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Comparative Evaluation of the Anti-Proliferative, Apoptotic, and Migratory Effects of Aronia melanocarpa Fruit Extract on Breast Cancer Cells and Healthy Endothelial Cells

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ABSTRACT

Purpose: *Aronia melanocarpa* fruit, known for its antioxidant and cytotoxic properties, is recognized as a potential natural agent in cancer research. This investigation evaluated the in vitro effects of aronia fruit extract on cytotoxicity, cell migration, and the expression of apoptotic genes in MDA-MB-231 breast cancer cells and HUVEC healthy endothelial cells were examined.

Methods: Cytotoxicity analyses were performed using the MTT assay, cell migration was assessed by scratch assay, and apoptotic gene expression was evaluated by RT-qPCR in MDA-MB-231 and HUVEC cells. Experiments were carried out in triplicate. For statistical evaluation, all analyses were performed using ANOVA, and differences with a p value of less than 0.05 were considered significant.

Results: The cytotoxicity experiments demonstrated that the extract reduced the viability of MDA-MB-231 cells in a manner dependent on both exposure time and concentration, while in contrast, HUVEC cells exhibited an increase in viability after 24 and 48 hours of treatment. The IC_{50} value was 148.92 µg/mL. cratch assays showed that the extract suppressed migration in MDA-MB-231 cells with no effect on HUVEC cells. RT-qPCR revealed increased Bcl-xL and decreased Bak, Caspase-3, and Caspase-8 in HUVEC cells, whereas MDA-MB-231 cells exhibited reduced Bcl-xL and elevated Bak, Caspase-3, and Caspase-8.

Conclusion: These findings indicate that aronia fruit extract exhibits selective pro-apoptotic and cytotoxic effects in breast cancer cells while maintaining a safe profile in healthy cells. Further studies are required to elucidate molecular mechanisms related to apoptosis and evaluate the preclinical and clinical potential of aronia fruit extract.

Keywords: *Aronia melanocarpa*, Apoptosis, Cell migration, Cytotoxicity, Gene expression

ÖZET

Amaç: *Aronia melanocarpa* meyvesi, antioksidan ve sitotoksik özellikleri nedeniyle kanser araştırmalarında potansiyel doğal bir ajan olarak kabul edilmektedir. Bu çalışmada, aronya meyve ekstresinin MDA-MB-231 meme kanseri hücreleri ve HUVEC sağlıklı endotelyal hücrelerde sitotoksisite, hücre göçü ve apoptotik gen ekspresyonu üzerindeki in vitro etkileri incelenmiştir.

Yöntem: MDA-MB-231 ve HUVEC hücrelerinde sitotoksisite analizleri MTT testi ile, hücre migrasyonu scratch testi ile ve apoptotik gen ekspresyonları RT-qPCR ile değerlendirilmiştir. Tüm deneyler üç tekrarlı olarak gerçekleştirilmiştir. Tüm istatistiksel değerlendirmeler ANOVA yöntemi kullanılarak gerçekleştirilmiş olup, p değeri 0,05'in altında olan farklar anlamlı kabul edilmiştir.

Bulgular: Sitotoksisite deneyleri, ekstresin MDA-MB-231 hücrelerinin canlılığını hem uygulama süresine hem de konsantrasyona bağlı olarak azalttığını gösterirken, buna karşılık HUVEC hücrelerinin 24 ve 48 saatlik uygulama sonrası canlılıklarının arttığı gözlenmiştir. IC_{50} değeri 148,92 µg/mL olarak hesaplanmıştır. Scratch testleri, ekstrenin MDA-MB-231 hücrelerinde göçü baskıladığını, HUVEC hücrelerinde ise belirgin bir etkisi olmadığını göstermiştir. RT-qPCR sonuçları, HUVEC hücrelerinde Bcl-xL seviyelerinin arttığını, Bak, Caspase-3 ve Caspase-8'in azaldığını; buna karşılık MDA-MB-231 hücrelerinde Bcl-xL'in azaldığını, Bak, Caspase-3 ve Caspase-8'in ise arttığını ortaya koymuştur.

Sonuç: Bu bulgular, aronya meyve ekstraktının meme kanseri hücrelerinde seçici pro-apoptotik ve sitotoksik etkiler gösterirken sağlıklı hücrelerde güvenli bir profil sergilediğini göstermektedir. Aronya meyve ekstraktının apoptozla ilişkili moleküler mekanizmalarının daha kapsamlı şekilde aydınlatılması ve preklinik ile klinik potansiyelinin değerlendirilmesi için ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Aronia melanocarpa*, Apoptoz, Hücre migrasyonu, Sitotoksisite, Gen ekspresyonu

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As one of the most prevalent cancers in women, breast cancer is regarded as a significant public health concern because of its high potential for metastasis (1,2). The limited efficacy of current treatment approaches, coupled with the substantial economic burden on patients and society, underscores the need for more effective and sustainable strategies to combat breast cancer.

