Cytotoxic Potential of Crataegus orientalis Fruit Extract: Expression Profiles of Apoptosis - Related Genes (Bax, Bcl2, p53)

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Research Article

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Abstract

Scientists are becoming more interested in the possibility of natural bioactive chemicals as supplemental medicines in cancer treatment. Investigating the biological effects of Crataegus orientalis fruit extract on different human cell lines was the goal of this investigation. HepG2 (liver), MCF-7 (breast), HEK293 (embryonic kidney), HCT-116 (colon), and A549 (lung) cell lines were used to evaluate cytotoxic characteristics using the MTT test. The extract was made with a variety of solvents and tested at different doses to assess dose-dependent effects. The results demonstrated that the cytotoxic effects were varied by the cell line, concentration, and extraction solvent. Notably, the extract's low IC_{50} value demonstrated high cytotoxicity against the HCT-116 cell line. The mRNA expression levels of important apoptosis-related genes, specifically Bcl2, Bax, and p53, were quantitatively examined using qRT-PCR in human HepG2 and MCF-7 cancer cell lines in order to obtain additional understanding of apoptotic mechanisms. Gene expression was found to have decreased statistically significantly in HepG2 cells (p 0.0002 for Bcl2, p 0.0344 for Bax, and p 0.0035 for p53). All of these results suggest that the fruit extract of C. orientalis may have anticancer properties via influencing the expression of genes linked to apoptosis in cancer cells.

Highlights

• The cytotoxic effects of C. orientalis fruit extract were evaluated in five human cell lines

• HCT-116 cells showed the highest sensitivity with a low IC_{50} value

• The extract was prepared using different solvents and tested at various doses

• Apoptosis-related genes Bcl2, Bax, and p53 were analyzed by qRT-PCR

• Significant downregulation of these genes was observed in HepG2 cells.

INTRODUCTION

Serious health issues include hypertension, liver-related disorders, blood sugar abnormalities, and cardiovascular disease affect a large number of people worldwide. The most dangerous of these illnesses is cancer, for which there is now no known cure [1]. According to the World Health Organization’s 2019 report, cancer ranks among the primary causes of premature mortality in 112 out of 183 countries, affecting individuals before the age of 70. In 23 countries, it is identified as the third or fourth leading cause of early death [2]. It is anticipated that 15.5 million individuals will be battling cancer globally by 2030, and 11.5 million will lose their lives to the illness. It is anticipated that 15.5 million individuals will be battling cancer globally by 2030, and 11.5 million will lose their lives to the illness [3]. Even though there are many medications available to treat cancer, a totally safe and effective therapeutic approach has not yet been created [4]. Among the most popular methods for combating cancer are include radiotherapy, bone marrow transplants, surgery, immunotherapy, and hormone

therapy [5, 6]. Cancer treatment with chemotherapy can harm healthy cells and result in severe side effects like anemia, hair loss, and exhaustion. Therefore, creating novel methods to minimize side effects and lower treatment expenses is crucial. Patients frequently resort to traditional therapy approaches in areas with limited access to modern care [4]. For ages, herbal items have been used extensively in various countries and have served as the foundation for traditional medical systems [7]. According to recent studies, 63% of cancer medications are sourced from plants, and traditional herbal extracts have anti-cancer properties. It has been discovered that certain herbal ingredients have a high response to treatment and few adverse effects. Thus, research into the potential of herbal remedies in the battle against cancer is still ongoing. According to recent studies, 63% of cancer medications are sourced from plants, and traditional herbal extracts have anti-cancer properties. It has been discovered that certain herbal ingredients have a high response to treatment and few adverse effects. Thus, research into the potential of herbal remedies in the battle against cancer is still ongoing [8, 9, 10]. According to the World Health Organization, herbal medicines account for almost 80% of the economies of developing nations [11]. Additionally, since about 20% of current medications are derived from traditional medicinal plants, there is growing interest in researching herbal items for the treatment of cancer [2, 12]. The fact that 80% of synthetic medications used globally are plant-based further supports this tendency [13]. Therefore, many plants have been found to have anti-cancer capabilities in addition to their beneficial effects as medical plants [14, 15]. *Crataegus*, a member of the *Rosaceae* family, is regarded by some as a prickly tree or shrub [16, 17]. The other names of this plant, which is native to the Mediterranean, Crimea, Turkey and Western Iran, are carpets, nuts, hawthorn, wild roses and aktiken [17, 18]. Since ancient times, extracts made from hawthorn leaves, blossoms, and fruits have been used to treat respiratory issues, diarrhea, abdominal pain, palpitations, cardiovascular illnesses, and hypertension [19, 20]. Because of its historical use, Hawthorn has also caught the attention of scientists [21]. In addition to being a strong source of vitamin C, hawthorn fruit is said to include sugars and carbs and is especially rich in important minerals including calcium, potassium, phosphorus, magnesium, and iron [22]. Flavonoids, proanthocyanidins ,organic acids, and amines are its primary constituents [23]. It is well known that phytochemicals derived from a variety of plants can effectively treat cancer. Research is being conducted worldwide to find novel plant extracts with potent antibacterial and anticancer effects [24]. In the context of cancer research, oncogenes and tumor suppressor genes have recently drawn a lot of attention for their functions in regulating the cell cycle and apoptosis. Since resistance to apoptosis is a significant treatment issue for cancer, a thorough understanding of apoptosis, a type of programmed cell death, is crucial for the development of effective anticancer medicines. [12, 25]. There have been some studies on *C.orientalis*'s antibacterial and antioxidant properties, but none that have looked closely at how it affects human cells.

