CMJ, 2024;46(4): 280-291
DOI: 10.7197/cmj.1601162

Cumhuriyet Medical Journal

| cmj.cumhuriyet.edu.tr | Founded: 2004 Available online, ISSN:1305-0028 Publisher: Sivas Cumhuriyet Üniversitesi

Investigation of the Synergistic Effect of *Allium Polyanthum Schult* Extract and Docetaxel on Apoptosis-Related Genes in Colorectal Cancer Cells and Bioinformatics Analysis

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Research Article

History

Received: 13/12/2024
Accepted: 25/12/2024

ABSTRACT

Objective: Colorectal cancer (CRC) is a type of cancer with high mortality and widespread metastasis. The aim was to determine the expression levels of apoptosis-related genes in CRC cells treated with *Allium polyanthum Schult* plant extract and the combination of this plant extract with docetaxel.

Methods: Expression analyses of *caspase-2* (*CASP2*), *nuclear factor NF-kappa-B1* (*NFKB1*), apoptosis regulator *BAX* and proto-oncogene *MYC* genes that play a role in apoptosis in the CRC cell line HT-29 and the healthy colon cell line CCD-18Co, which were treated with only *Allium polyanthum Schult* plant extract and docetaxel together with this plant extract, were performed using the real-time polymerase chain reaction (RT-PCR) method. Bioinformatics analysis of relevant genes was performed using various databases.

Results: *CASP2*, *MYC*, *NFKB1* and *BAX* gene expression was significantly decreased in CRC cells treated with the combination of *Allium polyanthum Schult* extract and docetaxel compared to healthy cells. Accordingly, only the extract of *Allium polyanthum Schult* plant significantly reduced the expression of apoptosis-related genes in HT-29 cells compared to the extract combined with docetaxel. As a result of bioinformatics analysis, it was found that CASP2, MYC, NFKB1 and BAX proteins interact with each other and the expression levels of their genes are associated with survival. In addition, the methylation status of *CASP2* and *NFKB1* has the potential to change protein levels by affecting epigenetic mechanisms in CRC.

Conclusion: According to the information in the literature, it has been reported that *Allium* species affect genes in apoptotic pathways. Accordingly, *Allium polyanthum Schult* alone and in combination with docetaxel should be supported by further studies.

Keywords: *Allium polyanthum Schult*, apoptosis, colorectal cancer, docetaxel, gene expression

Allium Polyanthum Schult Ekstraktı ve Dosetakselin Kolorektal Kanser Hücrelerindeki Apoptozla İlgili Genler Üzerindeki Sinerjik Etkisinin Araştırılması

Araştırma Makalesi

Süreç

Geliş: 13/12/2024
Kabul: 25/12/2024

ÖZET

Amaç: Kolorektal kanser (CRC), yüksek mortalite ve yaygın metastazlı bir kanser türüdür. CRC'nin erken teşhis edildiği durumlarda kemoterapi ve cerrahi uygulamalar esastır. Docetaxel, taksan grubunda bir antikanser ajandır. Ayrıca, tıbbi aktiviteye sahip bitkilerin CRC üzerinde koruyucu bir etkiye sahip olduğu bilinmektedir. Amaç, Allium polyanthum Schult bitki özütü ve bu bitki özütünün dosetaksel ile kombinasyonu ile tedavi edilen kolorektal kanser hücrelerinde apoptozisle ilişkili genlerin ifade düzeylerini belirlemekti.

Yöntem: Sadece Allium polyanthum Schult bitki ekstresi ve bu bitki ekstresiyle birlikte docetaxel uygulanan CRC hücre hattı HT-29 ve sağlıklı kolon hücre hattı CCD-18Co'da apoptozda rol oynayan kaspaz-2 (CASP2), nükleer faktör NF-kappa-B1 (NFKB1), apoptoz düzenleyici BAX ve proto-onkogen MYC genlerinin ekspresyon analizleri, gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) yöntemi kullanılarak gerçekleştirildi. Çeşitli veritabanları kullanılarak ilgili genlerin biyoinformatik analizi gerçekleştirildi.

Bulgular: CASP2, MYC, NFKB1 ve BAX gen ekspresyonu, Allium polyanthum Schult özütü ve docetaxel kombinasyonu ile tedavi edilen CRC hücrelerinde sağlıklı hücrelere kıyasla önemli ölçüde azaldı. Buna göre HT-29 hücrelerinde sadece Allium polyanthum Schult bitkisinin ekstraktı, dosetakselle kombine olan ekstrakta göre apoptozla ilgili genlerin ekspresyonunu oldukça düşürmüştür. Biyoenformatik analiz sonucunda, CASP2, MYC, NFKB1 ve BAX proteinlerinin birbirleriyle etkileşime girdiği ve genlerinin ekspresyon seviyelerinin hayatta kalma ile ilişkili olduğu bulundu. Ayrıca, CASP2 ve NFKB1'in metilasyon durumu, CRC'de epigenetik mekanizmaları etkileyerek protein seviyelerini değiştirme potansiyeline sahiptir.

Sonuç: Literatürdeki bilgilere göre, Allium türlerinin apoptotik yollardaki genleri etkilediği bildirilmiştir. Buna göre, Allium polyanthum Schult'un tek başına ve docetaksel ile kombinasyonu daha fazla çalışma ile desteklenmelidir.

