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Original Article

Caffeic acid stimulates breast cancer death through Reactive oxygen species (ROS) formation, Caspase activation and mitochondrial membrane potential depletion

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ABSTRACT

Objectives: The aim of this study is to evaluate the potential effect of caffeic acid (CAF) on the growth of breast cancer cells, in addition to determining the contributing role of caspases, mitochondria, and oxidative status.

Methods: MCF-7 and MDA-MB-468 breast cancer cells were exposed to varying concentrations of CAF for different periods of time. The potential cytotoxic effect was measured using the MTT assay. The activities of caspase 3 and caspase 8, as well as the cellular level of reactive oxygen species (ROS) and the level of mitochondrial membrane potential (Δψm), were evaluated in different groups of cells.

Results: The findings showed that CAF decreased the percentage of MCF-7 and MDA-MB-468 cells in a manner that depended on the dose and duration of exposure. The death of breast cancer cells induced by CAF was associated with an increase in ROS level in both cell lines. The decrease in mitochondrial membrane potential (Δψm) following CAF treatment suggests that mitochondrial dysfunction may be involved in the death of breast cancer cells induced by CAF. Importantly, the activity of caspase 8 increased after treatment, indicating the potential involvement of the extrinsic apoptosis pathway in the inhibition of breast cancer cell growth by CAF. The dosage of 20µm of CAF following 48 hours of incubation appeared to have the most significant impact on breast cancer cells.

Conclusion: The study highlights the potential pro-apoptotic effect of CAF in both estrogen-positive and estrogen-negative breast cancer cells. This, in conjunction with other evidence, may lead to new insights for more effective therapeutic approaches in breast cancer.

Keywords: Caffeic acid, Cytotoxicity, Reactive oxygen species, Caspase 3, Caspase 8, mitochondrial membrane potential (Δψm).

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Introduction

Breast cancer, one of the leading causes of cancer-related deaths in women, originates from the epithelial lining of medium to large ducts (ductal type) or the epithelium of the terminal ducts of lobules (lobular type), and can be invasive or non-invasive (1). Epidemiological studies have shown that genetic, environmental, and hormonal factors play a significant role in the development of this cancer (2, 3). Prognostic factors such as the patient's age, early stage diagnosis, absence of nodal involvement, and the presence of estrogen and progesterone receptors in cancer tumors are important for diagnosis and disease management (4-6). Despite extensive research, there is still a need to develop effective pharmacological compounds to control cancer cells and inhibit their growth (7, 8). Cancer cell homeostasis relies on the balance between cell proliferation and apoptosis (9, 10). Apoptosis, a programmed cell death process, diminishes during the development and spread of cancer cells (11). Understanding the molecular mechanism of apoptosis and identifying the biochemical factors involved in this process can be an effective molecular tool for evaluating the sensitivity and significance of cancer prevention compounds (12, 13). There is an increasing need to identify compounds with high efficiency and specificity to suppress cancer cell growth with minimal side effects (12, 14). In this context, natural compounds such as caffeic acid have been considered and recommended as potential anti-cancer agents. Caffeic acid (CAF), an organic compound classified as a hydroxycinnamic acid, contains two functional groups, phenolic and acrylic (15, 16). It mediates lignin biosynthesis and is present in many plants, obtained from the hydroxylation of coumaric acid and has been used due to its medicinal features (17). It was evident that CAF has bioactivity with anti-inflammatory and anti-oxidant properties, and it exhibits immunomodulatory effects by down-regulating nuclear factor kappa B (NF-κB) and inflammatory cytokines (18, 19). Research demonstrated that the intake of CAF by prostate cancer patients was associated with a reduced risk of cancer progression (20). Additionally, studies showed that CAF induced apoptosis in lung cancer cells (H1299 and A549 cells) through mitogen-activated protein kinases (MAPK) signaling (21). Moreover, CAF demonstrated an inhibitory effect on the proliferation and migration of cancer cells, which may occur through caspase activation (22). Therefore, a more comprehensive investigation of the molecular mechanisms of caffeic acid's effects on various cancer cells could further demonstrate the anticancer potential of this compound and enhance the possibility of its medicinal applications. Consequently, this study aims to investigate the effect of different doses of CAF on the growth of breast cancer cells, both estrogen receptor positive and negative, as well as its impact on the activity of caspases 3 and 8, intracellular ROS levels, and mitochondrial membrane potential.

