Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: www.jacb.journals.ekb.eg

Ginger Extract as Potent Anti-Cancer Agent against Breast Cancer

Mona I. M. Ibrahim[1]; Donia M. Wafaa[2] and Ghada H. El Nady[3*]

[1]Agricultural biotechnology Department. Faculty of Biotechnology, Misr University for Science and Technology (MUST).
[2]Environmental Biotechnology Department. Faculty of Biotechnology, Misr University for Science and Technology (MUST).
[3]Medical Genetic Center, Faculty of Medicine, Ain Shams University

ABSTRACT

Ginger has been renowned for its therapeutic powers, such as immuno-modulatory, antioxidant, anti-inflammation, and anti-carcinogenic qualities. It is well known that anti-cancer therapies are unable to differentiate between cancer and normal cells, that could lead to subpar therapeutic outcomes and serious side effects. In this regard, we investigated the anti-cancer features of ginger extract in MCF-7 cell line for breast cancer. In order to achieve our goal, MTT assay was carried out to study the Cell growth viability with various concentrations of ethanolic ginger extract (0, 12, 25, 50, 100 and 200 µg/ml) for 24 hours and IC50 was 49.7 µg/ml. Also, flow cytometry followed by Annexin V-FITC was carried out to study the cell cycle analysis and confirm the apoptosis induced by ginger extract. According to the IC50, the (0, 25, 50 and 100 µg/ml) concentrations for 24 hours were chosen. The cell cycle analysis showed that treatment 3 is the best concentration from ginger ethanolic extract to arrest cell at G2/M stage (37.72%) comparing to control (8.4%). Data obtained from the Annexin V-FITC revealed that there was significant difference in early and late apoptosis between control and treatment, which increase in treatments. Finally, at the molecular level, the study of the gene expression demonstrated that with increasing the ginger dose, the P53 gene expression was significantly up-regulated, and the Bcl-2 gene expression was down-regulated with significant difference. Our data demonstrated that ginger extract has the ability to promote apoptosis in MCF-7 cells.

Keywords: Ginger, Breast cancer, Apoptosis, Cell cycle analysis, Expression

INTRODUCTION

One of the leading causes of mortality is cancer. In 2022, cancer statistics predicted that there will be 1,918,030 incidence rate and 609,360 cancer-related deaths. The estimated number of new instances of breast cancer among them is 287,850 persons (31%) (Siegel *et al*., 2022).

All cancers are diseases that develop when abnormalities in a collection of healthy cells within the body cause uncontrolled growth, resulting in a lump termed a tumor. Cancer is a complex genetic disease though, environmental, chemical, physical, metabolic, and genetic factors all have an impact on how tumors originate and progress (Fresco *et al*., 2006)

Cancer is caused by a variety of reasons, but one that stands out is the disruption of the equilibrium between cell propagation and cell death, which leads to an increase in cell replication and the failure of damaged cells to be eliminated via apoptosis (Hanahan and Weinberg 2011). Apoptosis is an energetic form of cell self-destruction regulated by a complex of genes, plays a remarkable role in the pathophysiology of disorders like cancer, and is an essential operation during development (Wong, 2011).

Breast cancer is defined by the unchecked proliferation of abnormal cells in the milk-producing glands of the breast or in the channels (ducts or passage) that transport milk to the nipples (ACS, 2014).

For a long time, the standard treatment options for breast cancer were surgery, chemotherapy, and radiotherapy. Even though many of these medicines have demonstrated impressive advantages, the prevalence of illness return caused by remaining cancerous cells is still a significant issue. Additionally, the best anticancer medication is free of the typical side effects of surgery, chemotherapy, and radiotherapy and thoroughly destroys all tumor cells while causing the least amount of damage to normal cells (Xin *et al*., 2017). As a substitute medicine for the treatment of numerous diseases including cancer, there has recently been a rise of interest in the development of inexpensive, well-tolerated, and natural items that are not poisonous. For thousands of years, natural goods were the only source of medications and have significantly improved human health. In the 1950s, the U.S. National Cancer Institute recognized the promise of employing natural compounds as anticancer medicines (NCI) (Bauml *et al*., 2015).

