

The Effect of Chrysin Nanoparticles in Preventing the Growth of MCF-7 Cancer Cells

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ABSTRACT

BACKGROUND AND OBJECTIVE: Chrysin is a natural and biologically active compound extracted from plants, honey and propolis. Since chrysin has pharmacological activities such as antioxidant, anti-inflammatory and anti-cancer properties, this study was conducted to compare the effect of chrysin and its nanoparticle on breast cancer cells (Michigan Cancer Foundation-7).

METHODS: In this study, chrysin nanoparticles were prepared using chitosan, and then the prepared nanoparticles were dissolved in water, and the cytotoxic effects of 20, 40 and 80 μ M chrysin and chrysin dissolved in dimethyl sulfoxide (at the same concentrations) on MCF7 cells were investigated. Cell viability was measured using MTT assay after 24, 48 and 72 hours, and was compared with viability of the control samples.

FINDINGS: The results of this study showed that chrysin and chrysin nanoparticles prevented the growth and proliferation of MCF7 cell line. These effects depend on the concentration of chrysin and its nanoparticles, and it has been shown that the effect of chrysin nanoparticle on cells at a concentration of 40 μ M in 72 hours is significant with $p < 0.001$ while it is significant with $p < 0.01$ at the same concentration in 24 and 48 hours. The IC₅₀ value of chrysin nanoparticle was approximately 40 μ M.

CONCLUSION: The results of the study showed that the chrysin nanoparticle exhibits anti-proliferative activity in breast cancer cells in the laboratory and its effect is greater than that of chrysin.

KEY WORDS: Chrysin, Nanoparticle, MCF7, MTT, Breast Cancer.

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Introduction

Cancer is one of the leading causes of death in the world that occurs as a result of uncontrollable proliferation of cells (1, 2), which is affected by environmental factors such as chemicals, short waves, viruses, or a genetic mutation (3 – 5). Antioxidants are associated with physiological and pathological processes in which free radicals and active species are produced. Excessive production of these species causes oxidative stress in the cell, while an intracellular antioxidant system cannot counteract the destructive effects of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and radical hydroxyl (OH) (6, 7). Oxidative stress involves damage to biological molecules such as DNA, membrane lipids and enzymes, which ultimately lead to diseases like cancer and age-related neurodegenerative diseases (8, 9). In 2012, breast cancer was reported as one of the leading causes of death from cancer among women worldwide, specified with 1.7 million cases and about half a million deaths (10). This type of cancer occurs through gradual transformation of normal cells to cancer cells. Estrogen plays a major role in the growth of mammary epithelial cells and as a result, breast cancer (7, 11). Treatment for this type of cancer is different and includes methods such as chemotherapy, radiotherapy, gene therapy and surgery, but these methods have several side effects for patients, and many patients are encountered with drug resistance (12). Flavonoids are a group of natural compounds that are extensively found in plants. These compounds are known as one of the largest groups of natural compounds and they are considered as strong antioxidants with significant effects on cellular biology. One of these important effects is the inhibition of free radicals, which play a significant role in the spread of cancerous tumors in addition to causing damage to cells. Research has shown that these substances play an important role in preventing gene mutations and the spread of cancerous tumors (7, 13). Chrysin (5,7 – dihydroxyflavone) is a natural flavonoid extracted from propolis, which is also found in natural honey and has anti-bacterial, anti-inflammatory, anti-oxidant and anti-cancer properties (14). In addition, medical reports have introduced honey as a cover (cure) for wound and burn

(15). The potential apoptotic effect of chrysin is a key molecular mechanism responsible for anticancer activity in breast and gastric cancers, which has been reported in several studies (16). In spite of the favorable effects of chrysin, its insolubility in water leads to its poor penetrability into cancer cells and the lack of biological access, which has led to a reduction in its efficacy. Therefore, in this study we increased its solubility in water by synthesizing the chrysin nanoparticle, and with the help of nanotechnology, we increased the effectiveness of chrysin, decreased their toxicity and increased the half-life of nanoparticles. This study was conducted to compare the effect of chrysin and its nanoparticles on breast cancer cells (MCF7).

