Antitumor efficacy of Ellagic acid against MCF-7 using Nanotechnology

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Abstract: Breast cancer is the most frequent cancer among women worldwide. Nanotechnology has various useful applications in cancer biology and invades the field of breast cancer specially. Ellagic acid (EA) a naturally occurring polyphenolic constituent that is contained in ellagitannins in fruits and nuts such as grapes, pomegranate, red raspberry, strawberry, blueberry, walnuts and Cashew nuts. The main purpose of this study is to investigate the effect of Ellagic acid (EA) synthesized by nanotechnology on human breast cancer cell line (MCF-7) proliferation. Firstly Ellagic acid (EA) was synthesized using nanotechnology as the nanoparticles (NPs) are usually non-toxic, biocompatible, non-immunogenic and biodegradable. And they may not be recognized by the host's defense mechanisms. The cytotoxicity of Ellagic acid nanoparticles (EA-NPs) was investigated on the human breast cancer (MCF-7) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay which showed the reduction of cell viability by reducing MCF-7 cell count to 50% (IC50) (3.54 0.2 µg/ml). So, Ellagic acid nanoparticles (EA-NPs) could be promising agent in breast cancer treatment. Further studies with in vivo models, are needed to confirm the antitumor efficacy of EA-NPs in inhibition or prevention of breast cancer growth.
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1. Introduction

Breast cancers is the most frequently diagnosed cancer in women worldwide (Bray et al., 2012) which making it the only cancer that is common among women in all regions of the world (Bray et al., 2012). In 2012, it accounting for 25.2% of cancers diagnosed in women resulted in 1.68 million cases and 522,000 deaths (WHO, 2014). In Egypt, breast cancer is estimated to be the most common cancer among females accounting for 37.7% of their total with12, 621 new cases in 2008. It is also the leading cause of cancer-related mortality accounting for 29.1% of their total with 6546deaths. The incidence to mortality ratio is poor (1.9:1) (Zeeneldin et al., 2012). The conventional breast cancer therapies (surgery, chemotherapy and radiotherapy) have limitations as they are not usually successful in curing metastatic stages (Skandarajah and Bruce, 2013) or they may be not targeted sufficiently. Nanotechnology has recently invaded the field of breast cancer which may overcome the limitations of the conventional breast cancer therapies which attributed to the advantages of nanoparticles of having high surface area to volume ratio which allowing many functional groups to be attached to a nanoparticle, which also can seek out and bind to certain tumor cells (Seleci et al., 2016). Ellagic acid (EA) naturally occurring polyphenolic constituent that is contained in many fruits and nuts as grapes, pomegranate, red raspberry, strawberry, blueberry, walnuts and Cashew nuts (Zhang et al., 2014), is known by its wide-range of health benefits however it has limited use due to its low oral bioavailability (Avachat et al., 2015) and those health benefits are due to its ability to act as antioxidant, anti mutagenic, anti cancer and anti metastatic, Cardio and hepatic – protective. In addition to its anti-inflammatory and anti-aging activities (Avachat et al., 2015). EA elicits anti-carcinogenic effects by in habiting tumor cell proliferation, inducing apoptosis, breaking DNA binding to carcinogens, blocking virus infection, and disturbing inflammation, angiogenesis, and drug-resistance processes required for tumor growth and metastasis (Zhang et al., 2014). In the current study, Ellagic nanoparticles (EA-NPs) were synthesized by the mean of green nanotechnology a clean approaches to minimize potential environmental and human health risks associated with the manufacture and use of nanotechnology products. So EA-NPs were synthesized by lactic acid bacteria Lactobacillus strain according to method described by Philip, 2009. Then the size and zeta potential of the EA-NPs were measured and their cytotoxicity was investigated in vitro against the human breast cancer cell line (MCF-7) using MTT assay.

2. Materials and methods

1. Chemicals

Ellagic acid was purchased from Sigma-Aldrich Company, Saint Louis, Missouri, USA.

Cell line

Human breast cancer (MCF-7) cell line was obtained from VACSERA Tissue Culture Unite.

3. Synthesis and characterization of EA-NPs

Lactobacillus strain will be inoculated into sterile MRS and 2 gm of wet biomass will be collected in a 500-ml Erlenmeyer flask mixed with 100 ml of 1 mM of Ellagic acid (EA) and incubated at 37^{O}C under agitation (200 rpm) for 24 h. Ultrasonic disruption of cells will be carried out with an ultrasonic processor (Sonics & Materials Inc, vibra cell T.M., USA). The sonicated samples will be centrifuged at 15,000 rpm for 30 min at 4°C to remove cell-debris. The resulting aqueous solution will be filtered through a 0.22 µm Millipore filter before use (Philip, 2009).

4. The zeta potential and size distribution of the EA-NPs

Were characterized using the Malvern Zetasizer Nano ZS (United Kingdom).

5. EA-NPs cytotoxicity on MCF-7 cell line

Cytotoxicity of EA-NPs was investigated on the human breast cancer (MCF-7) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Wilson et al., 1990) which based on the mitochondrial dehydrogenase conversion of the MTT to a blue formazan product in the viable cells by an enzyme present in the mitochondria of viable cells. The colorimetric changes of blue formazan dissolved in dimethyl sulfoxide (DMSO) were measured spectrophotometrically at 590 nm using enzyme-linked immunosorbent assay (ELISA) plate reader DV990BV4 microplate reader from Gio. de Vita E C. S.r.l (Rome, Italy) and data were analyzed using 990 win 6 software.

