RESEARCH ARTICLE

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Triggering of Endoplasmic Reticulum Stress by Tannic Acid Inhibits the Proliferation and Migration of Colorectal Cancer Cells

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Abstract

Introduction: Due to the pivotal role of endoplasmic reticulum (ER) stress in cancers, interfering with its function can cause the accumulation of unfolded proteins, which ultimately leads to the activation of the unfolded protein response (UPR) signaling pathway and apoptosis. Therefore, the use of plant compounds such as tannic acid with UPR-inducing properties can be proposed as a possible treatment method for cancer. In this study, we investigated the effect of tannic acid on cell migration, colony formation, growth, and UPR-induced apoptosis in the SW48 colorectal cancer cell line. **Methods:** The MTT assay was performed to investigate the cytotoxic effect of tannic acid. We performed the qPCR method to elucidate the effect of tannic acid on the expression of Bim, MMP-9, Bcl-xL, cyclin D1, CHOP, and ATF4 genes. We also used the colony formation and migration experiments to investigate the effect of this compound on the colony formation and migration ability of tumor cells. Finally, we used Hoechst staining to measure cell apoptosis. **Results:** Tannic acid inhibited the cell survival, clonogenic, and migration of colon cancer cells. This compound increased the expression of ER stress-mediated UPR genes, ATF4 and CHOP. Moreover; tannic acid increased the expression of pro-apoptotic proteins like Bim, while at the same time causing a sharp decline in the expression of anti-apoptotic protein Bcl-xL. A decline in MMP-9 expression confirmed the anti-metastatic role of this compound. **Conclusion:** Taken together, tannic acid can induce apoptosis via ER stress-mediated UPR pathway, and has a suppressive effect on cell viability, growth, migration, colony formation, and metastasis, suggesting it may be a potential drug in colorectal cancer treatment.

Keywords: ATF4- CHOP- Colorectal cancer- Endoplasmic reticulum- Tannic acid

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Introduction

Colorectal cancer is the third most prevalent malignancy in the world and the fourth leading cause of cancer-related mortalities, which has an almost equal prevalence in both sexes (Aiello et al., 2019; Selvam et al., 2019). Although the current chemotherapy drugs are effective, they can affect some healthy cells as well, which cause many side effects, even in some cases make failure in the chemotherapy process (Aiello et al., 2019; Kim et al., 2019). Today, we can use other treatment methods such as phytotherapy, the use of herbal compounds for treatment of cancers (Aiello et al., 2019; Esmeeta et al., 2022). Herbal medicines can be used as alternative or complementary methods that increase the effectiveness of the medicine through synergism and reduce its side effects (Kim et al., 2019).

Tannic acid is a hydrolysable polyphenol which presents in several plant sources such as many types of trees, green tea, coffee, and fresh fruits like pomegranate (Djakpo and Yao, 2010; Guo et al., 2021). This drug inhibits tumor growth due to its various properties, including inhibition of free radicals, antioxidant, and anti-inflammatory systems (Jo et al., 2015; Perumal et al., 2019; Yeo et al., 2020). Several studies have demonstrated that tannic acid can inhibit survival, migration, and colony formation in various cancer cells through different signaling pathways such as TGF-β, MAPK, and VEGFR (Nagesh et al., 2018; Pattarayan et al., 2018; Sp et al., 2020). Furthermore, it has been proven that this compound inhibits growth and induces apoptosis in cancer cells by affecting some proteins such as Bax, Bcl-2, P53, and cyclin D1 (Nagesh et al., 2018). Other studies have shown that tannic acid inhibits the growth of colon cancer cells under in vivo and in vitro conditions and increases the effectiveness of chemotherapy (R et al., 2021).

The endoplasmic reticulum (ER) is an organelle with several major activities including synthesis, folding,

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maturation, and transfer of proteins (Storm et al., 2016). Under stress conditions and disturbed homeostasis, many misfolded or unfolded proteins accumulate in the ER, called ER stress (Díaz-Villanueva et al., 2015; Maurel et al., 2015). There is a mechanism to overcome these conditions and restore ER homeostasis, which is called unfolded protein response (UPR) (Mori, 2015). The main purpose of this mechanism is to restore ER homeostasis and cell survival. If the stress is long-term or severe and it is not possible to restore homeostasis, it causes cell death (Maurel et al., 2015). Three transmembrane proteins, Inositol-requiring enzyme 1 (IRE1), Protein kinase RNA-like endoplasmic reticulum kinase (PERK), and Activating transcription factor 6 (ATF6) detect misfolded or unfolded proteins and activate this pathway. This activation can lead to changes in the expression levels of various targets of this pathway, including transcription factors such as C/EBP Homologous protein (CHOP), ER-resident chaperones such as Binding immunoglobulin protein (Bip) and Protein disulfide-isomerase (PDI), and transcriptional activating factors such as ATF4, which can lead to survival or apoptosis depending on the conditions (Ma et al., 2002; Ma and Hendershot, 2003; Maurel et al., 2015; Perri et al., 2015).

