Amygdalin anti-proliferative impact on HT-29 colorectal cancer cell line

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Abstract

Background: Amygdalin, a naturally occurring glycoside, has been utilized in traditional Chinese medicine. This naturally occurring cyanogenic chemical has historically been utilized for therapeutic purposes, encompassing the management of diverse ailments such as cough, asthma, nausea, leprosy, and leukoderma. Significantly, there has been a growing focus on the antitumor effect of Amygdalin in recent years. **Objective**: The aim of this study was to examine the potential chemoprotective and cytotoxic properties of amygdalin separately or in combination with cisplatin on the Human colorectal cancer (HT-29) cell line. **Methods and Materials:** The cytotoxic impact of amygdalin and cisplatin on both normal and HT-29 cells was assessed using the MTT assay. Additionally, the apoptotic effect of amygdalin was evaluated by examining the gene expression levels of P53, BAX, Caspase-3, and BCL-2. **Results**: The findings indicated that amygdalin alone or in combination with cisplatin exhibited a discerning cytotoxic impact specifically on human HT-29 cells while demonstrating no cytotoxicity towards normal cells. Simultaneously, the administration of amygdalin alone or in combination with Cisplatin results in an upregulation of P53, BAX, and Caspase-3 gene expression, while downregulating the expression of the BCL-2 gene. In conclusion, it can be observed that amygdalin alone or in combination with cisplatin exhibits a chemo-modulatory effect, offering protection to human HT-29 cells. This suggests a notable selective chemoprotective capability, potentially enhancing the overall well-being of individuals with cancer.

Keywords: Amygdalin, Cisplatin, antitumor effect, Colorectal cancer cell lines, Cytotoxicity, Apoptosis

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1. Introduction

Colorectal cancer (CRC) ranks third in terms of prevalence, accounting for 6.1% of all cancer cases, and second in terms of death, with a mortality rate of 9.2%. According to projections, the cumulative mortality rates for rectal and colon cancer are expected to rise by around 60% and 71.5% respectively, by the year 2035 [1]. The chance of acquiring this particular form of cancer is associated with poor dietary habits, tobacco use, inflammatory bowel disease, the presence of polyps, genetic predisposition, and the natural process of aging [2, 3]. Among the cohort of individuals diagnosed with colorectal cancer, it is seen that 90% of patients are aged 50 or above, with a median age of 64 years [4].

In the year 1950, Ernst Theodore Krebs discovered a novel vitamin, designated as B-17, which was afterwards named laetrile or amygdalin [5]. Amygdalin is a type of cyanogenic complex that belongs to the category of aromatic cyanogenic glycosides [6]. It is present in various plant species, particularly in the seeds of rosaceous plants such as apricot, cherry, plum, and peach [7]. The seeds of rosaceous fruits, such as apricot kernels and other bitter nuts, exhibit the highest concentration. The compound forms a diglucoside with the cyanide radical (CN), which has a high degree of bio-accessibility [8].

Amygdalin is a molecule that is considered to be non-toxic. However, it has the ability to undergo hydrolysis under the influence of glucosidase enzymes, such as prunase and amygdalase [9]. This hydrolysis process ultimately leads to the production of mandelonitrile, which further decomposes into benzaldehyde and hydrogen cyanide (HCN) [10]. The HCN is a toxic compound that can undergo decomposition through enzymatic processes. Consequently, certain investigations have indicated that amygdalin has antitussive and anti-asthmatic properties [11]. Additionally, amygdalin has been found to have several pharmacological actions, including anti-atherogenic properties [12], inhibition of kidney interstitial fibrosis [13], prevention of pulmonary fibrosis [14], immunological suppression [15], resistance to hyperoxia-induced lung damage [16], immune regulation, anticancer activity, anti-inflammatory effects, and antiulcer properties [17].

Nevertheless, the primary impact of amygdalin identification is its antitumor or anticancer properties, achieved through the breakdown of carcinogenic compounds within the body, hindering the nutrient supply to tumor cells, inducing cancer cell death, inhibiting cancer cell

proliferation, and potentially reducing the occurrence of breast cancer, prostate cancer, and lung cancer [18-21].