Anahtar Kelimeler: *Allium polyanthum Schult*, apoptoz, docetaksel, gen ekspresyonu, kolorektal kanser

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How to Cite: Tunçbilek Z, Kabak G, Siliğ Y. Investigation of the Synergistic Effect of Allium Polyanthum Schult Extract and Docetaxel on Apoptosis-Related Genes in Colorectal Cancer Cells and Bioinformatics Analysis. Cumhuriyet Medical Journal, 2024;46(4):280-291.

Introduction

CRC is a cancer with high mortality and incidence worldwide. According to GLOBOCAN statistics for 2022, CRC ranks fourth in terms of incidence and third in terms of mortality.[1] Due to the increasing incidence of CRC, which is usually diagnosed at an advanced age, it is predicted that these cases will increase according to the estimates for the year 2035. It is estimated that CRC cases will increase especially in underdeveloped countries.[2, 3] The term CRC refers to the uncontrolled proliferation of glandular epithelial cells within the colon, including the rectum.[4] CRC is diagnosed by staging. These stages are classified as Stage 0-IV. While surgical treatment is applied in Stage 0 to II, adjuvant therapy is recommended in addition to surgery in Stage III. In Stage IV, advanced surgical applications and the use of targeted therapies are included.[5] For CRC, as in all cancers, there is a strong relationship between apoptosis signaling and cell survival. Abnormal functioning of apoptosis pathways in CRC disrupts homeostasis of colorectal epithelial cells. Suppression of apoptotic pathways has been explained by resistance to chemotherapeutic agents. The fact that apoptosis is different in normal cells compared to cancer cells and that apoptosis increases the cytotoxic response in cancer has helped develop various strategies in this regard.[6] In addition, apoptosis includes genetic factors that may be effective in the proliferation and recurrence of cancer cells Investigating the genes and proteins involved in apoptotic pathways has the potential to help shed light on the disease.[7]

Although there are various chemotherapeutic applications that increase the survival of CRC, drug resistance is a possibility. In addition, the controversial reliability of synthetic drugs directs attention to traditional medical applications. In this context, the effectiveness of many plants with medical importance for many diseases, including cancer, has been reported from past to present. The fact that natural medical practices are safer in addition to chemotherapy has helped increase studies on this subject. [8-10] Medicinal aromatic plants are important in terms of their medical effectiveness in cancer. The anti-cancer properties of plants in the *Allium* genus have been investigated and in addition, their anti-microbial, anti-oxidant and anti-inflammatory activities have been revealed. There are many plants identified as belonging to the *Allium* genus, such as garlic, leek and onion.[11-13] *Allium* plants belong to the *Amaryllidaceae* family and contain many bioactive components, which explains their biological activity. The discovery of many therapeutic activities of *Allium* plants has made the research of plants belonging to this genus important.[14]

Materials and Methods

Preparation of Allium polyanthum Schult extract

Allium polyanthum Schult plant was obtained from Zara, Sivas, Turkey. The plant was collected in May and September. Essential oils were extracted according to the method determined by Alkan et al. According to the procedure, the essential oils of *Allium polyanthum Schult* plant were obtained by hydrodistillation for three hours from flowering stem parts and only stem parts with the help of Clevenger apparatus. The temperature of the cooling water was adjusted to 4°C by connecting the Clavenger apparatus to the microhiller device. The isolated essential oil was purified from water by Na_2SO_4. The samples were stored at -20°C.[15]

Cell culture

HT-29 (ATCC, HTB-38) was used within the scope of CRC cell line. In addition, CCD-18Co (ATCC, CRL-1459) cell line is healthy colon epithelial cells. These cells were cultured at 37ºC in a humidified environment containing 5% CO_2. Roswell Park Memorial Institute 1640 medium (RPMI, Biological Industries) was used as HT-29 medium, and Minimum Essential Medium (MEM, Sigma-Aldrich) was used as CCD-18Co cell line medium. 10% Fetal bovine serum (FBS, Capricorn Scientific) and 1% penicillin/streptomycin (Sigma-Aldrich) were applied to the media.

Expression of apoptosis-related genes

RT-PCR method was used for expression analysis of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in HT-29 and CCD-18Co cells. First, cell lines were seeded in six-well plates for gene expression analysis. The cytotoxic dose of *Allium polyanthum Schult* plant extract and additionally the cytotoxic dose (IC_{50}) of docetaxel drug together with extract were applied to these cells.[16] IC_{50} doses of 0.190 µM for docetaxel, 0.043 µM for *Allium polyanthum Schult* + docetaxel, and >300 µM for *Allium polyanthum Schult* alone were applied to the CCD-18Co cell line separately. IC_{50} doses of 0.077 µM for docetaxel, 0.009 µM for *Allium polyanthum Schult* + docetaxel, and >300 µM for *Allium polyanthum Schult* alone were applied to the HT-29 cell line. After 48 hours of incubation, RNA isolation from cells was performed according to the kit procedure. cDNA was generated from the RNAs obtained using the cDNA synthesis kit. Primers for genes are provided in the form

of primer assays. Gene expression analysis was performed using Syber Green Master mix.