So far, various studies have demonstrated that plant polyphenolic compounds such as tannic acid can arrest the cell cycle in the G1/S phase or induce apoptosis through ER stress in some cancer (Kotecha et al., 2016; Garrido-Armas et al., 2018; Heo et al., 2018; Nagesh et al., 2018). This has been proven by increased expression of genes involved in the UPR pathway and cell cycle such as IRE1, ATF6, PERK, BiP, CHOP, PDI, p21, and p18 and decreased expression of some genes such as cyclin D1 in some cancers (Wang et al., 2011; Heo et al., 2018; Nagesh et al., 2018).Apoptosis is considered as one of the major protective mechanisms against the initiation and progression of cancer. Studies has shown that tannic acid increases the expression of CHOP protein, which caused a reduction of anti-apoptotic proteins and enhancement of apoptotic proteins, that leading to apoptosis. In addition, there is evidence of the effect of tannic acid on increasing the expression of proteins involved in the classical pathway of apoptosis such as caspase-3 in some cancers (Tsukano et al., 2010; Iurlaro and Muñoz-Pinedo, 2016; Nagesh et al., 2018). Furthermore, the inhibitory effect of tannic acid in some metastatic cancers has been proven by reducing the expression of some mesenchymal markers such as MMP-2 and MMP-9 (Nagesh et al., 2018).

So far, the effect of tannic acid on the UPR signaling pathway has rarely been studied. Moreover, the exact molecular mechanisms of the effect of tannic acid on colon cancer cells have not been determined. The purpose of this study was to investigate the effect of tannic acid on the cell cycle, colony formation, metastasis, and apoptosis via ER stress pathway in SW48 colorectal cancer cells.

Materials and Methods

Cell culture

The human SW48 cell line was provided by the Iranian Pasteur institute (Tehran, Iran). This cell line was cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% of fetal bovine serum (V/V, Sigma-Aldrich) and 1% of penicillin-streptomycin (V/V, Sigma-Aldrich). Cell culture was incubated at 37°C in a humidified atmosphere with 5% CO_2 (Figure 1).

MTT assay

MTT assay was used to investigate the cytotoxic effect of tannic acid on SW48 cells. The test was performed in 3 groups: blank control, solvent control, and tannic acid. First, the cells were grown in 96-well culture plates with an initial density of 2×10^4 cells per well. Then, the cells were treated with the different concentration of tannic acid (Sigma-Aldrich, Figure1) and were allowed to incubate for 24 h. Next, 10 µl MTT solution (Sigma-Aldrich) (5 mg/ml) was added to each culture. After 4 h of incubation at 37°C, the mediums were removed, and 150 µL of DMSO was added to the wells. Absorbance (A) at 540 nm was quantified with an ELISA reader (Awareness Technology, Palm City, FL, USA). The survival rate (SR) was determined from the following formula: SR (%) = (A test /A Control) × 100%. IC_{50} (the concentration that reduced 50% of cell viability) and IC_{30} (the concentration that reduced 30% of cell viability) were calculated by GraphPad software (GraphPad Software Inc., San Diego, CA, USA). In the following experiments, treatment was performed with these two doses.

Real-time PCR assay

Total RNA was extracted from the SW48 cell line by using the Parstous RNA isolation kit (Tehran, Iran), as mentioned in manufacturer company instructions. Complementary DNA (cDNA) was synthesized from 2 µg of purified total RNA by using the cDNA synthesis kit (Parstous) and following the manufacturer's protocol. QRT-PCR was performed on a LightCycler 96 System (Roche Diagnostics GmbH, Mannheim, Germany) by use of SYBR Premix Ex Taq (Parstous). RT-PCR reactions were performed in a total volume of 20 µl containing of 1 µL cDNA, 12 µl of SYBR green reagent, 0.2 µM of each of the oligonucleotides primers, and 6 µl distilled water. The PCR conditions were as followed: 40 cycles at 95 °C for 10 sec and 55°C for 30 sec after the initial denaturation step at 95°C for 5 min. Relative quantification of gene expression was measured with the $2^{-(\Delta\Delta Ct)}$ method (Alamdari-Palangi et al., 2020; Shahverdi et al., 2020; Shahverdi et al., 2021), using β-actin as an internal control. Forward and reverse primers used in real-time PCR are listed in Table1.

Wound healing assay

Colorectal cancer cell migration was assessed using the wound healing assay. SW48 cells were allowed to grow into 100% confluency in 6 well plates. Then, a scratch was made in the center of each well by a sterile pipette tip. The cells were washed with PBS and the fresh RPMI-1640 medium was added in the wells and was treated with two doses (IC_{30} and IC_{50}) of tannic acid for 24 h. Finally, photograph imaging was carried out from random-selected fields along the scratch in each well by using the inverted microscope (Nikon, Japan) at 24 h time points for 4 days. Any changes in the width of the scratch were compared

to the control groups.

