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Cytotoxic and Pro-apoptotic Effects of Malvidin Anthocyanin in Berry Fruits on Colon Cancer

Üzümsü Meyvelerdeki Malvidin Antosiyanininin Kolon Kanseri Üzerindeki Sitotoksik ve Pro-apoptotik Etkileri

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Öz

Giriş ve Amaç: Tüketici bilincinin artması, taze meyve ve sebze tüketimini yükseltmiştir. Meyveler arasında en çok tüketilenler arasında kırmızı-mor meyveler bulunmaktadır. Meyvelerin rengini, flavonoidlerin bir alt grubu olan antosiyaninler sağlar. Meyvelerde bulunan altı antosiyanin türü; siyanidin, pelargonidin, peonidin, delfinidin, petunidin ve mavimsi rengi veren ve en az miktarda bulunan (%7) malvidindir. Malvidinin anti-kanserojen ve pro-apoptotik etkileri, meyvelerdeki düşük oranı nedeniyle literatürde yeterince araştırılmamıştır.
Gereç ve Yöntemler: Bu çalışmada malvidinin HT-29 insan kolon kanseri hücre hattı üzerindeki sitotoksik ve pro-apoptotik etkileri ölçülmüştür. Malvidin (0, 25, 50, 75 ve 100 µM), HT-29 hücrelerine 24, 48 ve 72 saat boyunca uygulanmış ve hücre canlılığı CellTiter-Glo ve MTT metodları ile ölçülürken, apoptoz göstergeleri Caspase-Glo 3/7 testi ile değerlendirilmiştir.
Bulgular: Malvidin, hücreler üzerinde doz ve zamana bağlı sitotoksik etki göstermiş ve 72 saatlik IC50 değeri 62,22 µM olarak belirlenmiştir. Malvidinin sitotoksisitesinin apoptoz yoluyla olup olmadığını belirlemek için Caspase-Glo 3/7 testi kullanılmış ve 25, 50, 75 ve 100 µM malvidin uygulamalarının 72 saatte sırasıyla 1,26, 1,47, 1,94 ve 2,21 kat artışla kaspaz 3/7 aktivasyonunu yükselttiği görülmüştür.
Sonuç: Malvidinin kolon kanseri hücreleri üzerinde sitotoksik etkiler gösterdiği ve bunun apoptoz yolunun aktive edilmesiyle gerçekleştiği belirlenmiştir. Bu nedenle, antosiyaninler açısından zengin meyvelerin diyete dahil edilmesinin, gastrointestinal malignitelere karşı faydalı olabileceği düşünülmektedir.

Anahtar kelimeler: antosiyaninler, üzümsü meyveler, hücre kültürü, hücre büyümesi, apoptoz

Abstract

Aim: Increasing consumer consciousness elevated the consumption of fresh fruits and vegetables. Berries are among the most consumed fruits. The colorful nature of berries comes from the anthocyanins, a subgroup of flavonoids. The six anthocyanins in berries are cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin, which gives blue color and is the least abundant (7%). Anti-carcinogenic and pro-apoptotic effects of malvidin have been studied insufficiently in literature due possibly to its small presence in berries.
Method: In this study, the cytotoxic and pro-apoptotic effects of malvidin was measured on HT-29 human colon cancer cell line. Malvidin (0, 25, 50, 75, and 100 µM) was applied on HT-29 cells for 24, 48, and 72 hours, and cell viability was measured using MTT and CellTiter-Glo methods, while apoptosis indicators were measured using Caspase-Glo 3/7 assay.

Results: Malvidin has showed dose- and time-dependent cytotoxicity on the cells with 72-hour IC50 value as low as 62.22 μM. To find out whether malvidin induced cytotoxicity through apoptosis, Caspase-Glo 3/7 assay was used, which showed that 25, 50, 75, and 100 μM malvidin applications elevated the caspase 3/7 activation at 72 hours by 1.26, 1.47, 1.94, and 2.21 times, respectively.
Conclusion: As a result, it has been determined that malvidin has cytotoxic effects on colon cancer cells and this occurs via the activation of apoptosis pathway. Therefore, inclusion of berry fruits rich in anthocyanins in diet may be beneficial against gastrointestinal malignancies.

Keywords: anthocyanins, berries, cell culture, cell proliferation, apoptosis

1. Introduction

Being the third common diagnosed cancer, colon cancer is the second leading cause of cancer-related deaths worldwide. It was reported that over 100,000 new cases of colon cancer are expected to be diagnosed and around 50,000 deaths from colorectal cancer are expected in 2023 [1]. Despite advances in treatment, the high incidence and mortality rates underscore the urgent need for novel preventive and therapeutic strategies. Epidemiological evidence consistently supports the protective role of dietary fruits and vegetables against colon cancer, largely attributed to their rich content of bioactive phytochemicals [2].