Bioinformatics analyses

Analysis of protein-protein interactions of CASP2, NFKB1, BAX and MYC was provided by the Stringv12 database (https://version-12-0.string-db.org/cgi/network?networkId=bBuQ7cSnMJMh). The GEPIA database is an effective tool for analyzing the expression levels of many genes in tumor tissues compared to normal tissues (http://gepia2.cancer-pku.cn). UALCAN is a database that provides transcriptomic data for all cancer types (http://ualcan.path.uab.edu/index.html). Using this database, survival rates and methylation levels of *CASP2*, *BAX*, *NFKB1* and *MYC* genes were analyzed separately in colon and rectum compared to normal tissues.[17] According to the database, methylation levels between 0.7-0.5 indicate hypermethylation, and between 0.3-0.25 indicate hypomethylation.[18, 19] Using the MuTarget database, which identifies mutations in cancer, the somatic mutations in *CASP2*, *BAX*, *NFKB1* and *MYC* genes were analyzed to determine which genes' expression was affected in colon adenocarcinoma (https://www.mutarget.com).[20]

Statistical Analysis

RT-PCR data were obtained using Rotor-Gene 6000 Series Version 1.7 software. The $\Delta\Delta C_T$ method was used for gene expression analysis. Expression analysis was completed with the RT^2 profiler RT-PCR Array Data Analysis version 3.5 application (https://geneglobe.qiagen.com/us/analyze). ANOVA tests was applied and Tukey's multiple comparison test was used. Three replicates were used to obtain data. Significance value was accepted as $p<0.05$.

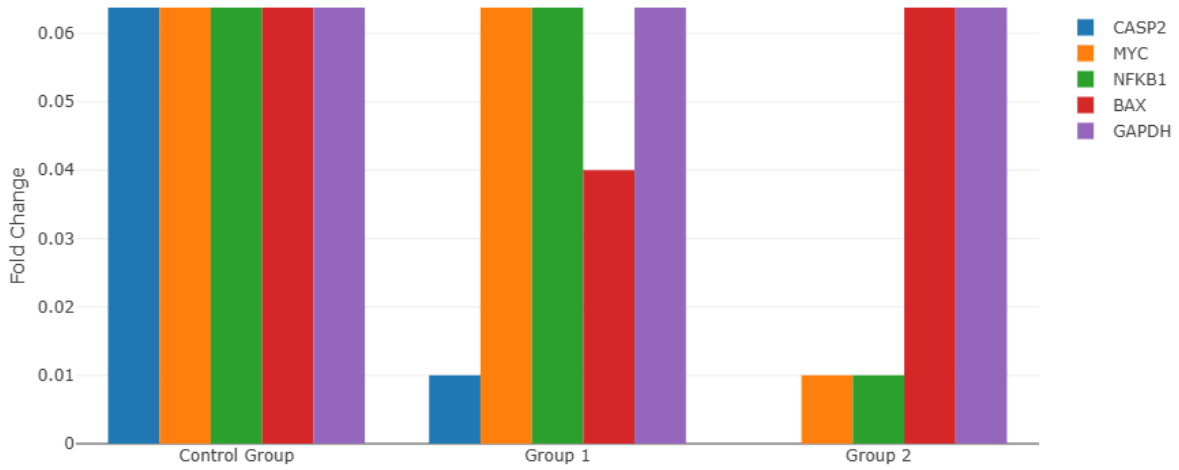

Figure 1. Fold change graph for expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in HT-29 cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

Table 1. Fold change, fold regulation and p values of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in HT-29 cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

Genes	**Group 1**			**Group 2**		
	Fold change	**Fold regulation**	***p*-value**	**Fold change**	**Fold regulation**	**p-value**
CASP2	0.01	-117.78	0.001*	0.00	-310.83	0.001*
MYC	0.08	-13.27	0.001*	0.01	-97.01	0.001*
NFKB1	0.07	-14.42	0.001*	0.01	-78.25	0.001*
BAX	0.04	-27.67	0.001*	0.42	-2.39	0.001*

Results

Expression level of apoptosis-related genes

The expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes were evaluated in HT-29 and CCD-18 cells with CRC. The effectiveness of *Allium polyanthum Schult* plant extract and its combination with docetaxel was evaluated. Accordingly, *CASP2*, *NFKB1*, *BAX* and *MYC* gene expressions were significantly decreased in HT-29 cells to which *Allium polyanthum Schult* plant extract was applied and the combination of plant extract and docetaxel was applied compared to HT-29 cells to which nothing was applied (Figure 1) (Table 1).

The combination of plant extract and docetaxel increased *CASP2*, *MYC* and *BAX* gene expression and decreased *NFKB1* gene expression in CCD-18Co cells compared to cells without any treatment. In addition, *CASP2* and *NFKB1* expressions were decreased, while *MYC* and *BAX* gene expressions were decreased in cells treated with only plant extract (Figure 2) (Table 2).

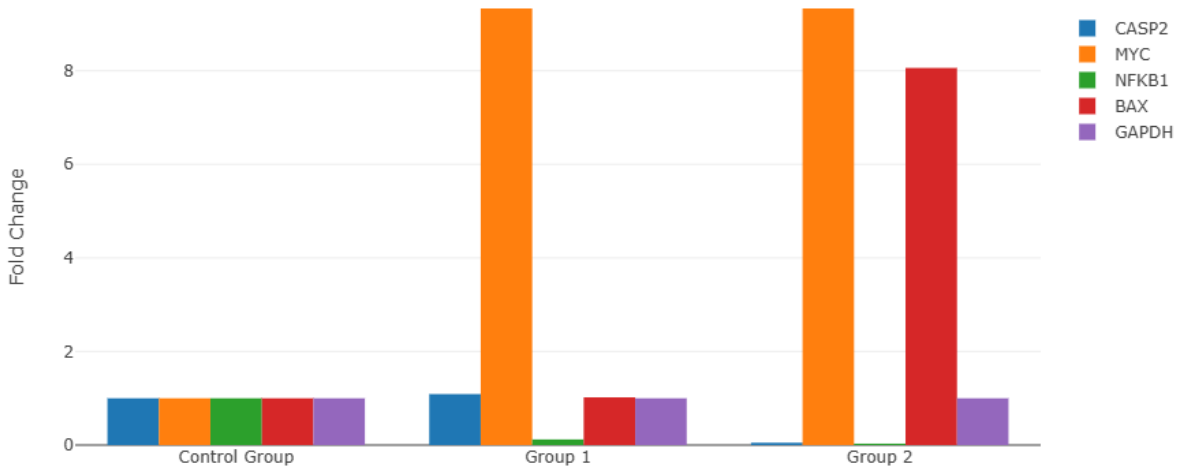

Figure 2. Fold change graph for expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in CCD-18Co cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