Cisplatin, also known as cis-diammininedichloroplatinum (II), is an inorganic chemical that functions as an alkylating agent. It is widely utilized as a prominent therapeutic medicine for inhibiting the proliferation of cancer cells in several types of human cancers, such as breast, testicular, ovarian, colon and lung cancers [22]. Cisplatin has the ability to create adducts between and between DNA strands, resulting in a strong capacity to induce cell cycle arrest, ultimately leading to apoptosis in a majority of cancer cell types [23]. The interactions between cisplatin and DNA result in the formation of crosslinks, which effectively hinder the processes of replication, transcription, and other nuclear functions [24]. These inhibitory effects play a crucial role in impeding the proliferation of cancer cells and the growth of tumors. The effectiveness of cisplatin is contingent upon the cellular capacity to either repair DNA damage or undergo apoptosis. Hence, the signaling pathways governing apoptosis play a pivotal role in determining cellular response to cisplatin [25].

Hence, the objective of this study was to conduct additional research on the benefits of amygdalin and the signaling mechanism involved in its anti-tumor effects on HT-29 colorectal cancer cell line.

2. Materials and methods

2.1 Chemicals

The amygdalin was purchased from Sigma-Aldrich (St. Louis, Missouri, United States), and dissolved in water (stock 1M). The cisplatin compound was purchased from a local pharmacy located in Egypt (EIMC United Pharmaceuticals, Egypt). The contents of each vial, which contained 50mg of the substance in a 50ml solution, were dissolved in physiological saline consisting of 0.9% sodium chloride. The HT-29 cell line and normal retina cell line (RPE1) were purchased from Vacsera, AL Giza, Egypt. All additional chemicals utilized in this experiment were purchased from Sigma Aldrich (St. Louis, Missouri, United States).

2.2 Cell-line preparation

The cell line was cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/mL streptomycin.

The culture was maintained in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at a temperature of 37ºC. HT-29 cells at a concentration of $5x10^5$ cells per ml were cultured in a six-well culture plate with 2 mL of DMEM. After a 24-hour incubation period, the media was replaced with fresh medium containing different concentrations of amygdalin and cisplatin separately and combination group of Amygdalin+Cisplatin (1.25, 2.5, 5, 10, 20 mg/mL). One well was left untreated as a reference [26].

2.2 Cytotoxic effect on human cell lines using MTT assay

The MTT assay was employed to evaluate the cytotoxic impact on human cell lines. The cytotoxic activity test was performed and assessed by the Bioassay Cell Culture Laboratory at the National Research Centre in Dokki, Egypt. The MTT assay is a colorimetric assay that relies on the reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [27]. In this study, RPE1 and HT-29 cells were subjected to treatment with different doses of amygdalin and cisplatin separately and combination group of Amygdalin+Cisplatin. Following a 48-hour incubation period, a concentration of 2.5 µg/ml of MTT was introduced to each well and subsequently incubated at a temperature of 37°C for a duration of 4 hours. The formazan crystals that were generated were solubilized with the addition of 200 µl per well of a 10% solution of Sodium dodecyl sulphate. In this study, a positive control was employed to ensure the reliability of the experimental circumstances. This positive control has been previously established to result in 100% mortality under the identical conditions [28]. The absorbance of the samples was measured at a wavelength of 595 nm. The calculation of the viability change percentage was performed using the following formula:

Cytotoxicity %= (Extract reading/Negative control reading) x100

Viability %= 100- Cytotoxicity%,

The efficacy of each treatment is assessed through the determination of its IC_{50}, which represents the concentration at which 50% of the cellular viability is impeded.

2.3 Molecular determination

The extraction of total RNA from human HT-29 cells was performed using the PureLink® RNA Mini Kit, which was obtained from Ambion by Life Technologies and manufactured by Thermo Scientific. The specific catalog numbers for the kit were 12183018A. The extraction procedure

followed the instructions provided by the manufacturer. The assessment of RNA sample purity was conducted using the NanoDrop® ND-1000 Spectrophotometer, manufactured by NanoDrop Technologies in Wilmington, Delaware, USA. The synthesis of complementary DNA was performed using the High Capacity cDNA Reverse Transcription Kit, which was acquired from Thermo Scientific under the product code 4374966. The gene expression of P53, BAX, BCL-2, and Caspase-3 was detected using real-time PCR amplification. This was achieved using the Maxima SYBR Green qPCR Master Mix (2X) kit, which was obtained from Thermo Scientific (catalog number K0251). The quantification of target gene expression levels was performed using the formula $2^{-\Delta\Delta ct}$ [29]. The housekeeping GADPH was used as a standard. The primer sequences for the target genes were developed in accordance with the specifications outlined in Table 1.