With an emphasis on apoptosis-related gene expression, this work intends to examine the cytotoxic and pro-apoptotic effects of *C. orientalis* fruit extract on HepG2 and MCF-7 cell lines. The extract was also tested in other human-derived cell lines, such as A549, HCT-116 and HEK293 to investigate its effects across a variety of cellular models and deepen our understanding of its biological activity. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was employed as a housekeeping gene to

normalize the expression data and ensure analytical accuracy. Quantitative real-time PCR (qRT-PCR) was conducted to measure the mRNA expression levels of apoptosis-related genes, including *p53, Bax*, and *Bcl2*.

MATERIALS AND METHODS

Obtaining C.orientalis and cell lines used in the experiment

C.orientalis species collected from Cip town in Elazığ province of Turkey in September and November. Assoc. Prof. Dr. Semih Dalkılıç, one of the faculty members of the Molecular Biology and Genetics Programme of the Department of Biology, Fırat University that has HepG2, MCF-7, HEK293, HCT-116 and A549 cell lines in his laboratory.

Preparation of the extract

C. orientalis fruits were dried at room temperature and ground to obtain a homogeneous particle size. For the extraction process, 1 gram of dry plant sample was weighed on a precision balance, and the process was carried out using two different solvents. In the first step, extraction was performed with 10 mL hexane, followed by 10 mL methanol. The extraction process was carried out in a shaking oven at 37°C for 72 hours, after which the extracts were filtered with Whatman No. 1 filter paper to remove the solvents. After drying the extracts, yield calculation was performed, and extraction efficiency was determined. When evaluated on a solvent basis, the yield of hexane extract was 31.3%, and the yield of methanol extract was 29.4%, as shown in Table 1. It was dissolved in 10 mL dimethylsulfoxide (Dimethyl Sulfoxide Merck 116743.1000) The extracts were stored at +4 °C for the duration of the study. Hexane and methanol solvents, which are widely preferred for the identification of phenolic compounds, were chosen for the cytotoxicity evaluation of *C. orientalis* extract in this study.

Cell culture and determination of cytotoxic activity

MCF-7, HepG2, A549, HCT-116 and HEK 293 cell lines were cultured in 75 cm² flasks at 37°C with 5% CO_2 in RPMI 1640 Medium, with L-Glutamine (Cat-No: RPMI-A) medium containing 10% FBS (Cat-No: FBS-11A) and 1% Penicillin-Streptomycin (Cat-No:PS-B). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a technique used to evaluate cytotoxicity, proliferation and cell viability. The basis of this technique is that MTT and active mitochondria in living cells combine to form water-insoluble purple formazan crystals [26]. MTT method was used to evaluate the effect on *C.orientalis* cell viability. After dissolving formazan crystals in appropriate solvents, the amount of viable cells was counted using spectrophotometry. 90% confluent cells were removed from the medium in 75 cm² flasks and washed with 5 ml sterile PBS. After adding 1 ml trypsin-EDTA (Lot: 325043240, Cat:325-043-EL) to the flasks, they were incubated at 37˚C for two minutes in an incubator containing 5% CO_2. Trypsin-EDTA was inactivated and 5 ml RPMI was added after the cells were detached from the surface. After the cells were removed from the flask, the supernatant was discarded after centrifugation at 2000 rpm for five minutes. Countess II automated cell counter was used to count the cell pellet after