Material and Methods

Cell culture

The human breast cancer cell lines MCF-7 and MB-MDA-468 were acquired from the Pasture Institute of Iran and cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum, 100 U/ml of penicillin, and 100 µg/ml of streptomycin. The cells were maintained at 37 ºC with 5% CO2 and 100% humidity, and regularly checked for transparency and absence of bacteria and fungi. When the cells reached 80% confluence, they were washed with PBS, detached using trypsin, and then incubated in culture medium containing FBS to neutralize the trypsin. After centrifugation and counting, the cells were transferred to new flasks.

Cell viability assay

The MTT assay was used to assess the cytotoxicity of CAF on breast cancer cells. MCF-7 and MDA-MB-468 cells were seeded at a density of 5×10^3 cells/well in 96-well plates containing RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/ml of penicillin, and 100 µg/ml of streptomycin. The cells were treated with various concentrations of CAF (1, 5, 10, and 20 µm) and incubated for different time periods (12, 24, and 48 hours). After the specified incubation time, 20 µl of MTT (5mg/ml in PBS) was added to each well and incubated for 4 hours at 37 ºC. The cell supernatant was removed, and 200 µl of dimethylsulfoxide (DMSO) was added and incubated for 15 minutes. The absorbance of each well was measured using a microplate reader (Bio-Rad, Hercules, CA, USA) at 580 nm, and the cell viability was calculated by comparing the absorbance of the treated groups with the control group.

Caspase-3 activity assay

The Caspase-3 colorimetric Assay Kit (BioVision Inc. Milpitas, CA USA) was utilized to assess caspase 3 activity in MCF-7 and MDA-MB-468 cells treated with CAF. In brief, the cells were treated with 5, 10, and 20 µm of CAF and incubated for 48 hours. Following incubation, the cells were lysed using cold lysis buffer. After centrifugation, each cell group was treated with 50 µM of reaction buffer containing 10mm DTT and 5 µM of DEVD-pNA substrate (200 µM) and incubated at 37 ºC for 120 min. The emission of each cell group was then measured at 405 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Caspase-8 activity assay

For the measurement of Caspase-8 activity, the Caspase-8 Detection Kit (BioVision Inc. Milpitas, CA USA) was employed. The cells were treated with 5, 10, and 20 µm of CAF and incubated for 48 hours. Then, 1 µl of FITC-IETD-FMK was added to each cell group and incubated for 1 hour. Notably, FITC-IETD-FMK is a cell-permeable compound that serves as a labeled inhibitor of caspase 8 in viable cells. Subsequently, the cells were centrifuged, suspended in wash buffer, and the fluorescence intensity was measured at an excitation of 485 nm and emission of 535 nm using a microplate reader.

Intracellular reactive oxygen species (ROS) level assay

The fluorescent probe 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) is utilized to measure the level of intracellular ROS formation, including hydrogen peroxide (H2O2), hydroxyl radical (OH•), and hydroperoxides (ROOH). To conduct the assay, cells were cultured in a 96-well plate and treated with DCFH-DA (20µM) in the culture medium, followed by incubation at 37°C for 45 minutes. After the incubation period, the cells were treated with 5, 10, and 20 µm of CAF and incubated for 48 hours. The level of ROS was then determined based on the fluorescence generated and detected using a microplate reader (BioTek Synergy Ht, Winooski, Vermont, USA) at 485 nm and 528 nm.

The mitochondrial membrane potential (Δψm) assay

The JC-1 staining method was utilized to assess the potential involvement of mitochondria after the cells were exposed to CAF. JC-1 is a lipophilic and fluorescent dye capable of accumulating in healthy and intact mitochondria, forming red-fluorescent "j-aggregates." In the absence of aggregation, it emits green fluorescence, and a decrease in the green/red fluorescence ratio indicates mitochondrial depolarization. To conduct the experiment, cells were treated with 5, 10, and 20 µm of CAF and incubated for 48 hours. After removing the culture medium, the cells were exposed to 10 µl of staining solution and incubated for 15 minutes at 37°C. Subsequently, the fluorescent intensity was measured using a microplate reader (BioTek Synergy Ht, Winooski, Vermont, USA) at 485 nm excitation/ 528 nm emission (green) and 530 nm excitation/ 590 nm emission (red), and the calculated ratio was determined.