Clonogenic assay

First, SW48 cells were seeded at a density of 5 × 10^3 cells per well in 12 well plates and incubated overnight at 37 °C for 24 h. Next, the cells were treated with IC_{30} and IC_{50} doses of tannic acid for 24 hours. The medium was replaced with the fresh medium and after 4 days of incubation, the cells were washed twice with ice PBS and fixed with acetic acid/ methanol. Next, fixed cells were stained with 2% crystal violet for 20 minutes. Finally, we took photos of fixed colonies.

Cell apoptosis analysis by Hoechst 33342 staining

Tannic acid-induced apoptosis in SW48 cells was assessed by Hoechst 33,342 staining. The cells were cultured in 12 well plates and incubated for 24 h. Then, the cells were treated with IC_{30} and IC_{50} doses of tannic acid. After 24 h, the cells were washed with PBS two times, fixed in 3.7% formaldehyde, and stained with 5 µg⁄mL Hoechst 33,342 (Sigma-Aldrich) for 30 min. Finally, stained cells were observed by using fluorescence microscope (Nikon, Japan). The cells in which the nucleus is seen in the condensed or fragmented form are regarded as apoptotic cells.

Statistical analysis

All of the quantitative data in this study were presented as mean ± standard deviation (SD) of three experiments. Statistical analysis was performed with the analysis of variance (ANOVA) and t-test using GraphPad Prism version 5.0 software. A p-value<0.05 imply that the difference is statistically significant.

Results

Tannic acid inhibited the survival rate of the SW48 cells

To investigate the effect of tannic acid on survival of the colorectal cancer cells, the MTT assay was performed. The result of the MTT assay revealed that treatment with tannic acid markedly decreased the survival rate of SW48 cells dose-dependently ($p< 0.05$, relative to the blank control). The IC_{30} and IC_{50} doses of tannic acid for 24 h treatment were 35.2 and 46.7 µM, respectively (Figure 1). As expected, the solvent had no significant effect on cell survival relative to the blank control group ($p> 0.05$).

Tannic acid altered the expression of cell cycle, apoptotic, metastatic, and ER stress related genes

The qPCR was performed to analyze the effect of tannic acid on expression of cell cycle, apoptotic, metastatic, and ER stress related genes in SW48 cells. The results showed that 24 h treatment with IC_{30} dose of tannic acid decreased the expression levels of cyclin D1, Bcl-xL, and MMP-9, while the expression levels of CHOP, ATF4 and Bim were increased compared to the blank control group ($p< 0.05$, Figure 2). The IC_{50} dose of tannic acid had

Table 1. The Sequence of Primers Used in Real-Time PCR

Genes	Sequences (5′→3′)
β-actin FW	GACATCCGCAAAGACCTGTA
β-actin RV	GGAGCAATGATCTTGATCTTCA
Bim FW	CAAGAGTTGCGGCGTATTGGAG
Bim RV	ACACCAGGCGGACAATGTAACG
MMP-9 FW	AGACCTGGGCAGATTCCAAAC
MMP-9 RV	CGGCAAGTCTTCCGAGTAGT
Bcl-xL FW	CAGAGCTTTGAACAGGTAG
Bcl-xL RV	GCTCTCGGGTGCTGTATTG
Cyclin D1 FW	CAATGACCCCGCACGATTTC
Cyclin D1 RV	CATGGAGGGCGGATTGGAA
CHOP FW	GCCTTTCTCCTTTGGGACACTGTCCAGC
CHOP RV	CTCGGCGAGTCGCCTCTACTTCCC
ATF4 FW	GCATGCTCTGTTTCGAATGGA
ATF4 RV	CCAACGTGGTCAAGAGCTCAT

