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Evaluation of the Chemotherapeutic Potential of Medicinal Plant *Mespilus germanica* Fruit Extract: Cell Death Pathways and DNA Damage Mechanism

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ABSTRACT

Plant extracts are a mixture of natural complex compounds containing various biological activities, including anticancer properties. The fact that they have fewer side effects than synthetic drugs has made plant extracts an important strategy in cancer treatment The purpose of this study was to explore the chemotherapeutic potential of Mespilus germanica (medlar) fruit extract. The compound content of the extract was determined by HPLC. The proliferative concentration (PRO) and the concentration inhibiting the proliferation of half of the cells (IC50) were determined by the MTT viability test. PRO and IC50 concentrations were treated to A549 lung cancer cells for 48 hours. The study groups were determined as 3 groups: control, PRO, and IC50. Total mRNA was obtained from the cells by using the Trizol Reagent-chloroform method. cDNA synthesis was performed from total mRNA. mRNA gene expression levels of programmed cell death markers were detected by RT-qPCR. For all group studies, $p<0.05$ was considered statistically significant. It was detected in the extract content analysis, chlorogenic acid, ellagic acid, quercetin, and gallic acid polyphenolic compounds. As a result of MTT, IC50 was detected at 540 µg/ml, and PRO was 100 µg/ml. It was revealed that IC50 concentration significantly increased ($p<0.05$) the expression of *ATG5* (autophagic) and *RIPK1* (necrotic) genes. In addition, it was observed that the expression of proteases C*aspase-8, BAX, Apaf-1, Caspase-9, Caspase-3, and Caspase-7*, as well as genes associated with genotoxic damage, *PARP-1* and *P53*, increased significantly ($p<0.05$). As a result, it was determined that *Mespilus germanica* triggered the programmed cell death pathways in the A549 cancer cell line. It was concluded that adequate consumption of *Mespilus germanica* fruit can reduce or inhibit cancer cell proliferation. An experimental administration with an in vivo phase should be administered to reveal these results definitively.

Keywords: *Cancer, Cell death pathway, DNA damage, Mespilus germanica.*

ÖZ

Tıbbi Bitki *Mespilus Germanica* Meyve Ekstraktının Kemoterapötik Potansiyelinin Değerlendirilmesi: Hücre Ölüm Yolakları ve DNA Hasar Mekanizması

Bitki ekstarktları antikanser dahil olmak üzere çeşitli biyolojik aktiviteler içeren doğal kompleks bileşiklerin bir karışımıdır. Sentetik ilaçlara göre daha az yan etkiye sahip olmaları, bitki ekstraktlarını kanser tedavisinde önemli bir strateji haline getirmiştir. Bu çalışmanın amacı, *Mespilus germanica* (Muşmula) meyve ekstraktın kemoterapötik potansiyelinin araştırılmasıdır. Elde edilen ekstraktın bileşik içeriği HPLC ile tespit edildi. MTT canlılık testi yoluyla proliferatif konsantrasyon (PRO) ve hücrelerin yarısının çoğalmasını inhibe eden konsantrasyon (IC50) belirlendi. PRO ve IC50 konsantrasyonlar 48 saat boyunca A549 akciğer kanser hücresine uygulandı. Çalışma grupları kontrol, PRO ve IC50 olmak üzere 3 grup olarak belirlendi. Elde edilen hücrelerden Trizol reagent-kloroform yöntemi kullanılarak toplam mRNA elde edildi. Toplam mRNA'dan cDNA sentezi yapıldı. Programlanmış hücre ölümü belirteçlerinin mRNA gen ekspresyon seviyeleri RT-qPCR ile tespit edildi. Tüm grup çalışmaları için $p<0.05$ istatistiksel olarak anlamlı kabul edildi. Ekstrakt içeriği analizinde klorojenik asit, elajik asit, kuersetin ve gallik asit polifenolik birleşikler tespit edildi. MTT sonucu IC50 540 µg/ml ve PRO 100 µg/ml olarak tespit edildi. IC50 konsantrasyonunun, *ATG5* (otofajik) ve *RIPK1* (nekrotik) genlerin ekspresyonunu önemli ölçüde arttırdığı ($p<0.05$) ortaya konuldu. Ayrıca *Kaspaz-8, BAX, Apaf-1, Kaspaz-9, Kaspaz-3* ve *Kaspaz-7* proteazlarının yanı sıra, genotoksik hasarla ilişkili genler olan *PARP-1* ve *P53*'ün ekspresyonunun da önemli ölçüde arttığı gözlendi ($p<0.05$). Sonuç olarak *Mespilus germenica* A549 kanser hücrelerinin ortadan kaldırılması için istenilen programlanmış hücre ölüm yollaklarını tetiklediği tespit edildi. *Mespilus germenica* meyvesinin yeterli oranda tüketilmesinin kanser hücre çoğalmasını azaltabileceğini veya tamamen ortadan kaldırabileceği kanaatine varıldı. Elde edilen bu sonuçların kesin olarak ortaya konması için *in vivo* fazlı deneysel bir uygulamanın yapılması gerekeceği düşünülmektedir.