Statistical analysis

The cell culture data was evaluated using non-parametric one-way analysis of variance (ANOVA) with Dunnett's post hoc test and Tukey´s post hoc. The cell line experiments were conducted in triplicate and repeated at least three times to ensure specificity and accuracy. Graph Pad Prism version 6 (Graph Pad Software, San Diego California) and Statistical Package for Social Science (SPSS v.20) were utilized for statistical calculations. Results were presented as mean±SD and significance was determined for $P<0.05$, $P<0.01$, and $P<0.001$, indicated by asterisks in the corresponding figures.

Results

Caffeic acid reduced breast cancer cell viability in a dose and time-dependent manner

To determine the cytotoxic effect of CAF on breast cancer cells, MCF-7 and MDA-MB-468 cells were treated with 1, 5, 10, and 20 µm of CAF and incubated for 12, 24, and 48 hours. The percentage of viable cells in different time intervals was determined using the MTT assay. According to the results, the percentage of MCF-7 cells following exposure to CAF decreased significantly compared to the control group (Fig. 1.A). It was evident that by increasing the dose of CAF and extending the incubation time, the number of viable cells decreased significantly. A similar pattern of cell death was detected in MDA-MB-468 cells, and a significant reduction in the percentage of viable cells was detected following CAF treatment. This reduction occurred in a time and dose-dependent manner (Fig.1.B).

Caffeic acid induced the activity of caspase-3 and 8 in breast cancer cells

In relation to the crucial role of caspase enzymes as effectors that lead cells to apoptosis, the potential impact of CAF on caspase 3, a key player in apoptosis that interacts with caspase 9 and 8, as well as caspase 8 as a significant effector of the extrinsic pathway of apoptosis, was assessed. The findings revealed that different concentrations of CAF did not have a significant effect on the activity of caspase 3 in MCF-7 cells (Fig. 2.A). However, treatment of MDA-MB-468 cells with 5, 10, and 20 µm of CAF for 48 hours resulted in the induction of caspase 3 activity in a dose-dependent manner (Fig.2.B), with the highest activity of caspase 3 detected following treatment with 20 µm of CAF ($P<0.0001$

). It was also found that CAF induced the activity of caspase 8 in MCF-7 cells in a dose-dependent manner (Fig.2.C). The data showed that following treatment of MCF-7 cells with 5 ($P=0.001$), 10 ($P<0.0001$), and 20 µm ($P<0.0001$) of CAF, the activity of caspase 8 significantly increased. Additionally, treatment of MDA-MB-468 cells with 5 ($P=0.048$), 10 ($P=0.0087$), and 20 µm ($P=0.009$) of CAF resulted in a significant induction of caspase 8 activity in a concentration-dependent manner (Fig.2.D).

