FABAD J. Pharm. Sci., 48, 3, 513-522, 2023
Doi: 10.55262/fabadeczacilik.1351725
RESEARCH ARTICLE

In Silico and *In Vitro* Anticancer Effects of Caffeic Acid Phenethyl Ester on Pancreatic Adenocarcinoma Cells

Zübeyde TANRIVERDİ[*], Burak KUZU[**], Ergül EYOL[***], Fuat KARAKUŞ[****°]

In Silico and In Vitro Anticancer Effects of Caffeic Acid Phenethyl Ester on Pancreatic Adenocarcinoma Cells

SUMMARY

Pancreatic adenocarcinoma is an aggressive and fatal malignancy due to the lack of early diagnosis and poor therapeutic response. At this point, determining the anticancer potential of non-toxic natural compounds is essential. Caffeic acid phenethyl ester is a bioactive compound with different activities. In this study, the toxicity of caffeic acid phenethyl ester was estimated by in silico methods in 14 pancreatic cancer cells, and its anticancer activity was evaluated in rat adenocarcinoma cells. According to the in silico results, caffeic acid phenethyl ester had anticancer properties without causing severe toxicity. Subsequently, we investigated the effects of caffeic acid phenethyl ester on rat pancreatic cancer (ASML) cells. Caffeic acid phenethyl ester reduced ASML cell viability by up to 27% in a dose- (5, 10, 20, 40, and 80 μM) and time-dependent (24, 48, and 72 h) manner. In the scratch assay, only 80 μM caffeic acid phenethyl ester statistically considerably inhibited ASML cell migration at 24 h. On the other hand, at 48 hours, all doses of caffeic acid phenethyl ester statistically remarkably decreased cell migration. Caffeic acid phenethyl ester also decreased ASML colony numbers at 5 μM and 10 μM compared to the control and completely suppressed colony formation at ≥ 20 μM. Our results revealed that caffeic acid phenethyl ester showed anticancer potential against human and mouse pancreatic cancer cells in silico and significantly inhibited the viability, migration, and colony formation of ASML cells in vitro.

Key Words: *pancreatic cancer, caffeic acid phenethyl ester, ASML cells, anticancer activity, cytotoxicity.*

Kafeik Asit Fenetil Ester'in Pankreas Adenokarsinom Hücreleri Üzerine In Silico ve In Vitro Antikanser Etkileri

ÖZ

Pankreas adenokarsinomu erken tanı eksikliği ve tedaviye verilen zayıf yanıt nedeniyle agresif ve ölümcül bir malignitedir. Bu noktada toksik olmayan doğal bileşiklerin antikanser potansiyelinin belirlenmesi önem taşımaktadır. Kafeik asit fenetil ester, farklı aktivitelere sahip biyoaktif bir bileşiktir. Bu çalışmada kafeik asit fenetil esterin toksisitesi in siliko yöntemlerle 14 pankreas adenokarsinomu hücresinde tahmin edildi ve antikanser aktivitesi sıçan adenokarsinom hücrelerinde değerlendirildi. İn siliko sonuçlara göre kafeik asit fenetil ester, tehlikeli toksisiteye neden olmadan antikanser özelliklere sahipti. Daha sonra kafeik asit fenetil esterinin sıçan pankreas kanser (ASML) hücreleri üzerindeki etkilerini araştırdık. Kafeik asit fenetil ester, ASML hücre canlılığını doz (5, 10, 20, 40 ve 80 μM) ve zamana bağlı (24, 48 ve 72 saat) bir şekilde %27'ye kadar azalttı. Yara iyileşme deneyinde yalnızca 80 μM kafeik asit fenetil ester, 24 saatte ASML hücre göçünü istatistiksel olarak anlamlı düzeyde inhibe etti. Öte yandan hücre migrasyonu 48. saatte tüm kafeik asit fenetil ester dozlarında istatistiksel olarak anlamlı düzeyde inhibe edildi. Kafeik asit fenetil ester ayrıca kontrole kıyasla 5 μM ve 10 μM dozlarda ASML koloni sayısını azalttı ve ≥ 20 μM dozda koloni oluşumunu tamamen bastırdı. Sonuçlarımız, kafeik asit fenetil esterin in siliko olarak insan ve fare pankreas kanseri hücrelerine karşı antikanser potansiyel gösterdiğini ve in vitro olarak da ASML hücrelerinin canlılığını, hücre göçünü ve koloni oluşumunu önemli ölçüde inhibe ettiğini ortaya çıkardı.