Recently, researchers have increasingly focused on natural products and edible medicinal plants as valuable sources of bioactive compounds that may provide sustained and reliable cancer protection (3,4). These plants, containing multiple bioactive compounds, may provide broader protective effects than a single pharmacological agent and offer potential benefits in breast cancer prevention (5). The antioxidant effects of plant-derived compounds are proposed to impede cancer progression by mitigating oxidative stress–related damage, a contributor to tumorigenesis. Therefore, taking antioxidant supplements has been proposed as a possible approach to decrease breast cancer recurrence and enhance survival outcomes (6).

Several naturally occurring antioxidant compounds have demonstrated anticancer potential, and diets abundant in anthocyanins, catechins, and flavonols have been linked to a decreased probability of cancer occurrence. (7,8). Other bioactive phenolic constituents include stilbenes, flavonoids, curcuminoids, lignans, quinones, phenolic acids, tanins, and coumarins (9).

Aronia melanocarpa is a fruit increasingly recognized for its health-promoting components, including phenolic compounds, flavonoids, antioxidants, vitamins, anthocyanins, proanthocyanidins, pectins, and tannins (10,11). Due to its high antioxidant and phenolic content, aronia stands out among natural products with potential growth-suppressive effects on cancer cells (12).

The effects of aronia fruit extract, particularly rich in flavonoids and polyphenols, on proliferation, migration, and apoptotic processes in MDA-MB-231 human breast cancer cells and healthy human umbilical cord vein endothelial (HUVEC) cells were evaluated in our study, providing new insights into the cytotoxic mechanisms in cancer cells and highlighting its potential therapeutic relevance.

Material and Methods

Cell Lines and Culture Conditions

MDA-MB-231 (ATCC HTB-26) and HUVEC (ATCC PCS-100-010) were cultured in Dulbecco's Modified Eagle Medium (Cat. No: 41966-029; Gibco, USA) supplemented with 10% fetal bovine serum (Cat. No: A5256701; Gibco, USA). Cells were routinely subcultured every 3–4 days prior to reaching confluence and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

HUVEC cells were designated as the primary control group to assess selective cytotoxicity in comparison with cancer cells, while untreated control cells (0 µg/mL) in both HUVEC and MDA-MB-231 cultures were used as the secondary control group for comparisons with extract-treated cells (0–800 µg/mL).

Preparation of Aronia Fruit Extract

Aronia fruits were collected from the Gönen district of Balıkesir, Turkey. Following multiple rinses with deionized water, the samples were kept at –80°C for one day. After freezing, 10 g of fruit was ground in a mortar and subjected to maceration in 100 mL of 70% ethanol-methanol mixture (3:2, v/v) for 48 hours. After paper filtration, the plant extracts were concentrated at 40°C under vacuum conditions employing a rotary evaporation process. The composition of the extract was previously validated by HPLC analysis, ensuring reproducibility and standardization of the extract. Resulting extracts were stored at –20°C until use.

Stock solutions of aronia fruit extract were prepared for cell culture experiments. The extract was first mixed with the medium to reach a concentration of 100 mg/mL. Then, a 10 mg/mL solution was prepared from it and passed through a 0.22 µm filter for sterilization. A single intermediate stock solution was used in all experiments, ensuring consistency, reducing variability, and allowing reliable comparison of different concentrations.

Cytotoxicity Assay

The impact and biological responses triggered by the extract in HUVEC and MDA-MB-231 cell lines were assessed by treating the cells with concentrations ranging from 0 to 800 µg/mL for 24, 48, and 72 hours. All experiments were performed in 96-well microplates

with at least three technical replicates, and the cells were incubated under standard culture conditions. The MTT assay (GoldBio, USA) was used to assess cytotoxic effects, with 0.5 mg/mL of MTT solution added to each well after the designated incubation periods, followed by a further 4-hour incubation at 37°C. To assess cell viability, the resulting formazan crystals were first thoroughly mixed in isopropanol containing 0.008 M HCl to ensure complete dissolution, and then absorbance was read at 570 nm on a Thermo Multiskan GO spectrophotometer, with values expressed as a percentage of the untreated control.

Scratch Assay

HUVEC and MDA-MB-231 cells were added to each well of 6-well plates at a density of 1 million cells per well and then left to incubate overnight at 37°C in a humidified atmosphere with 5% CO_2. When the cells reached approximately 90% confluency, cross-shaped scratches were made on the cell monolayer using a sterile 200 µL pipette tip. After washing with PBS to remove cell residues and smooth the scratched area, fresh culture medium was added to each well. The control group received no additional treatment, while the treatment groups were exposed to aronia fruit extract at concentrations determined from the MTT assay. Cell movement was observed at 0, 24, and 48 hours under an inverted microscope, and the percentage of migration was determined by comparing how much of the scratch remained relative to its original size. All experiments were performed in triplicate, and the results were analyzed using ImageJ software.