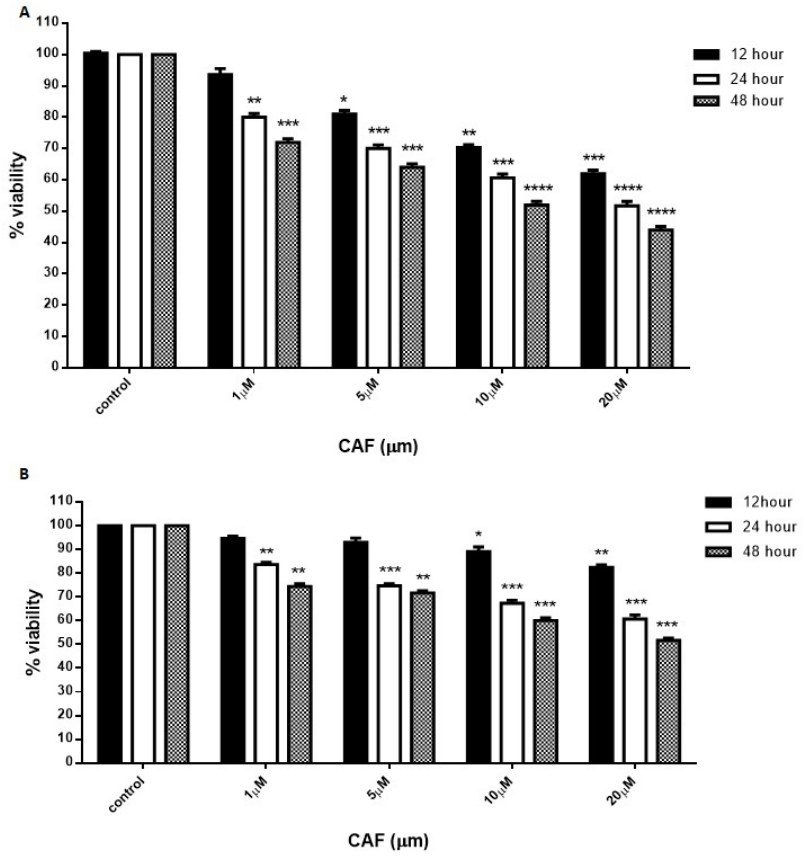

Figure 1: CAF reduced the viability of breast cancer cells

MCF-7 and MDA-MB-468 cells were exposed to varying concentrations of CAF (1, 5, 10, and 20 µm) for different time periods (12, 24, and 48 hours), and the resulting cell death rates were assessed using the MTT assay. The Mean±SD values from three separate experiments are provided. Statistical differences between groups were analyzed and indicated by asterisks on the bars (*= P <0.05, **= P <0.01, ***= P <0.001, ****= P<0.0001).

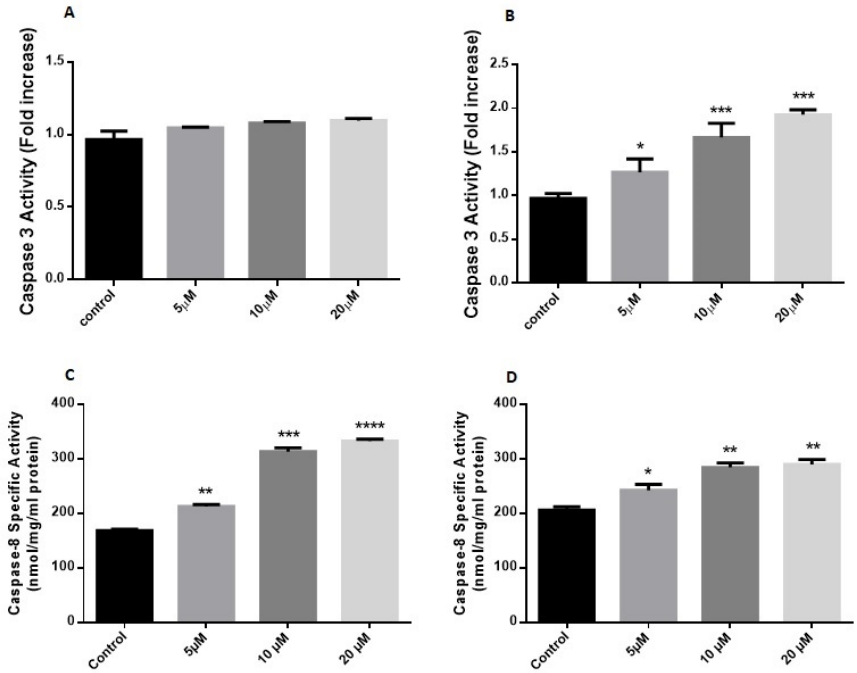

Figure 2: CAF induced the activity of caspase 3 and caspase 8 in breast cancer cells

After treating cells with 5, 10, and 20 µm of CAF for 48 hours, the activity of caspase 3 and caspase 8 were measured The data represents the mean±SD of three separate experiments, and statistical differences between groups were analyzed and indicated by asterisks on the bars (**= P <0.01 and ***= P <0.001).

Caffeic acid induced mitochondrial membrane potential (ΔΨm) depletion

In order to determine the possible involvement of mitochondria in the CAF-induced breast cancer cell death, MCF-7 and MDA-MB-468 cells were treated with 5, 10, and 20 µm of CAF for 48 hours and JC-1 staining was performed to track changes in mitochondrial membrane potential. Based on data, CAF reduced the level of mitochondrial membrane potential significantly in a concentration of 20 µm (P=0.023) (Fig 3.A). Additionally, CAF decreased the level of mitochondrial membrane potential significantly in a concentration of 20 µm (P=0.049) in MDA-MB-468 cells (Fig. 3.B).

