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Quercetin conjugated with silica nanoparticles inhibits tumor growth in MCF-7 breast cancer cell lines

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ABSTRACT

Background: Quercetin is a plant polyphenol from the flavonoid group that plays a fundamental role in controlling homeostasis due to its potent antioxidant properties. However, quercetin has extremely low water solubility, which is a major challenge in drug absorption.
Method: In this study, we described a simple method for the synthesis of quercetin nanoparticles. The quercetin nanoparticles had an average diameter of 82 nm and prominent yellow emission under UV irradiation. Therefore, we used an in vitro model treated with quercetin and quercetin nanoparticles to investigate the effects of quercetin nanoparticles on MCF-7 breast cancer cell line.
Finding: MCF-7 cells were cultured with different concentrations (1−100 μM) of quercetin nanoparticles at the 24th, 48th and 72 nd hours, and cell cycle and apoptosis assays were detected by flow cytometry (FCM). In this study, we found that quercetin nanoparticles (1−100 μM) could significantly reduce cell vitality, growth rate and colony formation of MCF-7 cells.
Conclusion: Quercetin nanoparticles can inhibit cell growth by blocking the cell cycle and promoting apoptosis in MCF-7 cells more than quercetin. *As a result, quercetin nanoparticles may be useful therapy or prevention on breast cancer.*

1. Introduction

Breast cancer is one of the most common malignancies and has a serious impact on women's health. The prevalence of illness and mortality in women with malignant tumors is high [1]. According to the World Health Organization, about 1.3 million women develop breast cancer every year [2]. The incidence of breast cancer in women is at the first rank of cancer in all malignant tumors and breast cancer mortality in women is second only to lung cancer [3,4].

Hormone-dependent MCF-7 human breast cancer cell line has been studied widely. These cells show a pleotropic response to estrogen since they contain functional estrogen receptors [5–7]. Estrogen motivates proliferation of these cells under in vitro conditions [8,9]. In contrast, MDA-MB-231 cell line does not display estrogen receptor and it shows an estrogen-independent breast cancer model [10].

Despite the development of surgical techniques and chemotherapy regimens designed to treat cancer patients, relapse in patients with advanced cancer is almost inevitable. Although various chemotherapy drugs are used to treat breast cancer, which can kill tumor cells or inhibit tumor cells growth, they are usually associated with side effects. Therefore, the discovery and development of new anti-tumor chemicals is crucial for improving integrated treatment programs.

Recent studies show that quercetin inhibits the growth of cancer cells [11,12]. Quercetin (3, 3, 4, 5, 7-pentahydroxyflavone) is a bioflavonoid compound capable to scavenge ROS. Due to its ion chelating and iron stabilizing properties, quercetin can regulate lipid peroxidation.

Quercetin is ubiquitously present in fruits, vegetables and oils, and can be easily extracted. Important applications of quercetin can be treatment of any soft tissue injury using various quercetin

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products [13,14]. Moreover previous studies have shown promising use of quercetin in cancer treatment [15].

Quercetin has been reported to inhibit the growth of cancer cells by inducing apoptosis [16]. Previous studies also have shown that quercetin is capable of inhibiting the spread of gastric, esophageal and ovarian cancer [17]. However, the effects of quercetin on breast cancer have rarely been studied.

Nanotechnology in biochemical programs has focused on the synthesis of nanoparticles with improved antioxidant activity against different diseases such as cancer and Alzheimer's [18].

By reducing the size of the particle, the contact surface between the particle and the surrounding area increases, therefore the nanoparticles perform better than the larger particles in terms of absorption and effectiveness. In this study quercetin nanoparticle were prepared and their effect in inhibiting the growth and inducing the apoptosis of MCF-7 cells has been evaluated.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grade (or highest available purity). HPLC solvent, acetonitrile, methanol, and water for extraction were purchased from Merck, Germany. Quercetin ≥95% (HPLC), DMSO and Propidium iodide were purchased from Sigma Aldrich. Ultrapure Milli-Q water (18.2 MW) was used in all experiments. Other chemicals were purchased from Sigma.

2.2. Synthesis of quercetin nanoparticle

2.2.1. Synthesis of biogenic silica nanoparticles from rice husk

The method for synthesis of silica nanoparticle from rice husk was taken from the study of Athinarayanan et al. [19] with some minor modifications. For this purpose, 40 g of rice husk was mixed with 200 ml of 1 N HCl and was pressurized under 15 psi and autoclaved at 121 °C for 45 min. Then, the acid pretreatment sample for removing the remainders of hydrochloric acid was repeatedly filtered and washed with distilled water; measurement of pH was assured in this work. The rice husk was dried in the oven at 45 °C and then for obtaining a white powder, the dried rice husk was calcined in a muffle furnace at 700 °C for 1 h.