Methods

Materials: The chemicals used in this test were of analytical type. Chitosan and chrysin were purchased from Sigma Aldrich. The solvents acetonitrile, methanol and HPLC water were prepared from Merck (Germany). The MCF7 cancer cell was also obtained from the Tehran Pasteur Institute. Fetal bovine serum (FBS), EDTA trypsin and RPMI-1640 were purchased from Gibco (UK). Other materials used in this experiment were purchased from Sigma. The Labconco freeze dryer (USA) was used to remove the solvent and dry the chrysin nanoparticles, and high performance chromatography (Knauer, Germany) was used to determine the loading capacity of the chrysin nanoparticles.

Chrysin nanoparticle synthesis: Low molecular weight chitosan was used to prepare chrysin nanoparticles. Chitosan was dissolved in water containing 0.01% acetic acid in stirrer condition, and 1% Tween 80 was added to the solution to prevent mass formation and was placed on stirrer for 24 hours and was then centrifuged at 12000 rpm for 15 minutes, and the precipitate was stored in the freezer at -80°C for 24 hours. Then, the precipitate was dissolved in water and was mixed with dissolved chrysin drop by drop at the lowest amount of DMSO. A solution of 0.1% tripolyphosphate was added to the above sample drop

by drop and again centrifuged at 14000 rpm for 15 minutes, and then the precipitate was dried using a freeze dryer (17).

Identification and determination of the efficiency of chrysin nanoparticle by high performance liquid chromatography (HPLC): The amount of chrysin loaded on chitosan nanoparticles was determined using reversed-phase high-performance liquid chromatography (RP-HPLC). For analysis, the HPLC protocol was used with the mobile phase including acetonitrile and water with a 20/80 ratio, and the stationary phase of C18, 5 µm and a length of 25 cm with mobile phase velocity of 0.8 ml/min. The absorption rate was read by UV detector at a wavelength of 270 nm.

Determination of chrysin nanoparticle size: A scanning electron microscope (SEM) (JEOL JBX-6300FS) was used to determine chrysin nanoparticle size. The spectrophotometer (UV-vis, 7205, Jenway Co., United Kingdom) was used to determine the amount of chitosan in the nanoparticle synthesis process. Then, the FT-IR device (Nicolette, USA, Thermo Fisher Scientific) was used to identify functional groups.

Evaluation of cell viability and cell proliferation (toxicity) by MTT Assay: In this experimental study (ethics code: IR-MUBABOL.HRI.1397.039), the MCF7 cell line, which was purchased from the cell bank of Tehran Pasteur Institute, was cultured in RPMI medium containing 10% FBS and 1% penicillin-streptomycin in CO₂ incubator at 37 °C with sufficient moisture. The MTT colorimetric assay was used to investigate the effect of chrysin and chrysin nanoparticles on growth and proliferation of cancer cells.

In this method, 104 cells were cultured in each well of the 96-well plate. After 24 hours of incubation, different concentrations of chrysin (dissolved in DMSO) and chrysin nanoparticle (dissolved in water) were added to the cells at 20, 40, 80 µg / ml and were incubated at 24, 48 and 72 hours. After this period, the cells were exposed to MTT dye (5 mg / ml) for 4 hours and the DMSO solution was added to cells to dissolve formazan crystals. The absorption rate of the cells and

the color change of the MTT compound added to them were read at a specific wavelength (570 nm) using ELISA reader. Each of these experiments was repeated three times and cell viability was calculated according to the following equation:

$$100 \times \frac{\text{test optical absorption}}{\text{control optical absorption}} = \text{Cell viability (\%)}$$

Statistical analysis: The results were calculated using SPSS 16 and the relationship between cell viability and nanoparticle size was obtained by one-way ANOVA and Tukey's post-test and P < 0.05 was considered significant.

Results

The chrysin nanoparticles were successfully synthesized and were identified using SEM and FT-IR techniques. The results of the experiments showed that the chrysin nanoparticles were cylindrical shaped and had a mean particle size of 150 nm (Fig. 1, 2).



Figure 1. Chrysin nanoparticle image by electron microscope (SEM)

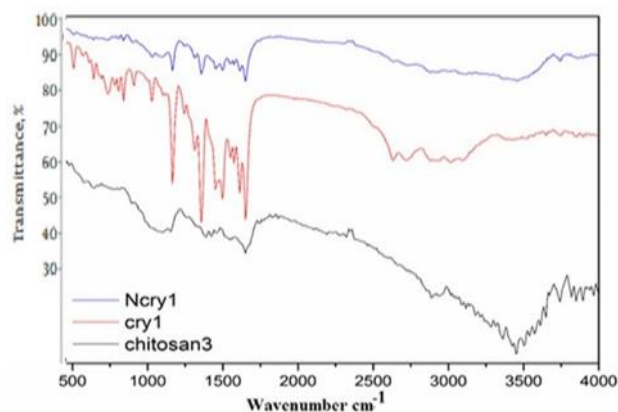


Figure 2. Chromatogram obtained from FTIR for chitosan, chrysin and chrysin nanoparticle