Table 2. Fold change, fold regulation and p values of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in CCD-18Co cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel; Group 2: *Allium polyanthum Schult* extract)

Genes	**Group 1**			**Group 2**		
	Fold change	**Fold regulation**	***p*-value**	**Fold change**	**Fold regulation**	**p-value**
CASP2	1.09	1.09	0.001*	0.05	-21.26	0.001*
MYC	19.03	19.03	0.001*	163.14	163.14	0.001*
NFKB1	0.12	-8.57	0.001*	0.03	-33.13	0.001*
BAX	1.02	1.02	0.001*	8.06	8.06	0.001*

A significant difference was observed in terms of *CASP2*, *NFKB1*, *BAX* and *MYC* expressions in CCD-18Co cells and HT-29 cells to which the combination of *Allium polyanthum Schult* extract and Docetaxel was applied. When CCD-18Co cells and HT-29 cells were compared, it was found that only *Allium polyanthum Schult* extract caused a change in the expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes ($p<0.05$) (Figure 3). It was determined that *CASP2*, *MYC*, *NFKB1* and *BAX* gene expressions were significantly decreased in HT-29 cells to which only *Allium polyanthum Schult* extract was applied compared to control cells. In addition, a decrease in *CASP2*, *MYC*, *NFKB1* and *BAX* gene expressions was observed in HT-29 cells to which *Allium polyanthum Schult* was applied together with docetaxel compared to control cells. However, the decreases in the expression of these genes were more pronounced in cells to which *Allium polyanthum Schult* was applied alone.

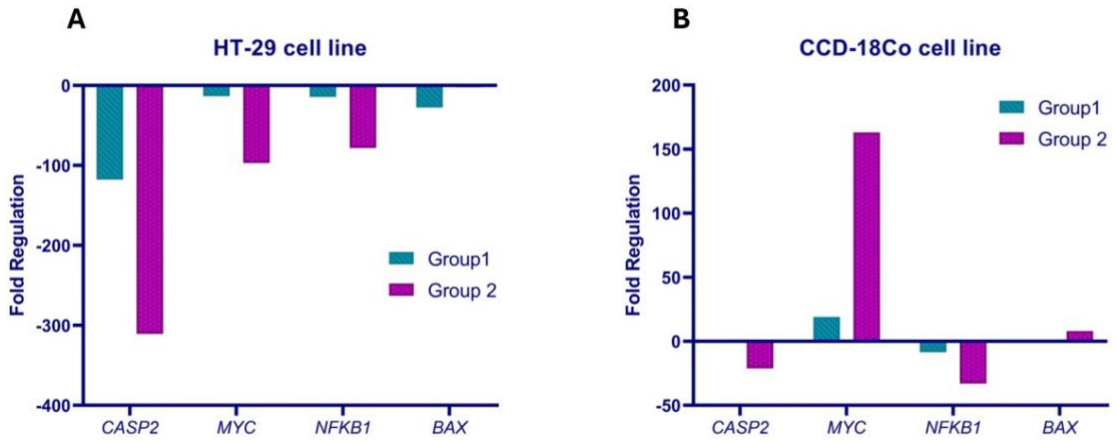

Figure 4. The combination of *Allium polyanthum Schult* extract and Docetaxel and only *Allium polyanthum Schult* extract increased apoptosis genes expression in HT-29 (A) and CCD-18Co (B) cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

As a result, *CASP2*, *MYC*, *NFKB1* and *BAX* gene expression was significantly decreased in CRC cells treated with the combination of *Allium polyanthum Schult* extract and docetaxel compared to healthy cells. It was determined that the decrease in these genes was more in the cells given only the plant extract than in the CRC cells to which the plant extract and drug combination was applied.

Bioinformatics analyses

Bioinformatic analyses were performed within the scope of the study. The protein-protein interactions of CASP2, MYC, BAX and NFKB1 were analyzed using the String v12 database. Accordingly, it was determined that CASP2, MYC, BAX and NFKB1 were related to each other. In addition, CASP2, MYC, BAX and NFKB1 were seen to interact with many other proteins given in Figure 5. The interaction levels of these proteins were found to be significant. When the interaction scores of the proteins were examined, the highest interaction was found to be between NFKB1 and MYC proteins (Table 3).