FW, Forward; RV, Reverse

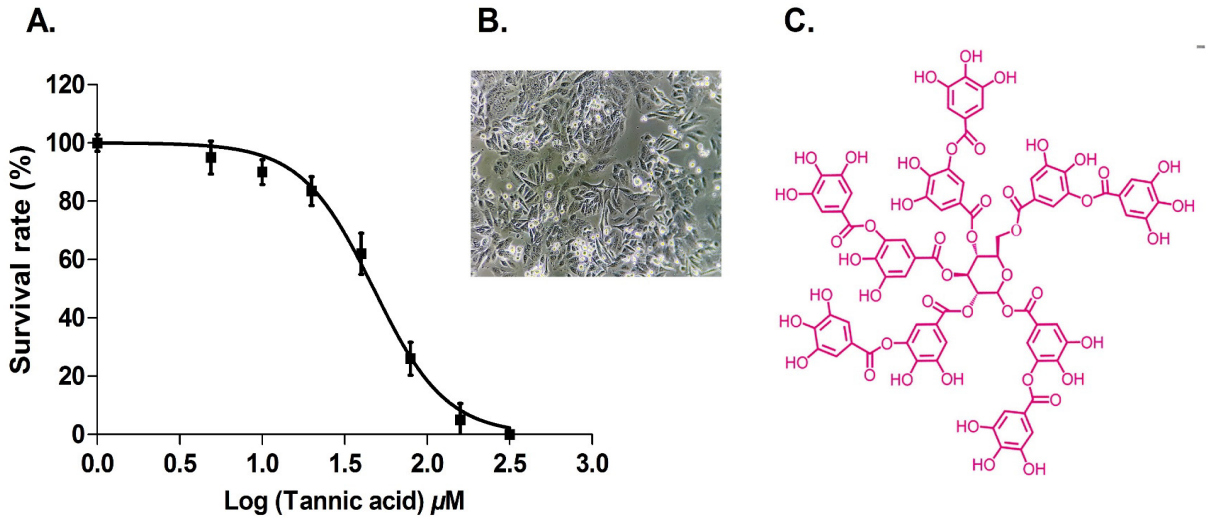

Figure 1. The Effect of Tannic Acid on Cell Survival. The SW48 cells were treated with tannic acid at indicated concentrations. After 24 h, the cell survival rate was measured by the MTT assay. The cell survival curves were plotted using GraphPad 6.1 software. Data are expressed as mean ± SD of three independent experiments. (A) MTT graph; (B) the morphology of SW48 cells; (C) the chemical structure of tannic acid.

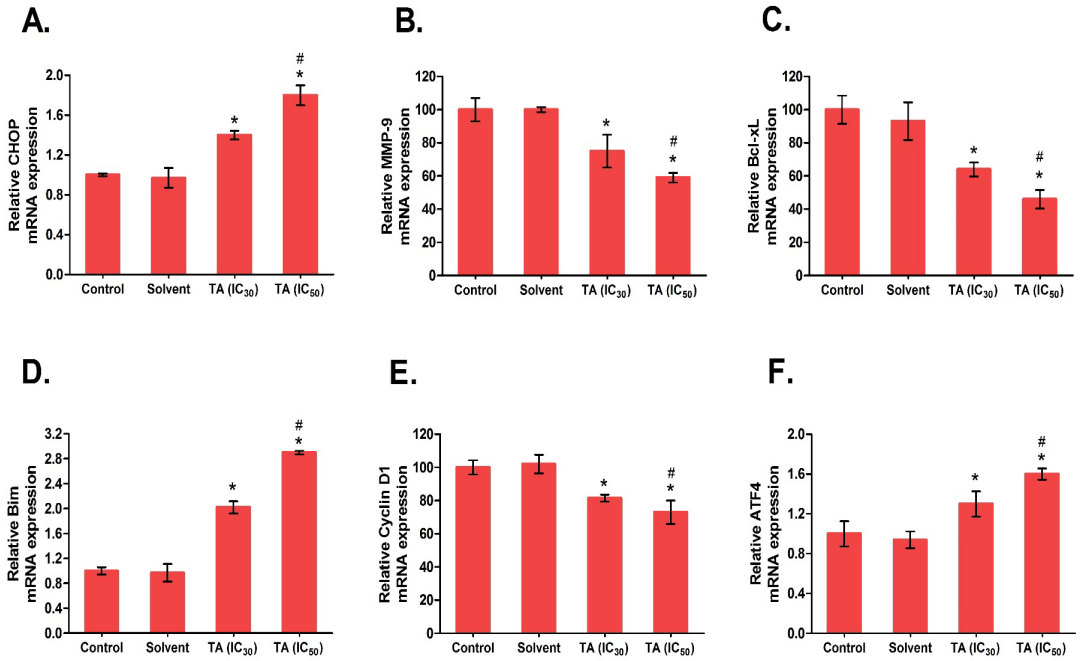

Figure 2. RT-qPCR Analysis of SW48 Cells. The SW48 cells were treated with tannic acid (IC30 and IC50 doses) for 24 h. Relative expression levels of CHOP (A), MMP-9 (B), Bcl-xL (C), Bim (D), cyclin D1 (E), and ATF4 (F) mRNAs were measured by RT-qPCR using $2^{-(\Delta\Delta Ct)}$ method and β-actin as an endogenous control. Data are presented as mean ± SD (n=3). #p< 0.05 relative to IC_{30}; *p < 0.05 relative to blank control. TA, tannic acid.

a bigger effect on gene expression relative to the IC_{30}. As expected, the solvent did not have a notable effect on gene expression relative to the blank control group (p> 0.05).

