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Thesis Title: Co-loading of berberine and hesperidin by poly(lactic-co-glycolic acid) nanoparticles and determination of its effects on viability and induction of apoptosis in cancer cells.

Abstract:

Prostate cancer is still one of the main causes of cancer deaths among men, which shows the necessity of developing new treatment approaches. Recently, the use of therapeutic agents of natural origin, especially phytochemicals such as hesperidin and berberine, are increasingly considered as a promising strategy to prevent malignancy and treat cancer. Despite the health benefits associated with berberine and hesperidin, achieving effective therapeutic results depends on improving the pharmacokinetic properties of these compounds after oral administration. Therefore, recent studies have focused on nanotechnology for the development of new drug delivery systems. Therefore, in this study, the simultaneous encapsulation of berberine and hesperidin in poly(lactic-co-glycolic acid) (PLGA) nanoparticles and the evaluation of their effects on the survival and induction of apoptosis in prostate cancer cells were investigated. Nanoparticles containing hesperidin, nanoparticles containing berberine and nanoparticles containing both substances in half the concentration used were synthesized using double emulsion-solvent evaporation (W/O/W) method. The produced nanoparticles were characterized in terms of size, dispersion index, morphology, encapsulation efficiency and drug release profile. Prostate cancer cells (LNCaP) were cultured and treated with different concentrations of nanoparticles containing berberine, nanoparticles containing hesperidin and nanoparticles containing both hesperidin and berberine. Cytotoxicity of produced nanoparticles was evaluated by MTT and LDH tests. The rate of apoptosis and necrosis was evaluated using flow cytometry and Annexin V/PI staining. The expression level of genes involved in apoptosis was investigated by qRT-PCR method. The results showed that all three types of produced nanoparticles had an average diameter below 200 nm, a uniform size distribution below 0.1, and a favorable encapsulation efficiency. As shown by the MTT test, the treatment with nanoparticles compared to the free form of hesperidin and berberine led to a significant decrease in cell viability in a dose-dependent manner. 35/µg/ml), berberine nanoparticles with doxorubicin (at a concentration of 37.43±3.6 µg/ml) and combined nanoparticles group with Doxorubicin was (at a concentration of 22.67±2.05 µg/ml). Flow cytometry analysis showed an increase in the population of apoptotic cells after treatment with nanoparticles, compared to the free form of hesperidin and berberine. Also, the results showed that berberine had a higher percentage of apoptosis induction than hesperidin, both in the free form and in the nano form. The use of these two substances together increased the percentage of apoptotic cells and this percentage increased again with the simultaneous use of nanoparticles combined with

doxorubicin. In the clonogenic test, treatment with all treatment groups decreased the ability of colony formation in LNCaP cells compared to the control group. But in general, it can be said that berberine compared to hesperidin, nanoparticles compared to the free form, and combined groups compared to single ones, inhibited colony formation more. In the evaluation of LDH, the level of LDH in the supernatant of all treated cells increased. However, the highest percentage of cytotoxicity against LNCaP cells was related to combined nanoparticles with doxorubicin and berberine nanoparticles with doxorubicin. The results showed that all treatment groups were involved in the apoptosis of LNCaP cells by inducing caspase-3 activity in treated cells compared to untreated cells. However, combined nanoparticles with doxorubicin caused a 2.35-fold increase, combined nanoparticles with a 2.05-fold increase, and berberine nanoparticles with doxorubicin caused a 1.92-fold increase in caspase 3 levels compared to the control group. The PCR results also showed that berberine compared to hesperidin, nanoparticles compared to free form, and combined groups compared to single ones, increased the expression of BAX gene (pro-apoptotic) and decreased the expression of Bcl-2 gene (anti-apoptotic gene). These findings indicate that PLGA nanoparticles containing berberine and hesperidin have great potential as a therapeutic strategy for the treatment of prostate cancer and provide a dual approach to reduce cell viability and promote apoptosis. To confirm these results and fully investigate the therapeutic potential of this nanoparticle formulation, further laboratory studies and clinical trials are necessary.

Keywords: Berberine, Hesperidin, Poly(lactic-co-glycolic acid), Cance