Among these phytochemicals, anthocyanins, which is a subclass of flavonoids responsible for the vibrant red, purple, and blue hues in berry fruits, have attracted considerable attention due to their antioxidant, anti-inflammatory, and anticancer properties [3,4]. Anthocyanins exist predominantly as glycosides of six anthocyanidins: cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin [5]. While cyanidin and delphinidin have been extensively studied for their anticancer effects, malvidin, despite being less abundant (~7% of total anthocyanidins in berries), has recently emerged as a promising compound with distinct bioactivities [6,7].

Malvidin exhibits notable cytotoxic and pro-apoptotic effects in various cancer cell models, including colon cancer cell lines such as HT-29, by inducing apoptosis and inhibiting cell proliferation [8,9]. A study conducted on human colorectal HCT-116 cancer cells revealed that malvidin has an IC50 of 15 μM, while higher concentration of IC50 value (65 μM) on FR-2 normal human cells (10). Its mechanisms of action involve modulation of apoptosis-related pathways, including upregulation of p53 and caspase-3, which are critical regulators of programmed cell death [11]. However, research focusing specifically on malvidin's effects in colon cancer remains limited compared to other anthocyanidins, warranting further investigation [12-15].

This study aims to elucidate the cytotoxic and pro-apoptotic effects of malvidin extracted from berry fruits on the HT-29 human colon cancer cell line. By focusing on malvidin, we seek to expand the understanding of its potential as a natural therapeutic agent in colon cancer management.

2. Method

2.1. Cell culture

HT-29 human colorectal adenocarcinoma cells were cultured in RPMI-1640 (Sigma-Aldrich, St. Louis, MO, USA) medium supplemented with 10% fetal bovine serum (Sigma-Aldrich) (RPMI+10%FBS). The cells were maintained in a humidified incubator at 37°C under 5% CO_2 atmosphere. Cell passages were performed using Trypsin–EDTA (Sigma-Aldrich) when cultures reached approximately 80% confluency [16]. For all experiments, cells were seeded in appropriate plate formats and allowed to adhere overnight before treatment.

Malvidin chloride (Cayman Chemical Co., Ann Arbor, MI, USA) was dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich) to prepare a stock solution and stored at −20°C in the dark until use. During treatment, the final concentration of DMSO in the culture medium did not exceed 0.5%, and the same amount of DMSO was added to control wells. All experimental conditions were performed in at least three biological replicates.

2.2. Cell viability assays (MTT and CellTiter-Glo)

The effect of malvidin on cell viability was measured using two different methods: colorimetric MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich) assay and luminometric CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA). The MTT assay is based on mitochondrial dehydrogenase activity converting MTT to insoluble formazan crystals, whereas CellTiter-Glo® quantifies intracellular ATP as an indicator of metabolic activity.

HT-29 cells were seeded at a density of 6×10^3 cells/well in transparent 96-well cell culture plates in 200 μl of RPMI+10%FBS. Next day, for the MTT assay [17], given that malvidin is a naturally pigmented compound with a deep purple color, it was initially dissolved in DMSO and further diluted in RPMI+10%FBS. Malvidin was applied on the cells at concentrations of 0, 25, 50, 75, and 100 μM. The cells were incubated for 24, 48, and 72 h. Each treatment was performed in three replicate wells. Control wells contained only RPMI+10%FBS and 0.5% DMSO but did not contain malvidin. Also wells with RPMI+10%FBS and without cells were prepared to control for the media effect on MTT. After incubation, the used RPMI+10%FBS was

removed from the wells and 100 µl of sterile MTT (1 mg/ml) dissolved in RPMI+10%FBS was added into each well followed by 3 h of incubation at 37°C CO_2 incubator. After 3 hours, MTT containing media was discarded again and 100 µl of DMSO was placed into each well and the plate was shaken for 15 minutes to dissolve the purple formazan crystals in the cells. The optical density of the resulting color in the wells was measured at 570 nm using a Biotek Epoch microplate spectrophotometer (Winooski, VT, USA).

