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Comparative Investigation of the Cytotoxic Effects of Carnosic Acid and Gallic Acid on the HNF1A Gene in PANC-1 Pancreatic Cells

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ABSTRACT

Objective: Pancreatic cancer is one of the most aggressive malignancies and is associated with a high mortality rate due to late diagnosis and limited therapeutic options. Natural phenolic compounds have attracted considerable attention because of their potential anticancer activities. This study aimed to comparatively investigate the cytotoxic and apoptotic effects of gallic acid (GA) and carnosic acid (CA) on HNF1A gene expression in PANC-1 pancreatic cancer cells, and to evaluate their combined effects in the presence of mesenchymal stem cells (MSCs).

Methods: Different experimental groups consisting of PANC-1 cells, MSCs, GA, and CA were established using an indirect co-culture model. Cell viability was evaluated using the WST-1 assay, while apoptosis levels were determined by Annexin V-PI flow cytometry analysis. Changes in HNF1A gene expression were analyzed using quantitative real-time PCR (qPCR).

Results: The results demonstrated that treatment with GA and CA significantly reduced the viability of PANC-1 cells and increased apoptotic cell populations. The combined treatment of GA (50 µM) and CA (25 µM) in the presence of MSCs showed the most pronounced inhibitory effect on cancer cell proliferation. Furthermore, gene expression analysis revealed that this combination significantly downregulated HNF1A expression, suggesting a potential regulatory effect on pancreatic cancer progression.

Conclusion: The findings indicate that gallic acid and carnosic acid exert antiproliferative and pro-apoptotic effects on PANC-1 pancreatic cancer cells, particularly in combination with MSCs. The observed decrease in HNF1A gene expression suggests that these compounds may contribute to the modulation of molecular mechanisms involved in pancreatic tumor progression. These results highlight the potential of GA and CA as promising candidates for the development of novel therapeutic strategies for pancreatic cancer.

Keywords: Carnosic acid, Gallic acid, HNF1A, Mesenchymal stem cells, Pancreatic cancer

ÖZET

Amaç: Pankreas kanseri, geç tanı konulması ve sınırlı tedavi seçenekleri nedeniyle yüksek mortalite oranına sahip en agresif malignitelerden biridir. Doğal fenolik bileşikler, potansiyel antikanser etkileri nedeniyle son yıllarda önemli ilgi görmektedir. Bu çalışmanın amacı, gallik asit (GA) ve karnosik asidin (KA) PANC-1 pankreas kanseri hücrelerinde HNF1A gen ekspresyonu üzerindeki sitotoksik ve apoptotik etkilerini karşılaştırmalı olarak incelemek ve mezenkimal kök hücreler (MKH) varlığındaki birleşik etkilerini değerlendirmektir.

Yöntem: PANC-1 hücreleri, MKH, GA ve KA içeren farklı deney grupları indirekt ko-kültür modeli kullanılarak oluşturuldu. Hücre canlılığı WST-1 testi ile değerlendirilirken, apoptoz düzeyleri Annexin V-PI akım sitometrisi ile analiz edildi. HNF1A gen ekspresyonundaki değişiklikler ise kantitatif gerçek zamanlı PCR (qPCR) yöntemi ile incelendi.

Bulgular: Elde edilen sonuçlar, GA ve KA uygulamasının PANC-1 hücrelerinde hücre canlılığını anlamlı şekilde azalttığını ve apoptotik hücre oranını artırdığını göstermiştir. Özellikle GA (50 µM) ve KA (25 µM) kombinasyonunun MKH varlığında uygulanması, kanser hücre proliferasyonu üzerinde en belirgin inhibitör etkiyi göstermiştir. Ayrıca gen ekspresyon analizleri bu kombinasyonun HNF1A gen ekspresyonunu anlamlı düzeyde azalttığını ortaya koymuştur.

Sonuç: Bu bulgular, gallik asit ve karnosik asidin PANC-1 pankreas kanseri hücreleri üzerinde antiproliferatif ve pro-apoptotik etkiler gösterdiğini ortaya koymaktadır. Özellikle MKH ile birlikte uygulandığında HNF1A gen ekspresyonundaki azalma, pankreas kanseri progresyonunda rol oynayan moleküler mekanizmaların düzenlenmesinde bu bileşiklerin potansiyel rol oynayabileceğini düşündürmektedir. Bu sonuçlar, GA ve KA'nın pankreas kanseri tedavisi için yeni terapötik stratejilerin geliştirilmesinde umut verici adaylar olabileceğini göstermektedir.

Anahtar Kelimeler: Karnosik asit, Gallik asit, HNF1A, Mezenkimal kök hücre, Pankreas kanseri

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Pancreatic cancer, despite its low incidence, has a high mortality rate and generally has a poor prognosis due to late diagnosis. The survival rate has remained unchanged over the last decade, with one-year survival rates of 20% and five-year survival rates of 7%. It is the 14th most common type of cancer worldwide and ranks 7th in cancer-related deaths. It is more common in men and between the ages of 40-85 (1,2). The poor prognosis of this cancer is due to its insidious progression, nonspecific symptoms, and lack of sensitive methods for early diagnosis. It predicted that deaths from pancreatic cancer will increase in the future (3,4).

HNF1A is a transcription factor that plays a crucial role in glucose metabolism and the development of pancreatic β-cells. The gene, located on chromosome 12, involved in pancreatic carcinogenesis, has not been fully elucidated. Some studies have shown that inhibition of HNF1A with siRNA promotes cancer cell proliferation, prevents apoptosis, and activates the Akt/mTOR signaling pathway (5). However, Abel et al. determined that HNF1A is an oncogene that regulates the properties of pancreatic cancer stem cells. This has been supported by other studies (6,7).