Anahtar Kelimeler: *pankreas kanseri, kafeik asit fenetil ester, ASML hücreleri, antikanser aktivite, sitotoksisite.*

Received: 29.08.2023
Revised: 22.09.2023
Accepted: 26.09.2023

[*] ORCID:0000-0002-1170-181X: Independent Researcher, Gaziantep/Türkiye.
[**] ORCID: 0000-0002-7305-7177: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Van Yuzuncu Yil University, 65080 Tuşba-Van/Türkiye.
[***] ORCID: 0009-0000-3699-6725: Independent Researcher, Mannheim /Germany.
[****] ORCID: 0000-0002-5260-3650: Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Van Yuzuncu Yil University, 65080 Tuşba-Van/Türkiye.

[°] Corresponding Author; Fuat Karakuş
Tel.: +90 432 444 5065 Extension: 2118, E-mail:fuatkarakus@yyu.edu.tr

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive lethal malignancy due to the lack of early diagnosis and limited treatment response. It is the most prevalent type of pancreatic neoplasm, and it is developed in the exocrine compartment and accounts for more than 90% of pancreatic cancer cases (Sarantis et al., 2020). PDAC is the fourth most frequent cause of cancer-related deaths worldwide, with a 5-year overall survival of less than 8% (Orth et al., 2020).

In traditional treatment, chemotherapeutics cause many adverse effects, and the patients could resist drugs. Therefore, the studies on whether bioactive compounds obtained from natural sources can be used in the treatment are continuing rapidly. One of these bioactive compounds, caffeic acid phenethyl ester (CAPE) (Figure 1A), has been identified as the main active component of honeybee hive propolis and has many biological properties, including antiviral, anti-inflammatory, antibiotic, antifungal, antioxidant, immune-modulator activities and also anticancer efficacy by suppressing the growth of some tumor cell types and triggering apoptosis (Tang et al., 2017; Karakuş et al., 2019).

In this study, we used *in silico* methods to determine CAPE's toxicity profile and anticancer activities. Then, we evaluated CAPE's antiproliferative, antimetastatic, and anti-clonogenic effects on rat pancreatic cancer (ASML) cells.

MATERIALS AND METHODS

Toxicity profile of CAPE

Key toxicity characteristics of CAPE, including hepatotoxicity and cardiotoxicity, were identified using the web-based tools ADMETLab 2.0 and ProTox-II. With the aid of ADMETLab 2.0, which efficiently calculates and predicts 27 toxicity endpoints and eight toxicophore rules (751 substructures), the predictive toxicity of compounds can be found. ProTox-II employs a thorough methodology to predict a variety of toxicity endpoints, including acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways, and toxicity targets. This methodology includes molecular similarity, fragment propensities, and machine learning (with 33 models) (Banerjee et al., 2018; Xiong et al., 2021).

Anticancer activity prediction of CAPE

The anticancer properties of CAPE were studied using PASS (Prediction of Activity Spectra for Substances) Online. Based on the structural characteristics of chemical molecules, it is intended to predict over 4,000 different types of biological activity. A large dataset of over 250,000 physiologically active compounds, including medicines, drug candidates, lead compounds, and toxic substances, was used to analyze the correlations between structure and activity for the prediction. After entering CAPE into PASS Online, we used the "probable activity (Pa, probability of being active)" metric to evaluate the likelihood of potential anticancer effects linked to substantial anticancer processes, such as Tyrosine-protein kinase (JAK2) expression inhibition, antimutagenic activity, Hypoxia-inducible factor 1-alpha (HIF1A) expression inhibition, Cellular tumor antigen p53 (TP53) expression enhancement, and apoptosis agonism. Pa values vary from 0.0001 to 1.000 and are given as a percentage of likelihood (%) (Filimonov et al., 2014). In addition, the anticancer activity of CAPE against 13 human pancreatic cancer cells and one mouse pancreatic cancer cell was evaluated by the DiPCell web server. DiPCell uses the SMOreg (support vector machine for regression) machine learning algorithm to apply QSAR models developed from high-throughput drug screening data (Kumar et al., 2014).

ASML cell line and reagents

The ASML cell line, rat pancreatic ductal adenocarcinoma cells, was kindly obtained from Prof. Martin R. Berger (German Cancer Research Center,

Germany) via Dr. Ergül Eyol. MTT powder was purchased from Applichem, and Fetal Bovine Serum (FBS) was purchased from Biowest (France). RPMI-1640 medium and all other cell culture reagents were purchased from Lonza (Basel, Switzerland). Caffeic acid phenethyl ester was kindly provided by Prof. Yılmaz Çiğremiş (Inonu University, Türkiye), and the stock solution was prepared in DMSO.