Anahtar Kelimeler: *DNA hasarı, Hücre ölüm yolağı, Kanser, Mespilus Germanica.*

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INTRODUCTION

The side effects of therapeutic plants are less than those of artificial drugs. The synergistic effects of therapeutic plant ingredients are becoming increasingly important in the treatment of diseases. Therefore, the therapeutic effects of herbs are necessary to reveal (Dar et al. 2017). The chemotherapeutic effects of plants containing high amounts of phenolic compounds have been intensively investigated in recent years. At this point, studies conducted with plant extracts have yielded promising results. Intensive research is being carried out on this subject in various countries worldwide (Oruganti and Meriga 2021; Ng et al. 2022). Metabolites such as alkaloids, tannins, flavonoids, and phenolics synthesized in plants are beneficial therapeutically (Rawat et al. 2018; Ginwala et al. 2019; Top et al. 2019). *Mespilus germanica* is a plant belonging to the family *Rosaceae* (Shulaev et al. 2008), mainly growing on rocks. The edible fruits of the *Rosacea* family are rich in compounds including potent antioxidant activities such as L-ascorbic acid, phenolics, flavonoids, and other phytochemicals beneficial for health (Rop et al. 2011). *Mespilus germanica* is popularly consumed as a fruit and used to treat enteritis (Glew et al. 2003), constipation, diuretics, and kidney and bladder stones (Baytop 1999). It has been reported in previous studies that Mespilus germanica has abundant mineral and trace elements (Ayaz et al. 2002; Glew et al. 2003), its fruits are rich in organic acids, amino acids and tannins (Rop et al. 2011) and it has high polyphenol content (Ercisli et al. 2008). This study was carried out to evaluate the anticancer effect of the extract obtained from the fruit of *Mespilus germanica*, which is popularly consumed in Türkiye and also used in the treatment of various diseases *in vitro*. Despite the disease-curing properties and rich content of Mespilus germanica, studies on its effects on cancer cell lines remain limited.

MATERIAL AND METHODS

Materials

The proposed research project does not need Animal Research Ethics Committee Approval (Date: 29/08/2024 Decision number: 2024/08-12). This study was performed using the A549 (ATCC® CCL-185™) lung cancer cell line. Fruits of *Mespilus germanica* were collected from Van province, Türkiye, during the young fruiting period in July. *Mespilus germanica* trees were grown without insecticides or herbicides for two fruiting seasons. Lyophilized extracts obtained from the fruits of *Mespilus germanica* were extracted with solvents of different polarities and applied to cancer cell lines.

Preparation of Plant Extract

Mespilus germanica fruits were treated with liquid nitrogen and lyophilized at -51 °C and 50 millitorr pressure for 96 hours. Sequentially lyophilized hydrophilic fractions were prepared according to a modified version of the methods (Dai and Mumper 2010; Dalar et al. 2013). The mixture was kept in a sonicator at +40 °C for half an hour, then at +4 °C for 24 h. Then it was centrifuged (Hitachi-High speed refrigerated centrifuge-CR22N) at 15.320 x *g* (10.000 rpm) for 30 min at 4 °C. The supernatant obtained was removed from the solvent by the evaporator and lyophilized. Acidified ethanol was added to the remaining pellet and centrifuged. The ethanol extract obtained was kept in a lyophilizer at -51 °C and 50 millitorr pressure for three days and then stored at -20 °C until analysis. Lyophilized extracts were filtered through a 0.45 μm filter and made ready for analysis.

HPLC Conditions

A quantity of 1000 μg/mL methanol was added to *Mespilus germanica* extract for HPLC analysis. Fruit extracts were made with slight modifications in compliance with earlier investigations (Dalar et al. 2012). Phenolic compounds were detected by using HPLC system that was equipped with a C18 column (5 μm particle size, 150 mm L x 4.6 mm I.D, Kromasil, Nouryon SE–445 80 Bohus, Sweden) and a Thermo Scientific Finnigan Surveyor diode array detector (system controler SCL-10A, LC-10ADVP pump, DGU-12A degasser, CTO-1-ADVP column oven). 1.25 ml/min was the flow rate. The HPLC analyses were conducted using an injection volume of 20 μL and an oven temperature of 40 °C. 28% (v/v) of methanol, 2% (v/v) of acetic acid, and 70% (v/v) of purified water made up the mobile phase. The elution profile of the HPLC was isocratic. The gradient employed to elude the sample was 1 ml/min for 1–12 min, 1.25 ml/min for 12–15 min, and 1.5 ml/min for 15–40 min afterward. Before injection, all standards were dissolved in HPLC-quality methanol. They employed concentration ranges of 1.0–40.0 ng/μl. By contrasting the retention periods of extracts with those of pure standards, phenolic components in the extracts were identified. The percentage of each compound from the phenolic compounds was used to clarify the results.