Carnosic acid (CA) is a phenolic compound obtained from plants belonging to the Lamiaceae family and has many biological activities, such as antioxidant, anti-tumor, and anti-inflammatory. It inhibits cancer cell proliferation and migration, promotes apoptosis, and suppresses tumor angiogenesis (8–10).

Gallic acid (GA) is a compound with strong anti-tumor and antioxidant properties and induces cell cycle arrest, apoptosis, and autophagy in cancer cells. It also prevents invasion and metastasis by reducing the expression of matrix metalloproteinases (11–13). It induces apoptosis and suppresses metastasis in leukemia and lung cancer (14).

Mesenchymal stem cells (MSCs) vary in phenotype and differentiation capacities due to the effects of different microenvironments. These cells can show pro-tumor or anti-tumor effects. Anti-tumor effects include suppression of Wnt and Akt pathways, reduction of NF-κB signaling, and regulation of TRAIL expression (15–17).

This study examines the combined effects of gallic acid and carnosic acid, natural agents that have not been previously evaluated, on cancer. GA and CA have shown success individually in various cancer studies. Therefore, studies were conducted on the hypothesis that combining these two compounds could inhibit the proliferation of PANC-1 pancreatic cancer cells. In addition, mesenchymal stem cells (MSCs) with anti-tumor potential were also included in the study. Within the scope of the study, an experimental model was created by combining PANC-1 cells, MSC, GA, and CA. The HNF1A gene, which plays a crucial role in pancreatic cancer development, is also a key focus of the study. HNF1A is a gene reported to have an oncogenic role in recent studies, and therefore, it has become a critical target in pancreatic cancer. Our study investigated the cytotoxic, anti-cancer, and apoptotic effects of the combination of GA and CA on the expression of the HNF1A gene in PANC-1 cells, using MSC as a mediator.

Material and Methods

PANC-1 cells used in this study were obtained from the ATCC (Biological Resource Center) cell collection. Human bone marrow–derived mesenchymal stem cells (hBM-MSCs) used in this study were obtained from the Stem Cell and Gene Therapies Research and Application Center (KÖGEM), Kocaeli University, Türkiye. The cells are maintained in the KÖGEM cell archive under standardized laboratory conditions. Prior ethical approval for the establishment and storage of these cells had been obtained by the center.

Characterization Studies of PANC-1 Pancreatic Cancer Cell Line

The PANC-1 cell line used in our study was obtained from ATCC. PANC-1 cells were cultured in a medium consisting of 10% fetal bovine serum (FBS), 1% antibiotic (penicillin/streptomycin), 200 mM L-glutamine, and RPMI 1640 (Roswell Park Memorial Institute medium), and incubated at 37 °C in a 5% CO_2.

For PANC-1 characterization analysis, when the cells reached passage three, they were removed by trypsinization. Approximately 1×10^5 PANC-1 cells were homogenized in PBS. Fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies specific to the determined cell surface markers (Table 1) were prepared using the appropriate protocol. The analysis of the prepared cell suspension was performed using the BD FACS Calibur device and the BD CellQuest™ software program.

Authors' proof

hBM-MSC Characterization Studies

Frozen cells were stored at −150 °C and thawed rapidly in a 37 °C water bath before culture. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin–streptomycin, 10 mM FGF-β, and 200 mM L-glutamine. Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2, and the culture medium was replaced every three days. When the cells reached 70–80% confluence, they were detached using 0.25% trypsin-EDTA and subcultured. Cells at passage 3 (P3) were used for characterization and subsequent experimental procedures. When we reached passage 3, flow cytometry was performed for characterization analyses.

Table 1. Positive and negative markers of PANC-1 cells

MARKER	POSITIVE/NEGATIVE
CD18	+
CD24	-
CD31	-
CD34	-
CD38	-
CD44	+
CD133	-
CD166	+
CD309	-
CD324	-
CD325	-
CD338	-
KI-67	+
PCNA	+

For characterization, passage-3 hBM-MSCs were analyzed by flow cytometry according to the criteria defined by the International Society for Cell and Gene Therapy (ISCT). Briefly, cells were harvested using trypsin-EDTA and resuspended in phosphate-buffered saline (PBS). Surface marker expression was evaluated using fluorochrome-conjugated monoclonal antibodies against CD13, CD29, CD44, CD73, CD90, CD105, CD14, CD15, CD34, CD45, CD117, and HLA-DR.

Flow cytometry analysis was performed using a BD FACSCalibur flow cytometer, and data were analyzed with BD CellQuest™ software. Cells showing positive expression for CD13, CD29, CD44, CD73, and CD90, and negative expression for CD14, CD15, CD34, CD45, CD117, and HLA-DR, were considered to exhibit typical mesenchymal stem cell characteristics.

The analysis results of the MSCs obtained according to the criteria determined by the International Society for Cell Therapies (ISCT) are shown in Table 2.