Caffeic acid increased ROS formation in breast cancer cells

The data revealed a significant increase in ROS levels following treatment of MCF-7 cells with 5 (P=0.008), 10 (P=0.003), and 20 µm (P=0.038) of CAF after 48 hours of incubation (Fig. 4.A). Similarly, there was a significant increase in ROS levels following treatment of MDA-MB-468 cells with 5 (P=0.013), 10 (P=0.009), and 20 µm (P=0.007) of CAF after 48 hours of incubation (Fig. 4.B).

Discussion

Breast cancer ranks as one of the most prevalent cancers and the leading cause of death in women (23). Consequently, numerous studies have been undertaken to explore the clinical associations of various biomarkers and factors impacting the progression of this disease (7, 12). The goal of these investigations is to develop an improved drug and more effective method, with the aim of enhancing treatment through new therapeutic approaches (24). Meanwhile, CAF and its derivatives have been considered due to their herbal nature, biocompatibility, availability, and wide range of biological properties (21). The anti-inflammatory, antioxidant, and immunomodulatory bioactivities of CAF have been illustrated in previous studies (24). The potential impact of CAF on cancer cells was

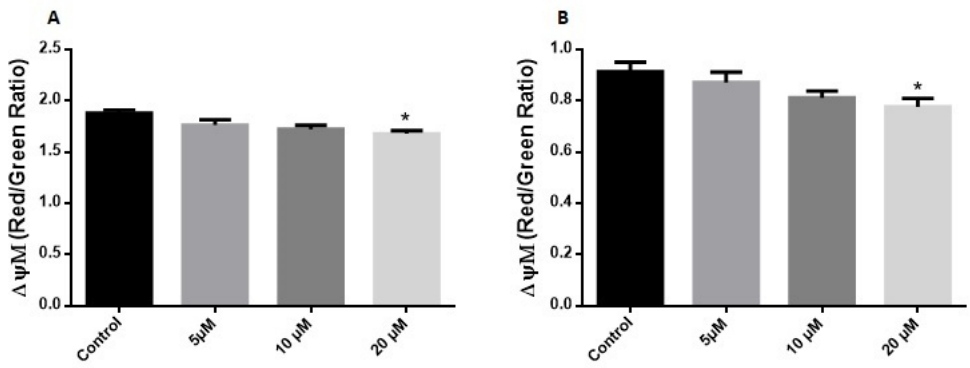

Figure 3: CAF reduced mitochondrial membrane potential (ΔΨm) in breast cancer cells.
MCF-7 and MDA-MB-468 breast cancer cells were exposed to 5, 10, and 20 µm of CAF for 48 hours, and the mitochondrial membrane potential (ΔΨm) was assessed using JC-1 staining. The data represents the mean±SD of three separate experiments, and statistical differences between groups were analyzed and indicated by asterisks on the bars (*= P <0.05).

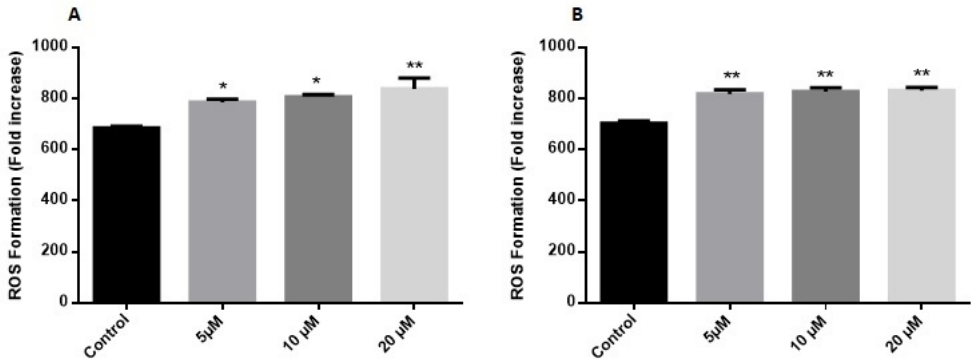

Figure 4: CAF stimulated the level of ROS in breast cancer cells.
Breast cancer cells were exposed to 5, 10, and 20 µm of CAF for 48 hours, and the level of ROS was measured using the fluorescent probe 2′, 7′-dichlorofluorescin diacetate (DCFH-DA). The data represents the mean±SD of three separate experiments, and statistical differences between groups were analyzed and indicated by asterisks on the bars (*= P <0.05**= P <0.01).