resuspension with 1000 µL RPMI. After cell dilution was calculated, 96-well plates containing 5x10³ cells and 100 µL RPMI per well were applied. The positive control was 2.5 µg/mL doxorubicin, while blank and negative controls were media only. Cells were incubated at 37.0 C with 5% CO_2 for 24 hours. Methanol and hexane extracts of *C.orientalis* fruit produced in RPMI were administered to the cells in six replicates at four different concentrations (100, 200, 400 and 800 µg/mL) after the medium was removed after incubation. Cells were incubated at 37˚C with 5% CO_2 for 72 hours. After completion, 10 µL of MTT solution (5 mg/mL) was added to each well and incubated at 37˚C with 5% CO_2 for 4 hours. Once incubation was over, the medium was taken out, formazan crystals were dissolved in 100 µL DMSO, and an ELISA microplate reader (Allsheng) set to 450 nm wavelength was used to measure the anticipated color change [27]. The control wells' absorbance readings were calculated, averaged, and approved as representing a 100% viable cell ratio. The viability % was calculated by comparing the absorbance values from the plant extracts with the absorbance value of the control group. This is how cytotoxicity was computed:

% Live cell = (Sample absorbance) / (Control absorbance) x100

RNA isolation from cell and cDNA synthesis

The apoptotic pathway effect of *C.orientalis* on HepG2 and MCF-7 cell lines was evaluated by looking at the expression levels of *Bcl2, Bax* and *p53* genes. Based on the cytotoxicity results of *C.orientalis* , the best extract and dosage for the two cell lines were determined. HepG2 and MCF-7 cells were exposed to the highest concentration of *C.orientalis* hexane extract (800 µg/ml) for 48 hours. Following the manufacturer's instructions, total RNA was extracted from these cells using the Total RNA Mini Kit (Generaid-Cat. No. RB300). The quality of the extracted RNA was analyzed using the iBright FL 1000 gel imaging system. The quantity and quality of RNA molecules determine how reliable gene expression analysis is [11]. Following the manufacturer's instructions, the quality and quantity of total RNA isolated was assessed using a Qubit™ RNA HS Assay Kit (REF: Q32852, LOT 1913928) and a Thermo Fisher Scientific Invitrogen instrument. RNA concentration was measured in ng/ml. RNA samples determined to be optimal in both quality and quantity were used to generate cDNA. Following the manufacturer's instructions, cDNA was generated by reverse transcription using the WizScript™ cDNA Synthesis Kit (REF: W2211, LOT 4E0420-02). Two microliters of reverse transcription buffer, 0.8 µl 25x dNTPs, 2 µl 10x random primers, one microliter MultiScribe Reverse Transcriptase, 4.2 µl nuclease-free water and 500 ng total RNA (50 µl) were included in 20 microliters of reaction mix. Using a SimpliAmp Thermal Cycler (Applied Biosystems, Singapore), reaction mixtures were incubated at 25°C for 10 min, 37°C for 120 min and 85°C for 5 min for each sample.

Quantitative real-time polymerase chain reaction (qRT-PCR)

The expression levels of four different genes (*Bcl2, Bax, p53* and *GAPDH*) were evaluated using quantitative RT-PCR. As indicated by the manufacturer, reactions were performed using Amplifyme SYBR Universal Mix (AM02-020) and Applied Biosystems Step One Plus Real-Time PCR System. The following components were present in 10 µl of reaction mix for each sample: Each sample was run in duplicate

using 5 µl 2X Amplifyme SG Universal Mix, 0.3 µl forward primer, 0.3 µl reverse primer, 0.2 µl 50× High Rox solution, 2.2 µl PCR Grade Water and 2 µl cDNA sample. The following are the steps of the PCR cycling program: 3 minutes at 95°C followed by 40 cycles of 95°C for 5 seconds, 58°C for 30 seconds, 95°C for 15 seconds, 60°C for 1 minute and finally 95°C for 15 seconds. Untreated cells were used as an external control in qRT-PCR, while *GAPDH* was used as an internal control. (**Table 2)** lists the primer sequences used.

Statistical analysis

SPSS (version 22) and GraphPad Prism (version 8.0.2) software were used for experimental design and statistical analysis of the data. As a result of the analysis, the statistical difference between the groups was evaluated by ANOVA test and significance levels were determined as $p < 0.05$.

RESULTS

Extract yield

Fruits of C. orientalis were gathered in September and November from the Cip district in the Elazığ region of Turkey. The HepG2, MCF-7, HEK293, HCT-116, and A549 cell lines utilized in this investigation were acquired from the lab of Assoc. Prof. Dr. Semih Dalkılıç, a professor in the Department of Biology's Molecular Biology and Genetics Program at Fırat University. The following formula was used to determine the extraction efficiency [28].