Table 2. Positive and negative markers of MSCs

MARKER	POSITIVE/NEGATIVE
CD13	+
CD14	-
CD15	-
CD29	+
CD34	-
CD44	+
CD45	-
HLA ABC	-
CD73	+
CD90	+
CD105	-
CD117	-
HLA-DR	-

Cell Co-Culture and Investigation of Their Interactions

A total of two control groups and eight experimental groups were co-cultured for 0, 1, 3, and 7 days (Table 3). Indirect co-culture experiments were performed using a Transwell system, allowing paracrine interaction between MSCs and PANC-1 cells without direct cell–cell contact. The changes in cell viability, proliferation, and apoptosis were compared. The cell culture medium was supplemented with intercellular ratios of PANC-1 and hBM-MSC at 1:1 and 1:3. The ratios of gallic acid and carnosic acid substances are as follows. The studies were continued with the most appropriate value, as determined by the data obtained from these ratios.

As a result of WST-1 tests and in line with the literature, measurements that suppress PANC-1, which does not kill mesenchymal stem cells, were continued (18,19). Gallic acid ratios were determined as 0, 5, 10, 25, 50, 100, 150 µM. Carnosic acid levels were determined as 0, 5, 10, 25, 50, 100, and 150 µM.

Author's proof

Table 3. Experimental and control groups in the study

CONTROL GROUPS	EXPERIMENTAL GROUPS
hBM-MSC	hBM-MSC + CA
	hBM-MSC + GA
	hBM-MSC + CA +GA
PANC-1	PANC-1+ hBM-MSC
	PANC-1+ CA
	PANC-1+ GA
	PANC-1 + CA + GA
	PANC-1+ hBM-MSC + CA +GA

PANC-1: Human pancreatic cancer cell line
hBM-MSC: human bone marrow-derived mesenchymal stem cell

Determination of Gallic Acid and Carnosic Acid Concentration

In order to determine the lethal dose of GA and CA on Panc-1 cells and to determine the dose that provides a toxic effect on cancer cells but does not show a toxic effect on hBM-MSCs, WST-1 analyses were performed for GA and CA concentrations of 0, 5, 10, 25, 50, 100, 150 µM. It was repeated 3 times.

As a result of the tests performed, the concentrations were determined to be GA at 50 µM and CA at 25 µM, in line with the literature (18,19). All experiments used GA at a concentration of 50 µM and CA at 25 µM throughout the study. Our study's solutions with different concentrations were prepared by diluting the stock solution with RPMI 1640 medium.

WST-1 Viability Assay

Cells were seeded in 96-well plates with 200 µL of medium, 1×10^4 cells per well, and three replicates for each concentration. The groups in Table 4 were cultured for the specified days. WST-1 application was carried out according to the specified kit protocol. The absorbance values of the samples were measured at 450 nm wavelength in a microplate reader (Versamax, USA).

Annexin V-PI Capacity Determination

The Annexin V-PI kit (BD Biosciences) was used to determine the apoptosis levels in PANC-1 and MSC cells at the end of 0, 1, 3, and 7 days after co-culture. The cells were prepared according to the specified kit protocol. The prepared cell suspension was analyzed on the FACS Calibur flow cytometry device, and apoptotic cell analysis was performed using the BD CellQuest™ program.

Table 4. WST-1 experimental and control groups

MSC WST-1	PANC-1 WST-1
MSC-GA 1D	PANC-1-MSC 1,3,7D
MSC-GA 3D	PANC-1-GA 1D
MSC-GA 7D	PANC-1-GA 3D
MSC-CA 1D	PANC-1-GA 7D
MSC-CA 3D	PANC-1-CA 1D
MSC-CA 7D	PANC-1-CA 3D
MSC - 50µM GA - 25µM CA - 0,1,3,7D	PANC-1-CA 7D
	PANC-1 - 50µM GA - 25µM CA - 0,1,3,7D
	PANC-1-MSC- 50µM GA - 25µm CA - 0,1,3,7D

Gene Expression Analyses

A quantitative real-time PCR (qPCR) study was performed using the Roche LightCycler device to determine the effects of the GA and CA doses obtained from WST-1 on the expression levels of the HNF1A gene in PANC-1 cells that were co-cultured. The "Cancer PathwayFinder PCR Array (SABioscience)" panel for cancer cells was used to determine the changes in gene expression of the cells after co-culture. First, the RNAs of cells were purified with an RNA isolation kit (High Pure RNA Isolation Kit; Roche, Mannheim, Germany). After isolation, RNA concentration was measured using a spectrophotometer and converted to cDNA with a cDNA synthesis kit (Transcriptor High Fidelity cDNA Synthesis Kit; Roche, Mannheim, Germany) to a final concentration of 1 µg total RNA. Amplification of target genes was performed using SYBR Green dye in a LightCycler 480-II (Roche Diagnostics, Rotkreuz, Switzerland) real-time quantitative PCR device. Target genes and appropriately selected reference genes were amplified in the same panel.

PCR conditions were applied, consisting of 10 minutes of incubation at 95°C, followed by 45 cycles of 15 seconds of denaturation at 95°C, 1 minute of annealing at 60°C, and 1 minute of extension. Relative gene expression levels were calculated using the 2−ΔΔCt method, and GAPDH was used as the internal reference gene for normalization. The results were analyzed by calculating Cp values with Light Cycler software (version 4). HNF1A gene expression analyses were performed based on control groups.

Authors' proof

Statistical Analysis

Statistical results were analyzed using SPSS 10.0 (SPSS, Chicago, USA). Data were analyzed using paired t-tests and the Newman–Keuls method for multiple comparisons. All experiments were performed in three independent biological replicates, and each measurement was conducted in triplicate technical repeats. The difference between the experimental and control groups was considered significant when $p < 0.05$ and highly significant when $p < 0.01$.

