Natural products play an important role in investigation of novel anti-cancer agents and the development of more effective drugs. In elimination of cancer cells, the effective treatment models are suggested such as the stimulation of both apoptotic and autophagic cell death types in the same time by using different agents (1). Colchicine is a natural product which is obtained from plants of the genus Colchicum, a member of Colchicacea family (2). We studied with Colchicum baytopiorum CD Brickell (Cb) which is an endemic plant species of Antalya-Turkey (3). It contains colchicine and its derivatives including demecolcine, 2-demethyldemecolcine, 3-demethylcolcine and cornigerin. The aim was to determine the role in both apoptotic and autophagic cell death pathways of the Cb extract on HeLa cell line.

The cytotoxic effect of Cb plant extract (0.1mg/ml) on HeLa cells was determined by using MTT assay. Cell viability was measured by trypan blue. To identify its molecular targets, the expression of genes which are involved in apoptosis and/or autophagic cell death (Bif and BNIP3 for both apoptotic and autophagic cell death; Atg12, Atg5 and DAPk for autophagic cell death; Bcl-xL, Bad, Puma, Noxa, Fas, Akt, TNFR1 and caspase-3,-8,-9 for the apoptotic cell death) were analyzed by qRT-PCR. Immunocytochemical analysis was performed by using active caspase-3 and t-Bid antibodies, then evaluated semiquantitatively.

We observed that there is a significant difference by means of cell cytotoxicity values between the control and the Cb extract treatment groups after an incubation period of 48 hours (p<0.001). According to qRT-PCR results, the expression levels of Bif(2,5), BNIP3(5,4), Atg12(5,7), Atg5(14,3), DAPK(2,3), Beclin-1(31), Bcl-xL(3,1), Bad(2,2), Puma(2,5), Noxa(4,2), Fas(2,4), Akt(2,6), TNFR1(25,1) and Caspase-3(5,7), 8(2,1), 9(2,3) genes were significantly increased (as fold) after the extract treatment. Active caspase-3 and t-Bid immunopositive cells were detected higher number in the Cb extract treated group compare to the control group.

This study shows that the treated dose of the Cb extract induces the crosstalk mechanisms between apoptotic and autophagic cell death in HeLa cells, as well as upregulates some of genes in both of cell deaths. We suggest that this endemic plant extract seems to be a promising new therapeutic approach in cancer.


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Fig. 1: Immunohistochemistry of t-bid (a,b) and active caspase-3 (c,d). (C: Control group, Cb: Colchicum baytopirium group)

Fig. 2: Cytotoxic activity (MTT) of Cb extract throughout 48h on HeLa cell cultures. *p<0.001 compared to control group. (C: Control group, Cb: Colchicum baytopirium group)

Fig. 3: Analysis of distribution of t-Bid and active caspase-3 expression in HeLa cells. *p<0.001 compared to control group. (C: Control group, Cb: Colchicum baytopirium group)