Cell culture

ASML cells were maintained at 37^{0}C in a 5% CO_2 atmosphere in RPMI-1640 medium supplemented with 10% FBS, L-glutamine (2mM), and 1% Penicillin/Streptomycin. For subculturing, the medium was discarded, and the cells were washed with PBS, trypsinized (0.25% trypsin/EDTA), and pelleted at 1500 rpm for 5 min.

Cell proliferation assay

The effect of CAPE on ASML cell viability was evaluated by the MTT test as described by Mosmann with some modifications (Mosmann et al., 1983; Eyol et al., 2019). In brief, ASML cells were seeded in 96-well plates (5000 cells/well) and incubated for 24 h. After the attachment, the medium was replaced with a complete growth medium, which included various CAPE concentrations (at final concentrations ranging from 5 to 80 μM) as determined in our previous study (Karakuş et al., 2019), followed by further incubations for 24-, 48-, and 72 h. After incubation, the medium was removed and the cells washed with PBS, subsequently incubated with MTT (at final concentrations of 1 mg/mL) for 4 hours at 37°C. At the end of the incubation time, the MTT solution was discarded, and the formazan crystals were dissolved in DMSO (150 μL/well). The absorbance of formazan crystals was measured at 550 nm using a microplate reader. Cell viability was determined as the percentage of the treated cells compared to the control (untreated) at 24-, 48-, and 72 h.

Scratch (Wound-healing) assay

6.0×10^4 ASML cells were seeded in 24-well plates for 24 hours to determine the effect of CAPE on the migration of ASML cells. Subsequently, the confluent cell monolayers were scratched with a p200 pipette tip, rinsed with PBS to remove debris, and treated with different concentrations of CAPE (5, 10, 20, 40, and 80 μM). The scratched area was photographed at 0, 24, and 48 h intervals using an inverted microscope and calculated with ImageJ's freeware program (Liang et al., 2007; Karakuş et al., 2018).

Colony formation assay

500 ASML cells were seeded on a 6-well plate to examine the effect of CAPE on the colony formation of a single ASML cell. After 24 h, the cells were exposed to CAPE (at final concentrations ranging from 5 to 80 μM) and incubated for 14 days. Two weeks later, the media containing CAPE was discarded and washed with PBS. Afterward, the colonies were fixed with methanol for 20 minutes to make them adhere to the petri dish. Subsequently, the colonies were stained with crystal violet (0.05%). The cell colonies with a diameter of more than 50 cells were counted and plotted (Franken et al., 2006; Eyol et al., 2019).

Statistical analysis

The data were represented as mean values ± standard error of the mean (SEM) of at least three independent experiments. The statistically significant differences between the two groups (control versus treatment group) were determined by a Student's t-test in each assay. The wound area was calculated with free ImageJ software. The data were analyzed using GraphPad Prism® version 8 (GraphPad Software, San Diego, CA, USA; www.graphpad.com). A p-value ≤ 0.05 was considered statistically significant. The exact p values are presented in the figure legends.

RESULTS AND DISCUSSION

It is generally known that CAPE has anticancer properties against numerous types of human cancer cells, such as medulloblastoma, cervical cancer, lung cancer, leukemia, colorectal cancer, multiple myeloma, breast cancer, oral cancer, prostate cancer,

and head and neck squamous cell carcinoma cells (Hung et al., 2003; Chen et al., 2004; Lin et al., 2006; Avcı et al., 2011; Chiang et al., 2014; Yu et al., 2017; Marin et al., 2019; Fu et al., 2022; Yu et al., 2023). However, the effect of CAPE on ASML (pancreatic adenocarcinoma) cells is unknown. The present study highlights the anticancer properties of CAPE using *in silico* and *in vitro* methods.

Predicted results of the toxicity profile and anticancer activity of CAPE

In silico toxicity assessment using the ADMETLab 2.0 and Protox-II web tools showed that CAPE exhibited no severe toxic effects except for some skin sensitization and eye irritation. The acute oral LD_{50} of CAPE predicted by the Protox-II web tool was 5000 mg/kg. It showed no hepatotoxic, cardiotoxic (hERG blockers), carcinogenic, mutagenic, or cytotoxic effects (Figure 1B and 1C). Although it could not be available due to the absence of healthy pancreatic cells in this study, the theoretical toxicity results of CAPE indicate that it may not be toxic to healthy cells. In a study was conducted on BALB/c nude mice, Chen et al. (2008) found that CAPE was less effective than gemcitabine as an anticancer agent, but it did not cause significant toxicity while suppressing tumor growth.