Results

Characterization Studies of PANC-1 Pancreatic Cancer Cell Line

A flow cytometry device characterized the PANC-1 cells used in the study before they were used in the experiments. Characterization analysis of the cells was performed using flow cytometry according to the selected cell surface markers, and the results are presented in Figure 1.

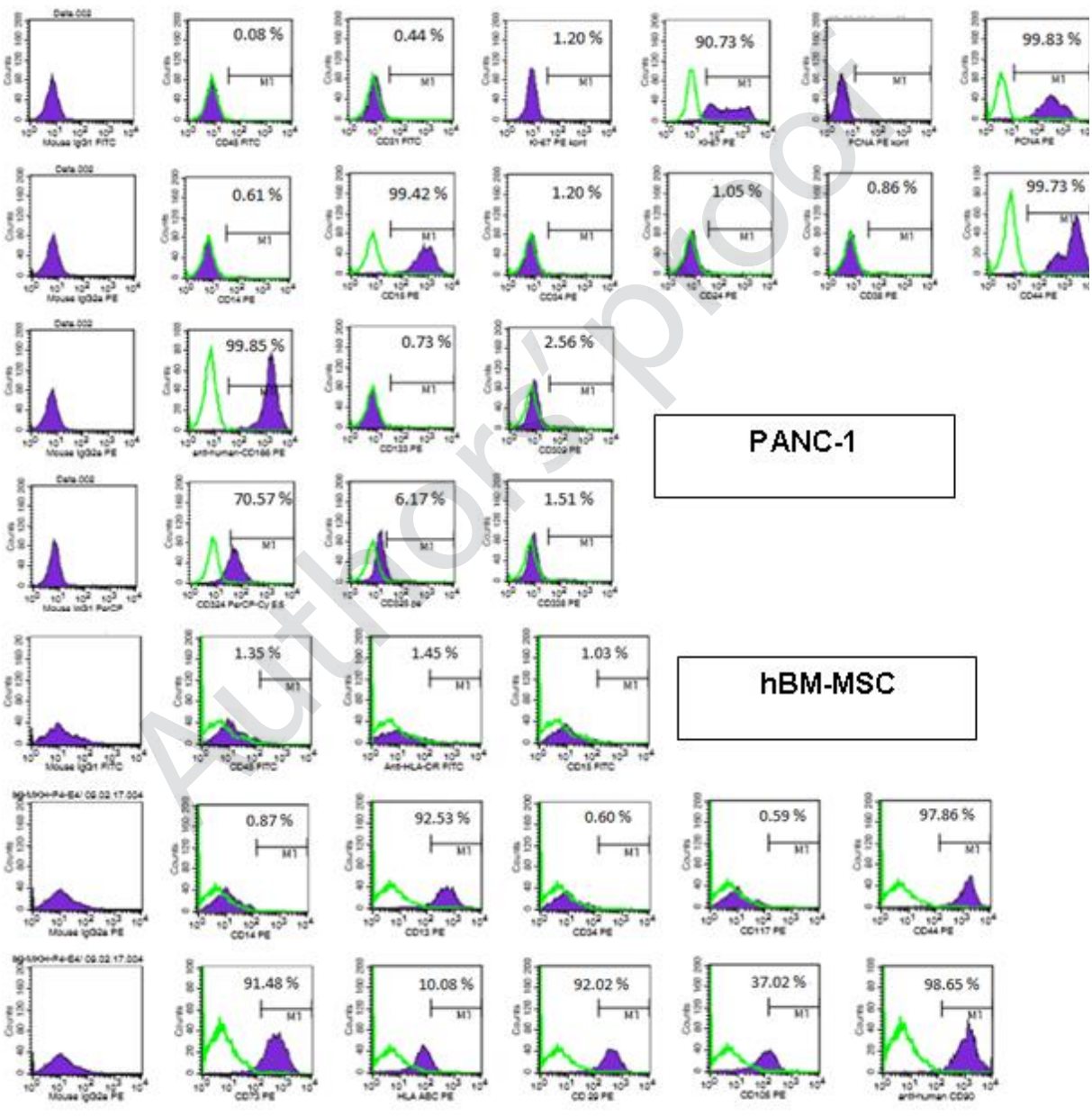


Figure 1. Flow cytometry characterization of PANC-1 pancreatic cancer cells and human bone marrow-derived mesenchymal stem cells (hBM-MSCs).

Author's proof

CD324, CD325, CD338, Ki-67, PCNA cell surface markers were found to be 99.42%, 1.05%, 0.44%, 1.20%, 0.86%, 99.73%, 0.73%, 99.85%, 2.56%, 70.57%, 6.17%, 1.51%, 90.73%, 99.83%, respectively (Figure 1).

After flow cytometry analysis, positive results for CD18, CD44, CD166, CD324, Ki67 and PCNA and negative results for CD24, CD31, CD34, CD38, CD133, CD309, CD325 and CD338 were detected in the flow cytometry device, indicating PANC-1 cell surface markers.

Morphological analyses of MSCs were performed under a light microscope. The cells' morphological structure and high proliferation characteristics were compatible with the mesenchymal stem cell phenotype. They maintained their high proliferative characteristics until advanced passages. In examinations performed under the microscope, it was observed that they had a spindle-shaped structure resembling fibroblast cells (Figure 2).

hBM-MSC Characterization Studies

Cell characterization analysis was performed using their floating markers, stem cell markers, and assessment of their differentiation ability. The surface markers they carry were found to be 92.53%, 1.03%, 92.02%, 97.86%, 91.48%, 98.65%, 37.02%, 10.08%, 0.87%, 0.60%, 1.35%, 0.59%, 1.45%, respectively, by looking at their CD13, CD15, CD29, CD44, CD73, CD90, CD105 and HLA A, B, C, CD14, CD34, CD45, CD117 and HLA-DR expressions (Figure 1).