RNA Isolation and RT-qPCR Analysis

MDA-MB-231 and HUVEC cells were seeded in 6-well plates at a density of 1 million cells per well and treated with aronia fruit extract at specific concentrations for 24 and 48 hours. After treatment, total RNA was isolated using the GeneJET RNA Purification Kit (Thermo Scientific, USA) and its quantity and purity were assessed with a NanoDrop spectrophotometer (samples with 260/280 ratios between 1.8 and 2.0 were used for cDNA synthesis). RNA quality was further validated by visualizing 18S and 28S bands on agarose gel electrophoresis.

cDNA was synthesized from 1 µg of RNA using the OneScript Plus Kit (Applied Biological Materials Inc., Canada) through a three-step process: primer annealing at 25°C for 10 min, synthesis at 50°C for 15 min, and enzyme inactivation at 85°C for 5 min. During cDNA synthesis, control PCR was performed using the Hβ2 housekeeping gene and DNA bands were observed on agarose gel; additionally, one positive and one negative control were included.

RT-qPCR was carried out in 20 µL reactions using BlasTaq 2× qPCR MasterMix (Applied Biological Materials Inc., Canada) on a CFX96 Touch system (Bio-Rad, USA) with cycling conditions of 95°C for 3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions, including negative controls, were performed in triplicate, and gene expression was normalized to β-actin according to the primer sequences listed in Table 1 and analyzed using the $2^{-\Delta\Delta CT}$ method.

Tablo 1: Primer sequences used for gene expression analysis

Primer Name	Primer Sequences (5′–3′)	
Bcl-xL	F	ATCCCCATGGCAGCAGTAAAGCAAG
	R	CCCCATCCCGGAAGAGTTCATCTACT
Bak	F	ACCAGCCTGTTTGAGAGTGG
	R	AGTGATGCAGCATGAAGTCG
Caspase 3	F	AGGACTCTAGACGGCATCCA
	R	CAGTGAGACTTGGTGCAGTGA
Caspase 8	F	AGAAGAGGGTCATCCTGGGAGA
	R	TCAGGACTTCCTTCAAGGCTGC
β-actin	F	CCAACCGCGAGAAGATGA
	R	CCAGAGGCGTACAGGGATA

Statistical Analysis

All data were obtained from at least three independent experiments and are presented as mean ± SD. Data analysis was carried out in SPSS 20 (IBM Corp., Armonk, NY, USA), applying one-way ANOVA for comparisons among groups. A p-value < 0.05 was considered statistically significant.

Results

MDA-MB-231 and HUVEC cells were treated with aronia fruit extract, and cytotoxicity was measured using the MTT assay at 24, 48, and 72-hour intervals. The results showed a significant reduction in MDA-MB-231 cell survival depending on dose and incubation time, while HUVEC cells maintained their viability (Figure 1).

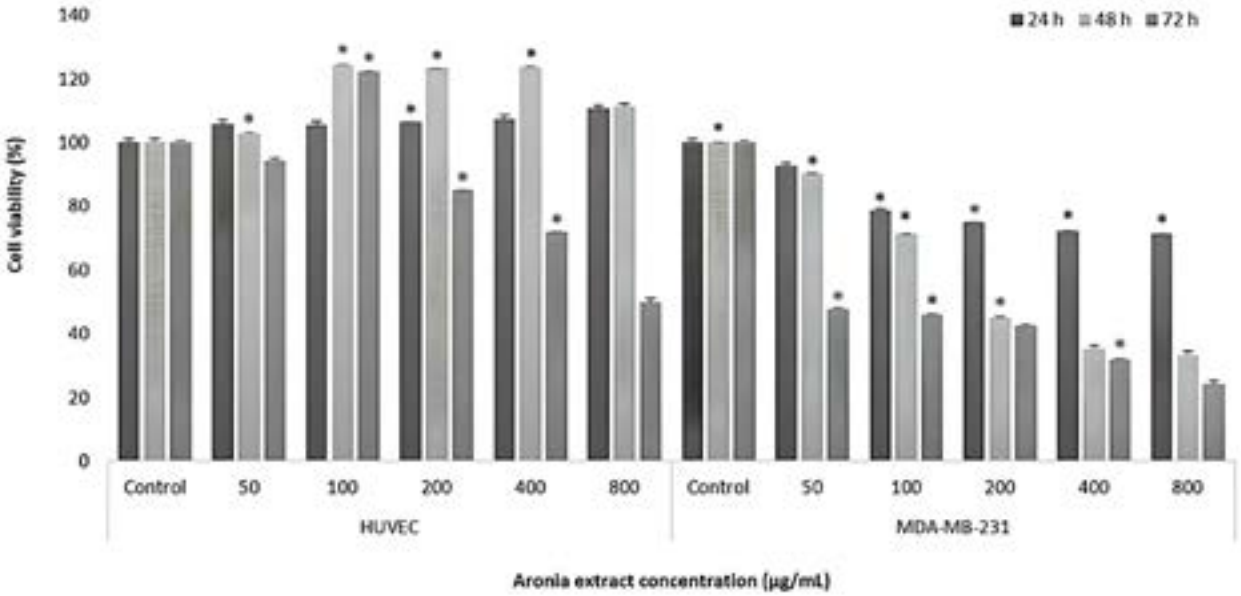

Figure 1: Dose- and time-dependent cytotoxic effects of aronia fruit extract on MDA-MB-231 and HUVEC cells. Cell viability was assessed using the MTT assay at 24, 48, and 72 hours after treatment. Data are presented as mean ± SD of three independent experiments. *p<0.05 is statistically significant.