For the CellTiter-Glo® Luminescent Cell Viability Assay [18], the HT-29 cells were seeded at a density of 3×10^3 cells/well in white half-area 96-well cell culture plates (Greiner, Austria) in 100 µl of RPMI+10%FBS. After overnight incubation, malvidin chloride was applied on the cells at different concentrations in triplicates for 24, 48, and 72 h. After the incubation, the CellTiter-Glo® reagent was added into the wells following the manufacturer's instructions and the plate was incubated for an additional 45 min at 37°C in the CO_2 incubator. The plate was then placed in a LuMate 4400 microplate luminometer (Awareness Tech. Inc., Palm City, FL, USA) and luminescence values as an indication of ATP levels in the cells were recorded.

Although similar trends are often expected between the colorimetric MTT and luminometric ATP-based assays, malvidin's intrinsic color may interfere with the MTT absorbance signal. Therefore, CellTiter-Glo® was also employed as a color-independent validation method. Any divergence in cytotoxicity values between the two assays may be attributed to this pigment-based optical interference, as well as differences in metabolic endpoints measured by each assay. The comparison between these two types of assays was informed by a previous work evaluating cytotoxicity of polyphenols using both tests [19], without reference to antioxidant activity.

2.3. Caspase 3/7 activity for apoptosis indication

Similar to the CellTiter-Glo assay, HT-29 cells were seeded as 3×10^3 cells/well in 100 µl of RPMI+10%FBS in white 96-well cell culture plates. Next day, malvidin chloride was applied on the cells for 24, 48, and 72 h. All applications were performed in triplicate wells. After the incubation, the Caspase-Glo® 3/7 (Promega) reagent was added into the wells and the plate was incubated for 45 min at 37°C in the CO_2 incubator according to the manufacturer's instructions. The luminescence in the wells was measured using LuMate 4400 microplate luminometer. The values measured were normalized using the cytotoxicity data obtained by both MTT and CellTiter-Glo methods to calculate the normalized caspase 3/7 signals.

2.4. Data Analysis

The statistical significance was analyzed with ANOVA and Student's t test with Bonferroni correction as post-hoc test, respectively. Differences with $P<0.05$ were regarded as statistically significant.

3. Results and Discussion

3.1. Cytotoxicity Measurements

MTT assay results revealed that malvidin has antiproliferative effect on HT-29 human colon cancer cells in a dose- and time-dependent fashion with the highest level of significant ($P<0.05$) growth inhibition (61.2%) at 100 µM dose by 72 h (Figure 1A). The 24 h and 48 h of 100 µM malvidin treatment resulted in 22.7% and 36.0% reductions in cell viability, respectively. The 72 h IC50 value of malvidin was calculated [20] as 62.22 µM after drawing 2nd order polynomial trendline on the 72-h cell viability graph (Figure 1B). The equation of the trendline was $y = 0.0054x^2 - 1.1438x + 100.26$ with an R^2 value of 0.9784.

CellTiter-Glo assay results also revealed that malvidin shows antiproliferative effect on HT-29 cells in a time and dose dependent manner. The highest level of growth inhibition measured was 28.5% at 100 µM dose by 72 h compared to the control treatment (Figure 1C). However, significant levels of growth reduction could not be obtained with the CellTiter-Glo method ($P>0.05$), which could be due to the differences between the two methods. Using the CellTiter-Glo result, the 72 h IC50 value of malvidin could not be calculated as it fell outside the highest malvidin dose applied (Figure 1D).

3.2. Apoptosis Measurements

The Caspase-Glo 3/7 assay resulted in relative apoptosis signals depending of the malvidin treatment. Since these signals depend on the number of living cells the apoptosis signals were normalized. Normalization was performed separately using either cytotocity results. When normalized against the MTT result (Figure 2A), apoptosis signal showed a dose- and time-dependent increase. The apoptosis signal measured on the cells not treated with malvidin (negative control) is used as the baseline level. The highest apoptosis signal (314% or 3.14 fold of negative control) was obtained at 72 h 100 µM malvidin treatment. The 48 h and 24 h of 100 µM malvidin treatments increased the apoptosis signal by 1.62 and 1.29 folds compared to the negative control.

Next, the raw apoptosis signals were normalized aginst the CellTiter-Glo results. A similar dose- and time-dependent increase was also observed (Figure 2B). However, the fold induction was lower than that seen in Figure 2A as the CellTiter-Glo assay resulted in less cytotoxicity in HT-29 cells treated with malvidin. The highest apoptosis signal (170% or 1.70 fold of negative control) was obtained at 72 h 100 µM malvidin treatment. The 48 h and 24 h of 100 µM malvidin treatments increased the apoptosis signal by 1.23 and 1.01 folds compared to the negative control.