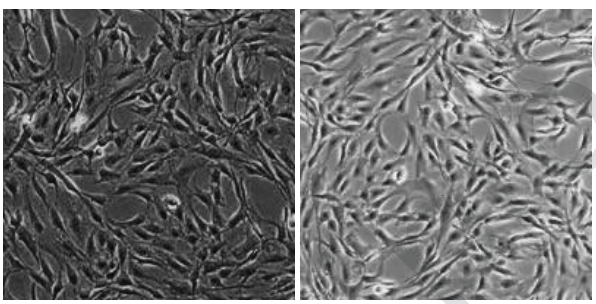


Figure 2. Light microscope images of hBM-MSCs (ATCC), (measuring bar: 100 µm)

After flow cytometry analysis, positive results for CD13, CD29, CD44, CD73, and CD90, and negative results for CD14, CD15, CD34, CD45, CD105, CD117, HLA-A, HLA-B, HLA-C, and HLA-DR were detected using the flow cytometry device. It has been demonstrated that hBM-MSC exhibit stem cell characteristics.

Viability Test (WST-1)

To determine the number of metabolically active cells, WST-1 proliferation assays were performed with samples taken on days 1, 3, and 7. The WST-1 assay results for the MSC-GA combination revealed an increase in viability up to a 50 µM GA concentration on days 1 and 3, followed by a decrease. On day 7, viability decreased to a 25 µM GA concentration, with the highest viability observed at a 50 µM GA concentration. All graphs showed a decrease in viability at concentrations of 100 µM and 150 µM GA (Figure 3). When examining the MSC-CA combination results, an increase in viability was observed at a 25 µM CA concentration on days 1, 3, and 7. In contrast, a decrease in viability was observed at higher concentrations. Furthermore, the results of the MSC-GA-CA group showed that the concentration ratios of 50 µM GA and 25 µM CA did not harm the MSCs on days 1, 3, and 7 (Figure 3).

The viability of the PANC-1 and MSC co-culture increased after day 3 (Figure 3). In the WST-1 test results for the PANC-1–GA combination, a decrease in viability was observed after the 25 µM GA concentration on days 1 and 3 and after the 10 µM concentration on day 7. However, when examining the PANC-1–CA combination, a decrease in viability was observed on days 1, 3, and 7 at the 10 µM CA concentration (Figure 3). While a regular decrease in viability was observed on the 1st, 3rd, and 7th days in the PANC-1-GA-CA combination, a regular decrease in viability was observed on the 0th, 1st, 3rd, and 7th days in the PANC-1-MSC-GA-CA combination (Figure 3). The treatment with gallic acid (GA) and carnosic acid (CA) resulted in a dose-dependent decrease in the viability of PANC-1 cells compared with the control group. Statistical analysis demonstrated that treatment with GA and CA significantly reduced PANC-1 cell viability compared with the control group ($p < 0.05$). The combined treatment of 50 µM GA and 25 µM CA in the presence of MSCs resulted in the most pronounced decrease in cell viability, which was statistically significant ($p < 0.01$).