Percent yield (w/w) = (weight of the dried extract (g))/ (weight of the dry seed material before extraction (g)) x100

Cytotoxic activity

The cytotoxic potential of MCF-7 and HepG2 methanol and hexane extracts obtained from Crataegus orientalis fruit. The MTT method was used to assess the cancer cell lines HCT-116 and A549 as well as the healthy cell model HEK293 cell line. Fruit extracts' cytotoxic activity on *C. orientalis* in vitro, based on dosage and solvent type (Figure 1(A-E)).

Cell viability in the MCF-7 cell line with the methanol extract was between 59-64%, while with the hexane extract it was between 57-66%. HepG2 cells show that both extracts have moderate cytotoxic effects. HCT-116 cell line showed the highest cytotoxic effect. With 400 g/mL hexane extract, cell viability decreased by 19%, while with 800 g/mL methanol extract it decreased by 15%. These results confirm that both extracts have strong antiproliferative effects in a dose-dependent manner.

A more limited cytotoxic effect was observed in A549 cells compared to other cell lines; the highest efficacy was obtained with 29% viable cell ratio in 400 µg/mL hexane extract treatment. In contrast, HEK293 cells were used as a reference to evaluate the possible toxic effects of the extracts on healthy cells and no significant cytotoxic effect was observed in this cell line. Therefore, the IC_{50} value for

HEK293 cells was not calculated. This suggests that *C. orientalis* fruit extracts have low toxicity on healthy cells and have selective anticancer potential.

These data were further supported by IC_{50} analyses, revealing that methanol and hexane extracts exhibited cytotoxic effects that varied depending on the cell type but generally showed anticancer potential (Table 3).

Effects of C. orientalis extract on apoptotic pathway

To assess the impact of the *C. orientalis* hexane extract on apoptosis-related genes in HepG2 and MCF-7 cell lines, gene expression analysis was carried out using quantitative real-time PCR (qRT-PCR). Following a 24-hour treatment period, there were observable changes in the expression levels of the *p53*, *Bax*, and *Bcl2* genes when compared to the control group. Notably, treatment led to a modest shift toward apoptosis, particularly in MCF-7 cells, as reflected by the calculated *Bax/Bcl2* ratio of 0.83 in MCF-7 and 0.67 in HepG2 cells.

As shown in Figure 2, *Bcl2* expression increased more markedly in HepG2 cells, whereas *Bax* expression exhibited a relative increase in both cell lines post-treatment. Similarly, *p53* expression showed a moderate elevation in treated groups. These results suggest that the *C. orientalis* hexane extract may modulate apoptotic gene expression patterns, though the overall pro-apoptotic shift appears limited.

In addition, the specificity of qRT-PCR amplification was confirmed by melting curve analysis, which revealed single and distinct peaks for all target genes, indicating the absence of non-specific products or primer-dimer formation.

DISCUSSION

Apoptosis, a regulated form of programmed cell death, plays a vital role in preserving cellular homeostasis and is a central mechanism in cancer biology. The intrinsic apoptotic pathway, which is mitochondria-mediated, is tightly controlled by the relative expression of pro-apoptotic (*Bax*) and anti-apoptotic (*Bcl2*) genes. This balance determines the release of cytochrome c from mitochondria into the cytosol, a critical event that triggers the activation of downstream apoptotic signaling cascades [29, 30]. A crucial stage in the intrinsic apoptotic process, the *Bax* protein stimulates the release of cytochrome c from the mitochondria. By blocking this release, *Bcl2*, which is attached to the outer mitochondrial membrane, stops apoptosis. Consequently, it is commonly acknowledged that a key factor influencing a cell's propensity to undergo apoptosis is the *Bax/Bcl2* expression ratio [31].

In this study, *C. orientalis* fruit extracts showed significant cytotoxic effects on various cancer cell lines. In particular, dose-dependent cytotoxic effects were observed in HCT-116 (colorectal cancer) and MCF-7 (breast cancer) cell lines. Methanol extract stood out as the most effective fraction with lower IC_{50} values in most cell lines. On the other hand, since no toxic effect was observed in HEK293 (human embryonic kidney) cells, IC_{50} values could not be calculated for these cells. This suggests that the

extracts have low toxicity on healthy cells and may have a selective effect against cancer cells. When the data obtained are evaluated in general, it can be said that *C.orientalis* has anticancer potential especially against colorectal and breast cancer cells.

The effect of the hexane extract on the expression of apoptosis-related genes (*p53, Bax*, and *Bcl2*) was further evaluated in MCF-7 and HepG2 cells. The results demonstrated an induction of apoptosis, characterized by a significant upregulation of *p53* and *Bax*, and a concurrent downregulation of the anti-apoptotic gene *Bcl2*. This pattern indicates activation of the mitochondrial (intrinsic) apoptotic pathway. Moreover, the increased *Bax/Bcl2* ratio, commonly considered a key marker for apoptosis initiation, reinforces this conclusion [31, 32].