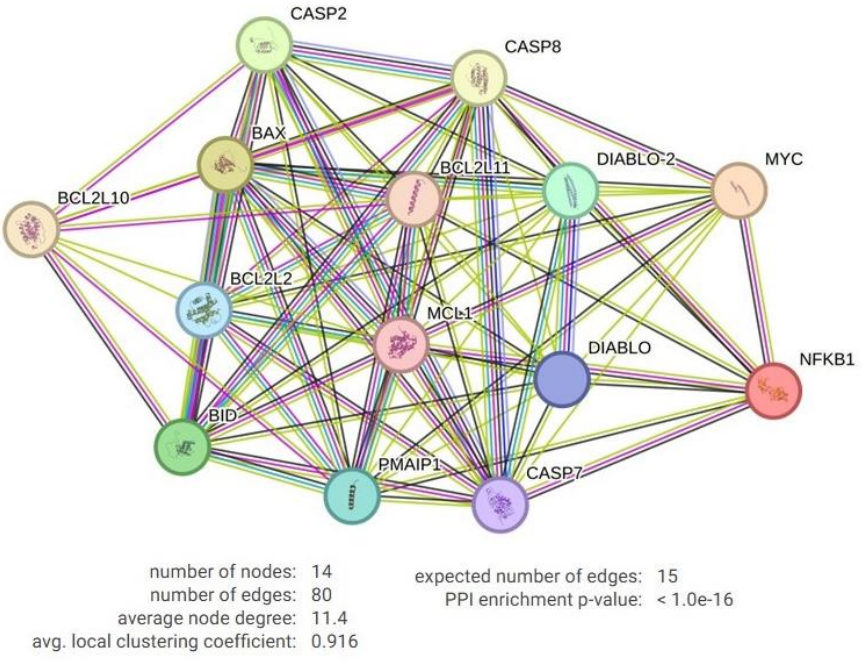

Figure 5. Illustration of interactions that CASP2, BAX, NFKB1, and MYC proteins share with other proteins

Table 3. Interaction scores of CASP2, BAX, NFKB1, and MYC proteins based on the STRING database

Node 1	Node 2	Node 1 accession	Node 2 accession	score
BAX	CASP2	ENSP00000293288	ENSP00000312664	0.447
BAX	MYC	ENSP00000293288	ENSP00000478887	0.415
MYC	NFKB1	ENSP00000478887	ENSP00000226574	0.917

The biological processes in which CASP2, BAX, NFKB1, and MYC proteins and additionally their associated proteins in 14 nodes participate according to the STRING database are given in Figure 6. Accordingly, it was determined that 12 proteins were highly related to extrinsic apoptotic pathways. In addition, it was recorded that 9 genes played a role in the intrinsic apoptotic pathway and 12 genes were generally effective in apoptotic pathways.

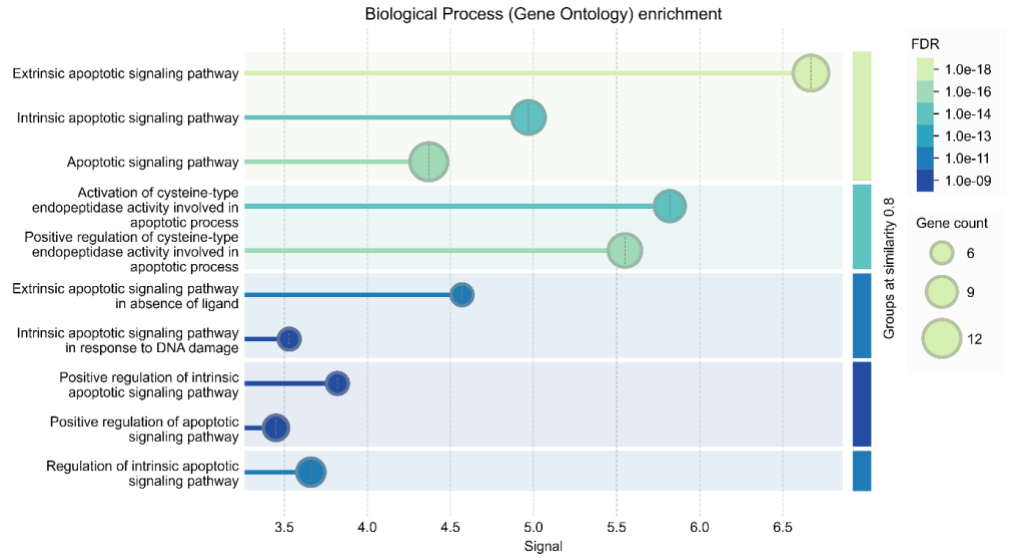

Figure 6. Common pathways of CASP2, NFKB1, BAX and MYC proteins in biological processes

Expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes were determined in 349 colon adenocarcinoma (COAD) patient tissues and 275 normal tissues within the scope of TCGA and GTEx data using GEPIA database. Accordingly, *CASP2*, *BAX* and *MYC* expression were significantly increased in COAD tissues compared to normal tissues. While *NFKB1* expression level was increased in COAD compared to normal tissues, this increase was not found to be significant (Figure 7).