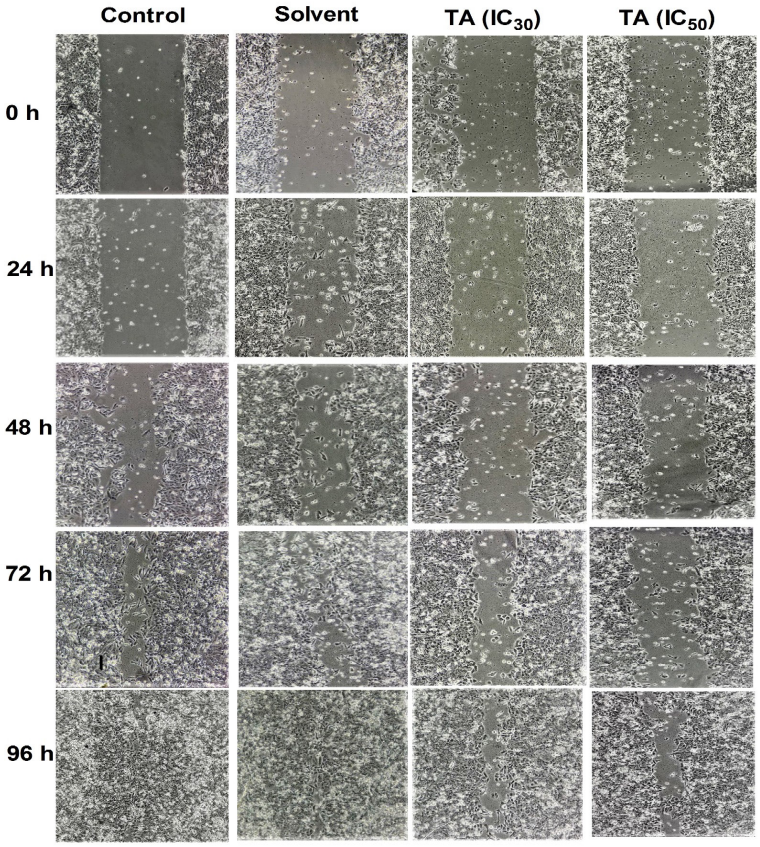

Figure 3. The Effect of Tannic Acid on Colorectal Cancer Cell Migration. Monolayer of SW48 cells were treated with IC30 and IC50 doses of tannic acid. The migration of SW48 cells was quantified by measuring wound closure areas at 0, 24, 48, 72, and 96 h. Data were representative of three independent experiments. TA, tannic acid.

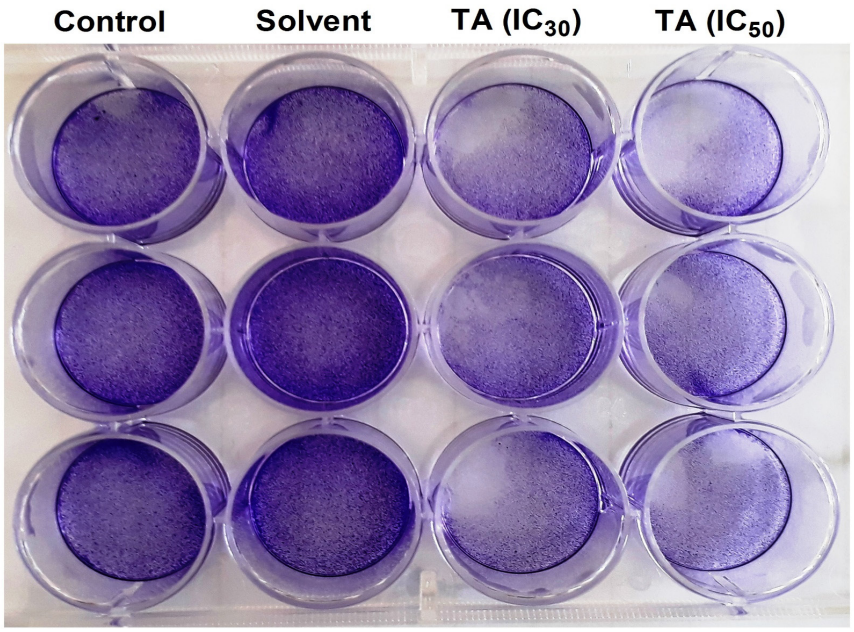

Figure 4. Effect of Tannic Acid on Colony Formation in SW48 Cell. Cells were treated with tannic acid for 24 h. Next, the cell colonies were stained with crystal violet and the number of colonies was observed after 7 days. TA, tannic acid.

Tannic acid decreased the migration of the SW48 cells dose-dependently

To evaluate the effect of tannic acid on the mobility of colorectal cancer cells, the wound healing assay was performed. Comparison of the wound closure rate for 4 days between the tannic acid-treated groups (IC_{30} and IC_{50}) and the blank control group demonstrated that tannic acid significantly decreased the migration of SW48 cells dose-dependently. Moreover, there was no difference between the migration of blank control and solvent control groups (Figure 3).

Inhibition of colony formation in SW48 cells by tannic acid

Colony formation assay was carried out to evaluate the anti-proliferative activity of tannic acid on colorectal cancer cells. In this experiment, we compared the effect of IC_{30} and IC_{50} doses of tannic acid on the number of colonies after 24 h treatment with the blank control group. The results demonstrated that the colony formation ability of SW48 cells was reduced in a dose-dependent manner compared to the control group (Figure 4). No marked difference between the solvent control and blank control groups was observed.