Cell Culture

Frozen cells war thawed rapidly (< 1 minute) in a 37 °C water bath. The cell series were grown in their specific media at 37 °C, 5% CO_2, and 95% humidity. The cell was purchased commercially. For MTT, 10^5 cells per well were seeded in plates (96 wells). 10^6 were seeded per flask (25 cm^2) for the study groups. The cells were allowed to adhere to the surface for 24 hours. At the end of 24 hours, plant extracts were treated to the cells according to the MTT result and the concentrations in the study groups.

Preparation of Plant Extract Solutions Treated

Since the extraction was prepared in a solvent, the stock solution was dissolved in DMSO. Final concentrations (100, 200, 400, 600, 800, and 1000 μg/ml) were prepared by dilution with the medium. In this dilution, the DMSO ratio was ready to have a nontoxic (≤0.005) effect (Sangweni et al. 2021).

Cytotoxicity (MTT Cell Viability) Test

In cell culture studies, IC50 (half maximal inhibitory concentration), i.e., the cytotoxic concentration that inhibits the proliferation of 50% of the cells, is used to determine the active substance or extract with the best potential. Among all drug candidates with this potency, the active substance with the lowest IC50 value is preferred. In this study, extracts prepared at different concentrations (0, 100, 200, 400, 400, 600, 800, and 1000 μg/ml) were treated to cancer cells for 48 hours, and their cytotoxic effects were determined. MTT viability test was performed at the end of the administration. The best proliferative concentration of the plant extract with the lowest IC50 value was also determined. The determined IC50 and proliferative concentrations were treated to the cell lines for 48 hours. The study was carried out in 3 groups in cell line: control, IC50, and PRO.

RNA Extraction and cDNA Synthesis

At the end of the administration periods, the cells were removed from the flask surface by using trypsinization. The cells were collected in 15 ml sterile falcon tubes. The collected cells were centrifuged at 1500 rpm for 5 minutes at 4 °C to remove the top medium, washed with sterile PBS, and centrifuged again to remove the PBS.

Total mRNA was obtained from the obtained cells by using the Trizol Reagent-chloroform method (Chomczynski and Mackey 1995). The amount and purity of the obtained mRNAs were measured in the spectrometer (BioDrop µLITE, England). Total mRNA 260/280 ratio was determined as 2 and above. No traces of DNA were found. Therefore, DNase I enzyme application could not be performed. cDNA synthesis was performed from total mRNA using a High-Capacity cDNA Reverse Transcription isolation kit (Applied Biosystems™ Cat: 4368814, Lithuania).

Real Time-qPCR Analysis

The mRNA transcription levels of the target genes, for which the primer sequences are provided in Table 1, were assessed by utilizing the complementary DNAs (cDNAs) acquired. The optimal conditions for primer design for each gene were found. The provided information on the reaction conditions for RT-qPCR may be found in Table 2. The reverse transcription quantitative polymerase chain reaction (RT-qPCR) was conducted using the ROTOR-GENE Q instrument manufactured by Qiagen in Germany. To ascertain the patterns of gene expression, the transcription levels of several key genes involved in autophagic and necrotic death pathways (*ATG5, ATG3*, and *RIPK1*), as well as genes belonging to the caspase enzyme systems implicated in the apoptotic pathway (*BCL-2, BAX, Apaf1, Caspase-8, Caspase-9, Caspase-3, and Caspase*-7), and the *TP53* gene, a marker for DNA damage, were assessed. Actin Beta (ACTB) was used as a control gene in expression analysis. SYBR Green master mix (ENZO Life Science cat: ENZ-NUC104–0200) was used for amplification detection in the study. The primer list of target genes is given in Table 1. Primers were purchased commercially. Each sample was repeated in 3 independent replicates, with one cycle threshold (Ct) determined at the beginning of the logarithmic phase of the amplifications. Evaluation of target gene products was performed according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2011). Differences between groups were evaluated according to the comparison of the increase-decrease fold changes in the expression of the control gene.

Statistical Analysis

SPSS version 22.0 package program was used for all statistical analyses. Since all our data showed normal distribution, One-way ANOVA and post-hoc Tukey test were used for descriptive statistics between groups. All parameters are presented as mean ± standard deviation. For all group studies, $p<0.05$ was considered statistically significant.