Authors' Proof

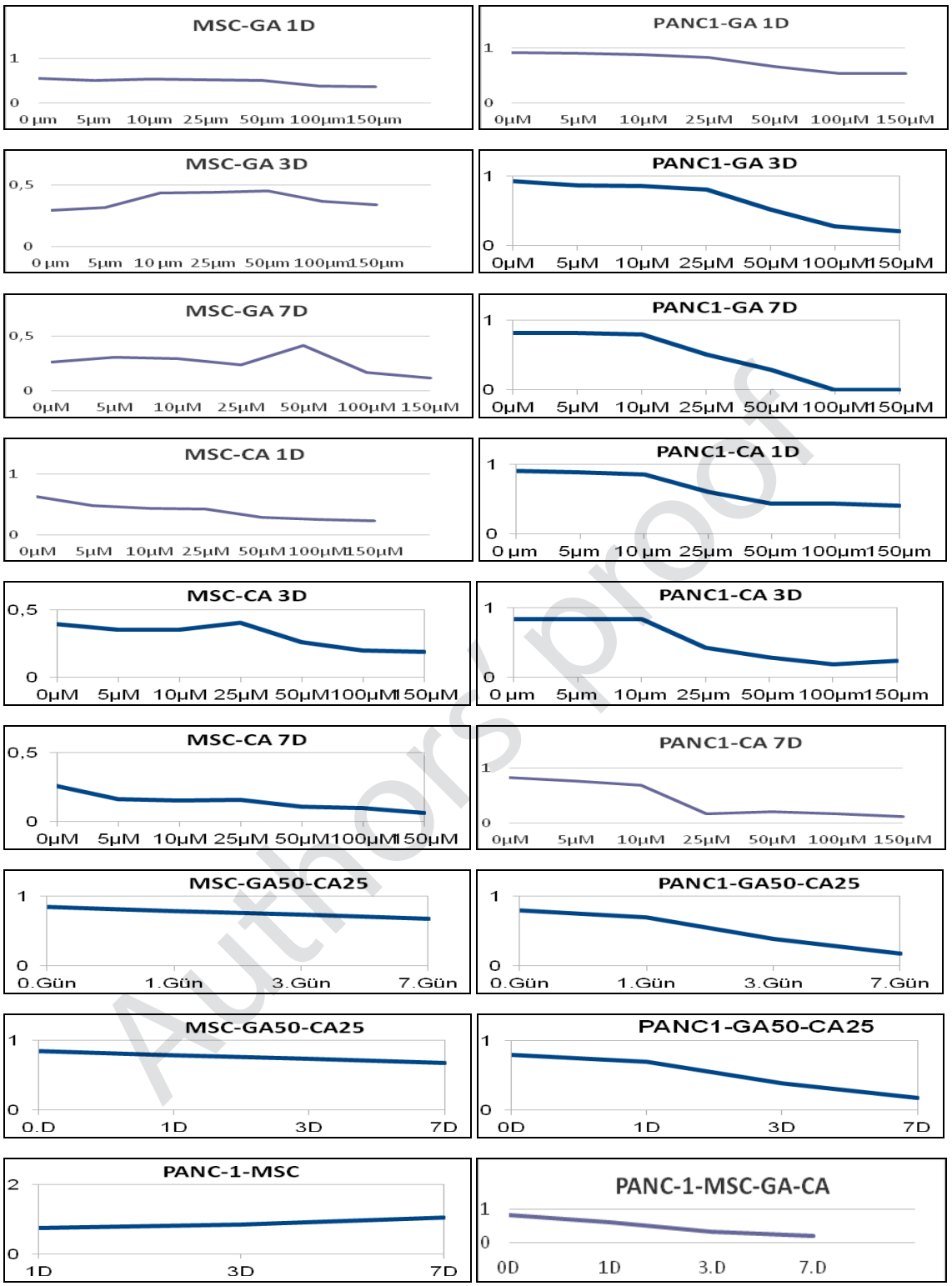


Figure 3. Graphical displays of WST-1 values

The effects of different concentrations of gallic acid (GA) and carnosic acid (CA), individually and in combination, on the viability of PANC-1 pancreatic cancer cells were evaluated using the WST-1 assay.

Authors' proof

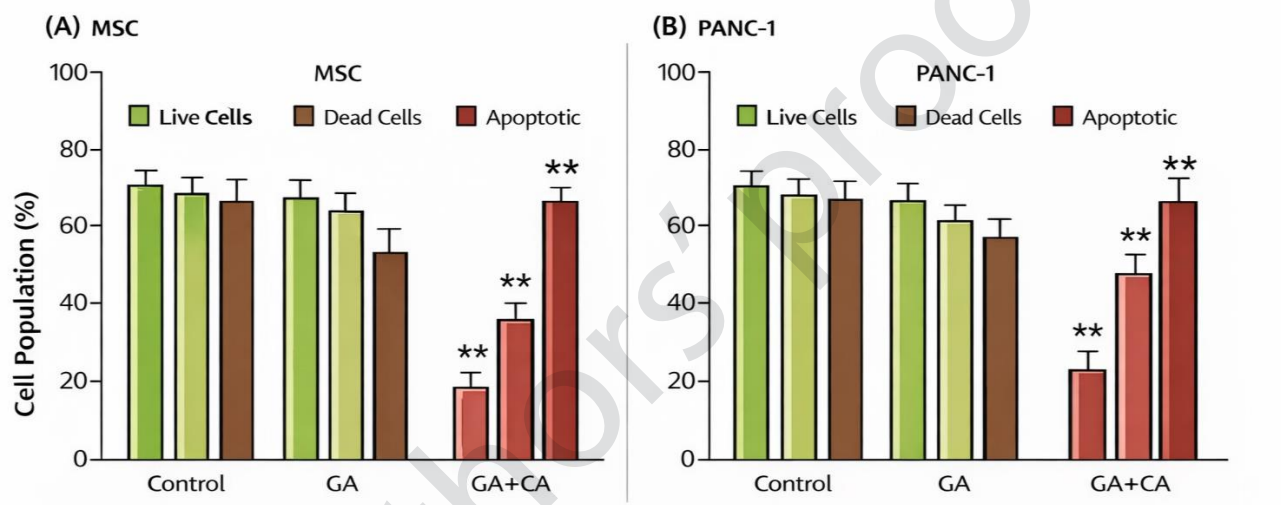
Annexin V-PI Capacity Determination

Annexin V-PI analysis revealed that hBM-MSC cell death rates at a 25 µM CA concentration were higher than those in the hBM-MSC-GA and hBM-MSC–GA–CA groups (Figure 4).

At a 50 µM GA–25 µM CA concentration, the viability of hBM-MSC cells was higher than that in the hBM-MSC-GA and hBM-MSC–CA groups. At the same concentration, the apoptosis rate of hBM-MSC cells was also higher than that in the hBM-MSC-GA and hBM-MSC–CA groups (Figure 4).

According to Annexin V-PI analysis results, the highest death rates in PANC-1 cells were observed in the PANC-1–MSC–25 µM GA–25 µM CA group, while the lowest viability rate was observed in the PANC-1 25 µM CA group. When the decreases in cell viability were examined, a similar decrease was observed in the MSC-50 µM GA-25 µM CA and PANC-1-25 µM CA groups (Figure 4).

Data are presented as mean ± standard deviation (SD) of three independent experiments. Statistical significance was determined using a paired Student's t-test followed by Newman–Keuls multiple comparison test. $p < 0.05$ and $p < 0.01$ were considered statistically significant compared with the control group.