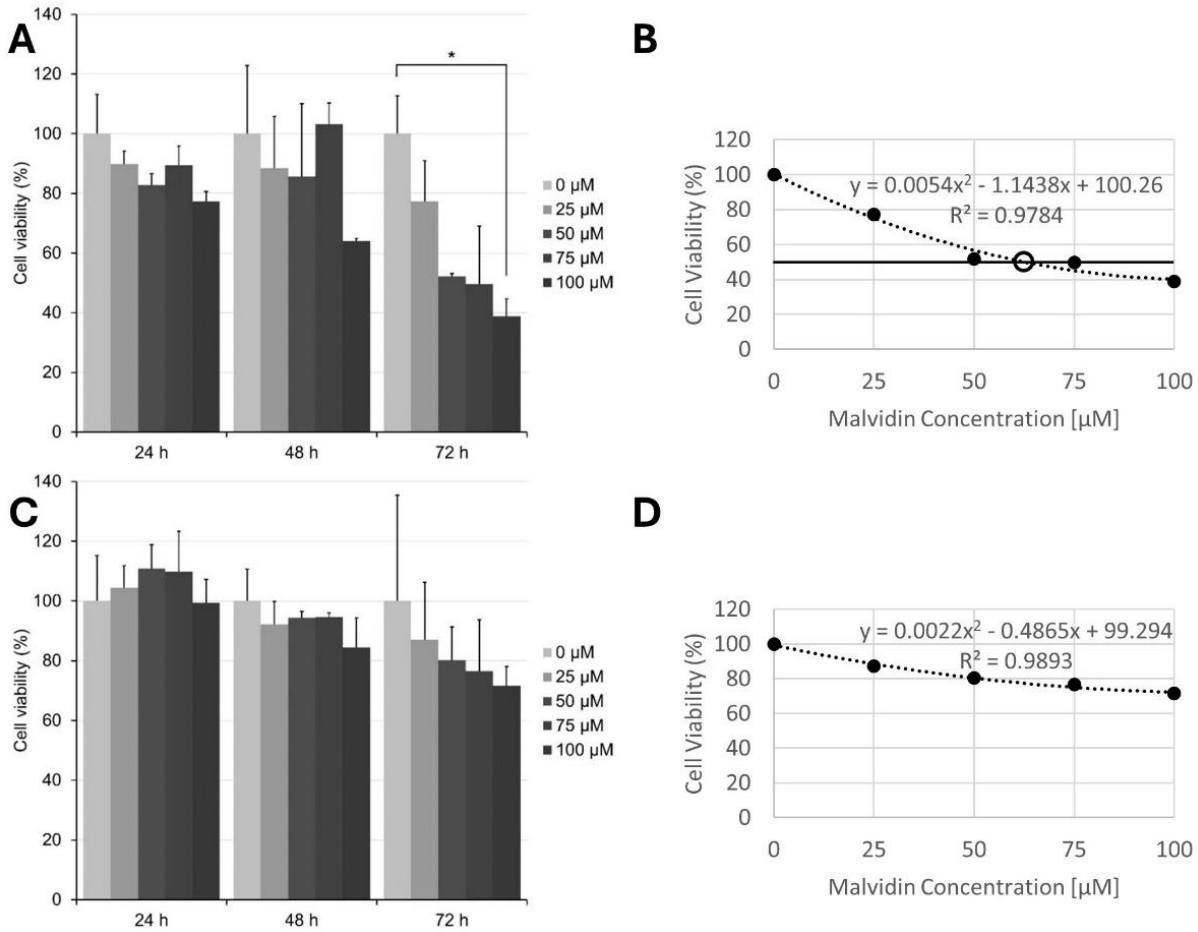

Figure 1. Cell viability results of HT-29 cells after malvidin treatment as measured by (**A**) MTT and (**C**) CellTiter-Glo methods. The 72-hour IC50 value was calculated based on the trendline in **B**. No IC50 could be calculated for **D**. (*: P<0.05)

Lastly, the raw apoptosis data was normalized against the average of two cytotoxicity results (Figure 2C). The highest apoptosis signal (221% or 2.21 fold of negative control) was obtained at 72 h 100 µM malvidin treatment. The 48 h and 24 h of 100 µM malvidin treatments increased the apoptosis signal by 1.40 and 1.13 folds compared to the negative control.