RESULTS

Ellagic acid and quercetin were detected at 254 nm, while gallic and chlorogenic acid were detected at 280 nm (Figure 1). Ellagic acid, chlorogenic acid, quercetin and gallic acid were determined in the extract. (Table 3).

The effect of the extracts obtained from the fruits of the *Mespilus germanica* plant collected during the seasonal period with solvents of different polarities on the A549 cell line was investigated. The results are given in Figure 2. The graph of cell viability values corresponding to each concentration treated to the cells was generated in CurveExpert (Professional 2.7.3). It was determined that IC50 was 540 µg/ml and proliferative concentration 100 µg/ml. These concentrations were treated to the cell line for 48 hours.

Figure 3 shows the effect of extract administration to A549 lines at both IC50 and proliferative concentrations on significant genes in *ATG3, ATG5* (autophagic), and *RIPK1* (necrotic) pathways compared to the control. Up and down-regulation of gene expression was done according to the control gene. It was determined that the *ATG3* gene was up-regulated 2 fold in the PRO group, and down-regulated 0,6 fold in the IC50 group. ATG5 gene was up-regulated 19 times in the IC50 group. It was determined that the *RIPK1* gene expression increased by 10 fold in the IC50 group. Differences were considered significant when $p<0.05$ compared to the control and PRO groups, there was an increase ($p<0.05$) in *ATG5* and *RIPK1* in the IC50-treated group.

A549 lung cancer cell lines were treated with *Mespilus germanica* extract for 48 hours. At the end of the treatment, the effect on the mRNA expression of apoptotic genes is given in Figure 4. Up and down regulation of gene expression was done according to the control gene. It was determined that *BAX* and *Apaf-1* were approximately up-regulated 8-fold compared to the control group in the IC50 group. It was detected that the *BAX/BCL-2* ratio increased 4 fold in the PRO group and 5-fold in the IC50 group. *Caspase-8, Caspase-9*, *Caspase-3*, and *Caspase-7* were also respectively determined to be up-regulated 7-fold, 4-fold, 9-fold and 7-fold. The mRNA levels of apoptotic genes *BAX, Caspase-9, Caspase-8, Apaf-1, Caspase-7* and *Caspase-3* significantly increased compared to the control group ($p<0.05$). It was determined that no change occurred in the level of *BCL-2*, which is antiapoptotic.

The effect of *Mespilus germanica* fruit extract applied to A549 lung cancer cell lines for 48 hours on the mRNA expression of DNA repair genes is given in Figure 5. *PARP-1* gene expression was down-regulated 0.6-fold in the PRO group and up-regulated 7-fold in the IC50 group. It was determined that *P53* was down-regulated 0.7-fold in the PRO group and up-regulated 8 fold in the IC50 group. The mRNA levels of *PARP1* and *P53* genes significantly increased ($p<0.05$) compared to the control group in IC50 group.

Table 1: Primer sequence of the target genes.

The name of the gene	Primer sequence	
	F. 5'-3'	R: 5'-3'
Actin Beta (ACTB)	ACTCTTCCAGCCTTCCTTC	ATCTCCTTCTGCATCCTGTC
ATG3	GAGATCACCTAGTCCACCAC	GCTTCCGTTATTCCTGTAATACC
ATG5	GAGACAAGAAGACATTAGTGAG	GATATTCCATGAGTTTCCGA
RIPK1	TGAGCTTCCGCTAGACA	CTGAAGCTCAACGCCCA
Caspase-8	GATGTTATTCCAGAGACTCCAG	GGTAGGTAATCAGCAAATCCA
Caspase-3	ATGGAAGCGAATCAATGGAC	AAACATCACGCATCAATTCC

Table 1 (continued): Primer sequence of the target genes.

The name of the gene	Primer sequence	
	F. 5'-3'	**R: 5'-3'**
BCL2	GTGGTGGAGGAACTCTTCAG	GTTCCACAAAGGCATCCCAG
Caspase-9	GGCTCTTCCTTTGTTCATCTCC	TCACCAAATCCTCCAGAACCA
BAX	AGCAAACTGGTGCTCAAGGC	CCACAAAGATGGTCACTGTC
Caspase-7	GGCTTGTATTGAAGAGCAGGG	CTGATCTTGTATCGAGGATTAGCA
Apaf-1	CCCTTTGTGTCCAGTAGTGGG	CTCTGTCTCGCCACATACCC
PARP-1	CACCAAAAAGGAGGTGGAAA	CAACTCCTGAAGGCTCTTGG
P53	CCCAGGTCCAGATGAAGCTC	CATGTAGTTGTAGTGGATGGTGGT

Table 2: Real-Time qPCR reaction conditions.