evident in the study, showing the anti-cancer effects on certain types of cancers and inhibitory effects on cancer cell invasion and migration (25). The data from the current study indicated that CAF caused cell death in both estrogen positive and negative breast cancer cells in a dose and time dependent manner. Additionally, research by Rosendahl et al. supported these findings, demonstrating that Caffeine and CAF induced breast cancer cell death, sensitized cells to tamoxifen, and suppressed their growth (26). Furthermore, CAF was found to induce cell death and apoptosis while suppressing melanin synthesis in SK-Mel-28 cells (22). The study also revealed that Caffeic acid phenyl ester inhibited oral cancer cell growth and prevented the expression of cycloxygenase-2 and EGFR, thereby mediating cancer cell progression (27). The findings also indicated that CAF treatment led to the activation of caspase 8 in MDA-MB-468 and MCF-7 cells, while the level of caspase 3 only increased in MDA-MB-468. It is well-known that caspases are endo-proteases that use cysteine-dependent hydrolysis to break down their substrates, playing a significant role in both intrinsic and extrinsic apoptosis pathways (28). The caspase-3-independent manner of cell death in MCF-7 cells has been previously explained (29), and the data supports this hypothesis. In terms of the molecular mechanism behind CAF-induced cell death in breast cancer, the potential effect of CAF on the cellular level of ROS was assessed, and the data showed a significant increase in ROS levels in both MDA-MB-468 and MCF-7 cells in a dose-dependent manner. It has been suggested that the accumulation of ROS in cancer cells triggers an apoptosis cascade and promotes cancer cell death, potentially impairing the function of subcellular organelles (30). One of the potential targets of ROS is the mitochondria, whose membrane may be affected by elevated ROS levels, leading to a decrease in its membrane potential ($\Delta\psi m$) and resulting in the suppression of cancer cell growth (31). The data demonstrated that CAF induced a depletion in the mitochondrial membrane potential in both MCF-7 and MDA-MB-468 cells in a concentration-dependent manner. According to the findings of this study, there was no notable contrast in the reaction of estrogen receptor-positive and negative cells to CAF, in terms of survival rate, caspase 8 activity, ROS levels, and mitochondrial membrane potential. Therefore, its pro-apoptotic effect may occur through an estrogen-receptor-independent pathway, but further mechanistic studies are necessary to clarify this aspect.

Conclusion

The findings of this study highlight the capability of CAF to trigger dose- and time-dependent cell death in two breast cancer cell lines. The results suggest that the activation of caspases 3 and 8, coupled with elevated ROS levels and reduced mitochondrial potential, may effectively hinder the growth of these cells when treated with CAF. These results suggest that the potential anti-cancer properties of CAF in breast cancer cells could lead to its biocompatible composition being effective in future treatment strategies.

Declarations

Competing interests

Authors declare that there is no conflict of interest.

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Author contributions

Ali Karami Robati: Investigation; Methodology, Zahra Shahsavari and Banafsheh Safizadeh: Validation; Methodology, Mohammad Amin Vaezi and Farzad Izak Shirian: Software; Analysis, Masoumeh Tavakoli-Yaraki: Funding acquisition; Project administration; Writing - review & editing.