Table 1 primer sequence of P53, BAX, BCL-2, Caspase-3.

Gene	Sequence	Accession number
P53	**F 5- CCTCAGCATCTTATCCGAGTGG-3** **R 5- TGGATGGTGGTACAGTCAGAGC-3**	**NM_000546**
BAX	**F 5- TCAGGATGCGTCCACCAAGAAG-3** **R 5- TGTGTCCACGGCGGCAATCATC-3**	**NM_004324**
BCL-2	**F 5-ATCGCCCTGTGGATGACTGAGT-3** **R 5-GCCAGGAGAAATCAAACAGAGGC-3**	**NM_000633**
Caspase-3	**F 5- GGAAGCGAATCAATGGACTCTGG-3** **R 5- GCATCGACATCTGTACCAGACC-3**	**NM_004346**
GADPH	**F 5-GTCTCCTCTGACTTCAACAGCG-3** **R 5-ACCACCCTGTTGCTGTAGCCAA-3**	**NM_002046**

Statistical analyses

The data were subjected to statistical analysis using the software SPSS 16.0 in order to calculate the mean values and standard errors. The data underwent analysis using a one-way analysis of variance (ANOVA) in order to ascertain the statistical significance of variations among the different experimental groups. A significance level of less than $P < 0.05$ was used.

3. Results

3.1 Cytotoxic effect of Amygdalin on HT-29 cell line

The MTT assay was conducted in order to evaluate the proliferation rate of HT-29 cells and the RPE1 following exposure to different doses of Amygdalin, Cisplatin and combination group of

Amygdalin+Cisplatin. The present study investigated the impact of several doses of amygdalin, cisplatin and combination group of Amygdalin+Cisplatin, ranging from 1.25 to 20 mg/mL, on the viability of HT-29 cells in an *in vitro* setting. The viability of the cells was assessed after a 48-hour incubation period using the MTT assay, as depicted in table 2, figure 1. The findings of the study indicated that amygdalin, cisplatin and combination group of Amygdalin+Cisplatin had a concentration-dependent inhibitory effect on HT-29 cells, while had no discernible impact on normal cells RPE1. A concentration of 12.14 mg/mL resulted in a 50% viability rate after a 48-hour treatment. While cisplatin concentration of 7.17 mg/mL resulted in a 50% viability rate after a 48-hour treatment. The combination group of Amygdalin+Cisplatin of 3.25 mg/mL resulted in a 50% viability rate after a 48-hour treatment. The amygdalin and cisplatin findings indicate that there was no observed impact on the viability of RPE1 cells as demonstrated in Table 2. The best results were observed in the combination group of Amygdalin+Cisplatin treated cells.

Table 2. LC50 of Amygdalin, Cisplatin, and Amygdalin+Cisplatin, on HT-29 & normal cells RPE1 by using MTT assay.

	Amygdalin IC_{50}	**Cisplatin IC_{50}**	**Amygdalin+Cisplatin IC_{50}**
HT-29 cells	**12.14 mg/mL**	**7.17 mg/mL**	**3.25 mg/mL**
RPE1 cells	**0**	**0**	**0**