Reaction content	For one sample	Reaction cycle
Buffer (2X)	10 µl	95 °C 2' denaturation
Primers	Forward : 0.5 µl Reverse : 0.5µl	95°C 5'' } 40 cycle
dH_2O	8.4 µl	*58 °C -60 °C } 40 cycle
cDNA	0.6 µl	
Total	20 µl	* The binding temperature varied according to the primers. Melting Curve Ramp: 50-99 (1 degree increment) 90 °C 5 seconds

Table 3: Retention time and concentrations of phenolics detected in *Mespilus germanica* fruit extract.

Phenolics	Retention time(min)	Concentration (mg/ml) mean ± SD
Chlorogenic acid	5.064	3.85±0.089
Ellagic acid	29.999	4.68±0.1701
Quercetin	47.00	3.68±0.096
Gallic acid	3.204	1.75±0.125

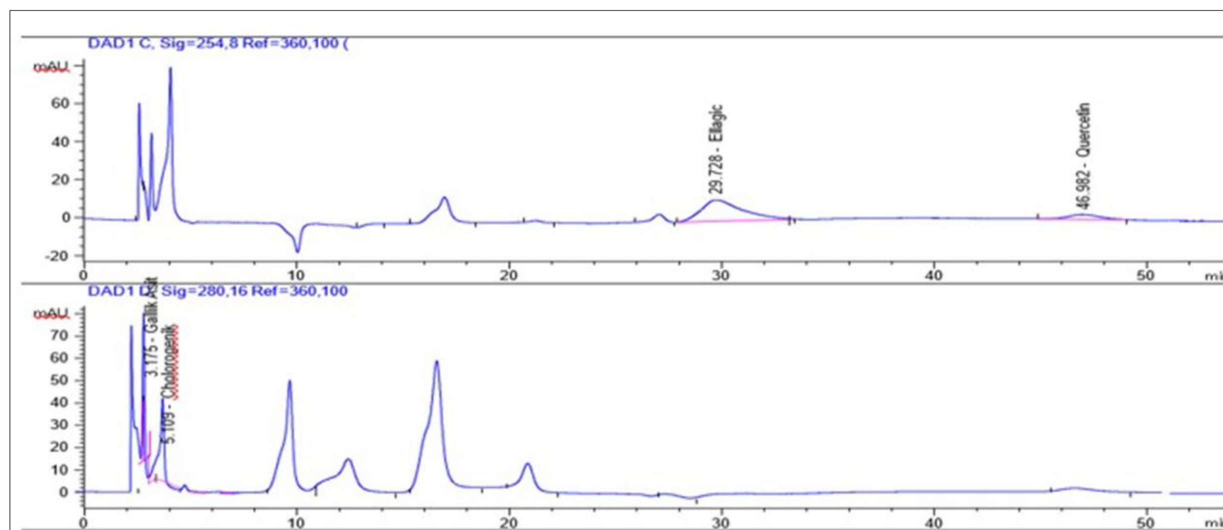

Figure 1: HPLC chromatogram of phenolic acid standards.

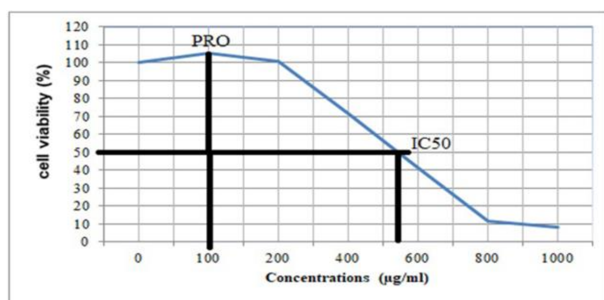

Figure 2: Graph of MTT viability test showing the effect of the extract treated at different concentrations on A549 cells.

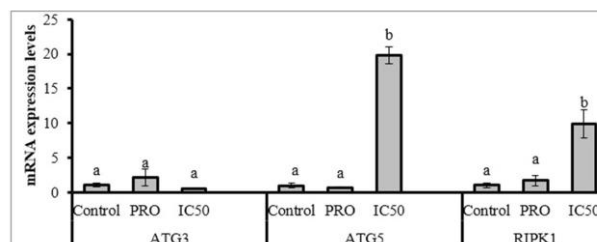

Figure 3: The mRNA transcript levels of *ATG3, ATG5* and *RIPK1* in the A549 cell line at the 48th hour: Represent the relative mRNA expression levels of *ATG3, ATG5* and *RIPK1* in the A549 cell line at the 48th hour. All data were expressed as mean±SD. Different letters (a–b) on the columns show a statistical difference ($p<0.05$).