Research in the literature regarding the effects of malvidin on cancer cells and its potential therapeutic benefits reveals notable similarities and differences. Zhang et al. (2005) applied malvidin to five different cell lines and reported that treatment with 100 µM malvidin for 72 hours reduced cell viability by 40% in the MCF-7 human breast cancer cell line. In contrast, the other four human cancer cell lines, AGS (gastric), HCT-116 (colon), NCI-H460 (lung), and SF-268 (central nervous system), exhibited cell death rates ranging from 65% to 80% [15]. Similarly, Saulite et al. (2019) treated human mesenchymal stem cells with malvidin for up to 72 hours and demonstrated that 100 µM malvidin reduced cell viability by 17–30%, indicating that malvidin can inhibit cell proliferation even at low doses [12]. Xu et al. (2018) reported that malvidin exerted anticancer effects in the HCT-116 cell line through apoptosis induction, G2/M phase cell cycle arrest, and upregulation of $p21^{WAF1}$ [21]. In a study by Sakthivel and Brindha (2020), malvidin was shown to inhibit the development of both solid and ascitic tumors by counteracting oxidative stress and inflammatory mediators [22]. Furthermore, Fritz et al. (2006) demonstrated that malvidin affects the phosphorylation of kinases such as ERK1 and ERK2 in the MAPK pathway through phosphodiesterase inhibition. However, this effect was reversible over time and had minimal impact on cellular targets in the G1 phase. Additionally, malvidin was found to inhibit cell progression in the G2/M phase [23]. When comparing the results of our study with those in the literature, it becomes evident that malvidin holds strong potential in cancer therapy, particularly in the treatment of various cancer types such as colorectal cancer. Malvidin appears to be associated with processes like oxidative stress and inflammation, and may inhibit cancer cell growth by targeting these pathways. Therefore, malvidin should be further investigated as a potential therapeutic agent in cancer treatment.

In addition to uncontrolled cell proliferation, resistance to apoptosis is considered one of the key characteristics of colorectal cancer cells. Apoptosis has been shown to be induced by anthocyanins, such as malvidin, through the following mechanisms; (*i*) cleavage of poly(ADP-ribose) polymerase (PARP), activation of caspase-3, and increased expression of the Bax/Bcl-2 ratio, (*ii*) downregulation of anti-apoptotic proteins, including cIAP-2, XIAP, and survivin, (*iii*) enhancement of cytochrome c release [24–26]. Malvidin has been observed to induce apoptosis in several cancer cell lines. A study conducted in 2004 reported that malvidin derived from *Oryza sativa* induced apoptosis in U937 human monocytic leukemia cells [27]. Wang et al. (2018) demonstrated that malvidin-3-galactoside triggered apoptosis in human hepatoma HepG2 cells [28]. Similarly, Dhivya et al. also reported that malvidin induced apoptosis in HepG2 cells [29]. In the study by Dahlawi (2022), the effects of malvidin on apoptosis induction and the inhibition of cell proliferation were investigated in myeloid and lymphoid leukemia cells [30]. Furthermore, Ma et al. (2020) reported that malvidin induced apoptosis in hepatic stellate cells by activating the endoplasmic reticulum stress pathway and the mitochondrial pathway [31]. The studies conducted by Yeh and Yen (2005) and Shih et al. (2005) explored the effects of anthocyanidins on apoptosis induction and inhibition of cell proliferation [32,33]. Despite these findings, Kobori (2003) reported that while malvidin induced apoptosis in HL-60 leukemia cell lines, it did not exhibit the same effect in HCT116 human colon carcinoma cells [34]. In this context, evidence in the literature regarding the ability of malvidin to induce apoptosis in colorectal cancer remains limited and inconclusive. In the current study, apoptosis induction by malvidin was demonstrated in HT-29 colorectal cancer cell lines. Following malvidin treatment, a significant decrease in the number of viable cells and a marked increase in apoptotic cell rates were observed. This finding suggests that different cell lines may exhibit varying sensitivities to malvidin.

Cell migration is a critical indicator in tumor progression and metastasis. However, the current study was primarily focused on evaluating the cytotoxic and pro-apoptotic effects of malvidin on HT-29 cells. Migration assays such as wound healing or transwell migration tests were not included due to the scope and design of the present study. Future studies may incorporate these assays to assess whether malvidin influences cell motility in addition to cell viability and apoptosis.

Our current study has demonstrated that malvidin exhibits a significantly strong effect on apoptosis, with these effects increasing in a dose-dependent manner. In this context, unlike previous studies in the literature, our research provides a substantial contribution by directly highlighting the cytotoxic and pro-apoptotic effects of malvidin on cancer cells. Furthermore, it more clearly emphasizes malvidin's therapeutic potential against gastrointestinal malignancies, particularly colorectal cancer.