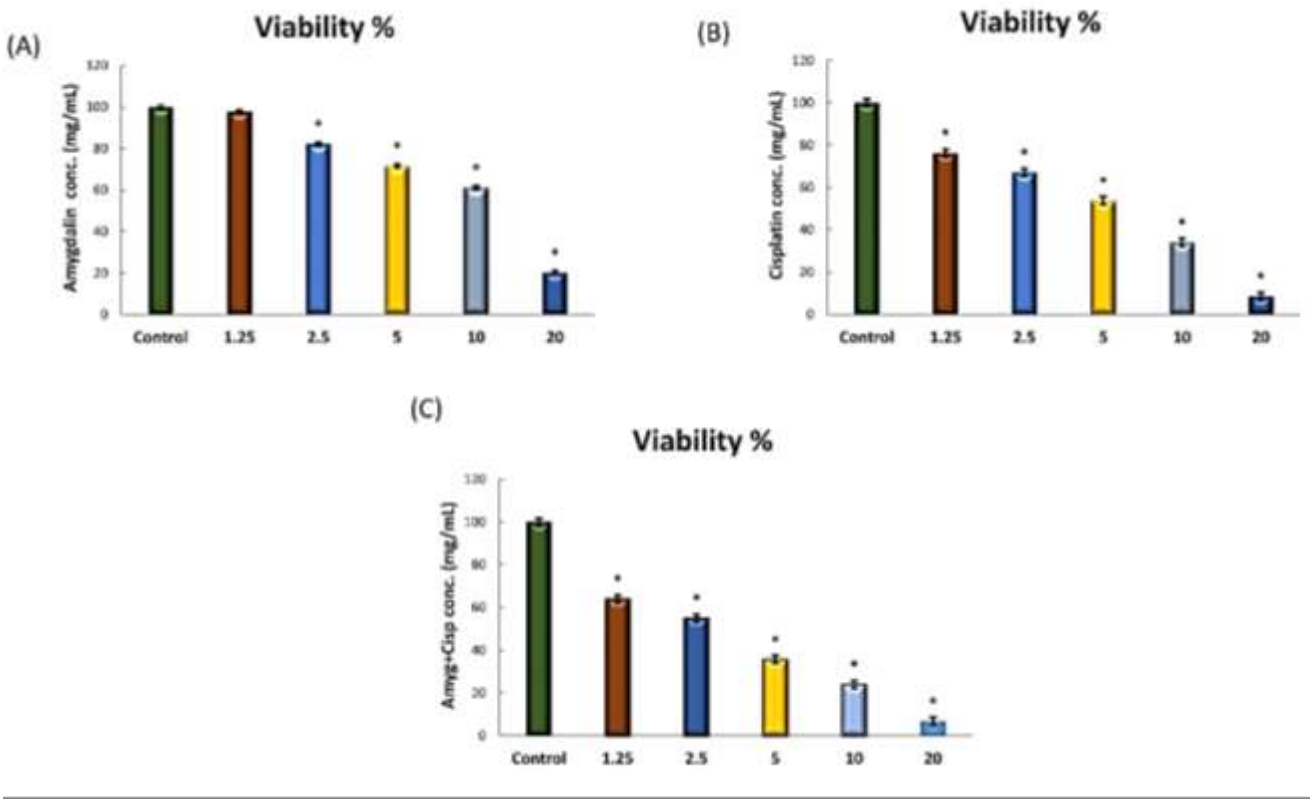

Figure 1. The effect of different concentration of Amygdalin (A), Cisplatin (B), and Amygdalin+Cisplatin (C), on HT-29 on cell viability by using MTT assay after 48 hr incubation. The results are presented as the mean ± SD. * Significant difference between control (untreated cancer cell) and treated groups ($p < 0.05$).

3.2 Effect of Amygdalin on the level of gene expression of p53, Bax, BCL-2, Caspase-3 by using real time PCR

The present study investigated the impact of amygdalin, cisplatin and combination group of Amygdalin+Cisplatin on the gene expression levels of p53, Bax, BCL-2, and Caspase-3 by the utilization of real-time PCR. The findings from the analysis of gene expression indicate that there was an observed upregulation in the levels of gene expression for P53, BAX, and Caspase-3 in HT-29 cells in amygdalin, cisplatin and combination group of Amygdalin+Cisplatin treated cells in comparison with untreated cells. Conversely, there was a downregulation in the expression level of the BCL-2 gene in HT-29 cells in amygdalin, cisplatin and combination group of Amygdalin+Cisplatin treated cells in comparison with untreated cells, as depicted in Figure 2. The best results were observed in the combination group of Amygdalin+Cisplatin treated cells.