Ginger (*Zingiber officinale*) has a long history of being used for medicinal and therapeutic purposes. Numerous health issues, including colds, swelling, high blood ulcers, diabetes, indigestion, and behavioral issues etc.. have all been treated with it as it has antiviral and antioxidant activity (Verma and Bisen 2022). These refer to the presence of compounds like gingerol, zingerone, shogaols, zingiberene, paradols, etc.,. Gingerols are the key substances in several bioactivities among these phytocompounds (Babasheikhali *et al*., 2019 and Verma and Bisen 2022).

In order to create new, low-toxic cancer prevention and treatment drugs, it may be essential to understand how dietary components affect cell proliferation and survival. Therefore, the goal of the current investigation was to determine whether whole ginger extract had any cytotoxic or anti-proliferative effects on the malignant MCF-7 cell line and to understand the effect of ginger extract on cancer cells.

* **Corresponding author.**
E-mail address: ghada.elnady@med.asu.edu.eg
DOI: 10.21608/jacb.2023.183821.1042

MATERIALS AND METHODS

Ginger Extract:

Using a magnet laboratory shaker, ethanol 96% (10:1; volume: weight) for 4 hrs, dry powdered from ginger rhizome was extracted. To obtain the ethanolic ginger extract, the alcohol was evaporated at 65 degrees Celsius (Sarami *et al*., 2020). The extraction was balanced and resolved in dimethylsulfoxide (DMSO) (Merck Company, Germany), which the maximum DMSO dilution in cultured cells did not surpass 0.1%. For each test, the extract was made fresh each test.

Cell culture and treatment

Breast cancer (MCF-7) cell line was purchased from VACSERA and maintained under standard lab conditions.

Cell growth viability assay (MTT assay)

Using MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], the cytotoxic impact of the ginger extract was investigated (Sigma, St. Louis, MO, USA). To carry out this test 5 × 103 MCF-7 cells were loaded into 96 well plate, which contains 200 µl of RPMI medium in each well, after a 24 incubation hours and letting on cells to bind, the medium was discharged delicately and displaced with fresh medium containing (our chosen concentrations of ginger extract (0.0, 12.5, 25, 50, 100 and 200 µg/mL) then 24 hours incubation at 37°C in an incubator contain 5 percentage CO2. Equal amount of the solvent (DMSO) was also added as a negative control cell. Twenty-four hours later, the medium was withdrawn then, 180 µl medium and 50 µl MTT solution was added to each well. Then after, incubate for 3 hrs at 37 °C. Dissolve blue formazan crystals in 100 µl of DMSO 30 min. after the plates have been agitated. The plate was then recorded at absorbance wavelength 570 nm using ELIZA reader Molecular Devices, Sunnyvake, USA). The experiment was duplicated three times and results were expressed as means ± SE.

The following calculation was made for cell viability: percentage of viability = [optical density of sample / optical density of control] 100 (ANWER el al., 2020).

Cell Cycle Investigation

DNA flow cytometry analysis was applied to examine each stage of the cell cycle. After being cultivated in 6-well plates for 24 hrs, MCF-7 cells were exposed to various concentrations of ginger extract (0, 25, 50, and 100 mg/mL). The ginger's doses were chosen according to the MTT results. Use of doxorubicin (0.40 mg/mL) as a positive control was performed. The cell-cycle phase distributions were determined using the Propidium Iodide Flow Cytometry kit (ab139418). Using the recommendations provided by the manufacturer, nuclei were extracted, stained with PI, and examined using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). To assess the proportions of cells in each cell-cycle phase, Modfit program (ModFit, Topsham, ME) was used (Kwan *et al*., 2016)

Assessment of apoptosis

The Annexin V-FITC Apoptosis Detection Kit (BD Pharmingen, Franklin Lakes, NJ, USA) was applied to identify cells going through apoptosis. After a 24-hour treatment period, cells including both adherent and floating cells were extracted. Following a cold PBS (pH 7.4) wash, the cells were labelled by staining and subjected to flow cytometric analysis. On a FACSCalibur flow cytometer, 1–5 x 105 cells per tube were instantly counted. Win MDI application version 2.8 was used to carry out the quantitative analysis (provided by the Flow Cytometry Core Facility, The Scripps Research Institute, La Jolla, CA, USA).