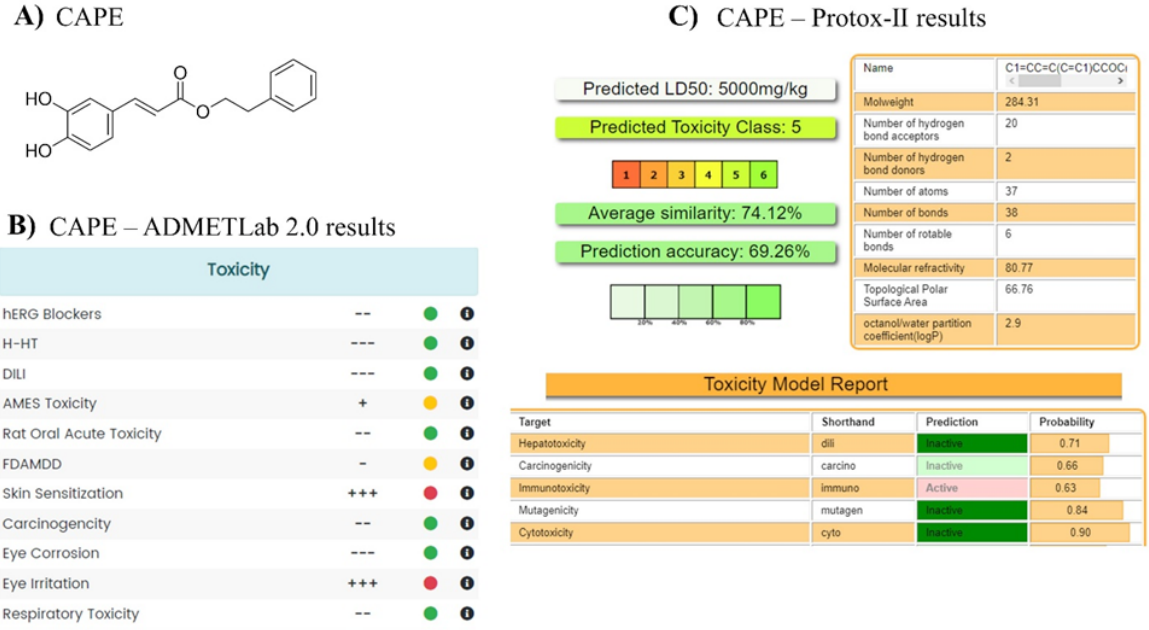

Figure 1. **A)** Molecular structure of caffeic acid phenethyl ester (CAPE) **B)** and **C)** predicted toxicity profiles of CAPE in ADMETLab 2.0 and in Protox-II, respectively.

PASS Online server results showed that CAPE exhibited some anticancer properties, with percentages ranging from 48.2% to 79.8%. Pa values in Table 1 range from 0.000 to 1.000 and are expressed as a percentage of likelihood (%). Chen et al. (2008) reported that CAPE acts as a potent apoptosis-inducing agent in human pancreatic cancer cells (BxPC-3 and PANC-1) by inducing mitochondrial dysfunction and activating caspase-3/caspase-7, aligning with the 'apoptosis agonist' result presented in Table 1.

In addition, the anticancer activity of CAPE against 14 pancreatic cancer cell lines is shown in Table 2, and the IC_{50} values of CAPE against these cells range from 0.03 µM to 4.31 µM.

Table 1. Fundamental anticancer properties of CAPE (%, PASS online)

Anticancer Properties	***Pa***
JAK2 expression inhibitor	79.8
Antimutagenic	76.7
HIF1A expression inhibitor	66.3
TP53 expression enhancer	61.5
Apoptosis agonist	48.2

CAPE decreased the proliferation of ASML cells

The results of cell viability determined by the MTT test are shown in Figure 2. A gradual decrease in cell viability was observed, both time- and dose-dependently. When CAPE concentration was increased from 5 to 80 µM, the cell viability gradually decreased from 88% to 68% at 24 h, from 81% to 45% at 48 h, and from 68% to 27% at 72 h compared to the control (expressed as 100%). Statistically, cell viability was significantly decreased when each group was compared with the control (*** $p \leq 0.001$). Duan et al. (2017) showed that CAPE significantly reduced cell viability in PANC-1 cells. This decrease was due to the inhibition of human neutrophil elastase, which induces cell growth and migration by CAPE. This study's dose range (27-90 µM) was comparable to the dose range used in our research.