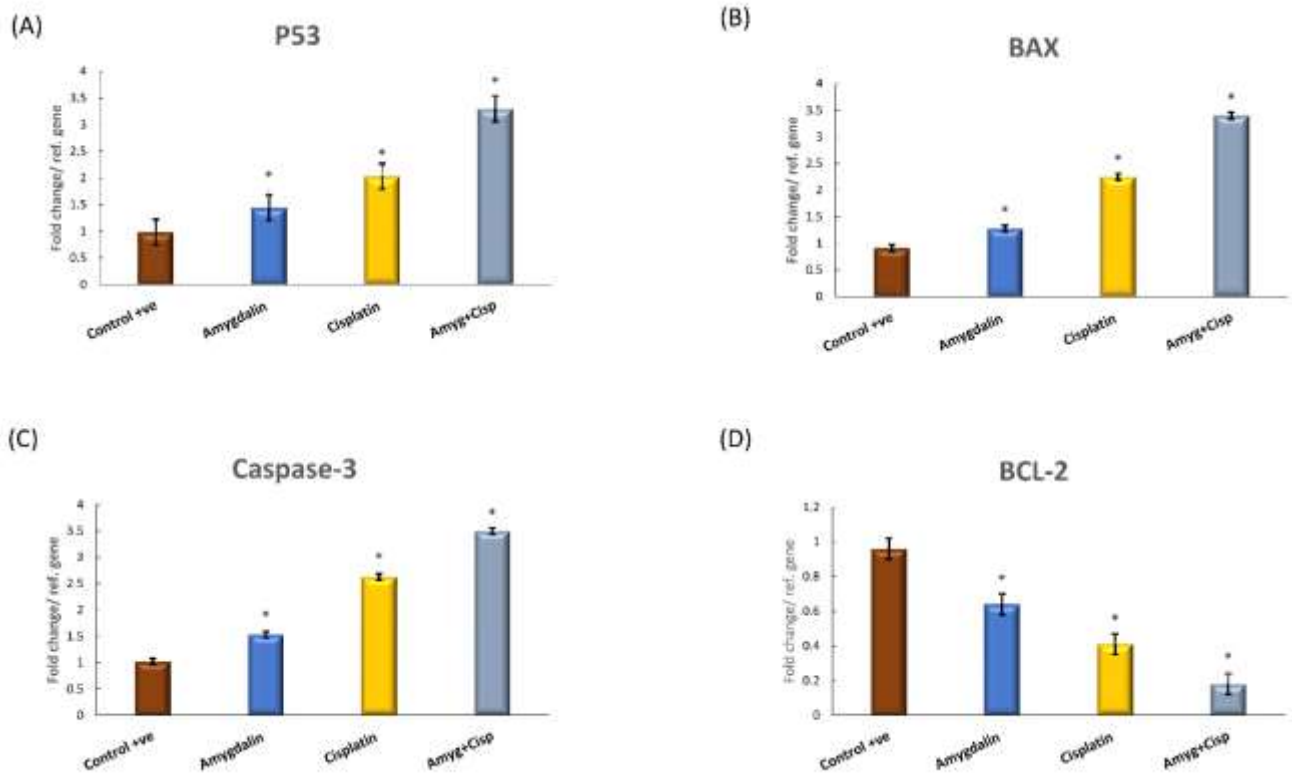

Figure 2. Analysis of PCR product of P53, BAX, Caspase-3 and BCL-2 genes in HT-29 cell line by using real time PCR. The results are presented as the mean ± SD. * Significant difference between control (untreated cancer cell) and treated groups ($p < 0.05$).

4. Discussion

The treatment of colon cancer involves the surgical excision of the tumor, which is afterwards followed by chemotherapy, with or without the addition of radiation therapy. Nevertheless, it is widely acknowledged that the adverse impact of these interventions has frequently worsened the general decline in the well-being and quality of life of the individuals receiving them. While the early identification of colon cancer has led to increased rates of survival, the efficacy of chemotherapeutic medicines employed in its treatment remains inconsistent across all patients [30].

Specific types of dietary fatty acids have the potential to induce irritation in the colon through the augmentation of pro-inflammatory reactions. The potential chemotherapeutic properties of kernels can be attributed to the presence of glycosides, including amygdalin [31]. The proliferation of

cancer cells can be attributed to their heightened need for lipids in order to build membranes. As a result, implementing dietary treatments that involve the consumption of fatty acids with anti-carcinogenic qualities offers a new, feasible, and relatively low-risk strategy to decrease the growth of cancer [32].

Upon interaction with the enzyme β-glucosidase, amygdalin undergoes degradation, resulting in the formation of two glucose molecules, one benzaldehyde molecule, and one molecule of HCN, which is known for its poisonous properties. Therefore, it is only the enzyme β-glucosidase that possesses the capability to produce HCN from amygdalin [33]. Within the human body, it is seen that the enzyme known as glucosidase is exclusively present in cancer cells, exhibiting a significantly higher concentration (about 3000 times more) compared to normal cells. Additionally, it is worth noting that amygdalin, which is found in vitamin B12 as well as in various berries such as blackberries, blueberries, and strawberries, contains the CN radical, which is not considered a harmful chemical [34].