References

1. Waks AG, Winer EP. Breast Cancer Treatment: A Review. JAMA. 2019;321(3):288-300. https://doi.org/10.1001/jama.2018.19323
2. Gonzalez-Angulo AM, Morales-Vasquez F, Hortobagyi GN. Overview of resistance to systemic therapy in patients with breast cancer. Adv Exp Med Biol. 2007;608:1-22. https://doi.org/10.1007/978-0-387-74039-3_1
3. Jafari-Hafshejani F, Shahanipour K, Kazemipiur M, Seif F, Jafari A, Behbahani MN, et al. Curcumin Attenuates Oxidative Stress-Induced Effects on TGF-β Expression and NF-κB Signaling in Bovine Aortic Endothelial Cells. Acta Biochim Iran. 2023;1(2):90-95. https://doi.org/10.18502/abi.v1i2.14106
4. Guarneri V, Conte PF. The curability of breast cancer and the treatment of advanced disease. Eur J Nucl Med Mol Imaging. 2004;31 Suppl 1:S149-61. https://doi.org/10.1007/s00259-004-1538-5
5. Ejlertsen B. Adjuvant chemotherapy in early breast cancer. Dan Med J. 2016;63(5).
6. Meshkani R, Saberi H, MohammadTaghvaei N, Tabatabaiefar MA. Estrogen receptor alpha gene polymorphisms are associated with type 2 diabetes and fasting glucose in male subjects. Mol Cell Biochem. 2012;359:225-233. https://doi.org/10.1007/s11010-011-1017-9
7. Yarahmadi S, Abdolvahabi Z, Hesari Z, Tavakoli-Yaraki M, Yousefi Z, Seiri P, et al. Inhibition of sirtuin 1 deacetylase by miR-211-5p provides a mechanism for the induction of cell death in breast cancer cells. Gene. 2019;711:143939. https://doi.org/10.1016/j.gene.2019.06.029
8. Jahangard RSSE, Vatannejad A, Meshkani R. Autophagy