In HUVEC cells, aronia fruit extract did not exhibit cytotoxic effects; on the contrary, cell viability was slightly increased at 24 and 48 hours of treatment. Considering the control group as 100% viable, at 24 hours, cell viability was 105.6% at 50 µg/mL, 105.5% at 100 µg/mL, 106.5% at 200 µg/mL, 107.3% at 400 µg/mL, and 110.6% at 800 µg/mL. At 48 hours, the corresponding viability values were 102.7%, 124.2%, 123.1%, 123.7%, and 111.3%, respectively. However, after 72 hours, cell viability decreased to 94.1%, 122.2%, 84.8%, 71.7%, and 49.8%, respectively, indicating that high doses and prolonged exposure induced cytotoxicity in HUVEC cells ($p<0.05$).

In MDA-MB-231 cells, aronia fruit extract exhibited pronounced cytotoxic effects, which increased with both concentration and incubation time. Considering the control group as 100% viable, at 24 hours, cell viability was 92.7% at 50 µg/mL, 78.7% at 100 µg/mL, 74.8% at 200 µg/mL, 72.1% at 400 µg/mL, and 71.3% at 800 µg/mL. At 48 hours, the values decreased to 90.1%, 71.1%, 44.9%, 35.1%, and 33.4%, respectively. The most pronounced cytotoxicity was observed at 72 hours, with cell viability decreasing to 47.8%, 45.9%, 42.5%, 31.8%, and 24.2%, respectively, indicating that, similarly to HUVEC cells, high doses and prolonged exposure induced cytotoxicity in MDA-MB-231 cells ($p<0.05$).

In the continuation of the study, cell migration analyses were performed using the scratch assay, and migration rates were quantitatively evaluated at 0, 24, and 48 hours (Figure 2). In HUVEC cells, the migration rate in the control group was 71.48% at 24 hours and 99.28% at 48 hours, while in the extract-treated group, migration was 71.79% at 24 hours and 99.72% at 48 hours (respectively, $p=0.048$ and $p=0.046$). In contrast, in MDA-MB-231 cells, the migration rate in the control group was 65.86% at 24 hours and 97.96% at 48 hours, whereas in the extract-treated group, migration decreased to 57.05% at 24 hours and 79.66% at 48 hours (respectively, $p=0.045$ and $p=0.016$). These findings indicate that aronia fruit extract did not affect migration in HUVEC cells but significantly inhibited migration in MDA-MB-231 cells.

