurpose class of magnetic resonance imaging (MRI) contrast agents [3]. Synthesized iron oxide nanoparticles also have various desirable qualities, such as improved blood circulation time, biocompatibility, and water solubility [4].

Breast cancer is the second leading cause of cancer-related death worldwide [5]. The American Cancer Society stated that women with breast cancer have a 2.9% probability of dying, and research is being conducted to prevent, treat and improve quality of life. Current cancer treatment procedures, such as chemotherapy and radiation therapy, are of particular concern as they can also kill healthy cells and healthy tissues inducing undesired gene alterations [6].

Breast cancer can also be caused by bacteria such as *Enterobacteriaceae*, *Staphylococcus*, and *Bacillus*. They are found in higher amounts in breast cancer cells than in normal cells [7]. Although it has not been scientifically shown that bacteria are the major cause of cancer, the bacterial mechanism for inducing cancer has been investigated [8]. Another cancer treatment option is to use antibacterial drugs with anti-proliferative and pro-apoptotic properties. However, previous research has indicated that antibiotics are one of the causes of cancer due to disrupted microbial balance [9]. Breast cancer caused by *Staphylococcus aureus* is a concern as it is resistant to antibacterial medications, increased infection in healthy cells and reduction in therapeutic alternatives is bothersome [10]. Therefore, novel antibacterial and anticancer medicines that kill only cancer cells must be discovered.

Arginine is basically an amino acid having the formula $(H_2N)(HN)CN(H)(CH_2)_3CH(NH_2)CO_2H$ and is encoded by the codons CGC, CGU, CGA, CGG, AGG, and AGA. L-arginine is the only enantiomer that occurs naturally [11], plays a major role in wound healing, cell division, immune function, and removal of ammonia from the body [12]. Silver nanoparticles conjugated with Poly-L-arginine enhances the antimicrobial properties of silver nanoparticles [13]. L-arginine coated nanoparticles are readily absorbed by cells that maximizes the therapeutic effect and