Analysis of gene expression

Total RNA was extracted from treated and control cells in accordance with the manufacturer's instructions using Qiagen RNeasy mini-Kit. A Beckman Coulter DU- 800 spectrophotometer was used to determine the levels of the isolated RNAs. The high-capacity cDNA transcription kit was used to create cDNA from ten ng of total RNA from each sample. The SYBR Green PCR Master kit (Fermentas, Thermo Fisher Scientific) was then used to amplify the cDNA in 48-well plates utilizing a Step One thermocycler - Applied Biosystems. The program included 10 minutes at 95 °C, then 40 cycles of 15 second at 95 °C, 20 second at 55 °C, and 30 second at 72 °C. Every reaction was repeated 3 times. After real-time PCR, melting curves were carried out to show the particular amplification of target individual gene product used. Amplification efficiencies of the primers used were evaluated by calibration curve using the comparative $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). According to Anwer *et al*., (2020), specific pairs of primer were used to study the expression of each of the following genes; P53 gene and Bcl-2, compared to the housekeeping β-actin gene.

Statistical Analysis

Data was analyzed using SPSS software version 16 using analysis of variance (ANOVA) and several comparisons were made with Tukey's HSD (Tukey 1949). P value less than 0.05 were considered as significant differences. The data presented as mean ±SE.

RESULTS AND DISCUSSIONS

Investigating the in vitro cytotoxicity of ginger extract:

To evaluate ginger's impact on the proliferation of the breast cancer cell line (MCF-7), the MCF-7 was treated with varying treatments of ginger extract (0,12.5, 25, 50, 100, 200 µg/ml) for 24 hrs. IC50 were plotted as shown in Figure (1), which its value is an effective dose needed to suppress the proliferative response by 50%. The viability of the cell was estimated. The IC50 estimated to be 49.7 µg/ml. The results of MTT test demonstrated that the activity of tumor cell is significantly lower after 24 hrs.

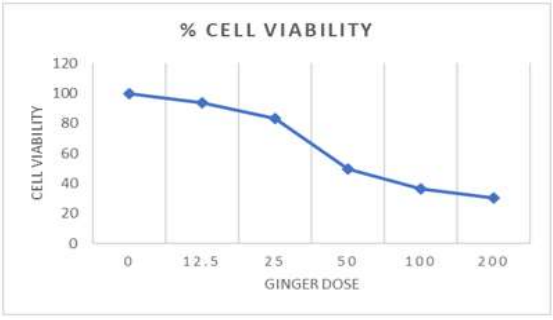


Figure 1. Cytotoxic effects of ginger extract on breast cancer (MCF-7) cell lines in 24 hours. The cell viability is expressed by percent.

Cell Cycle Investigation

The cell cycle was studied using flow cytometry when MCF-7 cells were subjected with ginger extract doses of 0, 25, 50, and 100 µg/ml for a day.

As shown in Figure 2 (A&B) and Table 1, there were significant differences in cell death in the different phases. The percentage of cell growth arrest at phase G2/M was 8.4%, 29.55%, 28.6% and 37.72% for control (C), T1, T2 and T3 respectively. While the percentage of cell death at phase pre

G1 was 2.31%, 9.57%, 16.19% and 19.43% for control (C), T1, T2 and T3 respectively. Therefore, the best concentration for ginger ethanolic extract is Treatment no. 3 (100 µg/ml) because it showed the highest percentage of growth arrest at G2/M and pre G1 phases. Increasing means that the cells lose its division ability. Numerous anti-cancer medications stop the cell cycle at the G0/G1, S, and G2/M phases before apoptosis induction (Murray, 2004 and Kwan *et al*., 2016).

Joo *et al.*, (2016) demonstrated that treatment with 10-gingerol a constitute of ginger for 24 hrs with different doses (0.1-10 µM) in breast cancer cells (MDA-MB-231) avoided the rise in G1 phase (55.3 versus 63.8%), and G2/M stage (29.8 versus 22.3%) and the reduce in S phase (14.9 versus 13.9%), these remarks recommend that 10-gingerol prohibits the conversion from G1 stage of the cell cycle to the S stage, causing in G1 arrest, which is highly associated with inhibition of cell multiplication.