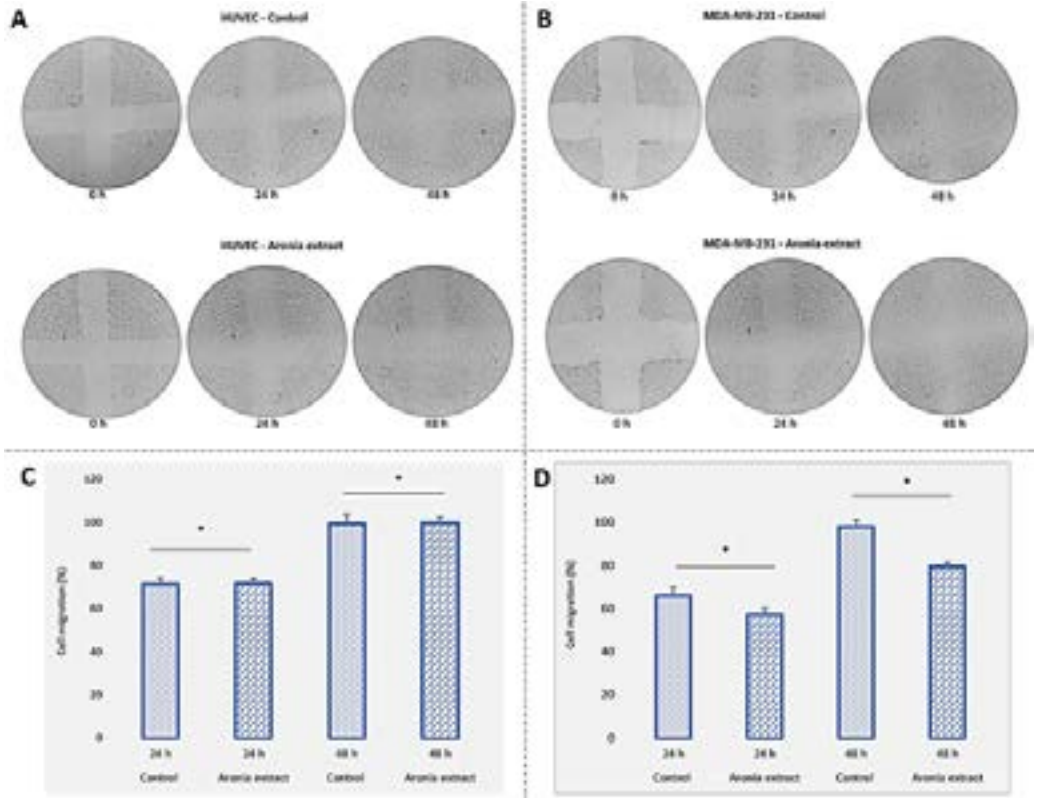

Figure 2: Cell migration assays were conducted on HUVEC and MDA-MB-231 cells. Following scratch formation, representative phase-contrast images of (A) HUVEC and (B) MDA-MB-231 cells in the control and aronia extract-treated groups were captured at 0, 24, and 48 hours using an inverted microscope at 4× magnification. Quantitative analysis of cell migration (%) at 24 and 48 hours was performed for (C) HUVEC and (D) MDA-MB-231 cells. *p<0.05 was considered statistically significant compared with the respective control group.

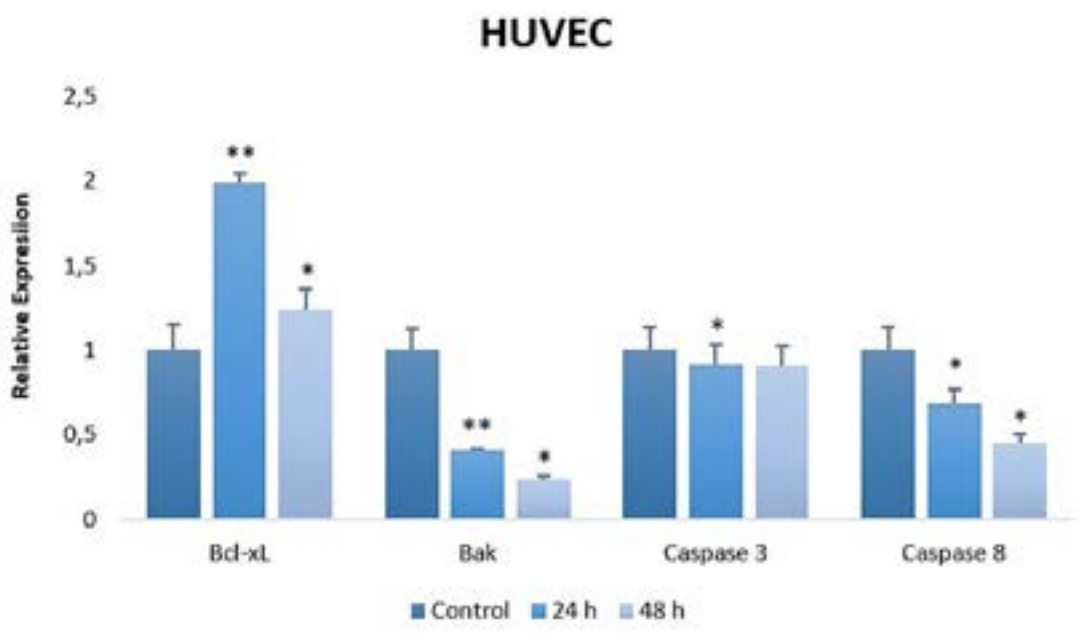

Figure 3: Effect of aronia fruit extract on apoptotic gene expression in HUVEC cells. Bcl-xL, Bak, Caspase-3, and Caspase-8 mRNA levels were measured by RT-qPCR at 24 and 48 hours post-treatment. Data are presented as mean ± SD of three independent experiments. *p<0.05 and **p<0.01 were considered statistically significant compared with the corresponding control group.

RT-qPCR analysis was carried out on HUVEC and MDA-MB-231 cells treated with aronia fruit extract to assess variations in apoptosis-associated gene expression. In HUVEC cells, Bcl-xL expression increased 1.99-fold at 24 hours (p=0.008) and 1.24-fold at 48 hours (p=0.021), remaining higher than control levels. Bak expression decreased to 0.41-fold at 24 hours (p=0.006) and 0.24-fold at 48 hours (p=0.047). Caspase-3 expression showed minimal change at both time points, while Caspase-8 expression was suppressed to 0.68-fold at 24 hours (p=0.027) and 0.45-fold at 48 hours (p=0.039). These results indicate that aronia fruit extract supports anti-apoptotic mechanisms in HUVEC cells, particularly by inhibiting the extrinsic apoptosis pathway, and this effect was found to be statistically significant with a p-value less than 0.05 (Figure 3).