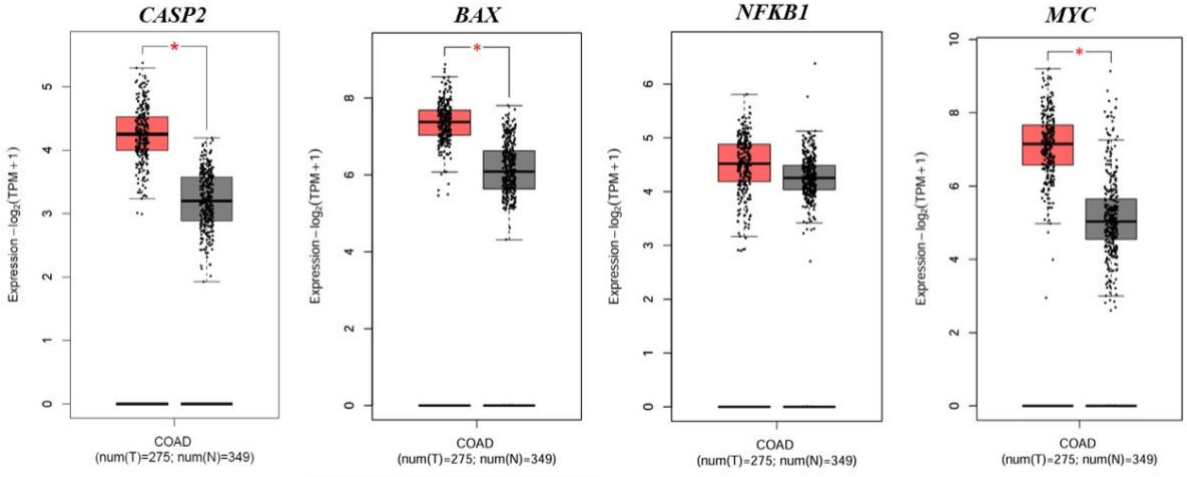

Figure 7. Box plot graph of *CASP2*, *BAX*, *NFKB1* and *MYC* gene expression in COAD compared to normal tissues obtained from GEPIA database (Red: Tumor tissue; Gray: Normal tissue) ($p<0.01$)

The expression of various genes has been associated with survival in cancer. According to the TCGA data of patients with COAD, the median of *CASP2*, *BAX*, *NFKB1* and *MYC* genes was taken and the effect of high and low expressions on survival was investigated. According to the findings, *BAX* and *CASP2* gene expression was closely associated with the survival of COAD patients, while *NFKB1* and *MYC* genes were not (Figure 8).

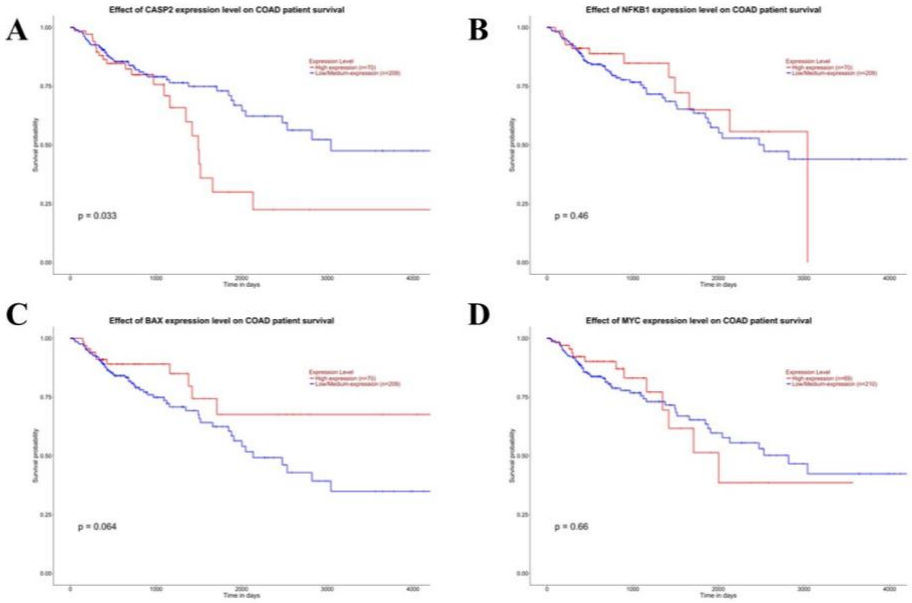

Figure 8. Kaplan–Meier curve showing the effect of *CASP2*, *NFKB1*, *BAX* and *MYC* gene expression on COAD survival ($p<0.05$)

Promoter methylation levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes were obtained from UALCAN database. Accordingly, methylation levels were examined in 313 primary COAD patient tissues and 37 normal tissues. Accordingly, it was found that *CASP2* gene was hypomethylated in COAD tissues compared to normal tissues, while *NFKB1* gene was hypermethylated. Promoter methylation levels of *BAX* and *MYC* genes were not found to be significant in COAD (Figure 9).

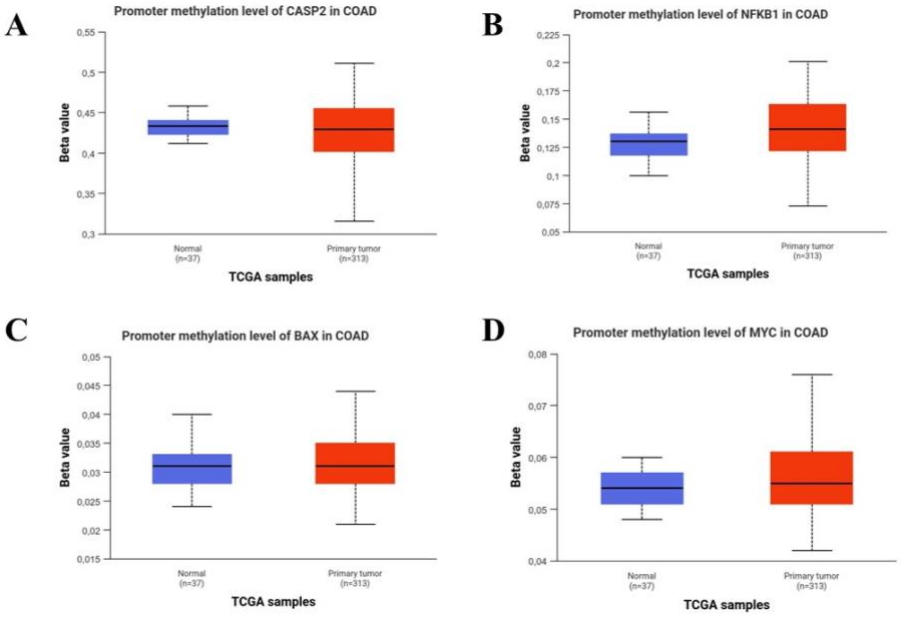

Figure 9. Levels of *CASP2* (A), *NFKB1* (B), *BAX* (C), and *MYC* (D) promoter methylation in COAD patients' tissue compared to normal tissues

