Summary of the DVM thesis NO 11986 Faculty of Veterinary Medicine, Urmia University.

The academic year: 2022-2023

Author: Mohammad Amin Bakhshan

Title of thesis: Evaluation of the cytotoxic effects of hesperidin-containing nanoparticles on prostate cancer cells (LNcap cell line)

Summary:

Introduction: Today, prostate cancer has become one of the most common cancers in men. Flavonoids are important compounds that have the strongest anticancer activities among other bioactive compounds. Hesperidin is a flavonone glycoside that is abundantly found in citrus fruits, and in addition to its anti-cancer properties, it is also anti-inflammatory and antioxidant. The purpose of this study was to investigate the anticancer effects of hesperidin nanoemulsion and free hesperidin on LNcap cell line as a prostate cancer cell line.

Materials and methods: Hesperidin nanoemulsion was investigated using the spontaneous oil-in-water emulsion method, the production, physicochemical properties and stability of the nanoemulsion. Then LNcap cancer cells treated with different concentrations of hesperidin nanoemulsion (0,10, 20, 30, 40, 50 and 60 ug/ml) and free hesperidin (0,10, 20, 30, 40, 50 and 60 ug/ml) and the cytotoxicity of the produced nanoemulsion was evaluated using neutral red, MTT, and trypan blue methods and evaluating the ability of colony formation as well as the amount of apoptosis and necrosis with acridine orange/propidium iodide staining method. In order to evaluate the biocompatibility of the produced nanoemulsion, MTT test was performed for PBMC cells and hemolytic test was performed on red blood cells.

Results: The produced nanoparticles had an average diameter of 197.2 ± 2.8 nm, zeta potential of -28 mV, uniform dispersion of 0.13, encapsulation efficiency of 84.04 ± 1.3%, and stable release. The stability of nanoparticles during a two-month storage period at two temperatures, 25 and 4 degrees Celsius, was investigated. The results showed that over time, the nanoparticles underwent certain changes in their physical and chemical properties, except for particle size and nanoparticles opacity, where the changes in other evaluated parameters were not statistically significant. This indicates the high stability of the produced nanoparticles. The results from cellular toxicity tests (MTT, neutral red, and trypan blue) revealed that cell viability in the groups treated with different concentrations of Hesperidin nanoparticles was significantly lower than the free Hesperidin group ($p < 0.05$). IC50 of hesperidin nanoemulsion in trypan blue test 31.25±2.5 ug/ml, in MTT test 31±2.08 ug/ml after 24 hours, 20.3±4.5 ug/ml after 48 hours, in The neutral red test was 38.4±2.6 ug/ml after 24 hours, and 29.4±3.5 ug/ml after 48 hours. As for free hesperidin, it could not kill at least 50% of cancer cells in the concentrations used in this

study in both the MTT and neutral red tests for 24 hours; But in 48 hours in MTT test at 47.6±1.5 ug/ml and in neutral red test at 52.08±2.1 ug/ml, it was able to kill at least 50% of cancer cells (IC50). The results of the acridine orange-propidium iodide test showed that the number of apoptotic cells increased significantly after treatment with hesperidin nanoemulsion compared to the control group and free hesperidin. The results of colony formation assay and cell morphology evaluation also showed the greater power of the nanoemulsion form of the drug than its free form. Biocompatibility evaluation tests of the produced nanoemulsion (hemolytic test and MTT test on PBMC cells) also showed that hesperidin nanoemulsion in low concentrations can be safely used inside the body.

Conclusion: In general, it can be concluded that hesperidin nanoemulsion not only showed stronger anticancer activity than free hesperidin, but also was very biocompatible and had minimal cytotoxic effects on healthy cells. And it can be further studied as an adjuvant treatment along with other treatment methods for the treatment of prostate cancer.

Key words: Prostate cancer, Hesperidin, LNcap cell line, Nanoemulsion