Tannic acid triggered apoptosis in colorectal cancer cells

We used Hoechst 33342 staining to identify the apoptotic morphological changes in colorectal cancer cells after 24 h treatment with tannic acid. As shown in Figure 5, after 24 h, the treated cells with tannic acid showed more nuclear fragmentation and chromatin condensation than the blank control group. The percentage of apoptotic cells increased in a dose-dependent manner. Furthermore, there was no difference in the number of apoptotic cells between the solvent control and the blank control groups.

Discussion

Although standard cancer treatment methods such as chemotherapy, radiotherapy, and surgery are effective, they have disadvantages and limitations (Aiello et al., 2019; Esmeeta et al., 2022). Following increasing demands for safe and efficient therapeutic molecules in

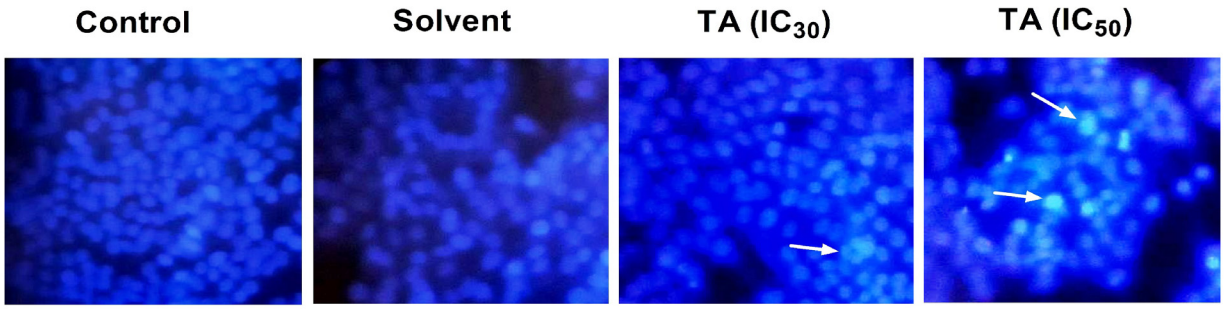

Figure 5. Morphologic changes of SW48 cells treated with tannic acid for 24 h. Representative images of cells treated with tannic acid after Hoechst 33342 staining. The apoptotic cells showed condensed nuclei or apoptotic bodies (indicated by arrows). TA, tannic acid.

cancer treatment, polyphenolic compounds such as tannic acid were discovered that inhibited tumor growth through different signaling pathways (Jo et al., 2015; Perumal et al., 2019; Yeo et al., 2020). ER stress pathway is one of these pathways which triggers apoptosis by the UPR mechanism. So far, the mechanism of the effect of tannic acid on apoptosis in colorectal cancer cells through the ER stress pathway has not been determined. In this study, we evaluated the effect of tannic acid on ER stress response in the SW48 colorectal cancer cells.

Our results demonstrated that tannic acid has a suppressive effect on colony formation, proliferation, and migration of SW48 colon cancer cells. Moreover, our study indicated that tannic acid stimulates the apoptosis in colon cancer cells. We also observed that the gene expression level of Bim was increased while the expression levels of Bcl-xL and cyclin D1 were decreased after treatment with tannic acid. Moreover, a significant decrease in mesenchymal marker MMP-9 was observed after tannic acid treatment. Also, the expression level of ATF4 and CHOP were reduced. So far, various studies have investigated the effect of tannic acid on cancer cells. Mhlanga et al., (2019) investigated the effect of tannic acid on the survival and apoptosis of liver cancer cells. They showed that tannic acid reduced the survival of liver cancer cells and increased the rate of cell apoptosis. Tannic acid also increases DNA fragmentation and reactive oxygen species in cells. In another study, Nagesh et al. (2018) investigated the effect of tannic acid on the growth, apoptosis, and migration of prostate cancer cells. They demonstrated that treatment with tannic acid activated ER stress and increased the expression of ATF4, Bip, and PDI proteins. As a result of the activation of this pathway, the expression of anti-apoptotic proteins Bcl-xL and Bcl-2 decreased, while the expression of pro-apoptotic proteins Bak and Bim increased. Tannic acid also decreased the expression of cell cycle proteins (cyclin D1) and metastatic proteins (MMP-2, MMP-9). Darvin et al., (2015) showed that tannic acid inhibited the cell cycle in the G1 phase and induced cell apoptosis in YD38 cells of the gums. Tannic acid suppressed cell cyclins, Bcl-xL, and Bcl-2 proteins through inhibiting the STAT-3 transcription factor. In addition, an increase in the amount of Bax and cellular caspases was observed after treatment with tannic acid. Sp et al., (2020) showed that treatment of A549 lung cancer cells with tannic acid inhibited growth, cell cycle, and invasion, and activated the intrinsic pathway of apoptosis. Also, the study of Pyrko et al., (2007) demonstrated that suppression of Bip protein expression by siRNA in glioblastoma cells induces CHOP and caspase 7 activation. Also, the suppression of Bip expression increased the sensitivity of the glioblastoma cells to temozolomide and 5-Fluorouracil. The results of the above studies are in line with the results of our study and show that tannic acid can inhibit the cell growth and induce apoptosis by stimulating ER stress.