Hexane extract from *C. orientalis* fruit was used to evaluate the expression of apoptosis-related genes in MCF-7 and HepG2 cells. The calculated *Bax/Bcl2* ratios were found to be 0.83 for MCF-7 cells and 0.67 for HepG2 cells. The fact that these ratios were below 1 in both cell lines indicates that the anti-apoptotic effect was dominant and the apoptotic response was limited. However, the up-regulation observed in the *Bax* gene and the increase in the *p53* gene reveal the potential of the extract to direct the cell to apoptosis. Indeed, the increase in the *Bax/Bcl2* ratio in our study supports that the extract activates the apoptotic process. The *p53* tumor suppressor gene, a critical regulator of apoptosis, becomes activated under cellular stress conditions, particularly those involving genomic instability [33]. In this context, increased *p53* expression is expected to trigger *Bax* transcription and initiate cell death [31]. In the literature, it is reported that some compounds of *Crataegus* species, especially triterpenoid ursolic and oleanolic acids, stimulate apoptosis via mitochondrial pathway and accelerate cell death by increasing caspase-3 and caspase-9 activation [34, 35]. The gene expression profile found in this investigation is consistent with earlier findings. In particular, the idea that triterpenoid chemicals in *C. orientalis* fruit may trigger apoptotic pathways is supported by the overexpression of *p53* and *Bax* and the downregulation of *Bcl2*. It is also noteworthy that compared to previous studies on the anticancer effects of nearby species such as *Crataegus aronia*, *C. orientalis* showed a higher cytotoxic effect at lower concentrations. For instance, it has been observed that extracts from *C. aronia* only significantly affect HepG2 cells at 500 µg/mL [36]. In this respect, it can be said that *C. orientalis* has a stronger anticancer potential.

CONCLUSION

According to the study's findings, fruit extracts from *C. orientalis* have cytotoxic effects on cancer cell lines like MCF-7 and HepG2, and they may also alter the expression of genes linked to apoptosis. The methanol extract's increased bioactivity emphasizes how important the extraction technique and solvent choice are in affecting the effectiveness of chemicals produced from plants. The *Bax, Bcl2*, and *p53* gene expression levels did not approach statistical significance, but the trends particularly in the MCF-7 cell line indicate a pro-apoptotic response. This possible effect may also be reflected molecularly in the rise in the *Bax/Bcl2* ratio. Important hints that *C. orientalis* might be a potential natural anticancer drug are provided by these early results. However, more thorough in vitro testing, mechanistic research, and in vivo investigations are required to completely illustrate this effect. Whether this plant represents a

pharmacologically evaluable therapeutic resource will be made clear by more thorough research in the future.

Declarations

CONFLICT OF INTEREST

The authors declare any conflicts of interest.

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AUTHOR CONTRIBUTIONS

LKD contributions to the manuscript's final editing and conceptual design. **SD** Contributed to the manuscript's structural revision and content development. **ŞA** experimental research, writing, data analysis, and manuscript preparation. **EA** contributions to the manuscript's revision.

References

1. Ahmed, F., Ijaz, B., Ahmad, Z., Farooq, N., Sarwar, M. B., & Husnain, T. *Phytomedicine*, **68**, 153168. (2020).
2. Assouyouti, A. *Master's thesis, Bursa Uludag University (Turkey)*, (2022).
3. Shin, S. A., Moon, S. Y., Kim, W. Y., Paek, S. M., Park, H. H., & Lee, C. S. *International journal of molecular sciences*, **19**(9), 2651, (2018).
4. Majolo, F., Delwing, L. K. D. O. B., Marmitt, D. J., Bustamante-Filho, I. C., & Goettert, M. I. *Phytochemistry Letters*, **31**, 196-207, (2019).