(A) Percentage of live, dead, and apoptotic MSC cells after treatment with GA and CA individually and in combination.

(B) Percentage of live, dead, and apoptotic PANC-1 pancreatic cancer cells under the same treatment conditions.

Authors' proof

Figure 4. Annexin V-PI capacity determination graph of MSC and Panc-1 cell groups
Apoptotic effects of gallic acid (GA) and carnosic acid (CA), individually and in combination, on PANC-1 pancreatic cancer cells were analyzed using Annexin V/propidium iodide (PI) flow cytometry.
Data are presented as mean ± standard deviation (SD) of three independent experiments. Statistical significance was determined using a paired Student's t-test followed by Newman–Keuls multiple comparison test. $p < 0.05$ and $p < 0.01$ were considered statistically significant compared with the control group.

The Annexin V-PI analyses revealed that the highest apoptosis rate of cancer cells was observed in the PANC-1-MSC-50 µM GA-25 µM CA group, while a similar increase was observed in the PANC-1-25 µM CA group on day 7 (Figure 4). Annexin V-PI analysis revealed changes in apoptotic and viable cell populations in both MSC and PANC-1 groups following treatment with GA and CA. Quantitative analysis indicated that the proportion of apoptotic cells significantly increased in the PANC-1–MSC–GA–CA group compared with the control group ($p < 0.05$). The highest apoptosis rate was observed on day 7, demonstrating a statistically significant increase compared with untreated cells ($p < 0.01$).

Gene expression analysis with PCR

Real-time PCR analysis was performed to evaluate changes in HNF1A gene expression following treatment. The relative expression analysis demonstrated that HNF1A gene expression was significantly downregulated in the PANC-1–MSC–GA–CA group compared with the control group ($p < 0.05$). The decrease in HNF1A expression observed on day 7 was statistically significant and correlated with the reduction in cell viability and increase in apoptotic cell populations. In the PANC-1 cell line, an increase in HNF1A gene expression was observed on day 7. PANC-1–MSC-50 µM GA- 25 µM CA combination decreased HNF1A gene expression (Figure 5).

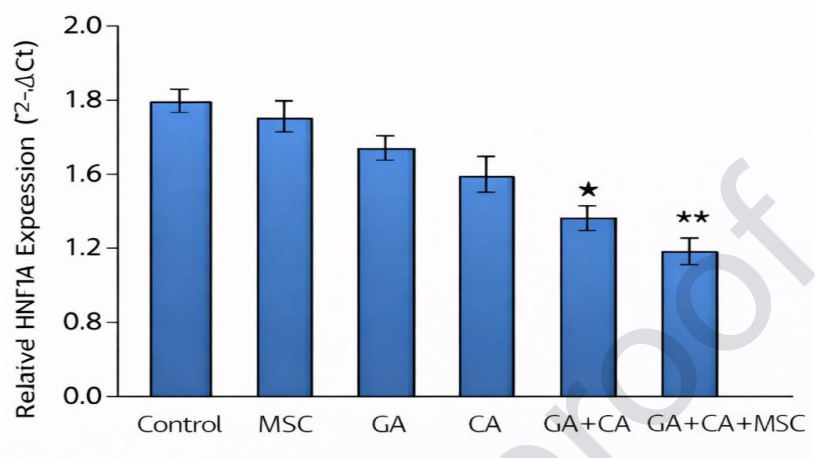


Figure 5. Determination of changes in HNF1A gene expression by Real-Time PCR analysis
Relative HNF1A gene expression levels in PANC-1 cells treated with gallic acid (GA) and carnosic acid (CA), individually and in combination, were determined using quantitative real-time PCR analysis.
Data are presented as mean ± standard deviation (SD) of three independent experiments. Statistical significance was determined using a paired Student's t-test followed by Newman–Keuls multiple comparison test. $p < 0.05$ and $p < 0.01$ were considered statistically significant compared with the control group.

Discussion

Pancreatic cancer ranks 4th among cancer-related deaths in developed countries and is a very aggressive type of cancer. Interest in natural products is increasing because these products have lower toxicity compared to chemotherapeutic drugs and possess properties that enhance the effects of drugs (20). Over 60% of chemotherapeutic drugs are derived from natural products, and the anti-cancer properties of plant-based natural products are also being investigated for the treatment of pancreatic cancer.

In recent years, it has been demonstrated that natural herbal products, including curcumin, resveratrol, lycopene, folic acid, beta-carotene, selenium, and flavonoids, can affect cancer cells through mechanisms such as inducing apoptosis and inhibiting carcinogenesis (21). The effects of herbal compounds, such as flavonoids, on pancreatic cancer cells are evaluated as potential therapeutic agents for treating pancreatic cancer (22). Zhang et al. (2020) demonstrated the anti-cancer effect of resveratrol (23). In our study, carnosic acid and gallic acid show anti-cancer effects.

The effects of these natural compounds on pancreatic cancer were examined in studies conducted on the PANC-1 cell line. In our study, a 50% decrease in HNF1A gene expression was observed after combining MSC with GA and CA in PANC-1 cells. Recent studies suggest that HNF1A may play a context-dependent role in the biology of pancreatic cancer. While some reports indicate tumor suppressor activity, other studies demonstrate that HNF1A regulates pancreatic cancer stem cell properties and tumor progression. Consequently, the downregulation of HNF1A observed in the present study may contribute to the suppression of tumor cell proliferation through modulation of transcriptional networks associated with cancer stemness and metabolic regulation. Mesenchymal stem cells (MSCs) can form a defense system in cancerous tissues. In our study, the combination of MSC+GA+CA prevented the growth of pancreatic cancer cells. Although MSC does not have an anti-cancer effect on pancreatic cancer cells, this effect was observed together with GA and CA.