2.2.2. Synthesis of quercetin conjugated silica nanoparticle

Ultrasound-assisted wet impregnation method was used for synthesis of silica: very simple and fast quercetin conjugation. One mg quercetin was mixed with 10 mg synthesized silica in a reaction flask containing 10 ml ethanol. After mixing, the suspension was sonicated in a sonicating water bath (Elma sonic, S10 H, Germany) at ambient temperature for 5 min. Then, the solvent was evaporated in an oven at 50 °C in the reaction flask. In addition, the synthesized quercetin conjugated silica was used for various structural analyses Fig. 1A.

2.3. Characterization of quercetin nanoparticles

2.3.1. Particle size analysis

Quercetin nanoparticles were characterized through scanning electron microscope (SEM, JEOL 6300 F, Tokyo Japan, Philips), Ultraviolet–visible spectroscopy (UV–vis, 7205, Jenway Co., United Kingdom), and Fourier transform infrared spectrometry (Nicolette is50, America, Thermo Fisher Scientific).

2.3.2. Morphology of the particles

The samples morphology was observed using a SEM (Quanta 3D FEG/FEI) with 20 kV, and a collector bias of 300 V. The powder samples were spread on a SEM stub and sputtered with gold before the SEM observations Fig. 1B.

2.3.3. FTIR spectroscopy analysis

The structure was analyzed by FTIR spectra (FTIR-Nicolet 6700). For making pellets, samples were powdered and mixed with Potassium bromide (KBr). For preparing pellets in a size of 10.0 mm and thickness of 1–2 mm, it was compressed under high pressure. The pellets were scanned over a range of 4000 cm^{-1} to 400 cm^{-1}. FTIR spectra in the transmission mode were recorded using a Nicolet Nexus, connected to a PC, in which the resolution was 4 Fig. 3A (Fig. 2).

2.4. Anticancer activity tests

2.4.1. MTT assay

The MCF7 cell line were obtained from the Cell Bank of the Pasteur Institute of Iran. Then, these cells were kept in Cell Culture Laboratory in School of Public Health University of Medical Sciences. The MCF7 cell line was cultured in RPMI-1640 medium (Gibco, USA) containing penicillin and streptomycin antibiotic mixture and 5% fetal calf serum (Gibco, USA) (Gibco, USA) in incubator at 5% CO_2 pressure, 37 °C and saturated moisture. After incubation, 10 ml MTT reagent (5 mg/mL) was added to each well and incubated for three more hours. The supernatant was disposed of and isopropanol was poured into the wells. Finally, the color of the medium was read at wavelength of 570 nm using ELISA reader. Different concentrations of quercetin and its nanoparticles were tested in parallel in triplicate, and the IC50 values were obtained from quercetin and concentrations of its nanoparticles versus the mean OD values of the triplicate test curves [17].

2.5. Flow cytometry for analysis of apoptosis in MCF-7 cancer cells

The MCF-7 human breast cancer cell lines (Pasteur Institute of Iran) were placed into 6-well plates at 2.5–3.$\underline{0 \times 10^5}$ cells/ml and incubated overnight without stimulation in RPMI-1640 medium (biowest) supplemented with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and were incubated at 37 °C in 5% CO2. Next day, depending on the experimental protocol, cells were treated with various levels of quercetin nanoparticles and were incubated for 48 h. After 48 h of treatment with quercetin nanoparticles, cells were fixed with 70% ethanol (final concentration) and incubated at 37 °C with 100 μg/ml RNas I for 30 min. Then, the cells were stained with propidium iodide (PI) (Sigma) and cell cycle was monitored by measuring the cells' DNA content with BD FACSCALIBUR (Becton Dickinson Company).

2.5.1. Statistical analysis

Data were reported as mean and standard deviation (SD). Statistical analysis were done using the SPSS Ver. 19.0. Multiple comparisons were carried out using one-way ANOVA followed by the post hoc test. Differences in the MTT cell viability were analyzed using the general linear model, followed by the frequent measures. $P < 0.05$ was considered significant.

3. Results

3.1. Characterization of nanoparticles

SEM was carried out to evaluate the shape and size of NPs. SEM data demonstrated that quercetin-loaded NPs show a spherical form and the mean size of quercetin NPs is 84 nm.

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