3. Results

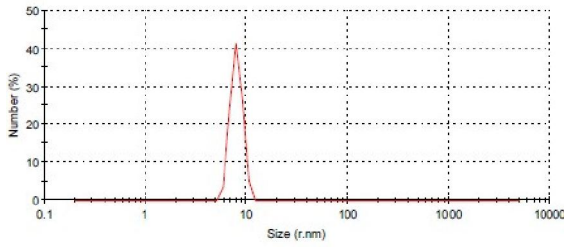

Figure: DLS analysis for size determination of EA-NPs. IV.2. Cytotoxicity of EA-NPs against MCF-7

Dynamic light scattering (DLS) of the EA-NPs EA-NPs were analyzed by DLS Zetasizer (ZS) for size determination and the results revealed that EA-NPs size ranged from 6.9-10.8 nm as shown in the Figure.

Cytotoxicity of EA-NPs against MCF-7 using MMT assay showed the reduction of cell viability relative to control and the concentration of EA-NPs which reduced MCF-7 cell count to 50% (IC50) was (3.54 0.2 µg/ml) and shown in the following table.

Table: Antitumor efficacy of EA-NPs against human breast cancer cell line (MCF-7).

EA-NPs conc. (µg/ml)	Viability % S.D.
500	4.24 0.21
250	7.07 0.13
125	9.80 0.35
62.5	17.70 0.27
31.25	26.52 0.76
15.6	32.67 0.48
7.8	37.09 1.07
3.9	49.08 2.65
2	52.81 1.72
1	65.34 0.84
0	100

4. Discussion

Breast cancer is the field of medicine with the greatest presence of nanotechnological therapeutic agents as it offers potential solutions to the historical challenge that has rendered breast cancer (Tanaka et al., 2009) as overcoming of the limitations of the conventional breast cancer therapies (surgery, chemotherapy and radiotherapy). surgery is effective only in the area of the primary tumor (Custodio et al., 2013), Chemotherapy kills both of normal and malignant cells as chemotherapeutic agents enter the blood stream and distribute throughout the body attacking many types of proliferating cells not only cancer cells (Aparo and Geol, 2012). So the medical applications of nanoparticles are growing, as they have the potential to offer novel methods of non-invasive cancer detection, diagnosis, and treatment. Tumor targeting ligands, such as antibodies, peptides, or small molecules, can be attached to nanoparticles for targeting of tumor antigens and vasculatures with high affinity and specificity. In addition, diagnostic agents (i.e. optical, radiolabels, or magnetic) and chemotherapeutic drugs can be integrated into their design for more efficient imaging and treatment of the tumor with fewer side effects (Deshpande, 2016).

Nanoparticles (NPs) can be synthesized by chemical and physical approaches that are often expensive, labor-intensive and potentially hazardous to the environment and living organisms (Makarov et al.,

2014) and since NPs revolutionize a series of medical tools and procedures, they became be synthesized biologically via Eco- friendly, cost-effective methods using microorganisms, enzymes, fungus and plants or plant extracts (Hasan, 2015) in a technology called green synthesis or biosynthesis.

All efforts in the world tend to use natural products for either protection (prevention) or treatment of several diseases. one of bioactive compound called Ellagic acid (EA) (3,7,8-tetrahydroxy [1]-benzopyrano [5,4,3-benzopyran-5,10-dione) Which is found in plants as one of Ellagitannis (ETs). The most rich dietary sources of (EA) include pomegranate, berries (including raspberries, blackberries, and strawberries) and nuts (including walnuts, pistachio, cashew nuts, and pecans) (shirode et al., 2015). (EA) has known by its potent antioxidant activity, radical scavenging capacity, chemopreventive and antiapoptotic properties (Hussein et al., 2014). EA decreased the incidence of chemically induced lung, mammary, and oral tumors, reduced the volume and multiplicity of estrogen-induced mammary tumors, and induced apoptosis in cancer cells in vitro (shirode et al., 2015). EA exerts adose- dependent impact on the metabolism of chemical carcinogens and drugs by affecting the enzymes involved in xenobiotics activation/detoxification and antioxidant pathways.

(Celik et al., 2013).

From the previous points of view, the current study was designed aiming to synthesize and evaluate the antitumor effect of Ellagic acid nanoparticles (EA-NPs) against human breast cancer (MCF-7) cell line.

EA-NPs were characterized by DLS Zetasizer (ZS) for determination of their size dimension which was ranged from 6.9-10.8 nm indicating their low scale (less than 100 nm) as the size of nanoparticles is an important factor which significantly influences their biological activity (Shin et al., 2015).

Regarding the cytotoxicity of EA-NPs against MCF-7, the result revealed a significant reduction of cell viability relative to control and that was in agreement with (Kim et al., 2009). Also Chen et al., 2015 showed that EA inhibits the proliferation of MCF-7 breast cancer cells mainly mediated by arresting cell cycle in the G0/G1 phase.

TGF-β/Smads signaling pathway was further found as the potential molecular mechanism of EA to regulate cell cycle arrest in vitro.

Conclusion

Ellagic acid nanoparticles (EA-NPs) could be promising agent in breast cancer treatment. Further studies with in vivo models, are needed to confirm the antitumor efficacy of EA-NPs in inhibition or prevention of breast cancer growth.

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