Table 2. Predicted IC_{50} values of CAPE on pancreatic cancer cell lines

Cell lines	Organism	Predicted IC_{50} (µM)
MIAPaCa2	Human	0.03
Capan-1	Human	1.04
Panc 10.05	Human	2.14
MZ-PC-1	Human	2.18
Capan-2	Human	2.53
BxPC-3	Human	2.55
HPAF-II	Human	2.56
KP-4	Human	2.58
Panc 08.13	Human	2.84
SW 1990	Human	3.02
AsPC-1	Mouse	3.77
HuP-T3	Human	3.90
YAPC	Human	3.98
PSN-1	Human	4.31

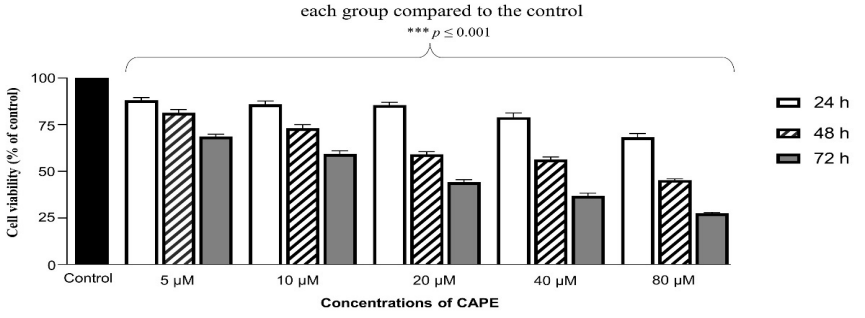

Figure 2. Time- and dose-dependent effect of CAPE on ASML cell viability. Cell viability was significantly reduced (*** p≤0.001) compared to control at all doses and times.

Papademetrio et al. (2016) also found that CAPE significantly reduced cell proliferation in MIAPaCa-2 and PANC-1 cells after 24 and 48 h. The researchers identified the simultaneous suppression of autophagy and NF-κB as the root cause.

CAPE reduced the migration of ASML cells

After being scratched and photographed, the confluent monolayers of ASML cells were calculated using the free software ImageJ. Untreated ASML cells closed almost the entire gap at 48 h. A statistically

significant difference was found only at 80 µM CAPE when compared at 0 and 24 h. The wound area was opened from 215 µm to 273 µm at 80 µM CAPE after 24h (Figure 3). On the other hand, a statistically significant difference was observed in all CAPE doses when comparing 0 and 48 h, with ASML cell migration inhibited by 21-45% at 48 h. Prasad and Katiyar (2013) also showed that CAPE inhibited cell migration by 40–80% in human pancreatic cancer cells (Miapaca-2, PANC-1, and AsPC-1) in 48 h. This effect was attributed to the hindrance of NF-κB, a key player in the epithelial–mesenchymal transition process and, consequently, in metastasis. Duan et al. (2017) have reported similar findings, demonstrating the potent inhibitory effect of CAPE on PANC-1 cell migration.