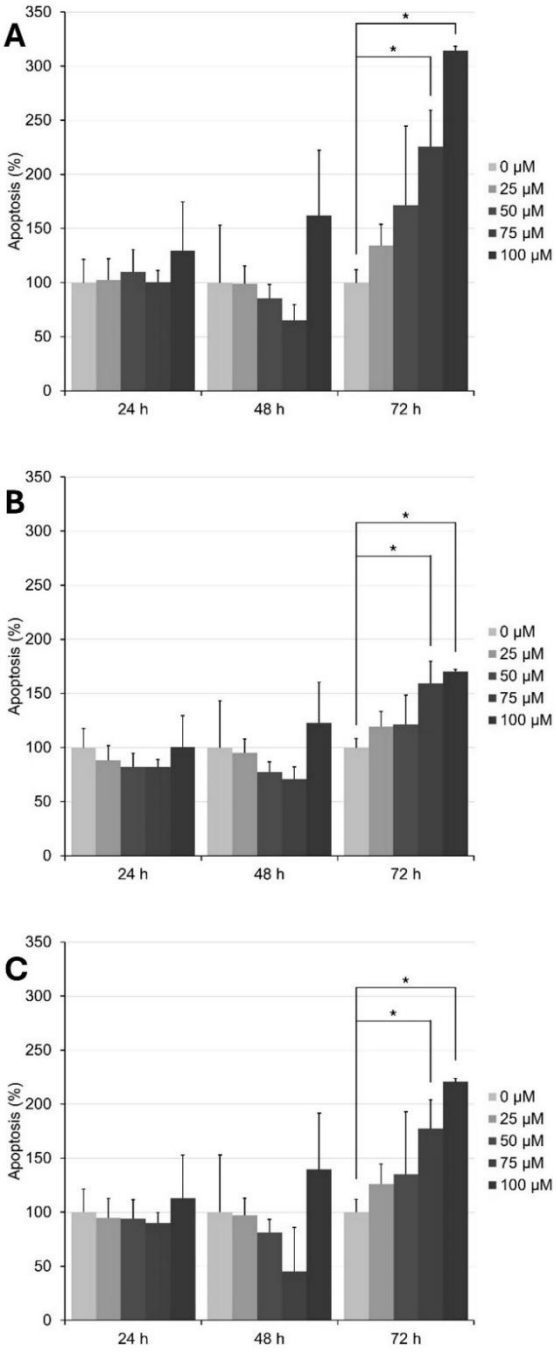

Figure 2. Apoptosis results of HT-29 cells after malvidin treatment as normalized against the (**A**) MTT, (**B**) CellTiter-Glo, and the (**C**) average of MTT and CellTiter-Glo results. (*: $P<0.05$)

Between the two cytotoxicity methods employed in the current study the MTT measured a higher rate of cell death (61.2%) after 72 h of 100 µM malvidin treatment than CellTiter-Glo, which measured only 28.5% cell death. This discrepancy was also

mentioned by Ulukaya et al. (2008) where the authors indicated that MTT method measures the dehydrogenase enzyme activity whereas the CellTiter-Glo method measures the amount of ATP present in the cells [35]. Although both methods are useful in measuring the cell viability the actual target they measure may result in this difference. In another study it is also mentioned that the chemical nature of the drug tested on the cells may interfere with the active ingredient of the viability tests especially the tetrazolium salts [36]. These results were supported by another study, which also pointed out that CellTiter-Glo method may provide better results than MTT method [19].

4. Conclusion

Our study demonstrated that malvidin exerts cytotoxic effects on colorectal cancer cells, primarily through the activation of the apoptotic pathway. Although research on malvidin's effects in colorectal cancer is limited, its potential anti-carcinogenic properties have been well-documented in various cancer models. There is a pressing need for further in-depth studies at advanced *in vitro*, *in vivo*, and clinical trial levels to fully understand its therapeutic potential. Additionally, comparing malvidin's efficacy and potency with other known anti-carcinogenic anthocyanins, polyphenols, and conventional chemotherapeutic agents could offer valuable insights into its advantages and possible applications in cancer treatment.