The utilization of combination therapy using cisplatin and other natural anti-cancer extracts, such as amygdalin medicines, has been employed as a potential therapeutic approach for many human cancers. In this study, we assessed the cytotoxicity of amygdalin, cisplatin, and a combination of amygdalin and cisplatin on the HT-29 cell line in an *in vitro* setting. The evaluation was conducted over a period of 48 hours. The viability of cells and the cytotoxic effects of cancer drugs were assessed at various concentrations using an MTT assay. The IC_{50} value of Amygdalin / Cisplatin mixture was found to be lower compared to that of amygdalin and cisplatin. This indicates that Amygdalin / Cisplatin mixture had a more cytotoxic impact on malignant cells at lower doses.

Amygdalin exhibits anticancer properties through the activation of apoptosis and the modulation of immunological function. Shi et al. [35] conducted a study. In order to ascertain the cytotoxicity effect of amygdalin, it is imperative to conduct *in vivo* investigations for the purpose of establishing the appropriate safe dosage. Our findings exhibit a strong correlation with the study conducted by Waseem and Nijoud [36], whereby they assessed the impact of amygdalin intervention on individuals diagnosed with breast cancer. According to Cassiem and de Kock [30], the authors reported that the amygdalin found in different types of apricot and peach kernels exhibited a reduction in the proliferation of cancer cells. The regulation of cell cycle progression and the production of a transient S-phase halt can effectively suppress proliferative activity. The findings

presented in this study were substantiated with the conclusions drawn by Moon et al. [37], which indicated that amygdalin effectively suppressed cell proliferation and reduced telomerase activity through the upregulation of ß glucosidase activity.

Consequently, amygdaline serves only as a source of glucose for healthy cells, facilitating energy provision. In contrast, it has been observed that malignant cells lack the presence of rhodanese, resulting in the accumulation of HCN within these cells [38, 39]. The significance of amygdalin in medical research is evident in its application as a complementary treatment for prostate cancer, where it is supplied alongside recognized medicines to enhance therapeutic outcomes. The ability to modify proteins involved in the regulation of the cell cycle, such as their expression, has been seen [40-42].

In the study conducted by Tsaur et al. [43] investigated the impact of cdk1 and its associated partner cyclin B on the growth rate of prostate cancer cell lines. The findings revealed that the presence of cdk1 and cyclin B resulted in a reduction in the growth rate of these cell lines [44]. Multiple studies have demonstrated that amygdalin exhibits anti-proliferative and pro-apoptotic characteristics in cells associated with promyelocytic leukemia, hepatoblastomacervical cancer, and bladder cancer [43, 45, 46].

Research studies have shown evidence that amygdalin has the ability to induce cell cycle arrest in many tumor cell lines, specifically in the G0/G1 phase. This effect is accompanied by the impairment of cells in the S- and G2/M phases [47]. Consequently, it induces growth inhibition and impedes proliferation by decelerating the cellular cycle. Several studies have demonstrated that two weeks following amygdalin therapy, there is a decrease in the expression of cell cycle [48].

Amygdalin has been shown to induce apoptosis in kidney fibroblasts and prostatic cancer cell lines. Nevertheless, the documentation of the signaling mechanism of apoptosis induced by amygdalin is currently lacking [49]. One of the activities of amygdalin is enhancing the immunological function of an organism. The activity of polyhydroxyalkanoates can be significantly enhanced by their ability to stimulate the proliferation of human peripheral blood T lymphocytes. This, in turn, leads to an increased secretion of interleukin-2 and interferon gamma, while inhibiting the secretion of tumor growth factor-β1 [10, 50].