Determination of the amount of chrysin loaded on the nanoparticle by HPLC technique: HPLC technique was used to determine the amount of chrysin loaded on the nanoparticle. The amounts of nanoparticles and drug loading content were calculated by equations (1) and (2) (18). Chrysin loaded on chitosan nanoparticles was calculated using equations (1) and (2) by analyzing HPLC, which was 5% (Fig. 3).

(1)

$$100 \times \frac{\text{amount of chrysin loaded on the nanoparticle}}{\text{total added chrysin}} = \text{drug loading content (\%)}$$

(2)

$$100 \times \frac{\text{total nanoparticle weight}}{\text{weight of the added chrysin and chitosan}} = \text{nanoparticle efficacy (\%)}$$

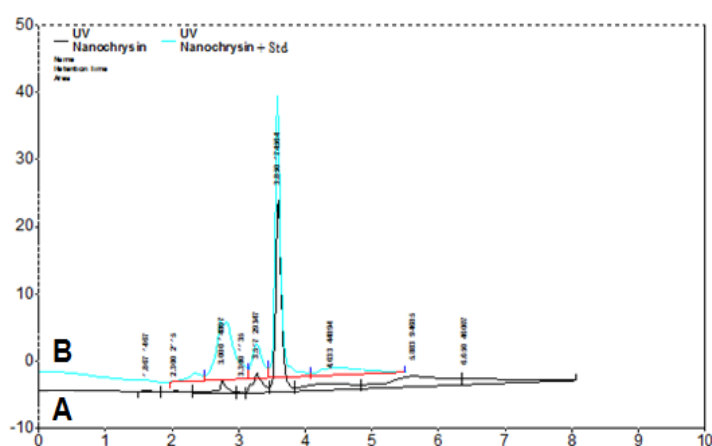


Figure 3. Chromatogram obtained by the HPLC method, A: chrysin nanoparticle; B: 5 PPM standard chrysin

Cytotoxicity measurement: The inhibitory effect of chrysin nanoparticle and chrysin on proliferation of MCF7 cells was demonstrated at concentrations of 20, 40 and 80 μM . According to the results of this experiment, the lack of growth and activity of the cancer cells showed a significant decrease in the chrysin nanoparticle compared to the chrysin at 40 μM ($p < 0.01$) and in 72 hours ($p < 0.001$) (Fig. 4). The IC_{50} value in 24 hours was $95 \pm 2.45 \mu\text{M}$, in 48 hours was $84 \pm 1.45 \mu\text{M}$, and in 72 hours was $58 \pm 1.3 \mu\text{M}$. Lack of cell growth at the same concentration of chrysin nanoparticle (80 μM) showed significant decrease, which was associated with different incubation time. Minimum cellular activity

was observed in treatment with 80 μM chrysin in 72 hours. The amount of inhibition was dose- and time-dependent and the highest inhibition was obtained at 82% (Fig. 5). The results of this study showed that the chrysin nanoparticle has greater effects on the growth inhibition and death of cancer cells compared to chrysin.

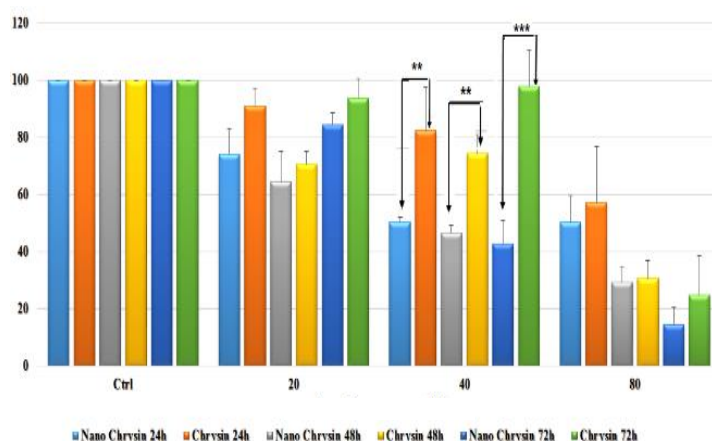


Figure 4. The cytotoxic effect of chrysin and chrysin nanoparticle on MCF7 cell line by MTT assay in 24 and 48 hours; there was a significant difference at the concentration of 40 μM ($p < 0.01$) in 72 hours ($p < 0.001$)