protects peripheral blood mononuclear cells from high glucose-induced inflammation and apoptosis. Acta Biochim Iran. 2023;1(1):40-49. https://doi.org/10.18502/abi.v1i1.14064
9. Kashyap D, Garg VK, Goel N. Intrinsic and extrinsic pathways of apoptosis: Role in cancer development and prognosis. Adv Protein Chem Struct Biol. 2021;125:73-120. https://doi.org/10.1016/bs.apcsb.2021.01.003
10. Gorgani-Firuzjaee sM, R. Resveratrol reduces high glucose-induced de-novo lipogenesis through mTOR mediated induction of autophagy in HepG2 cells. Acta Biochim Iran. 2023;1(1):32-39. https://doi.org/10.18502/abi.v1i1.14063
11. Barmoudeh Z, Shahraki MH, Pourghadamyari H, Borj MR, Doustimotlagh AH, Abbaszadeh-Goudarzi K. C1q tumor necrosis factor related proteins (CTRPs) in patients with cardiovascular diseases. Acta Biochim Iran. 2023;1(1):12-19. https://doi.org/10.18502/abi.v1i1.14060
12. Salimi V, Shabani M, Nourbakhsh M, Tavakoli-Yaraki M. Involvement of 15-lipoxygenase-1 in the regulation of breast cancer cell death induced by sodium butyrate. Cytotechnology. 2016;68(6):2519-2528. https://doi.org/10.1007/s10616-016-9972-3
13. Ghahremani H, Bahramzadeh A, Bolandnazar K, Emamgholipor S, Hosseini H, Meshkani R. Resveratrol as a potential protective compound against metabolic inflammation. Acta Biochim Iran. 2023;1(2):50-64. https://doi.org/10.18502/abi.v1i2.14101
14. Salimi V, Tavakoli-Yaraki M, Mahmoodi M, Shahabi S, Gharagozlou MJ, Shokri F, et al. The Oncolytic Effect of Respiratory Syncytial Virus (RSV) in Human Skin Cancer Cell Line, A431. Iran Red Crescent Med J. 2013;15(1):62-7. https://doi.org/10.5812/ircmj.4722
15. Prasad NR, Karthikeyan A, Karthikeyan S, Reddy BV. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Mol Cell Biochem. 2011;349(1-2):11-9. https://doi.org/10.1007/s11010-010-0655-7
16. Goodarzi G, Tehrani SS, Panahi G, Bahramzadeh A, Meshkani R. Combination therapy of metformin and p-coumaric acid mitigates metabolic dysfunction associated with obesity and nonalcoholic fatty liver disease in high-fat diet obese C57BL/6 mice. J Nutr Biochem. 2023;118:109369. https://doi.org/10.1016/j.jnutbio.2023.109369
17. Arciszewska Ż, Gama S, Kalinowska M, Świderski G, Świsłocka R, Gołębiewska E, et al. Caffeic Acid/Eu(III) Complexes: Solution Equilibrium Studies, Structure Characterization and Biological Activity. Int J Mol Sci. 2022;23(2). https://doi.org/10.3390/ijms23020888
18. Kinra M, Arora D, Mudgal J, Pai KSR, Rao CM, Nampoothiri M. Effect of Caffeic Acid on Ischemia-Reperfusion-Induced Acute Renal Failure in Rats. Pharmacology. 2019;103(5-6):315-319. https://doi.org/10.1159/000497474
19. Zamani-Garmsiri F, Emamgholipour S, Rahmani Fard S, Ghasempour G, Jahangard Ahvazi R, Meshkani R. Polyphenols: potential anti-inflammatory agents for treatment of metabolic disorders. Phytother Res. 2022;36(1):415-432. https://doi.org/10.1002/ptr.7329
20. Russo GI, Campisi D, Di Mauro M, Regis F, Reale G, Marranzano M, et al. Dietary Consumption of Phenolic Acids and Prostate Cancer: A Case-Control Study in Sicily, Southern Italy. Molecules. 2017;22(12). https://doi.org/10.3390/molecules22122159
21. Min J, Shen H, Xi W, Wang Q, Yin L, Zhang Y, et al. Synergistic Anticancer Activity of Combined Use of Caffeic Acid with Paclitaxel Enhances Apoptosis of Non-Small-Cell Lung Cancer H1299 Cells in Vivo and in Vitro. Cell Physiol Biochem. 2018;48(4):1433-1442. https://doi.org/10.1159/000492253
22. Pelinson LP, Assmann CE, Palma TV, da Cruz IBM, Pillat MM, Mânica A, et al. Antiproliferative and apoptotic effects of caffeic acid on SK-Mel-28 human melanoma cancer cells. Mol Biol Rep. 2019;46(2):2085-2092. https://doi.org/10.1007/s11033-019-04658-1
23. Katsura C, Ogunmwonyi I, Kankam HK, Saha S. Breast cancer: presentation, investigation and management. Br J Hosp Med (Lond). 2022;83(2):1-7. https://doi.org/10.12968/hmed.2021.0459
24. Ben-Dror J, Shalamov M, Sonnenblick A. The History of Early Breast Cancer Treatment. Genes (Basel). 2022;13(6). https://doi.org/10.3390/genes13060960
25. Duan J, Xiaokaiti Y, Fan S, Pan Y, Li X, Li X. Direct interaction between caffeic acid phenethyl ester and human neutrophil elastase inhibits the growth and migration of PANC-1 cells. Oncol Rep. 2017;37(5):3019-3025. https://doi.org/10.3892/or.2017.5516
26. Rosendahl AH, Perks CM, Zeng L, Markkula A, Simonsson M, Rose C, et al. Caffeine and Caffeic Acid Inhibit Growth and Modify Estrogen Receptor and Insulin-like Growth Factor I Receptor Levels in Human Breast Cancer. Clin Cancer Res. 2015;21(8):1877-87. https://doi.org/10.1158/1078-0432.CCR-14-1748
27. Michaluart P, Masferrer JL, Carothers AM, Subbaramaiah K, Zweifel BS, Koboldt C, et al. Inhibitory effects of caffeic acid phenethyl ester on the activity and expression of cyclooxygenase-2 in human oral epithelial cells and in a rat model of inflammation. Cancer Res. 1999;59(10):2347-52.
28. Kumar S, Dorstyn L, Lim Y. The role of caspases as executioners of apoptosis. Biochem Soc Trans. 2022;50(1):33-45. https://doi.org/10.1042/BST20210751
29. Kagawa S, Gu J, Honda T, McDonnell TJ, Swisher SG, Roth JA, et al. Deficiency of caspase-3 in MCF7 cells blocks Bax-mediated nuclear fragmentation but not cell death. Clin Cancer Res. 2001;7(5):1474-80.
30. Luo Z, Xu X, Sho T, Zhang J, Xu W, Yao J, et al. ROS-induced autophagy regulates porcine trophectoderm cell apoptosis, proliferation, and differentiation. Am J Physiol Cell Physiol. 2019;316(2):C198-c209. https://doi.org/10.1152/ajpcell.00256.2018
31. Yuan LQ, Wang C, Lu DF, Zhao XD, Tan LH, Chen X. Induction of apoptosis and ferroptosis by a tumor suppressing magnetic field through ROS-mediated DNA damage. Aging (Albany NY). 2020;12(4):3662-3681. https://doi.org/10.18632/aging.102836