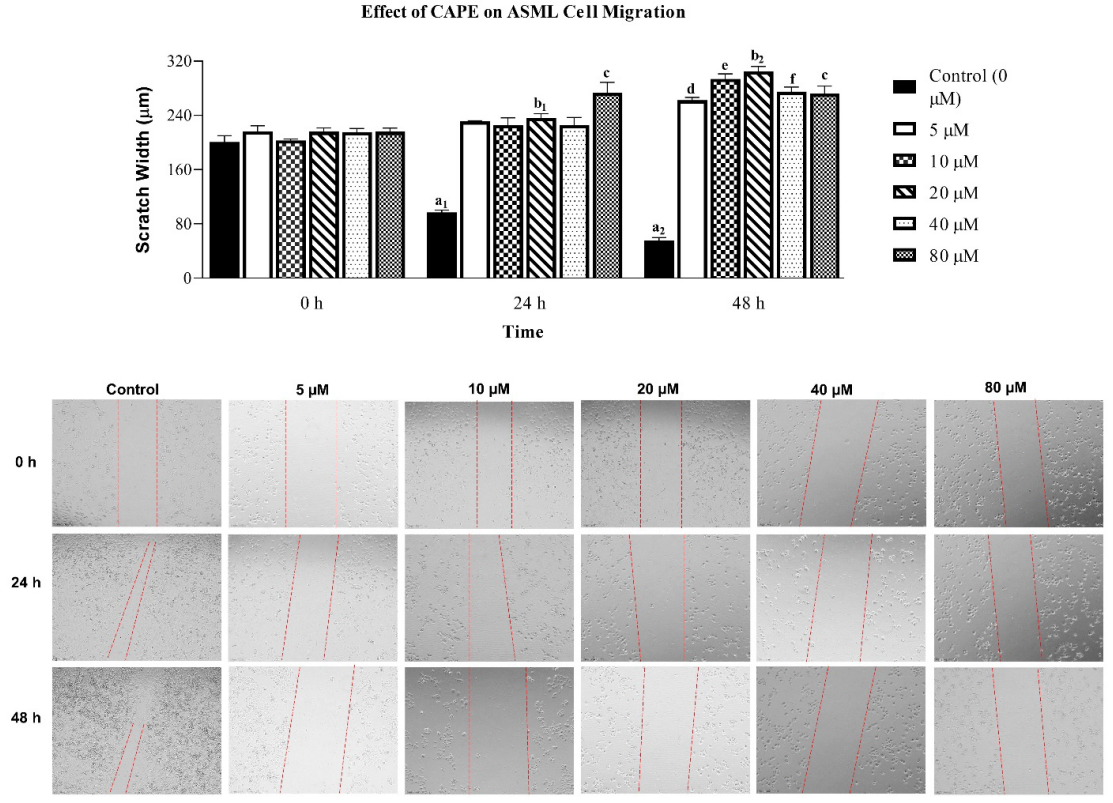

Figure 3. Effect of CAPE on ASML cell migration. After scratching the confluent ASML cell monolayer, the cells were photographed at 0, 24, and 48 h under an inverted microscope, and the scratched area was assessed with ImageJ's freeware program. All doses were compared with their controls at 0 h. Different levels of statistical significance are indicated by letters (a1: ** p≤ 0.01; a2: *** p≤ 0.001; b: ** p≤ 0.01; c: *** p≤ 0.001; d: *** p≤ 0.001; e: *** p≤ 0.001; f: *** p≤ 0.001).

CAPE prevented the formation of colonies of ASML cells

CAPE exposure leads to statistically significant inhibition of ASML cell colony formation at 5 µM (*p≤0.05) and 10 µM (**p≤0.01) compared to the control. The number of colonies decreased to 55 and 45 at 5 and 10 µM, respectively, compared to the control (60 colonies). In addition, CAPE suppressed colony formation 100% at concentrations of ≥20 µM. The inhibition of colony formation in ASML cells by CAPE is shown in Figure 4.

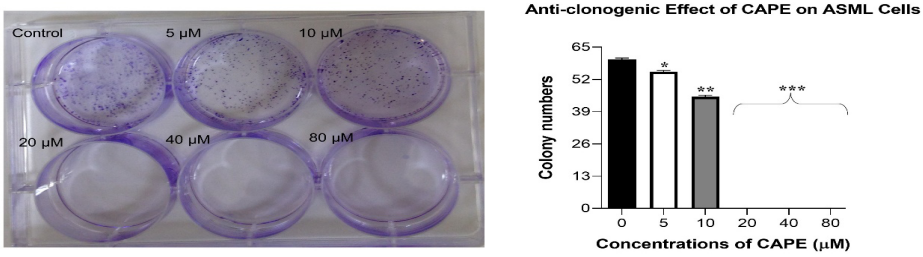

Figure 4. Effect of CAPE on colony formation in a single ASML cell. ASML colonies formed after incubation with CAPE for approximately two weeks were stained with crystal violet and counted under a microscope. Colony numbers decreased significantly at 5 µM (* $p \leq 0.05$) and 10 µM (** $p \leq 0.01$) CAPE compared to the control, and no colony formation was observed at ≥20 µM (*** $p \leq 0.001$).

As PDAC is highly aggressive and has a limited response to conventional chemotherapeutics, developing non-toxic bioactive compounds is essential. Since CAPE does not cause harmful toxicity *in silico* and its anticancer effect is determined by *in silico* and *in vitro* methods, it may stand out compared to conventional chemotherapeutics. This study could pave the way for developing bioactive molecules that will destroy pancreatic adenocarcinoma cells without causing toxicity to healthy cells. On the other hand, *in silico* results suggest that CAPE is not toxic to healthy pancreatic cells; the limitation of this study is the inability to determine the effect of CAPE on healthy pancreatic cells at the determined doses.