Mechanistically, under stress conditions such as tannic acid treatment, many unfold proteins accumulate in the ER lumen and initiate the UPR pathway (Corazzari et al., 2017). First, a chaperone called Bip (Grp 78) dissociates from UPR sensors such as PERK (Siegel et al., 2012; Youness and Gad, 2019). Activated PERK subsequently phosphorylates the eIF2α factor, which inhibits translation and activates the transcription activator factor ATF4. Activated ATF4 then increases the transcription of pro-survival proteins and increases CHOP protein, a pro-apoptotic transcription factor. Increased CHOP expression decreased the expression of pro-survival proteins like Bcl-xL, while, simultaneously increased the expression of pro-apoptotic ones such as Bim (Ma et al., 2002; Harding et al., 2003; Lu et al., 2004; Siegel et al., 2012). Therefore, this data demonstrate that tannic acid can trigger the cell death by overexpression of CHOP protein through UPR signaling pathway.

In conclusion, our results show that tannic acid inhibits the growth of colorectal cancer cells by arresting cell cycle process. Also, tannic acid induces apoptosis via ER stress-induced UPR in colorectal cancer cells, which can be proven by expressing CHOP and ATF4 as the indicators of UPR. The apoptotic role of tannic acid proved by decline in the expression of pro-survival genes like Bcl-xL and increase in the expression of pro-apoptotic ones like Bim. Moreover, our data elucidate the anti-metastatic role of tannic acid on colorectal cancer cells by EMT inhibition. Overall, our results suggest that tannic acid can inhibit colony formation and cell migration and induces apoptosis in colon cancer cells through activation of ER stress and UPR signaling pathway.

Author Contribution Statement

Study concept and design: HK; Acquisition of data: FSN; Analysis and interpretation of data: HK, FSN and MD; Drafting of the manuscript: HK, FSN and MD; Critical revision of the manuscript for important intellectual content: HK and MD; Funding recipients: HK.

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Ethical approval

This research was ethically wise approved from Deputy of research and technology, Arak University of Medical Sciences, Arak, Iran [Number 6674].

Conflict of interest

The authors have no conflict of interest to declare

References

Aiello P, Sharghi M, Mansourkhani SM, et al (2019). Medicinal Plants in the Prevention and Treatment of Colon Cancer.