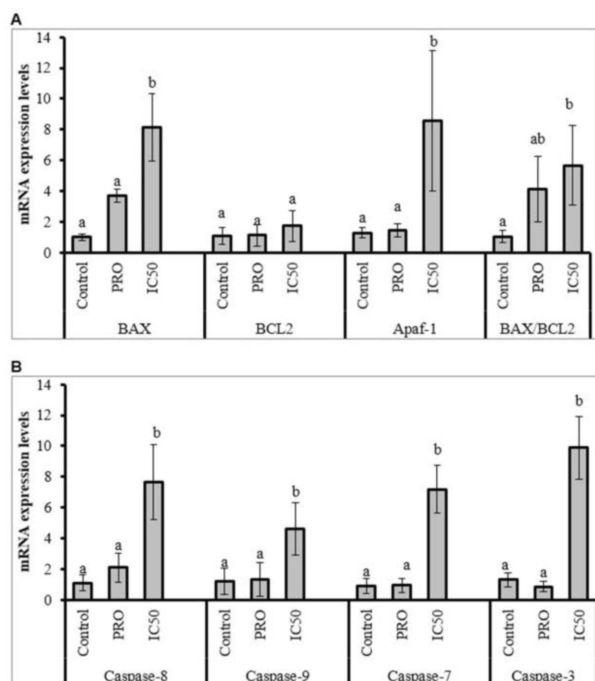

Figure 4: (A–B) The mRNA transcript levels of *BAX, BCL2, Apaf-1, Caspase-8, Caspase-9, Caspase-7* and *Caspase-3*, and *BAX/BCL-2* ration in the A549 cell line at the 48th hour: (A) Represent the relative mRNA expression levels of *BAX, BCL-2* and *Apaf-1,* and *BAX/BCL-2* ration in the A549 cell line at the 48th hour. (B) Represent the relative mRNA expression levels *Caspase-8, Caspase-9, Caspase-7* and *Caspase-3* in the A549 cell line at the 48th hour. All data were expressed as mean±SD. Different letters (a–b) on the columns show a statistical difference ($p<0.05$).

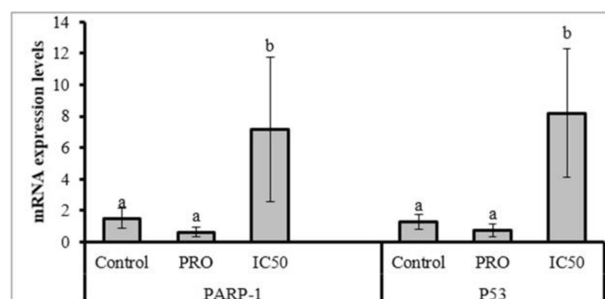

Figure 5: The mRNA transcript levels of *PARP-1* and *P53* in the A549 cell line at the 48th hour: Represent the relative mRNA expression levels of *PARP-1* and *P53* in the A549 cell line at the 48th hour. All data were expressed as mean±SD. Different letters (a–b) on the columns show a statistical difference ($p<0.05$).