ACKNOWLEDGMENTS

We want to thank Prof. Martin R. Berger (German Cancer Research Center, Germany) for ASML cells and Prof. Yılmaz Çiğremiş (Inonu University) for CAPE.

AUTHOR CONTRIBUTION STATEMENT

Z.T: *In vitro* experimental analysis, writing manuscript's draft. B.K: *In silico* experimental analysis, and interpretation of the data E.E: Developing hypothesis, and reviewing the manuscript. F.K: Developing hypothesis, *in vitro* and *in silico* experimental analysis and interpretation of the data, writing and reviewing the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Avcı, C. B., Gündüz, C., Baran, Y., Sahin, F., Yılmaz, S., Dogan, Z. O., & Saydam, G. (2011). Caffeic acid phenethyl ester triggers apoptosis through induction of loss of mitochondrial membrane potential in CCRF-CEM cells. *Journal of cancer research and clinical oncology, 137*(1), 41–47. https://doi.org/10.1007/s00432-010-0857-0

Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: a web server for the prediction of toxicity of chemicals. *Nucleic acids research, 46*(W1), W257–W263. https://doi.org/10.1093/nar/gky318

Chen, M. F., Wu, C. T., Chen, Y. J., Keng, P. C., & Chen, W. C. (2004). Cell killing and radiosensitization by caffeic acid phenethyl ester (CAPE) in lung cancer cells. Journal of radiation research, 45(2), 253–260. https://doi.org/10.1269/jrr.45.253

Chen, M. J., Chang, W. H., Lin, C. C., Liu, C. Y., Wang, T. E., Chu, C. H., Shih, S. C., & Chen, Y. J. (2008). Caffeic acid phenethyl ester induces apoptosis of human pancreatic cancer cells involving caspase and mitochondrial dysfunction. *Pancreatology : official journal of the International Association of Pancreatology (IAP), 8*(6), 566–576. https://doi.org/10.1159/000159843

Chiang, E. P., Tsai, S. Y., Kuo, Y. H., Pai, M. H., Chiu, H. L., Rodriguez, R. L., & Tang, F. Y. (2014). Caffeic acid derivatives inhibit the growth of colon cancer: involvement of the PI3-K/Akt and AMPK signaling pathways. *PloS one, 9*(6), e99631. https://doi.org/10.1371/journal.pone.0099631

Duan, J., Xiaokaiti, Y., Fan, S., Pan, Y., Li, X., & Li, X. (2017). Direct interaction between caffeic acid phenethyl ester and human neutrophil elastase inhibits the growth and migration of PANC-1 cells. *Oncology reports, 37*(5), 3019–3025. https://doi.org/10.3892/or.2017.5516

Eyol, E., Tosun, E., Karakuş, F., Adwan, H., Yılmaz, K., Kovacheva, M., Zepp, M., Berger, MR. (2019). Antineoplastic activity of cucurbitacin I in CC531 rat colorectal cancer cells. *Clinical Oncology and Research,* 2613-4942. https://doi.org/10.31487/j.COR.2019.01.105

Filimonov, D.A., Lagunin, A.A., Gloriozova, T.A., Rudik, A.V., Druzhilovskii, D.S., Pogodin, P.V., Poroikov, V.V. (2014). Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. *Chemistry of Heterocyclic Compounds, 50,* 444–457. https://doi.org/10.1007/s10593-014-1496-1

Franken, N. A., Rodermond, H. M., Stap, J., Haveman, J., & van Bree, C. (2006). Clonogenic assay of cells in vitro. *Nature protocols, 1*(5), 2315–2319. https://doi.org/10.1038/nprot.2006.339

Fu, Y. K., Wang, B. J., Tseng, J. C., Huang, S. H., Lin, C. Y., Kuo, Y. Y., Hour, T. C., & Chuu, C. P. (2022). Combination treatment of docetaxel with caffeic acid phenethyl ester suppresses the survival and the proliferation of docetaxel-resistant prostate cancer cells via induction of apoptosis and metabolism interference. *Journal of biomedical science, 29*(1), 16. https://doi.org/10.1186/s12929-022-00797-z

Hung, M. W., Shiao, M. S., Tsai, L. C., Chang, G. G., & Chang, T. C. (2003). Apoptotic effect of caffeic acid phenethyl ester and its ester and amide analogues in human cervical cancer ME180 cells. *Anticancer research, 23*(6C), 4773–4780.