Oxid Med Cell Longev, **2019**, 2075614.
Alamdari-Palangi V, Karami Z, Karami H, et al (2020). MiRNA-7 Replacement Effect on Proliferation and Tarceva-Sensitivity in U373-MG Cell Line. *Asian Pac J Cancer Prev*, **21**, 1747-53.
Corazzari M, Gagliardi M, Fimia GM, et al (2017). Endoplasmic Reticulum Stress, Unfolded Protein Response, and Cancer Cell Fate. *Front Oncol*, **7**, 78.
Darvin P, Baeg SJ, Joung YH, et al (2015). Tannic acid inhibits the Jak2/STAT3 pathway and induces G1/S arrest and mitochondrial apoptosis in YD-38 gingival cancer cells. *Int J Oncol*, **47**, 1111-20.
Díaz-Villanueva JF, Díaz-Molina R, García-González V (2015). Protein Folding and Mechanisms of Proteostasis. *Int J Mol Sci*, **16**, 17193-230.
Djakpo O, Yao W (2010). Rhus chinensis and Galla Chinensis--folklore to modern evidence: review. *Phytother Res*, **24**, 1739-47.
Esmeeta A, Adhikary S, Dharshnaa V, et al (2022). Plant-derived bioactive compounds in colon cancer treatment: An updated review. *Biomed Pharmacother*, **153**, 113384.
Garrido-Armas M, Corona JC, Escobar ML, et al (2018). Paraptosis in human glioblastoma cell line induced by curcumin. *Toxicol In Vitro*, **51**, 63-73.
Guo Z, Xie W, Lu J, et al (2021). Tannic acid-based metal phenolic networks for bio-applications: a review. *J Mater Chem B*, **9**, 4098-110.
Harding HP, Zhang Y, Zeng H, et al (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell*, **11**, 619-33.
Heo JR, Kim SM, Hwang KA, et al (2018). Resveratrol induced reactive oxygen species and endoplasmic reticulum stress-mediated apoptosis, and cell cycle arrest in the A375SM malignant melanoma cell line. *Int J Mol Med*, **42**, 1427-35.
Iurlaro R, Muñoz-Pinedo C (2016). Cell death induced by endoplasmic reticulum stress. *Febs J*, **283**, 2640-52.
Jo HJ, Chung KH, Yoon JA, et al (2015). Radical Scavenging Activities of Tannin Extracted from Amaranth (Amaranthus caudatus L.). *J Microbiol Biotechnol*, **25**, 795-802.
Kim J, Kim HY, Hong S, et al (2019). A new herbal formula BP10A exerted an antitumor effect and enhanced anticancer effect of irinotecan and oxaliplatin in the colon cancer PDTX model. *Biomed Pharmacother*, **116**, 108987.
Kotecha R, Takami A, Espinoza JL (2016). Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. *Oncotarget*, **7**, 52517-29.
Lu PD, Harding HP, Ron D (2004). Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *J Cell Biol*, **167**, 27-33.
Ma Y, Brewer JW, Diehl JA, et al (2002). Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J Mol Biol*, **318**, 1351-65.
Ma Y, Hendershot LM (2003). Delineation of a negative feedback regulatory loop that controls protein translation during endoplasmic reticulum stress. *J Biol Chem*, **278**, 34864-73.
Maurel M, McGrath EP, Mnich K, et al (2015). Controlling the unfolded protein response-mediated life and death decisions in cancer. *Semin Cancer Biol*, **33**, 57-66.
Mhlanga P, Perumal PO, Somboro AM, et al (2019). Mechanistic Insights into Oxidative Stress and Apoptosis Mediated by Tannic Acid in Human Liver Hepatocellular Carcinoma Cells. *Int J Mol Sci*, **20**.
Mori K (2015). The unfolded protein response: the dawn of a new field. *Proc Jpn Acad Ser B Phys Biol Sci*, **91**, 469-80.
Nagesh PKB, Hatami E, Chowdhury P, et al (2018). Tannic Acid Induces Endoplasmic Reticulum Stress-Mediated Apoptosis in Prostate Cancer. *Cancers (Basel)*, **10**.
Pattarayan D, Sivanantham A, Krishnaswami V, et al (2018). Tannic acid attenuates TGF-β1-induced epithelial-to-mesenchymal transition by effectively intervening TGF-β signaling in lung epithelial cells. *J Cell Physiol*, **233**, 2513-25.
Perri ER, Thomas CJ, Parakh S, et al (2015). The Unfolded Protein Response and the Role of Protein Disulfide Isomerase in Neurodegeneration. *Front Cell Dev Biol*, **3**, 80.
Perumal PO, Mhlanga P, Somboro AM, et al (2019). Cytoproliferative and Anti-Oxidant Effects Induced by Tannic Acid in Human Embryonic Kidney (Hek-293) Cells. *Biomolecules*, **9**.
Pyrko P, Schönthal AH, Hofman FM, et al (2007). The unfolded protein response regulator GRP78/BiP as a novel target for increasing chemosensitivity in malignant gliomas. *Cancer Res*, **67**, 9809-16.
R AY, Kamel R, N AE, et al (2021). Recent Advances in Tannic Acid (Gallotannin) Anticancer Activities and Drug Delivery Systems for Efficacy Improvement; A Comprehensive Review. *Molecules*, **26**.
Selvam C, Prabu SL, Jordan BC, et al (2019). Molecular mechanisms of curcumin and its analogs in colon cancer prevention and treatment. *Life Sci*, **239**, 117032.
Shahverdi M, Amini R, Amri J, et al (2020). Gene Therapy with MiRNA-Mediated Targeting of Mcl-1 Promotes the Sensitivity of Non-Small Cell Lung Cancer Cells to Treatment with ABT-737. *Asian Pac J Cancer Prev*, **21**, 675-81.
Shahverdi M, Amri J, Karami H, et al (2021). Knockdown of Myeloid Cell Leukemia-1 by MicroRNA-101 Increases Sensitivity of A549 Lung Cancer Cells to Etoposide. *Iran J Med Sci*, **46**, 298-307.
Siegel R, Naishadham D, Jemal A (2012). Cancer statistics, 2012. *CA Cancer J Clin*, **62**, 10-29.
Sp N, Kang DY, Kim DH, et al (2020). Tannic Acid Inhibits Non-small Cell Lung Cancer (NSCLC) Stemness by Inducing G(0)/G(1) Cell Cycle Arrest and Intrinsic Apoptosis. *Anticancer Res*, **40**, 3209-20.
Storm M, Sheng X, Arnoldussen YJ, et al (2016). Prostate cancer and the unfolded protein response. *Oncotarget*, **7**, 54051-66.
Tsukano H, Gotoh T, Endo M, et al (2010). The endoplasmic reticulum stress-C/EBP homologous protein pathway-mediated apoptosis in macrophages contributes to the instability of atherosclerotic plaques. *Arterioscler Thromb Vasc Biol*, **30**, 1925-32.
Wang FM, Galson DL, Roodman GD, et al (2011). Resveratrol triggers the pro-apoptotic endoplasmic reticulum stress response and represses pro-survival XBP1 signaling in human multiple myeloma cells. *Exp Hematol*, **39**, 999-1006.
Yeo J, Lee J, Yoon S, et al (2020). Tannic acid-based nanogel as an efficient anti-inflammatory agent. *Biomater Sci*, **8**, 1148-59.
Youness RA, Gad MZ (2019). Long non-coding RNAs: Functional regulatory players in breast cancer. *Noncoding RNA Res*, **4**, 36-44.