

Abstract

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Title of diploma thesis: *In vitro* study of molecular mechanisms of antiproliferative activity of extract from juniper berry in colorectal cancer cells

Colorectal cancer is one of the most diagnosed cancers and one of the leading causes of cancer-related death in developed countries. Its development is strongly influenced by environmental factors, particularly by diet that could provide cancer prevention and treatment with plant-derived substances possessing chemopreventive and/or antiproliferative ability with minimum of the undesirable side-effects, often seen with synthetic medicines. Phenolic compounds contained especially in various berries have displayed health-promoting properties including the anticarcinogenic effect.

In this study, the cytotoxic effects of juniper berry extract and the mechanisms of its antiproliferative activity were evaluated *in vitro*. Two colorectal cancer cell lines - HCA-7 and Caco-2 - were treated with different concentrations of juniper extract and the results were compared to untreated control cells. Cytotoxicity of the extract was examined by metabolic activity assay, using commercial kit, and by lactate dehydrogenase test. The molecular mechanisms of the effects of extract were assessed by Western blot to analyse the levels of specific proteins related to cell death.

Changes in cellular morphology after the treatments with selected concentrations of juniper extract were observed by confocal microscopy.

The results of cell viability assays showed time- and dose-dependent increase of antiproliferative activity in both cell lines in comparison to control, where the assessment by CCK-8 kit suggested that juniper extract has the significant antiproliferative activity already at concentration of 30 $\mu\text{g/ml}$; whereas the LDH test showed cytotoxicity of the extract up to the concentration of hundred times higher. Morphology studies of HCA-7 cells revealed typical hallmarks of apoptosis as membrane blebbing and pycnotic nuclei at the concentration of 30 $\mu\text{g/ml}$, proved that the juniper extract promotes programmed cell death. The results of Western blotting revealed the dose-dependent decrease of p53 abundance in HCA-7 cells after the treatment of juniper extract that can result in reduction of pro-oncogenic activity of its mutated form presented in HCA-7 cells. The levels of p27 were increased in dose-dependent manner in both cell lines that is associated with the inhibition of cellular growth and proliferation and it can play role in cytoskeleton reorganization observed by confocal microscopy. Juniper extract did not show any significant effect on β -catenin levels compared to control either in HCA-7 or Caco-2 cells. The effects on Akt inhibition were observed only in Caco-2 cells after the exposure of 1 $\mu\text{g/ml}$ juniper extract.

This study has indicated potential cytotoxic effect of the extract prepared from the juniper berries that is connected with activation of programmed cell death in colorectal cancer cells by different mechanisms of its initiation.