Table 1. Arrested cell growth at different phases of cell cycle during the treatment. According to Tukey's multiple range comparisons, means indicated by different letters are significantly different, meanwhile those indicated by the same letters are not.

treatment/ phase	G0/G1%	S%	G2/M%	Pre-G1%
C	49.29^{a}	42.31^{a}	8.4^{a}	2.31^{a}
T1	35.18^{b}	35.27^{b}	29.55^{b}	9.57^{b}
T2	37.51^{b}	33.89^{b}	28.6^{b}	16.19^{c}
T3	27.99^{c}	34.29^{b}	37.72^{c}	19.43^{c}

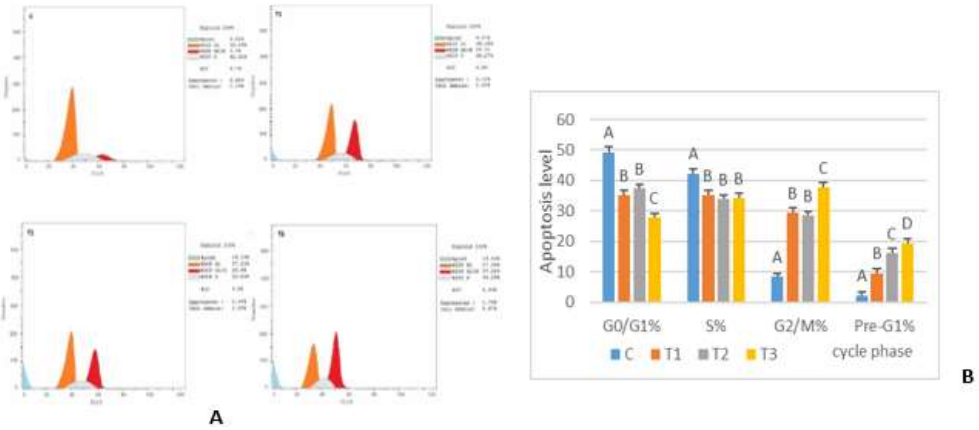


Figure 2. Propidium iodide (PI) staining was applied to assess the DNA content, and fluorescence was measured and examined. (A) Show flow cytometry for control and treated cells. (B) Chart show phases of cell cycle analysis after treatment with different ginger concentrations (C: control with 10µl ethanol, T1: Treatment 1 with 10µl of concentration 25 µg/ml, T2: Treatment 2 with 10µl of concentration 50 µg/ml, T3: Treatment 3 with 10µl of concentration 100 µg/ml) mean ±SE of the 3 replicates. p < 0.05. According to Tukey's multiple range comparisons, means indicated by different letters are significantly different, meanwhile those indicated by the same letters are not.

Apoptosis Detecting

The mechanism of cell death caused by ginger in MCF-7 cells treated with different concentrations of ginger (0, 25, 50 and 100 µg/ml) was confirmed by annexin V-FITC. As it is obviously shown in the obtained data, the apoptosis whether in early or late was increased, by increasing the ginger extract concentration apoptosis. As shown in [Table 2, Figure 3 (a & b)] showed the early apoptosis (Q4) was 1.12, 2.94, 6.33 and 6.79 in control (C), T1, T2 and T3 respectively. Whereas the late apoptosis level (Q2) 0.82, 1.19, 1.54 and 1.78 in control (C), T1, T2 and T3 respectively. Moreover, there are significant differences in the early apoptosis stage between control and treatment, which increase in treatments. That indicates that treatment 3 could be used as an anti-cancer as it causes apoptosis to the cancerous cells.

Table 2. Apoptosis stages after treatment with different ginger concentrations

Treatment/stage	Q1	Q2	Q3	Q4
C	0.37	0.82	97.69	1.12
T1	5.44	1.19	90.43	2.94
T2	8.32	1.54	83.81	6.33
T3	10.86	1.78	80.57	6.79