In MDA-MB-231 cells, Bcl-xL expression remained nearly unchanged at 24 hours (0.998-fold) (p=0.321) but decreased to 0.63-fold at 48 hours (p=0.044). Bak expression initially increased to 1.79-fold at 24 hours (p=0.011) and returned close to control levels (1.03-fold) at 48 hours (p=0.429). Caspase-3 expression was significantly upregulated to 1.91-fold at 24 hours (p=0.033) and 2.20-fold at 48 hours (p=0.004), while Caspase-8 expression increased to 1.58-fold at 24 hours (p=0.004) and remained above control levels (1.13-fold) at 48 hours (p=0.025). These findings suggest that aronia fruit extract strongly activates early pro-apoptotic signaling in MDA-MB-231 cells, with Caspase-3-mediated effector apoptosis continuing at later time points, and this effect was found to be statistically significant with a p-value less than 0.05 (Figure 4).

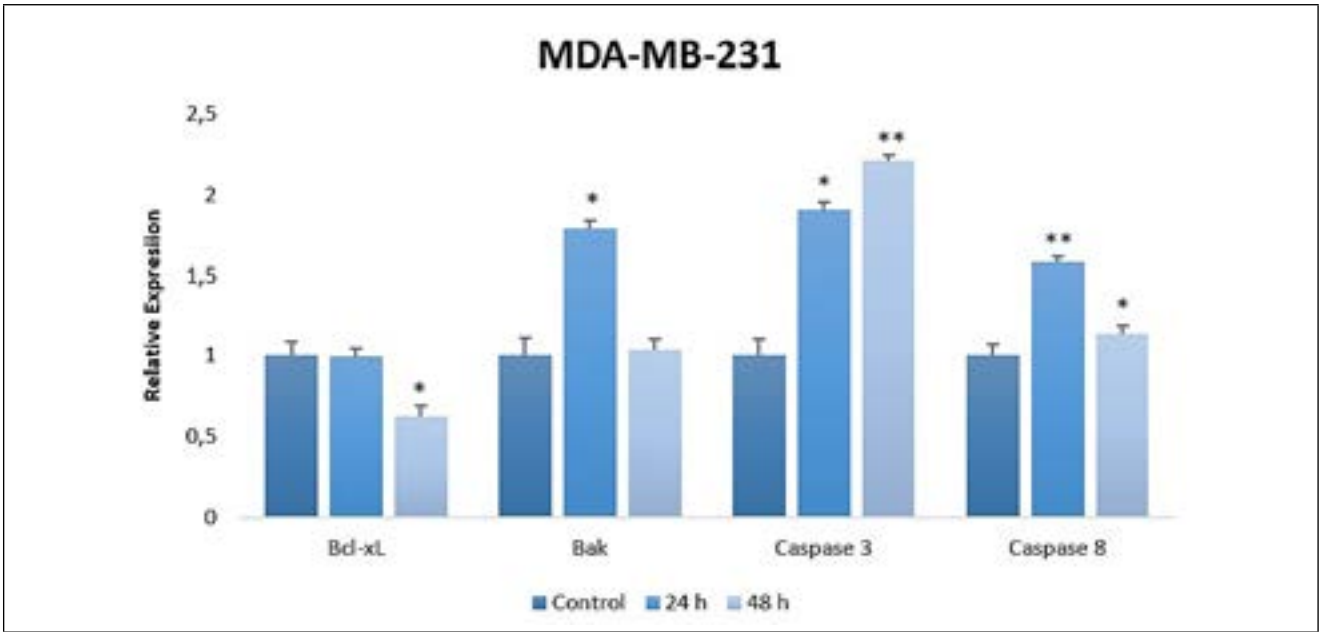

Figure 4: Effect of aronia fruit extract on apoptotic gene expression in MDA-MB-231 cells. Bcl-xL, Bak, Caspase-3, and Caspase-8 mRNA levels were measured by RT-qPCR at 24 and 48 hours post-treatment. Data are presented as mean ± SD of three independent experiments. *p<0.05 and **p<0.01 were considered statistically significant compared with the corresponding control group.

Discussion

Plant-derived phytochemicals, particularly phenolic compounds, flavonoids, and their subgroups such as anthocyanins, have emerged as molecules with significant anticancer potential in cancer research. Phenolic compounds exert effects on growth inhibition, cell cycle arrest, and apoptosis in cancer cells by not only scavenging free radicals but also modulating cellular signaling pathways (13). Flavonoids demonstrate anticancer activity through multifaceted mechanisms, including the reduction of cell proliferation, inhibition of migration and invasion, and upregulation of pro-apoptotic proteins (14). Specifically, anthocyanins, as part of the colored pigments in fruits and vegetables, have been shown to reduce cell viability, induce cell cycle arrest, and trigger caspase-mediated apoptosis in colon, breast, and other cancer types (15). Therefore, phenolic compounds and flavonoids are intensively studied in both in vitro and in vivo models, as they not only inhibit tumor growth and metastasis but also have the potential to enhance the efficacy of existing chemotherapeutic agents and overcome treatment resistance (16).