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA: A Cancer Journal for Clinicians 2023; 73(1): 17-48.
2. Wu H, Dai Q, Shrubsole MJ, Ness RM, Schlundt D, Smalley WE, Chen H, Li M, Shyr Y, Zheng W. Fruit and vegetable intakes are associated with lower risk of colorectal adenomas. The Journal of Nutrition 2009; 139(2): 340-4.
3. Nile SH, Park SW. Edible berries: Bioactive components and their effect on human health. Nutrition 2014; 30(2): 134-44. https://doi.org/10.1016/j.nut.2013.04.007
4. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food & Nutrition Research 2017; 61: 1361779. https://doi.org/10.1080/16546628.2017.1361779
5. Norberto S, Silva S, Meireles M, Faria A, Pintado M, Calhau C. Blueberry anthocyanins in health promotion: A metabolic overview. Journal of Functional Foods 2013; 5(4): 1518-28. https://doi.org/10.1016/j.jff.2013.08.015
6. Saulite L, Jekabsons K, Klavins M, Muceniece R, Riekstina U. Effects of malvidin, cyanidin and delphinidin on human adipose mesenchymal stem cell differentiation into adipocytes, chondrocytes and osteocytes. Phytomedicine 2019; 53: 86-95. https://doi.org/10.1016/j.phymed.2018.09.029
7. Lin J, Tian J, Shu C, Cheng Z, Liu Y, Wang W, Liu R, Li B, Wang Y. Malvidin-3-galactoside from blueberry suppresses the growth and metastasis potential of hepatocellular carcinoma cell Huh-7 by regulating apoptosis and metastases pathways. Food Science and Human Wellness 2020; 9(2): 136-45. https://doi.org/10.1016/j.fshw.2020.02.004
8. Lopez de las Hazas MC, Mosele JI, Macia A, Ludwig IA, Motilva MJ. Exploring the colonic metabolism of grape and strawberry anthocyanins and their *in vitro* apoptotic effects in HT-29 colon cancer cells. Journal of Agricultural and Food Chemistry 2017; 65(31): 6477-87. https://doi.org/10.1021/acs.jafc.6b04096
9. Zhang Y, Vareed SK, Nair MG. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. Life Sciences 2005; 76(13): 1465-72. https://doi.org/10.1016/j.lfs.2004.08.025
10. Xu H, Zhang J, Huang H, Liu L, Sun Y. Malvidin induced anticancer activity in human colorectal HCT-116 cancer cells involves apoptosis, G2/M cell cycle arrest and upregulation of p21WAFI. Int. J. Clin. Exp. Med. 2018;1 1(3): 1734-41.
11. Feng SH, Zhao B, Zhan X, Li RH, Yang Q, Wang SM, Li A. Quercetin-induced pyroptosis in colon cancer through NEK7-mediated NLRP3 inflammasome-GSDMD signaling pathway activation. American Journal of Cancer Research 2024; 14(3): 934.
12. Saulite L, Jekabsons K, Klavins M, Muceniece R, Riekstina U. Effects of malvidin, cyanidin and delphinidin on human adipose mesenchymal stem cell differentiation into adipocytes, chondrocytes and osteocytes. Phytomedicine 2019; 53: 86-95. https://doi.org/10.1016/j.phymed.2018.09.029
13. Lin J, Tian J, Shu C, Cheng Z, Liu Y, Wang W, Liu R, Li B, Wang Y. Malvidin-3-galactoside from blueberry suppresses the growth and metastasis potential of hepatocellular carcinoma cell Huh-7 by regulating apoptosis and metastases pathways. Food Science and Human Wellness 2020; 9(2): 136-45. https://doi.org/10.1016/j.fshw.2020.02.004
14. Lopez de las Hazas MC, Mosele JI, Macia A, Ludwig IA, Motilva MJ. Exploring the colonic metabolism of grape and strawberry anthocyanins and their *in vitro* apoptotic effects in HT-29 colon cancer cells. Journal of Agricultural and Food Chemistry 2017; 65(31): 6477-87. https://doi.org/10.1021/acs.jafc.6b04096
15. Zhang Y, Vareed SK, Nair MG. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. Life Sciences 2005; 76(13): 1465-72. https://doi.org/10.1016/j.lfs.2004.08.025
16. Cetin E, Boyacioglu O, Orenay-Boyacioglu S. An effective treatment approach of liposomally encapsulated metformin in colon cancer. Medical Oncology 2024; 41(4): 82.
17. Boyacioglu O, Kara B, Can H, Yerci TN, Yilmaz S, Boyacioglu SO. Leaf hexane extracts of two Turkish fig (*Ficus carica* L.) cultivars show cytotoxic effects on a human prostate cancer cell line. Agric Food Sci Res 2019; 6(1): 66-70.
18. Orenay Boyacioglu S. Role of PTEN in modulating preventive effect of 3, 4-DHPEA against oxidative stress. Sağlık Bilimleri Dergisi 2018; 27(1): 48-54.
19. Malinowski P, Skała K, Jabłońska-Trypuć A, Koronkiewicz A, Wołejko E, Wydro U, Świderski G, Lewandowski W. Comparison of the usefulness of MTT and CellTiterGlo tests applied for cytotoxicity evaluation of compounds from the group of polyphenols. Environmental Sciences Proceedings 2022; 18(1): 9.
20. Boyacıoğlu O. Zeytin karasuyu fenoliklerinden 3,4-dihidroksifenil etanolün farklı prostat kanseri hücre hatlarındaki sitotoksik etkileri. Food and Health 2019; 5(2): 95-100.
21. Xu H, Zhang J, Huang H, Liu L, Sun Y. Malvidin induced anticancer activity in human colorectal HCT-116 cancer