All somatic mutations in *CASP2, NFKB1, BAX* and *MYC* genes in colon adenocarcinoma were examined using the muTarget database. It was determined how the mutations in our genes in the database affected the cancer hallmark genes determined by the system.[21] Somatic mutations in *CASP2*, *NFKB1*, *BAX* and *MYC* genes increased the expression of *hypoxia-inducible factor-1* (*HIF1A*), *dipeptidyl peptidase IV* (*DPP4*), *Neuropilin 1* (*NRP1*) and *janus kinase 2* (*JAK2*) which are among the cancer hallmark genes, and decreased the expression of *chemokine (C-X-C motif) ligand 14* (*CXCL14*). Accordingly, it has been determined that mutations in these genes related to apoptosis increase and decrease the expression of various genes that play a role in cancer (Figure 10).

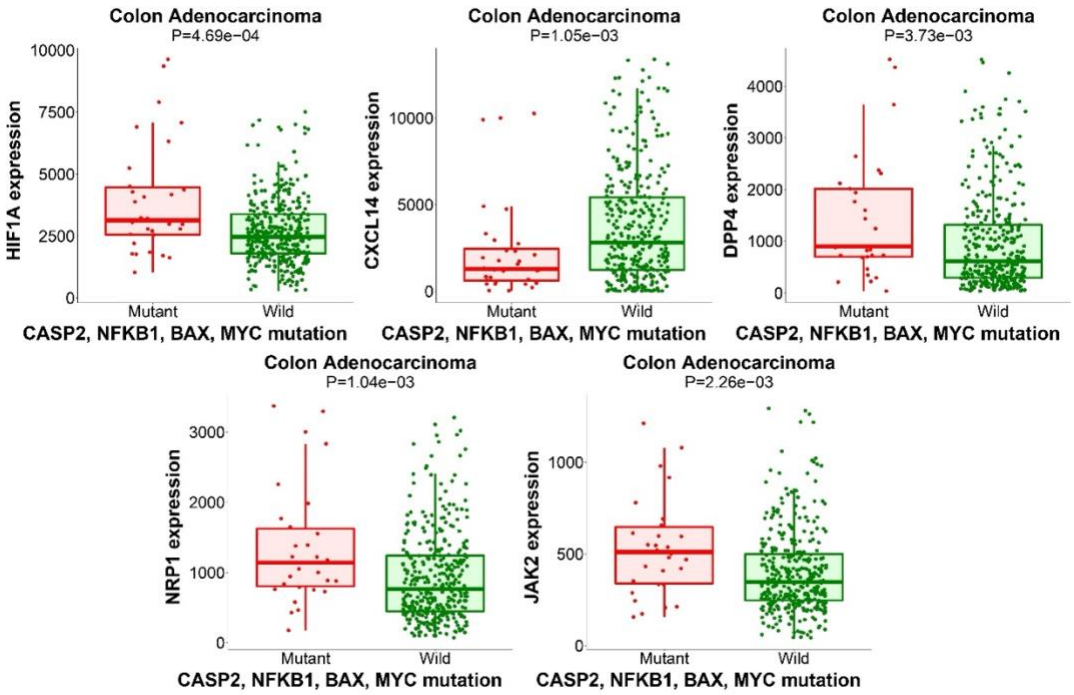

Figure 10. Effect of all somatic mutations of *CASP2*, *NFKB1*, *BAX* and *MYC* genes on the expression of certain cancer hallmark genes in colon adenocarcinoma ($p<0.05$)

Discussion

CRC has a high incidence rate globally and is the leading cause of cancer deaths.[22] CRC is a heterocomplex disease and therefore the effectiveness of recommended treatment options may vary.[23] In addition, studies have reported that the effectiveness of chemotherapeutic drugs applied for CRC is not fully effective due to many reasons, including drug resistance.[24] The drug docetaxel is also used in various types of cancer. Although this chemotherapeutic agent increases survival, it has been noted that it has many side effects.[25] Accordingly, within the scope of the study, the effect of *Allium polyanthum Schult* plant, which was extracted to create a synergetic effect by supporting the efficacy of docetaxel, on the expression of *CASP2*, *MYC*, *NFKB1* and *BAX* genes involved in apoptotic pathways was investigated.