Indeed, aronia fruit stands out as a plant source with a high content of polyphenols. Its main active constituents include anthocyanins (e.g., cyanidin glycoside derivatives), proanthocyanidins (oligomeric flavan-3-ol derivatives), and hydroxycinnamic acids (such as chlorogenic and neochlorogenic acids) (17). These components are thought to exert cytotoxic and anti-proliferative effects in cancer cells by inducing oxidative stress or acting through mitochondrial pathways and the extrinsic/apoptotic caspase system.

In this study, the effects of aronia fruit extract on cell viability, migration, and apoptosis-related gene activity were investigated in HUVEC and MDA-MB-231 cells. MDA-MB-231 cells, a model of triple-negative breast cancer (TNBC), are widely used due to the aggressive and often treatment-resistant nature of TNBC, providing a standard system for evaluating the cytotoxic effects of new therapeutic agents (18,19). HUVEC cells, representing normal endothelial tissue, serve as a standard model in in vitro angiogenesis studies, allowing preliminary assessment of the efficacy of targeted anti-angiogenic agents in cancer therapy (20).

Accordingly, in the present study, comparisons between HUVEC and MDA-MB-231 cells were performed to evaluate the selective cytotoxic effects of the extract on cancer cells. In addition, the experimental design allowed for the modeling of potential anti-angiogenic effects in HUVEC cells and the observation of differential responses between normal and cancer cells. Determining the differences in cytotoxicity between normal and cancer cells provides preliminary insight into the potential selectivity of the extract and is particularly important for evaluating the capacity of natural products or novel therapeutic agents to target tumor cells while minimizing harm to healthy tissues. Such information contributes to understanding underlying molecular mechanisms and optimizing the therapeutic window.

Both cell lines exhibited a pronounced reduction in viability following extended treatment with high-dose aronia extract, with the strongest effects observed at 72 hours. The IC_{50} value calculated from 24- and 48-hour data was 148.92 µg/mL, which was selected as a reference dose for examining apoptotic processes at the gene expression level. Consistently, aronia fruit extract has been reported to possess both antioxidant capacity and cytotoxic effects in, HCT-116, L929, HaCaT, and HT-29 cell lines (21). Caliskan et al. (2023) reported that aronia extract reduced HT-29 colon cancer cell survival in a manner proportional to the applied dose, with an IC_{50} value of 186 µg/mL. Importantly, the extract exhibited minimal toxicity in HUVEC cells, supporting the safety profile observed in our study (22). These findings provide strong evidence corroborating our cytotoxicity and cell migration results.

However, the observed reduction in viability can be interpreted within a time-dependent effect model, and this effect may be associated with oxidative stress, mitochondrial damage, or other cellular mechanisms induced by aronia fruit extract. Furthermore, the MTT assay measures cell viability indirectly via mitochondrial activity and does not directly reflect apoptosis; therefore, these mechanisms should be evaluated more comprehensively in gene expression studies related to apoptosis. Within this framework, in our study, the IC_{50} dose of aronia fruit extract in the MDA-MB-231 cell line was used to examine the expression of apoptosis-related genes, and the obtained data were evaluated in comparison with the HUVEC cell line.

RT-qPCR analyses revealed that in MDA-MB-231 cells, Bcl-xL levels were significantly decreased at 48 h, while Bak and Caspase-8 at 24 h and Caspase-3 at 24 h were significantly increased. These differential responses suggest that the extract promotes death signals in cancer cells while maintaining homeostasis in normal cells. Previous studies have reported that treatment of MDA-MB-231 cells with aronia fruit peel extract can induce apoptosis, increase Caspase-3/7 activity, cause cell cycle arrest, reduce tumor volume, modulate epigenetic regulation, and enhance chemosensitivity both in vitro and in vivo (23). These findings are consistent with the Caspase-3-mediated effector apoptosis observed in our study.

In HUVEC cells, RT-qPCR analyses showed that Bcl-xL levels were significantly increased at 24 h, while Bak, Caspase-3, and Caspase-8 exhibited their greatest statistically significant decreases at 48 h. A previous study using an anthocyanin-rich Aronox extract demonstrated inhibition of Caspase-3 activation and maintenance of anti-apoptotic Bcl-xL levels (24). These results parallel the anti-apoptotic responses and inhibition of Caspase-3 and Caspase-8 pathways observed in our experiments.

Scratch assays were performed to evaluate the effects of aronia fruit extract on cell migration. In HUVEC cells, wound closure was 0.31 % higher at 24 h and 0.44 % higher at 48 h compared to controls, indicating a slight

pro-migratory effect and maintenance of cell viability in normal endothelial cells. In contrast, MDA-MB-231 cells exhibited 8.81 % and 18.3 % reductions in wound closure at 24 h and 48 h, respectively, demonstrating that the extract inhibits migration and exerts anti-proliferative and anti-migratory effects in cancer cells. These statistically significant phenotypic findings are consistent with the gene expression changes observed in RT-qPCR analyses: in MDA-MB-231 cells, decreased Bcl-xL and increased Caspase-3/8 and Bak are associated with inhibition of cell migration and wound closure, whereas in HUVEC cells, increased Bcl-xL and suppression of pro-apoptotic genes support cell viability and migratory capacity.