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Recent studies have increasingly highlighted the anticancer potential of natural phenolic compounds in pancreatic cancer research. Specifically, gallic acid has been documented to impede proliferation and promote apoptosis in pancreatic cancer cell lines, including PANC-1 and MIA PaCa-2, through multiple molecular pathways. Mechanistic investigations have demonstrated that gallic acid promotes intracellular reactive oxygen species (ROS) generation and activates the p38 MAPK signaling pathway and endoplasmic reticulum (ER) stress responses, ultimately triggering apoptosis in pancreatic cancer cells (24). These findings align with the mounting evidence that phenolic compounds derived from plants can modulate critical signaling networks implicated in tumor progression and cell survival. Furthermore, recent experimental studies suggest that such compounds may exert synergistic effects when combined with other therapeutic agents or biological modulators, potentially enhancing anticancer efficacy while reducing toxicity (25). In this context, the present study contributes to the expanding body of literature by demonstrating that the combined application of gallic acid and carnosic acid, particularly in the presence of mesenchymal stem cells, significantly decreases the viability of PANC-1 cells and downregulates HNF1A gene expression. These findings suggest that natural polyphenols may influence pancreatic cancer progression through the modulation of apoptosis-related pathways and transcriptional regulators associated with tumor development.

Carnosic acid and gallic acid show anti-cancer effects on pancreatic cancer cells. Gallic acid induces apoptosis in pancreatic cancer cells (26), and in this study, GA caused a 3 times increase in cell death by day 7. The combination of carnosic acid and GA was also effective on pancreatic cancer cells. The combination of curcumin and carnosic acid reported by Pesakhov et al. was also found to be effective (27). In our study, the combination of PANC-1+MSC+GA+CA caused a 4.5 times decrease in cell viability.

Gallic acid and carnosic acid inhibit the growth of pancreatic cancer cells, increase the number of apoptotic cells, and prevent tumor development. In our study, GA and CA showed an antiproliferative effect on PANC-1 cells, reducing the viability of cancer cells and increasing the number of apoptotic cells. It was observed that carnosic acid is a more potent compound than gallic acid.

Although the present study provides evidence of the antiproliferative effects of gallic acid and carnosic acid on pancreatic cancer cells, several limitations should be acknowledged. The experiments were conducted in a single pancreatic cancer cell line and under in vitro conditions. Therefore, further studies involving additional pancreatic cancer models and in vivo systems are required to validate the therapeutic potential and molecular mechanisms of these compounds.

The present study provides novel insights into the potential anticancer effects of natural phenolic compounds in pancreatic cancer. Although the individual anticancer properties of gallic acid (GA) and carnosic acid (CA) have been reported in various cancer models, their combined effects in a mesenchymal stem cell (MSC)-mediated tumor microenvironment have not been previously investigated in pancreatic cancer cells. By establishing an indirect co-culture system between PANC-1 cells and MSCs, this study offers a more physiologically relevant in vitro model that partially mimics tumor–stromal interactions. The findings demonstrate that the combined administration of GA and CA significantly reduces cancer cell viability, increases apoptosis, and downregulates HNF1A gene expression, suggesting that natural polyphenols may influence transcriptional networks involved in pancreatic cancer progression. Therefore, this study contributes to the current literature by highlighting a potential synergistic interaction between plant-derived phenolic compounds and MSC-mediated tumor regulation, providing a basis for future mechanistic and translational studies aimed at developing novel therapeutic strategies for pancreatic cancer.

Conclusion

In this study, the anti-cancer effects of plant phenolic compounds carnosic acid (CA) and gallic acid (GA) were investigated in PANC-1 cells in conjunction with human bone marrow-derived mesenchymal stem cells (MSCs) in the treatment of pancreatic cancer. In the analyses, it was observed that MSCs showed antiproliferative and cytotoxic effects on PANC-1 cells. It was determined that the number of live cells decreased, and the number of apoptotic cells increased in PANC-1 cells.

The results indicate that MSCs may be a potential treatment option for pancreatic cancer, particularly when combined with GA and CA. However, for more effective results, both in vitro and in vivo studies should be conducted and

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repeated until statistically significant data are obtained. Additionally, it is crucial to investigate molecular pathways to comprehend the mechanisms by which MSCs eliminate cancer cells. According to our findings, the combination of GA+CA exhibits more substantial antiproliferative effects against pancreatic cancer cells, and Carnosic acid is a more effective compound than gallic acid.

In this study, the cytotoxic and apoptotic effects of GA and CA on pancreatic cancer cells were investigated for the first time. It was predicted that both compounds could be novel therapeutic agents for treating pancreatic cancer. As a result, it was observed that CA and GA, together with MSCs, reduced the number of live cells, increased the number of apoptotic cells in PANC-1 cells, and inhibited pancreatic cancer growth by decreasing HNF1A gene expression. This suggests the hypothesis that GA and CA may play a role in regulating cancer mechanisms.

Declarations

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Ethics Approval

Ethics committee approval was not required for the study due to the use of commercially purchased cell lines.

Availability of Data and Material

The data for this study are available from the corresponding author upon reasonable request.

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Authors' contributions

All authors have significantly contributed to this article's preparation, thoroughly reviewed the manuscript, and approved its final version for submission.

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