Cancer is a growing health problem and the second most important cause of death after cardiovascular disease worldwide. Colorectal cancer is the third most common cancer type among men and the second most common type of cancer among women. Apoptosis is a programmed cell death that occurs under normal physiological conditions and within genetic control. Abnormalities in cancer cells are usually caused by mutations in genes that encode proteins that regulate cell proliferation and apoptosis. 5-FU is frequently used in cancer therapy, but due to side effects, the development of non-toxic combination therapies has a major appeal. Achillea genus contains approximately 85 species and many of these species are endemic to Europe and the Middle East. Antimicrobial, antioxidant, antiinflammatory, antidiabetic and antitumor effects of different Achillea species have been reported. A. biebersteinii is used in the treatment of abdominal and stomach pain, injury and cancer in folk medicine.

In this study, anticancer and apoptotic effects of different solvent extracts of Achillea biebersteinii plant and the combined treatments of these extracts with 5-FU were investigated by in vitro cell culture analysis. A. biebersteinii was collected from north of Bingol province, 1150 meters altitude, in May 2014. Plant samples were dried in a moisture free conditions. Aerial parts of plant were extracted by sequential fractionation method using hexane, chloroform and methanol which have different polarity. A549 and MCF-7 cells were grown at 37 °C, 95% humidity and 5-6% CO2 using a high glucose DMEM medium containing 10% FBS, 1% L-glutamine and 1% penicillin-streptomycin. MTT assay was used to determine cell viability. Isobologram analysis was used to quantitatively determine the synergism of drugs and extracts. Cell Death Detection Elisa method was performed the determination of apoptotic cell death as colorimetric. The Human VEGF ELISA method was used to quantitatively determine the amount of VEGF in vitro. Changes in apoptotic genes and proteins (p53, Bax, Bcl-2, p38 MAPK, mTOR, PTEN and AKT) expression levels were assessed by qRT-PCR and Western blotting analysis. β-actin was used as housekeeping in optimization. Each experiment was performed independently in three repetitions.

Combined treatment of 5-FU with A. biebersteinii hexane, chloroform and methanol extracts, the cell viability was determined as 26%, 19.1% and 14.9% respectively (p<0.0001). A. biebersteinii hexane, chloroform and methanol extracts in combination with 5-FU increased the apoptosis 7.3, 9.7 and 9.5 fold, and decreased the amount of VEGF to 85.2, 78.5 and 69.7 pg/ml, when compared with the control group. Changes in apoptotic and anti-apoptotic genes and proteins expression levels were statistically significant in almost all combined treatment (p<0.0001). These findings reveal that the molecular mechanism of combined treatment of 5-FU and A. biebersteinii extracts in colorectal cancer cells. This combination can reduce the high cytotoxic effects of 5-FU.

Key words: Cancer, MTT, Apoptosis, Bel-2, Achillea