DISCUSSION AND CONCLUSION

Chlorogenic acid is a dietary phenolic acid compound synthesized by various plant species. Current reports have revealed that chlorogenic acid shows anticancer effects by inhibiting the cell cycle and initiating apoptosis (Hayakawa et al. 2022). In another study, chlorogenic acid inhibited the proliferation of the A549 cell line (Wang et al. 2020; Gupta et al. 2022). Ellagic acid is a polyphenol compound found naturally in various fruits and vegetables. This compound has been found to inhibit the growth of tumourigenic structures by reducing cell growth and inducing apoptosis (Duan et al. 2020). Another reported study stated that Ellagic acid induced apoptosis in A549 cells by inhibiting the PI3K/Akt signalling pathway (Liu et al. 2018). The anticancer potential of quercetin has been documented in numerous *in vivo and in vitro* studies involving various animal models and cell lines (Almatroodi et al. 2021). Quercetin was found to dose-dependently inhibit cell viability and induce mitochondria-dependent apoptosis in both A549 and H1299 cells. The mRNA levels of *LC3-II*, *beclin 1, Atg5, Atg7,* and *Atg12* were upregulated by quercetin treatment (Guo et al. 2021). In this study, the content analysis of the *Mespilus germanica* fruit extract we obtained was determined by the HPLC method. According to HPLC standard comparison, phenolic compounds such as chlorogenic acid, ellagic acid quercetin, and gallic acid were detected (Table 3). As a result of the application of this extract to the cells, it was determined that cell proliferation was stopped according to the MTT result, which is a cell viability test. It has been determined that the extract obtained from *Mespilus germanica* fruit is rich in molecules that reduce the proliferation of cancer cells and stop or inhibit the proliferation of cells. Therefore, these compounds prevent or inhibit the proliferation of cancer cells. The given information above is consistent with studies by Gupta et al. (2022), Almatroodi et al. (2021) and Wang et al. (2020). In the Sadeghinejad et al. (2022) study, different *Mespilus germanica* fruits were identified as suitable natural sources containing important antioxidants and phenolic compounds. Yunusa and Ozturk (2024) conducted a study on *Mespilus germanica* leaves and fruit, showing that this plant extract has a significant cytotoxic effect on cancer cell lines and may have an anti-cancer effect. In this study, it was determined that *Mespilus germanica* fruit contains important phenolic components. Our results were found to be compatible with the research conducted by Sadeghinejad et al. (2022). In this study, *Mespilus germanica* fruit extract caused significant cytotoxicity in the A549 cell line. The results are compatible with other studies (Yunusa and Ozturk 2024). Cytotoxic concentrations of *Mespilus germanica* extract obtained by MTT were treated to cells to determine the expression levels of genes that are markers of programmed cell death pathways and DNA damage. It was revealed that *ATG5*, an autophagic marker, reached a very high expression level. However, an increase in *RIPK1* mRNA level, a necrotic marker, was also found. The rise in *ATG5* level, in particular, leads the cells to prefer autophagy, a resting phase, to save themselves. Afterwards, it was revealed that this situation did not improve. The increase in the *BAX/BCL-2* ratio in the direction of *BAX* in the results of this study further supports the activation of caspase mechanisms involved in the programmed cell death pathway. The second process is initiated by loss of membrane integrity and mitochondrial depolarisation, regulated by members of the Bcl-2 protein family, which triggers the release of cytochrome c into the cytosol, activating caspase-3 as an effector. *Poly (ADP-ribose) polymerase-1 (PARP-1)* is another molecule involved in many critical biological processes, including apoptosis, cell proliferation control, replication, and DNA damage repair. *PARP-1* targets caspase protease activity and is associated with apoptosis (Calaf et al. 2018). Excessive DNA damage causes massive poly (ADP-ribosyl) action by *PARP-1*, which can activate death programs (Hong et al. 2013). According to the RT-qPCR results obtained in this study, it was determined that there was a significant increase in the expression of *PARP-1* and *P53* genes along with increased *BAX, Caspase-8, 9, 7*, and *3* and decreased *BCL-2*. The rise in *PARP-1* and *P53* mRNA expression levels parallels the effector and lethal proteases involved in apoptotic pathways. The literature review found no study on apoptotic, autophagic, necrotic, and

DNA repair mechanisms related to the *Mespilus germanica* plant or fruit. These increases were found to be consistent with the literature. Drugs used in cancer treatment are obtained by various chemical means. The medicines obtained in this way cannot completely stop the proliferation of cancerous cells and cause side effects on healthy cells. Studies that can be an alternative to treatment processes and eliminate or at least reduce these side effects should be continued rapidly. The most important active substances used in this alternative treatment are polyphenolic compounds and derivatives in other living species that can be compatible with human nature. In this study, the first of our research initiated for this purpose, we found that the fruit of *Mespillus germenica*, which we used in this study, has a rich polyphenolic content. As a result of the application of the extract to the A549 lung cancer cell line under in vitro conditions, it was determined that the cells died. Drugs used in cancer treatment target apoptosis, a programmed death pathway. This study determined that *Mespillus germanica* fruit can trigger both cytoplasmic and mitochondrial pathways of apoptosis. MTT viability test and mRNA expression results support each other at this point.

As a result, it can be concluded that adequate consumption of *mespillus germenica* fruit may be beneficial in preventing or treating cancer development. It plans to experiment with an in vivo phase to demonstrate these results definitively.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: VY, GG
Supervision / Consultancy: VY
Data Collection and / or Processing: VY, GG
Analysis and / or Interpretation: VY, GG
Writing the Article: VY
Critical Review: VY

REFERENCES

Almatroodi SA, Alsahli MA, Almatroudi A et al. (2021). Potential therapeutic targets of quercetin, a plant flavonol, and its role in the therapy of various types of cancer through the modulation of various cell signaling pathways. *Molecules,* 26 (5), 1315.

Ayaz FA, Glew RH, Huang HS, Chuang LT et al. (2002). Evolution of fatty acids in medlar (Mespilus germanica L.) mesocarp at different stages of ripening. *Grasas y Aceites*, 53 (3), 352–356.

Baytop T (1999). Therapy with medicinal plants in Türkiye (past and present) (pp. 118-119). 2nd Edition. Nobel Medicine Publication, İstanbul.

Calaf GM, Ponce-Cusi R, Carrión F (2018). Curcumin and paclitaxel induce cell death in breast cancer cell lines. *Oncol Rep,* 40 (4), 2381-2388.

Chomczynski P, Mackey K (1995). Short technical reports modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *Biotechniques,* 19 (6), 942-945.

Dai J, Mumper RJ (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15 (10), 7313-7352.

Dalar A, Konczak I (2013). Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. *Ind Crops Prod,* 44, 383-390.