Another study aimed to examine the chemoprotective and therapeutic effects of amygdalin in conjunction with the conventional chemotherapeutic drug, cisplatin. In order to assess the differential cytotoxicity between normal and cancerous breast cells, as well as fibroblasts, the impact of different concentrations of cisplatin and amygdalin was examined separately on MCF12F, MCF-7, MDA-MB-231 cell lines, and fibroblasts. The viability of all cell lines was seen to decline in a dose- and time-dependent manner upon exposure to cisplatin and amygdalin. It is noteworthy to mention that the administration of amygdalin at a concentration of 10 mM to both normal and cancer cells did not provide any statistically significant disparity in cell viability. Moreover, the viability rate remained consistently over 90% throughout all observed time-points. Based on the obtained IC_{50} data, it was determined that the concentration of amygdalin was established at 10 mM. This concentration did not exhibit any adverse effects on cell survival across all tested cell lines over a 24-hour timeframe. The concentration of cisplatin was established at 15 μM across all cell lines to ensure a cell survival of over 50%, hence facilitating a more accurate assessment of the impact of combination therapy on normal cells [22]. These results align with prior research that suggests some phytochemicals, such as amygdalin, curcumin, and sulphoraphane, have little toxicity towards non-cancerous, normal cells [51].

Cisplatin is a widely recognized chemotherapeutic agent utilized in the treatment of various types of human malignancies. However, its usage is accompanied by significant adverse effects, non-selective cytotoxicity, which results in harm to normal cells, and the emergence of drug resistance [52]. Several prior studies have shown evidence of the cytotoxic synergistic effects of cisplatin in combination with other substances, including bee venom, thiazolo[5,4-b] quinoline derivative D3CLP, and AT-101 medication, on different types of cancer cell lines [53]. A separate investigation showcased the synergistic efficacy of the phytochemical Epigallocatechin gallate when used in conjunction with cisplatin and tumor-active palladium compounds for ovarian cancer. This finding implies that the utilization of platinum-based drugs, such as cisplatin, and specifically engineered trans-palladiums, in combination with carefully selected phytochemicals, may potentially serve as a strategy to overcome drug resistance in the future [54].

In the context of cancer cells, our study demonstrated that cisplatin and amygdalin induces the activation of P53, BAX, caspase-3, and decrease BCL-2 which subsequently triggers apoptosis. The anticancer properties of benzaldehyde can be attributed to its activation of apoptosis through

caspases, resulting in the depletion of cellular energy. This activation occurs as a consequence of hydrocyanic acid's inhibition of mitochondrial cytochrome C oxidase in the respiratory electron transport chain, which subsequently reduces oxidative metabolism and the associated process of oxidative phosphorylation [55]. This process is facilitated by the downregulation of the anti-apoptotic protein BCL-2 and the elevation of the pro-apoptotic protein BAX. The activation of apoptosis is facilitated by both pro- and anti-apoptotic BCL-2 family proteins, which subsequently trigger the mitochondrial outer membrane permeabilization process. The process of mitochondrial outer membrane permeabilization leads to the liberation of cytochrome c from the mitochondria, which is then released into the cytosol. This event serves as a trigger for the activation of caspases, ultimately resulting in the induction of apoptosis.

According to a study the expression levels of pro-apoptotic genes PUMA, P53, and BAX were observed to be significantly reduced, while the expression levels of anti-apoptotic genes BCL-2 and BCL-xL were observed to be elevated in normal cells following the combined treatment compared to treatment with cisplatin alone. This particular observation and discovery provide evidence that aligns with the fundamental justification of the mechanism of action through the apoptotic pathway. In contrast, the expression levels of pro-apoptotic genes PUMA, P53, and BAX were found to be elevated, while the expression levels of anti-apoptotic genes BCL-2 and BCL-xL were observed to be reduced in cancer cells following the combined treatment as compared to treatment with cisplatin alone. The findings from the RT-PCR analysis conducted on breast cancer cell lines, specifically MCF7 and MDA-MB-231, indicate a potential synergistic impact of amygdalin in combination with cisplatin, hence emphasizing its anti-cancer properties [22].

Based on the aforementioned modifications seen in mRNA and protein expressions, it can be inferred that the concurrent administration of cisplatin and amygdalin has the potential to preferentially enhance the viability of normal cells by specifically impeding apoptosis solely in these cells. The aforementioned observations and findings corroborate our primary hypothesis and are consistent with previous studies that have demonstrated a similar mechanism of action of amygdalin in various cell lines and animal models [56-58].