cells involves apoptosis, G2/M cell cycle arrest and upregulation of p21WAFI. International Journal of Clinical and Experimental Medicine 2018; 11(3): 1734-1741.
22. Sakthivel KM, Brindha D. Malvidin abrogates oxidative stress and inflammatory mediators to inhibit solid and ascitic tumor development in mice. Journal of Environmental Pathology, Toxicology and Oncology 2020; 39(3): 247-260.
23. Fritz J, Kern M, Pahlke G, Vatter S, Marko D. Biological activities of malvidin, a red wine anthocyanidin. Molecular Nutrition & Food Research 2006; 50(4-5): 390-395
24. Bars-Cortina D, Sakhawat A, Piñol-Felis C, Motilva MJ. Chemopreventive effects of anthocyanins on colorectal and breast cancer: A review. In Seminars in Cancer Biology 2022 (Vol. 81, pp. 241-258). Academic Press.
25. Caponio GR, Cofano M, Lippolis T, Gigante I, De Nunzio V, Difonzo G, Noviello M, Tarricone L, Gambacorta G, Giannelli G, De Angelis M, Notarnicola M. Anti-proliferative and pro-apoptotic effects of digested aglianico grape pomace extract in human colorectal cancer cells. Molecules. 2022; 27(20): 6791.
26. Shi N, Chen X, Chen T. Anthocyanins in colorectal cancer prevention review. Antioxidants 2021; 10(10): 1600.
27. Hyun JW, Chung HS. Cyanidin and malvidin from *Oryza sativa* cv. Heugjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G2/M phase and induction of apoptosis. Journal of Agricultural and Food Chemistry 2004; 52(8): 2213-2217.
28. Wang Y, Lin J, Tian J, Si X, Jiao X, Zhang W, Gong E, Li B. Blueberry malvidin-3-galactoside suppresses hepatocellular carcinoma by regulating apoptosis, proliferation, and metastasis pathways *in vivo* and *in vitro*. Journal of Agricultural and Food Chemistry 2018; 67(2): 625-636.
29. Dhivya S, Khandelwal N, Abraham SK, Premkumar K. Impact of anthocyanidins on mitoxantrone-induced cytotoxicity and genotoxicity: an *in vitro* and *in vivo* analysis. Integrative Cancer Therapies 2016; 15(4): 525-534.
30. Dahlawi H. Effect of malvidin on induction of apoptosis and inhibition of cell proliferation on myeloid and lymphoid leukemia. Sch J Appl Med Sci 2022; 10: 150-156.
31. Ma Y, Li Y, Zhang H, Wang Y, Wu C, Huang W. Malvidin induces hepatic stellate cell apoptosis via the endoplasmic reticulum stress pathway and mitochondrial pathway. Food Science & Nutrition 2020; 8(9): 5095-5106.
32. Yeh CT, Yen GC. Induction of apoptosis by the Anthocyanidins through regulation of Bcl-2 gene and activation of c-Jun N-terminal kinase cascade in hepatoma cells. Journal of Agricultural and Food Chemistry 2005; 53(5): 1740-1749.
33. Shih PH, Yeh CT, Yen GC. Effects of anthocyanidin on the inhibition of proliferation and induction of apoptosis in human gastric adenocarcinoma cells. Food and Chemical Toxicology 2005; 43(10): 1557-1566.
34. Kobori M. *In vitro*-screening for cancer-suppressive effect of food components. Japan Agricultural Research Quarterly: JARQ 2003; 37(3): 159-165.
35. Ulukaya E, Ozdikicioglu F, Oral AY, Demirci M. The MTT assay yields a relatively lower result of growth inhibition than the ATP assay depending on the chemotherapeutic drugs tested. Toxicology In Vitro 2008; 22(1): 232-9.
36. Ulukaya E, Colakogullari M, Wood EJ. Interference by anti-cancer chemotherapeutic agents in the MTT-tumor chemosensitivity assay. Chemotherapy 2004; 50(1): 43-50.