Many plants belonging to the *Allium* genus have been reported to have various biological activities.[11] Because plants belonging to this genus contain many biologically active components, including high levels of sulfur, phenolic compounds and antioxidants.[26] Its biological activities can be listed as antiviral, antiasthmatic, antimotility,antidiabetic, antihypertensive, hypocholesterolemic, antiprotozoal, antiplatelet, antibacterial, antihelmintic, antiproliferative. In addition, the traditional use of this plant, which has cytotoxic roles in cancer, has highlighted its anticancer activity.[27-30] Abdel-Hady et al. reported anti-cancer activity of *A. ampeloprasum* strain in HepG2 and Caco-2 cells.[31] In another study, the cancer inhibitory properties of the affine type were demonstrated in OVCAR-3 cells, an ovarian adenocarcinoma cell line, due to its cytotoxic activity.[30] Cytotoxic activity of *A. atroviolaceum Boiss* methanol extract was proven by inducing apoptosis in MCF7, HeLa, MDA-MB-231 and HepG2 cells.[32, 33] In several studies, it has been reported that methanol or aqueous extracts of all parts of the *A. ursinum* plant have cytotoxic activity on gastric cancer cells, inhibiting cancer development in association with apoptosis, and have antioxidant properties.[34-37] Glycosides and saponin components isolated from *Allium schoenoprasum* plant

have been reported to have anticancer activity in HCT 116 and HT-29 colon cancer cells.[38] In the study conducted by Mskhiladze et al., the anticancer activity of furostanol and spirostanol and fractions obtained from *A. leucanthum* species was investigated in A549 lung cancer and DLD-1 colon cancer cell lines. Accordingly, it was discovered that *A. leucanthum* was cytotoxic in these cancer types.[39] Alshammari et al. proved the inhibitory effect of *Allium porrum* methanol extract on the proliferation of cancer cells via apoptotic pathways in colon cancer cells.[40] Since apoptotic pathways are associated with the development and progression of CRC cancer [41], the activity of *CASP2*, *MYC*, *NFKB1* and *BAX* genes was evaluated within the scope of the study.

Apoptosis, which is programmed cell death, ensures homeostasis of cells. However, impaired apoptotic processes can be activated by chemotherapy and targeted therapies. Caspases activate apoptosis. There are many members of the caspase family identified in mammals. CASP2, a member of the caspase family, is the caspase with the most conserved structure.[42] In a study involving 48-hour incubation of taxane group chemotherapeutic drugs, it was determined that *CASP2* activity in breast cancer cells increased 15-fold.[43] In this study, when *Allium polyanthum Schult* total extract was administered together with docetaxel, *CASP2* levels in CRC were decreased. In CRC cells administered only *Allium polyanthum Schult* total extract, a relatively greater decrease in *CASP2* expression was observed compared to healthy cells. The activation of *CASP2* indicates the activation of the apoptotic pathway.[44] Here, it is thought that the application of *Allium polyanthum Schult* extract alone may have anti-apoptotic activity. However, the fact that *CASP2* expression in CRC cells applied with only plant extract was relatively significantly more significant than in combined application suggests that combined application may have a more effective role in activating the apoptotic pathway. Additionally, bioinformatics analysis revealed that *CASP2* expression and promoter methylation increased with increasing COAD.

In the study conducted by Khazaei et al., the efficacy of the methanol extract of the flower parts of *Allium atroviolaceum* was tested on the apoptotic pathway in breast cancer cell line. Accordingly, it was reported that apoptosis was induced in the study in which various caspases were also included. NFKB plays a role in many cellular processes in cancer, both in the apoptotic pathway and in inflammation. NFKB inflammation has been frequently studied in relation to CRC. Upregulation of *NFKB* has been found to inhibit apoptosis and increase angiogenesis and cell proliferation.[45] Unlike cancer, it was determined that the *NFKB* gene was suppressed by *Allium sativum L.* extract in ulcerative colitis and had a protective effect on the colon.[46] According to the data obtained from the study, it was determined that there was a significant decrease in the *NFKB1* gene in CRC cells to which only *Allium polyanthum Schult* extract was applied. Accordingly, it is thought that extract may play a role in apoptosis by reducing *NFKB1* expression. According to bioinformatic data, it was determined that the expression of *NFKB1* increased in COAD and this increase was not significant. In addition, *NFKB1* was hypermethylated in COAD tumor tissues compared to COAD control tissues.

It is known that high expression of *BAX*, which has a proapoptotic function, helps in scavenging reactive species and inflicting cytotoxic damage on cancer cells. BAX has been reported to be suppressed in cancer.[47] In a study, it was reported that *BAX* was an effective prognostic factor in patients who underwent surgery for CRC. It was noted that higher mortality was seen in CRC patients in whom *BAX* expression was not observed.[48] In this study, it was determined that *BAX* expression was excessively decreased in CRC cells treated with only *Allium polyanthum Schult* and combined treatment with docetaxel. Accordingly, it is thought that there may be an adverse effect related to *BAX*-mediated apoptosis in CRC. According to bioinformatic data, *BAX* expression increased significantly in COAD tissues. Accordingly, these data show that the plant is effective experimentally.

MYC is generally associated with cell proliferation, depending on the cell cycle. There are data indicating that apoptosis is induced by MYC. While the increase in MYC levels due to the increase in growth factors causes the cell to proliferate, apoptosis can be observed in cells with reduced MYC in which growth factors are limited. Accordingly, the idea that MYC can induce apoptosis in cancer is exciting.[49] In this study, application of only *Allium polyanthum Schult* extract in CRC significantly reduced *MYC* expression due to combination therapy. In this case, it may be possible to say that the functioning of many mechanisms related to the cell cycle and apoptosis has changed. According to bioinformatic data, *MYC* gene expression was observed to increase in COAD tumors. The effects of *Allium polyanthum Schult* extract on the expression of apoptosis genes in CRC cells should be investigated more comprehensively.

Conclusion

Allium polyanthum Schult extract significantly reduced the expression of identified apoptosis genes. It is thought that the application of this plant extract alone in CRC cells

is due to its anti-apoptotic properties. It is predicted that the combination of *Allium polyanthum Schult* applied with docetaxel may have the effect of inducing apoptosis. The effects of this plant on apoptosis mechanisms in CRC cells should be investigated more extensively.

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