Dalar A, Türker M, Konczak I (2012). Antioxidant capacity and phenolic constituents of *Malva neglecta* Wallr. and *Plantago lanceolata L*. from Eastern Anatolia Region of Turkey. *J Herb Med*, 2 (2), 42-51.

Dar RA, Shahnawaz M, Qazi PH (2017). General overview of medicinal plants: A review. *J Phytopharmacol*, 6, 349-351.

Duan R, Du W, Guo W (2020). EZH2: a novel target for cancer treatment. *J Hematol Oncol,* 28,13 (1), 104.

Ercisli S, Akbulut M, Ozdemir O, Sengul M, Orhan E (2008). Phenolic and antioxidant diversity among persimmon (Diospyrus kaki L.) genotypes in Türkiye. *Int J Food Sci Nutr*, 59 (6), 477-482.

Ginwala R, Bhavsar R, Chigbu DI, Jain P, Khan ZK (2019). Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants (Basel*), 8 (2), 35.

Glew R. H, Ayaz, FA, Sanz C et al. (2003). Changes in sugars, organic acids and amino acids in medlar (*Mespilus germanica* L.) during fruit development and maturation. *Food Chem,* 83 (3), 363-369

Guo H, Ding H, Tang X et al. (2021). Quercetin induces pro-apoptotic autophagy via SIRT1/AMPK signaling pathway in human lung cancer cell lines A549 and H1299 in vitro. *Thorac Cancer*, 12 (9), 1415-1422.

Gupta A, Atanasov AG, Li Y, Kumar N, Bishayee A (2022). Chlorogenic acid for cancer prevention and therapy: Current status on efficacy and mechanisms of action. *Pharmacol Res,* 186, 106505.

Hayakawa S, Ohishi T, Oishi Y et al. (2022). Contribution of Non-Coding RNAs to Anticancer Effects of Dietary Polyphenols: Chlorogenic Acid, Curcumin, Epigallocatechin-3-Gallate, Genistein, Quercetin and Resveratrol. *Antioxidants* (Basel, Switzerland), 11 (12), 2352.

Hong SJ, Dawson TM, Dawson VL (2013). PARP and the release of apoptosis-inducing factor from mitochondria: Madame Curie Bioscience Database [Internet]. Austin (TX): *Landes Bioscience,* Bookshelf ID: NBK6179

Liu Q, Liang X, Niu C, Wang X (2018). Ellagic acid romotes A549 cell apoptosis via regulating the phosphoinositide 3-kinase/protein kinase B pathway. *Exp Ther Med*, 16 (1), 347-352.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods,* 25 (4), 402–408

Ng CX, Affendi MM, Chong PP, Lee SH (2022). The potential of plant-derived extracts and compounds to augment anticancer effects of chemotherapeutic drugs. *Nutr Cancer,* 74 (9), 3058-3076.

Oruganti L, Meriga B (2021). Plant polyphenolic compounds potentiates therapeutic efficiency of anticancer chemotherapeutic drugs: A Review. *Endocr Metab Immune Disord Drug Targets*, 21 (2), 246-252.

Rawat D, Shrivastava S, Naik RA et al. (2018). An overview of natural plant products in the treatment of hepatocellular carcinoma. *Anticancer Agents Med Chem,* 18 (13), 1838-1859.

Rop O, Sochor J, Jurikova T et al. (2011). Effect of five different stages of ripening on chemical compounds in medlar (*Mespilus germanica* L.). *Molecules,* 16 (1), 74-91.

Sadeghinejad Z, Erfani-Moghadam J, Khadivi A (2022). Bioactive content and phenolic compounds of common medlar (*Mespilus germanica L.*) and Stern's medlar (M. canescens Phipps). *Food Sci Nutr*, 10 (6), 1988-1993.

Sangweni NF, Dludla PV, Chellan N et al. (2021). The implication of low dose dimethyl sulfoxide on mitochondrial function and oxidative damage in cultured cardiac and cancer cells. *Molecules,* 26 (23), 7305.

Shulaev V, Korban SS, Sosinski B et al. (2008). Multiple models for Rosaceae genomics. *Plant Physiology,* 147 (3), 985-1003.

Top RY., Erden S, Tekin B (2019). The investigation of antioxidant and anticancer effects of some importance medical plants. *BEU Journal of Science*, 8(2), 435-442.

Wang L, Du H, Chen P (2020). Chlorogenic acid inhibits the proliferation of human lung cancer A549 cell lines by targeting annexin A2 in vitro and in vivo. *Biomed Pharmacother,* 131, 110673.

Yunusa UM, Ozturk Urek R (2024). Phenolic composition, antioxidant, and cytotoxic effects on HeLa and HepG2 cancer cell lines of Mespilus germanica grown in Turkey. *Nat Prod Res*, 38(11), 1972-1976.