5. Kayabaşı, Ç., Avcı, Ç. B., Süslüer, S. Y., Okcanoğlu, T. B., Yelken, B. Ö., Kurt, C. Ç., ... & Gündüz, C. *Ege Medical Journal,* **61**(2), 232-243, (2022).
6. Pekmezci, H., Köse, B. G., Akbal, Y., Özdemir, V. A., & Çol, B. K, *Journal of Health Academics,* **9(**3). (2022).
7. Top, R., Erden, Y., & Tekin, S. *Bitlis Eren University Journal of Science and Technology*, **8**(2), 435-442, (2019).
8. Gezici, S., Kocum, D., Yayla, F., & Şekeroğlu, N. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, **25**(5), 974-985, (2022).
9. Pekdemir, S., Çiftci, M., & Karatepe, M. *European Journal of Science and Technology,* **(18)**, 368-378, (2020).
10. Saraç, H., Daştan, T., Demirbaş, A., Daştan, S. D., Karaköy, T., & Durukan, H. *Journal of the Faculty of Agriculture,* **340-347***, (2018).*
11. Dalkılıç, S., Korkmaz, İ., Dalkılıç, L. K., Akay, G., & Fidan, S. *Food Bioscience*, **46,** 101501. (2022).
12. Özgüç, S., Kayalar, H., & Zeybek, U. *Journal of Faculty of Pharmacy of Ankara University,* **42**(2), 42-62. (2018).
13. Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, K. *International journal of molecular sciences*, **19**(6), 1578. (2018).
14. Aiello, P., Sharghi, M., Mansourkhani, S. M., Ardekan, A. P., Jouybari, L., Daraei, N., ... & Kooti, W. *Oxidative Medicine and Cellular Longevity*, **2019**(1), 2075614, (2019).
15. Aydoğmuş-öztürk, F. *Journal of the Institute of Science and Technology*, **11**(1), 645-653, (2021).
16. Murathan, Z. T., Erbil, N., & Arslan, M. *Bitlis Eren University Journal of Science and Technology,* **11**(1), 218-226, (2016).
17. Rocchetti, G., Senizza, B., Zengin, G., Mahomodally, M. F., Senkardes, I., Lobine, D., & Lucini, L. *Journal of the Science of Food and Agriculture*, **100**(5), 1998-2006 ,(2020).
18. Murathan, Z. T., Erbil, N., & Arslan, M. *Bitlis Eren Üniversitesi Fen Bilimleri Dergisi*, **11**(1), 218-226, (2016).
19. Hatipoğlu, M., Sağlam, M., Köseoğlu, S., Köksal, E., Keleş, A., & Esen, H. *H. PLoS One,* **10**(6), e0128134, (2015).
20. T.Ülger, T., Oçkun, M. A., Guzelmeric, E., Sen, N. B., Sipahi, H., Özhan, Y., ... & Yesilada, E. *Molecules,* **28**(18), 6520, (2023).
21. Özderin, S., Fakir, H., & Dönmez, İ. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, **19**(1), 120-123, (2015).
22. Sülüşoğlu, M., & Çavusoglu, A. *Journal of the Faculty of Agriculture,* **9**(1), 77-84, (2014).
23. Arslan, R., Bor, Z., Bektas, N., Meriçli, A. H., & Ozturk, Y. *Thrombosis Research*, **127**(3), 210-213, (2011).
24. Demirkaya, A. K., Gündoğdu, G., Dodurga, Y., Seçme, M., & Gündoğdu, K. *Atatürk University Journal of Veterinary Sciences*, **14**(1), 29-37, (2019).

25. Güneş, H., Oktay, M., Çelebi, F., & Tül, B. *Journal of Applied Biological Sciences,* **8**(2), 16-21 (2014).
26. Medhasi, S., Sriwarom, A., Permpalung, N., Torvorapanit, P., Plongla, R., Chindamporn, A., & Worasilchai, N. *Human Vaccines & Immunotherapeutics*, **20**(1), 2304372, (2024).
27. Dalkılıç, L. K., Dalkılıç, S., & Uygur, L. *International Journal of Plant Based Pharmaceuticals*, **3**(1), 62-67, (2023).
28. Ateşşahin, D. A., Dalkılıç, L. K., Özeren, Y., Dalkılıç, S., Çakmak, K., & Çiçek, T. A. *International Journal of Nature and Life Sciences*, **7**(2), 129-135, (2023).
29. Harandi, H., Falahati-Pour, S. K., Mahmoodi, M., Faramarz, S., Maleki, H., Nasab, F. B., ... & Nematollahi, M. H. (2022). *Nanoliposomal formulation of pistachio hull extract: preparation, characterization and anti-cancer evaluation through Bax/Bcl2 modulation. Molecular biology reports*, 1-9.
30. Opferman, J. T., & Kothari, A. (2018). *Anti-apoptotic BCL-2 family members in development.* Cell Death & Differentiation*,* **25**(1), 37-45.
31. Oltval, Z. N., Milliman, C. L., & Korsmeyer, S. J. (1993). *Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programed cell death. cell,* **74(**4), 609-619.
32. Adams, J. M., & Cory, S. (2007). *The Bcl-2 apoptotic switch in cancer development and therapy.* Oncogene, **26**(9), 1324-1337.
33. Aubrey, B. J., Kelly, G. L., Janic, A., Herold, M. J., & Strasser, A. (2018). *How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression?*. Cell death & differentiation, **25**(1), 104-113.
34. Li, T., Zhu, J., Guo, L., Shi, X., Liu, Y., & Yang, X. (2013). *Differential effects of polyphenols-enriched extracts from hawthorn fruit peels and fleshes on cell cycle and apoptosis in human MCF-7 breast carcinoma cells.* Food Chemistry, **141**(2), 1008-1018.
35. Jiang, X., Li, T., & Liu, R. H. (2016). *2α-Hydroxyursolic acid inhibited cell proliferation and induced apoptosis in MDA-MB-231 human breast cancer cells through the p38/MAPK signal transduction pathway*. Journal of Agricultural and Food Chemistry, **64**(8), 1806-1816.
36. Kmail, A., Lyoussi, B., Zaid, H., & Saad, B. (2015). *In vitro assessments of cytotoxic and cytostatic effects of Asparagus aphyllus, Crataegus aronia, and Ephedra alata in monocultures and co-cultures of Hepg2 and THP-1-derived macrophages.* Pharmacogn. Commun, 5, 165-172.

Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

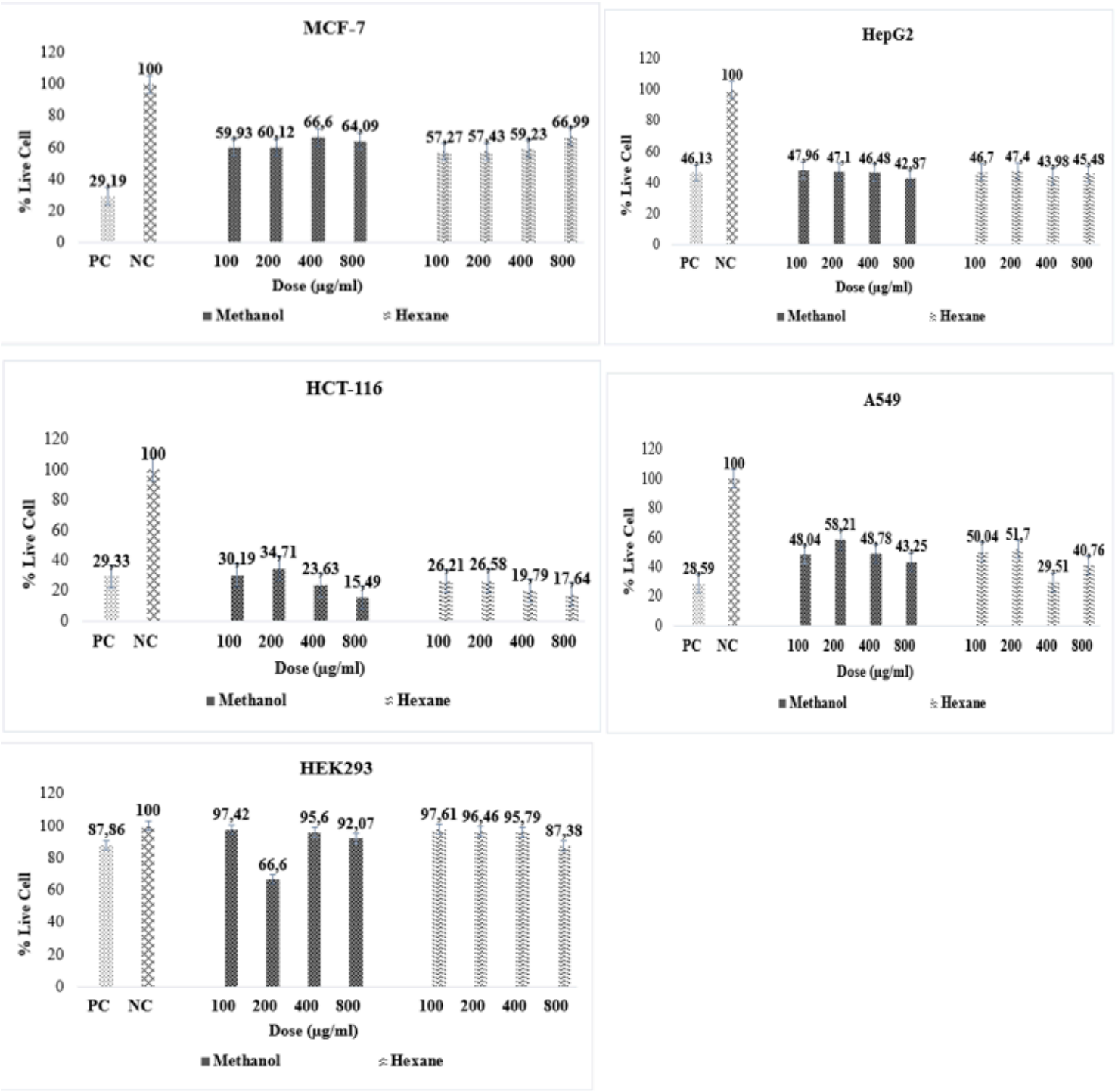

Figure 1

Cytotoxic effects of different concentrations of hexane and methanol extracts of *C. orientalis* on (A) MCF-7, (B) HepG2, (C) HCT-116, (D) A549 and (E) HEK293 cell lines. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant. PC = Doxorubicin (2.5 µg/ml); NC= Untreated cells (DMEM).

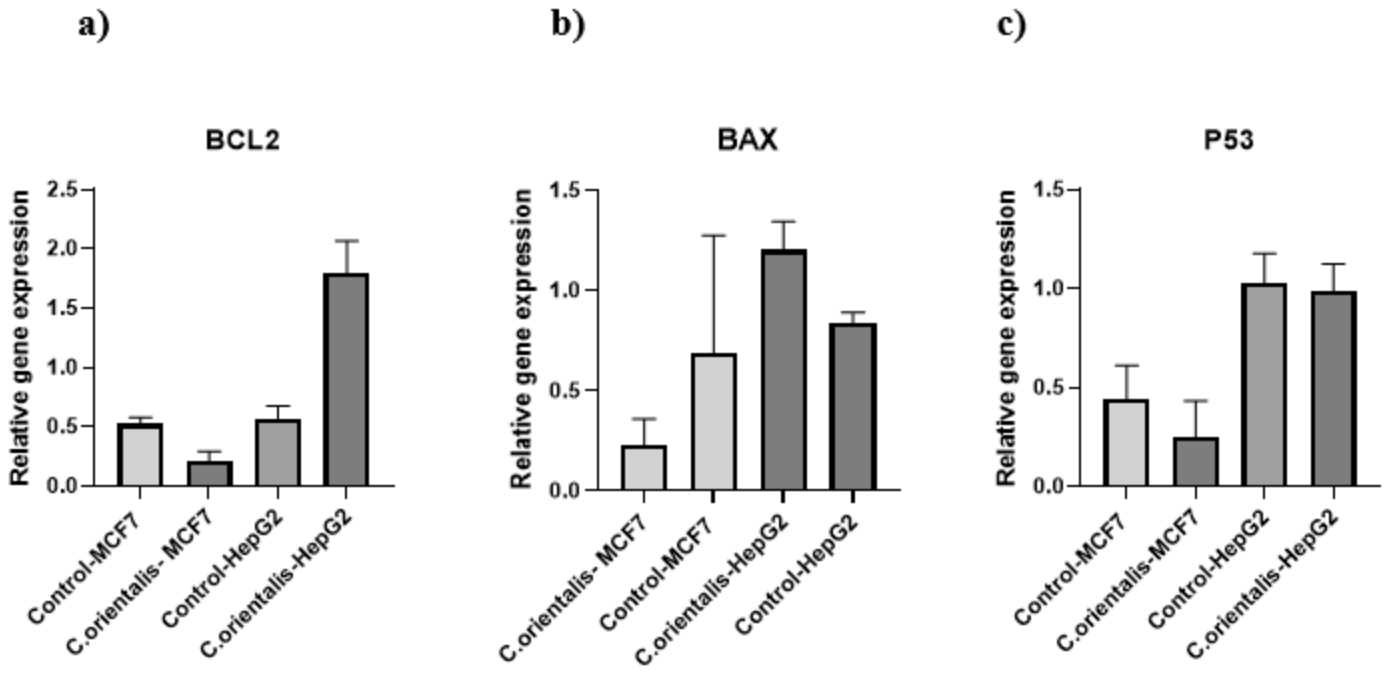

Figure 2

MCF-7 and HepG2 cell lines were treated with gypsum extract at a concentration of 800 µg/mL. Gene expression levels of (a) ***Bcl2***, (b) ***Bax***, and (c) ***p53*** were evaluated after 48 hours using the quantitative real-time PCR method. The results were compared with untreated cells, and gene expression levels were normalized to the *GAPDH* gene. **Statistical significance: $p < 0.0002$ for *Bcl2*, $p < 0.0344$ for *Bax*, and $p < 0.0035$ for *p53*.**

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.docx