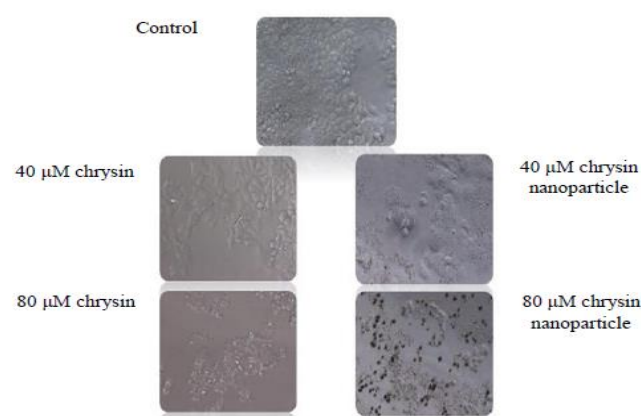


Figure 5. The cytotoxic effect of chrysin and chrysin nanoparticle on MCF7 cell line by MTT assay at 40 and 80 μM concentrations

Discussion

Based on the results of this study, the inhibitory effect of chrysin and chrysin nanoparticle on MTT cells was observed in 24, 48 and 72 hours after the effect. The MTT assay showed that the cytotoxic effect increases

with increasing dosage and time, which indicates that these drugs affect the breast cancer cells (MCF7) in a time- and dose-dependent manner. The results showed that pure chrysin had a half maximal inhibitory concentration (IC₅₀) of $95 \pm 2.45 \mu\text{M}$ in 24 hours, and this effect was $84 \pm 1.45 \mu\text{M}$ in 48 hours and $58 \pm 1.3 \mu\text{M}$ in 72 hours, while the results showed the effect of chrysin nanoparticle to be $40 \pm 1.2 \mu\text{M}$ in 24 hours, $38 \pm 2.8 \mu\text{M}$ in 48 hours and $35 \pm 1.4 \mu\text{M}$ in 72 hours. One of the new ways of treating cancer is direct transmission of anticancer drugs to the tumor. The use of nanotechnology in cancer treatment is one of the newest methods for targeted drug delivery (19).

Biodegradable nanocarriers for targeted drug delivery have been developed to reduce or minimize undesirable interactions or unwanted absorption in natural locations. The formulation of chemical nanoparticles is designed to provide efficient therapeutic effect at the right location and may be useful in treating cancer. Flavonoids are compounds that usually inhibit cell proliferation. The anti-proliferation effects of flavonoids on cancer cells have been reported in several studies (20, 21). Anti-proliferation and anti-growth effects in cancer cells are done by the induction of apoptosis or inhibition of cell cycle by flavonoids (22, 23). Chrysin is a natural flavonoid found in honey, plants and propolis. Various studies have shown that the use of flavonoid-containing foods reduces the risk of cancer (24 – 26). Based on this study, 40 and 80 μM concentrations of chrysin nanoparticles and 80 μM concentration of chrysin were effective against breast cancer cells. Chrysin nanoparticles were more effective

in inhibiting the growth of MCF-7 cells at 40 and 80 μM concentrations compared with chrysin. Our research provides evidence that chrysin nanoparticles reduce the proliferation of MCF-7 and prevent the growth of MCF-7 colonies compared with chrysin. These findings are usually consistent with the results of tests that consider the dose- and time-dependent cell proliferation. Various studies have shown that the use of chrysin nanoparticle reduces the proliferation of MCF-7 cancer cells (27 – 29), while the efficacy of the nanoparticle produced in this method is far more than other methods. In this experiment, chrysin nanoparticles at much lower concentrations proved to be more effective on MCF-7 cancer cells. Another advantage of this method is the use of biodegradable compounds that have no effect on healthy cells.

In this study, it was shown that IC₅₀ of chrysin nanoparticle is very low and would cause cell death at 40 μM concentration. As a result, the inhibitory effect of the chrysin nanoparticle on breast cancer cells is greater than pure chrysin. This effect proceeds by a mechanism that prevents the progression of the cell cycle from G1 phase to S phase and increases cell death and apoptosis in MCF7 cells. According to the results of this study, chrysin nanoparticle could be used in vitro protocols for the treatment of breast cancer.

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