Similarly, literature reports indicate that aronia leaf extracts significantly reduce migration and invasion in colon cancer cell lines SW-480 and HT-29; at 50–100 µg/mL, migration decreased by 41–80 % in SW-480 and 77–86 % in HT-29 cells, with reductions in MMP-2 and MMP-9 protein levels observed via ELISA and zymography (25). Additionally, aronia leaf extract reduced migration in SK-Hep1 hepatocarcinoma cells in scratch assays, with decreases in MMP-2/-9 gene and protein levels (26). Another study reported that aronia polyphenols induced apoptosis in U373 glioblastoma cells and downregulated invasion-related matrix metalloproteinase genes, including MMP-2, MMP-14, MMP-16, and MMP-17 (27).

Taken together, these findings, when considered alongside the gene expression changes observed in our study (e.g., Caspase/Bak/Bcl-xL pathway), suggest that aronia extract modulates not only cell death but also migration and invasion capacity in a cell type-specific manner. Specifically, the reduction in wound closure in MDA-MB-231 cells and the slight increase in migration in HUVEC cells confirm that phenotypic and gene-level data together contribute to understanding the therapeutic window of aronia polyphenols. These results align with our scratch assay findings, indicating that aronia extract inhibits migration in cancer cells while preserving migration and viability in normal cells. This effect can be phenotypically explained by a potential disruption of the cytoskeletal structure and reorganization of the actin/microtubule network, partially inhibiting cell migration in MDA-MB-231 cells, while preserving cytoskeletal integrity to maintain migration and viability in HUVEC cells. Therefore, aronia extract is thought to potentially modulate both apoptotic/invasion-related gene pathways and cytoskeletal and migration phenotypes in a cell type-specific manner.

Nevertheless, to more robustly evaluate the therapeutic potential of the extract, detailed investigations of apoptosis-related signaling pathways and cell cycle-related gene expression changes are warranted. Literature studies indicate that aronia affects apoptotic signaling pathways. In Caco-2 colon cancer cells, *Aronia melanocarpa* anthocyanins were reported to block the Wnt/β-catenin pathway and trigger programmed cell death (28). Similarly, *Aronia melanocarpa* juice (AMJ) activates p73 protein in p53-deficient Jurkat cells, triggering Caspase-3-mediated apoptosis and G2/M cell cycle arrest (29). Additionally, aronia fruit extract combined with oligomeric proanthocyanidins enhances apoptosis by inhibiting the PI3K/Akt pathway in colon cancer cells (30). These studies support the mechanistic basis of aronia-mediated apoptosis in cancer cells. In MDA-MB-231 cells, the observed apoptotic effects may relate to these pathways, emphasizing the necessity for further investigation into the extract's therapeutic value.

Future studies should include comprehensive in vitro and in vivo analyses supported by measurements of apoptotic protein expression, Caspase activity, and detailed cell cycle analyses. Such studies will help evaluate the clinical potential of aronia fruit extract and further confirm its selective anti-cancer efficacy while ensuring safety in healthy cells.

Limitations

This study has several limitations. Firstly, reference compounds used as positive controls were not included, which restricts the comparative evaluation of the extract's efficacy. Although healthy endothelial cells were used as controls, the inclusion of normal mammary epithelial cells would provide a more accurate assessment of the extract's selectivity toward cancer cells. The experiments focused solely on MDA-MB-231 cells, but testing other breast cancer lines, such as MCF-7, would enable verification of the findings in different cancer contexts. Furthermore, the results have not yet been confirmed in preclinical or clinical studies, limiting the generalizability of the findings in terms of in vivo efficacy and safety. In addition, although the data obtained from cytotoxicity assays, scratch tests, and gene expression analyses provide strong evidence, protein-level expression studies are necessary to fully understand the translational processes. Future research that takes these constraints into account could provide a more thorough and molecular insight into the effects of aronia fruit extract on cancer cells.

Conclusion

Overall, the results indicate that aronia fruit extract exerts potent anti-growth and apoptosis-inducing effects in MDA-MB-231 cells, while sparing HUVEC cells. Additional studies are needed to investigate the signaling mechanisms driving apoptosis, alterations in cell cycle-associated gene expression, and the extract's efficacy across other cancer models in vitro and in vivo.

Declarations

Ethics Approval and Funding

This study did not require ethical approval because it did not involve human or animal experiments and used open-source data.

Funding

The authors received no funding for this study.

Conflict of Interest

The author declared that there were no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of Data

The data sets used for the study and analyzed can be obtained from the author upon valid request.

Author Contributions

Concept: S.G.T, Literature Review: S.G.T, Design: S.G.T and A.S.A, Data acquisition: S.G.T and A.S.A, Analysis and interpretation: S.G.T, Writing manuscript: S.G.T, Critical revision of manuscript: S.G.T and A.S.A.

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