Karakuş, F., Eyol, E., Yılmaz, K., & Ünüvar, S. (2018). Inhibition of cell proliferation, migration and colony formation of LS174T Cells by carbonic anhydrase inhibitor. *African health sciences, 18*(4), 1303–1310. https://doi.org/10.4314/ahs.v18i4.51

Karakuş, F., Yılmaz, K., Eyol, E., & Ünüvar, S. (2019). Combination of 2 Bioactive Compounds for Treatment of Breast Cancer: Triterpenoid Cucurbitacin I and Phenolic CAPE. *Natural Product Communications, 14*(6), https://doi.org/10.1177/1934578X19857492

Kumar, R., Chaudhary, K., Singla, D., Gautam, A., & Raghava, G. P. (2014). Designing of promiscuous inhibitors against pancreatic cancer cell lines. *Scientific reports, 4,* 4668. https://doi.org/10.1038/srep04668

Liang, C. C., Park, A. Y., & Guan, J. L. (2007). In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nature protocols, 2*(2), 329–333. https://doi.org/10.1038/nprot.2007.30

Lin, Y. H., Chiu, J. H., Tseng, W. S., Wong, T. T., Chiou, S. H., & Yen, S. H. (2006). Antiproliferation and radiosensitization of caffeic acid phenethyl ester on human medulloblastoma cells. *Cancer chemotherapy and pharmacology, 57*(4), 525–532. https://doi.org/10.1007/s00280-005-0066-8

Marin, E. H., Paek, H., Li, M., Ban, Y., Karaga, M. K., Shashidharamurthy, R., & Wang, X. (2019). Caffeic acid phenethyl ester exerts apoptotic and oxidative stress on human multiple myeloma cells. *Investigational new drugs, 37*(5), 837–848. https://doi.org/10.1007/s10637-018-0701-y

Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods, 65*(1-2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Orth, M., Metzger, P., Gerum, S., Mayerle, J., Schneider, G., Belka, C., Schnurr, M., & Lauber, K. (2019). Pancreatic ductal adenocarcinoma: biological hallmarks, current status, and future perspectives of combined modality treatment approaches. *Radiation oncology (London, England), 14*(1), 141. https://doi.org/10.1186/s13014-019-1345-6

Papademetrio, D. L., Lompardía, S. L., Simunovich, T., Costantino, S., Mihalez, C. Y., Cavaliere, V., & Álvarez, É. (2016). Inhibition of Survival Pathways MAPK and NF-kB Triggers Apoptosis in Pancreatic Ductal Adenocarcinoma Cells via Suppression of Autophagy. *Targeted oncology, 11*(2), 183–195. https://doi.org/10.1007/s11523-015-0388-3

Prasad, R., & Katiyar, S. K. (2013). Grape seed proanthocyanidins inhibit migration potential of pancreatic cancer cells by promoting mesenchymal-to-epithelial transition and targeting NF-κB. *Cancer letters, 334*(1), 118–126. https://doi.org/10.1016/j.canlet.2012.08.003

Sarantis, P., Koustas, E., Papadimitropoulou, A., Papavassiliou, A. G., & Karamouzis, M. V. (2020). Pancreatic ductal adenocarcinoma: Treatment hurdles, tumor microenvironment and immunotherapy. *World journal of gastrointestinal oncology, 12*(2), 173–181. https://doi.org/10.4251/wjgo.v12.i2.173

Tang, H., Yao, X., Yao, C., Zhao, X., Zuo, H., & Li, Z. (2017). Anti-colon cancer effect of caffeic acid p-nitro-phenethyl ester in vitro and in vivo and detection of its metabolites. *Scientific reports, 7*(1), 7599. https://doi.org/10.1038/s41598-017-07953-8

Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T., & Cao, D. (2021). ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic acids research, 49*(W1), W5–W14. https://doi.org/10.1093/nar/gkab255

Yu, H. J., Shin, J. A., & Cho, S. D. (2023). Inhibition of focal adhesion kinase/paxillin axis by caffeic acid phenethyl ester restrains aggressive behaviors of head and neck squamous cell carcinoma in vitro. *Archives of oral biology, 146,* 105611. https://doi.org/10.1016/j.archoralbio.2022.105611

Yu, H. J., Shin, J. A., Yang, I. H., Won, D. H., Ahn, C. H., Kwon, H. J., Lee, J. S., Cho, N. P., Kim, E. C., Yoon, H. J., Lee, J. I., Hong, S. D., & Cho, S. D. (2017). Apoptosis induced by caffeic acid phenethyl ester in human oral cancer cell lines: Involvement of Puma and Bax activation. *Archives of oral biology, 84,* 94–99. https://doi.org/10.1016/j.archoralbio.2017.09.024