In a research, the researchers utilized a combination of cisplatin and amygdalin to assess the potential for a synergistic cytotoxic effect between these substances. The findings from the comparative analysis of the cytotoxic impact of amygdalin, cisplatin, and a combination of both

indicate that the cell toxicity exhibited by the mixture surpassed the cytotoxicity of amygdalin alone and cisplatin alone in the Hela cancer cell line, specifically in the context of human cervical cancer. The findings also indicate that the cytotoxicity of the mixture increased as its concentration, ranging from 1 to 1000 µg/ml, and incubation periods of 24 and 72 hours increased [23]. Cisplatin, for instance, contributes to the augmentation of amygdalin cytotoxicity by facilitating the provision of more glucosidase enzyme. This is achieved through cisplatin's ability to induce lysosomal membrane permeabilization, consequently leading to an increased availability of glucosidase enzyme in the cytoplasm. It is worth noting that lysosomes contain various hydrolytic enzymes, including glucosidase [23].

The capacity of benzaldehyde, released through the breakdown of amygdalin, to initiate apoptosis by activating caspase 3.8 and 9, as well as the capacity of cisplatin to induce the relocation of lysosomal components to the cytoplasm. This includes proteases associated with cell death, such as cathepsin B, as well as an enzyme linked to cell death known as cathepsin D. The proteases initiate a cascade of events that ultimately lead to the activation of apoptotic effectors, including mitochondria, caspases, and other hitherto unknown effectors [59].

In consistent with our study both amygdalin and cisplatin have demonstrated anti-cancer properties when tested individually on oral squamous cell carcinoma cells. Furthermore, when used in combination, they exhibited a synergistic inhibitory effect on oral squamous cell carcinoma cells [60].

The findings presented by Said et al. [61] were in alignment with the aforementioned information. In 2021, a research study was conducted to examine the potential anticancer and antioxidant properties of amygdalin in conjunction with platinum-based medicines in female rats with Ehrlich ascites carcinoma. The findings demonstrated that the administration of amygdalin, either alone or in conjunction with cisplatin or oxaliplatin, resulted in a reduction in tumor markers and an improvement in antioxidant indicators when compared to rats with Ehrlich ascites carcinoma.

In accordance with the findings of Sireesha et al. [62], it has been demonstrated that extracts derived from both almonds and apricots have anticancer properties when tested on the Oral cancer cell lines, which is a cell line commonly used to study oral cancer. The viability exhibited a decline that was depending on the dosage. The viability of the cells exhibited a negative correlation with the concentration of the extracts. The almond extract demonstrated optimal effectiveness at a

dosage of 50 μg/mL, resulting in the eradication of 78% of cells. In contrast, the apricot extract necessitated a higher concentration of 100 μg/mL to achieve 82% of its activity. The findings of this study indicate that amygdalin derived from almond and apricot seeds had cytotoxic properties against human oral cancer cell lines.

In the context of cancer cells, our study demonstrated that cisplatin and amygdalin induces the activation of P53, which subsequently triggers apoptosis. The anticancer properties of benzaldehyde can be attributed to its activation of apoptosis through caspases, resulting in the depletion of cellular energy. This activation occurs as a consequence of hydrocyanic acid's inhibition of mitochondrial cytochrome C oxidase in the respiratory electron transport chain, which subsequently reduces oxidative metabolism and the associated process of oxidative phosphorylation [55]. This process is facilitated by the downregulation of the anti-apoptotic protein BCL-2 and the elevation of the pro-apoptotic protein BAX. The activation of apoptosis is facilitated by both pro- and anti-apoptotic BCL-2 family proteins, which subsequently trigger the mitochondrial outer membrane permeabilization process. The process of mitochondrial outer membrane permeabilization leads to the liberation of cytochrome c from the mitochondria, which is then released into the cytosol. This event serves as a trigger for the activation of caspases, ultimately resulting in the induction of apoptosis.

5. Conclusion

The outcomes of our study indicate that Amygdalin operates through a specific process in HT-29 cells. These findings contribute to the growing body of evidence supporting the effectiveness of Amygdalin as a reliable preventive and therapeutic treatment for colorectal cancer. Based on the aforementioned findings, it can be inferred that Amygdalin+Cisplatin exhibits potential as a preventative and protective agent against colorectal cancer. The potential preventive effect of Amygdalin on colorectal cancer may be attributed to its apoptotic properties, which involve the upregulation of gene expression related to P53, BAX, and Caspase-3, as well as the downregulation of BCL-2.

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