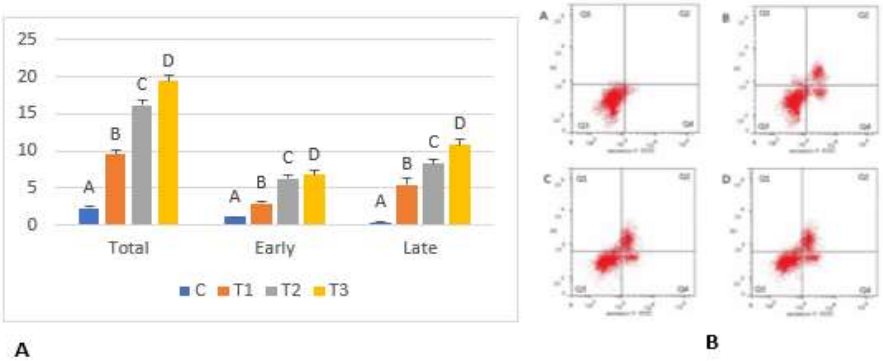


Figure 3. (A) Apoptosis stage analysis at different ginger treatment concentrations (B) Annexin V-FITC of MCF-7 cells treated with ginger extract. (C) control with 10µl ethanol, T1: Treatment 1 with 10µl of concentration 25 µg/ml, T2: Treatment 2 with 10µl of concentration 50 µg/ml, T3: Treatment 3 with 10µl of concentration 100 µg/ml). According to Tukey's multiple range comparisons, means indicated by different letters are significantly different, meanwhile those indicated by the same letters are not.

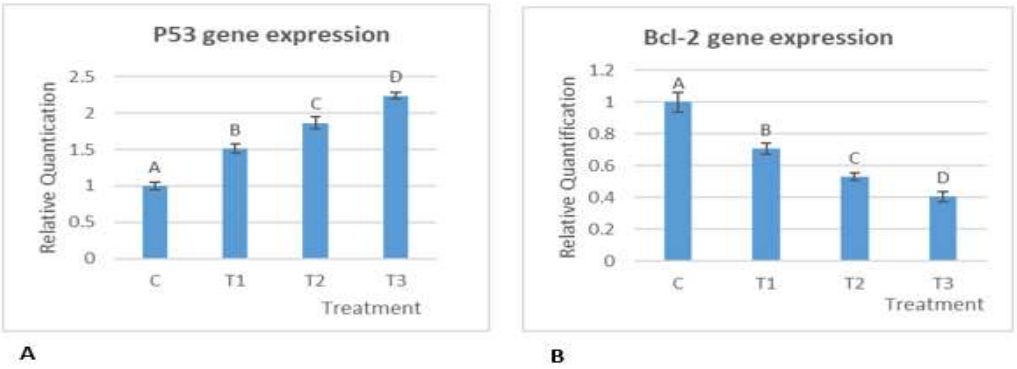
There are many factors that contribute to cancer, however one that stands out is the imbalance between cell proliferation and cell death being upset. This results in an increase in cell replication and a failure of damaged cells to be destroyed through apoptosis. Apoptosis is an active cell death process. A network of genes controls the regulation of suicide, which is a crucial process during development and has a big impact on the pathophysiology of diseases like cancer (Wong, 2011).

The regulation of cell proliferation is greatly influenced by the cell cycle and apoptosis. Cell cycle checkpoints safeguard the preservation of genomic integrity by shielding cells that divide from the potentially lethal effects of DNA damage. To prevent cells from replicating chromosomes with irreversible damage, cells may be forced to exit the cell cycle or die via apoptosis when unrepaired DNA damage is detected (Kwan *et al*., 2016). A Study conducted that ginger has an anti-proliferative effect on MCF-7 cells. This effect may be due to the activation of apoptosis, cell cycle inhibition and growth suppression. Treating MCF-7 cells with ginger for 24 hrs resulted in so obvious cell shrinkage and cellular detachment, also pro-apoptotic effect was shown as nuclear condensation and DNA fragmentation in MCF-7 (ElKady *et al.,* 2012).

Evaluation of apoptosis mechanism using real time PCR

To determine the molecular mechanism for ginger extract on apoptosis in MCF-7 cells, the mRNA expression levels of P53 and Bcl-2 genes were selected to study; P53 gene as a pro-apoptotic gene and Bcl-2 as anti-apoptotic gene.

Three doses of ginger extract (0, 25, 50, 100 µg/ml) were chosen for treatment for 24 hours after analyzing the MTT assay findings. As shown in Figure (5), Our result revealed that the expression of the gene P53 was markedly elevated, while the gene Bcl-2 was down regulated by increasing the ginger dose. In comparison with control versus treatment 3 (100 µg/ml), the P53 ratio increased from 1 to 2.239 with significant differences, while the Bcl-2 significantly decrease from 1 to 0.406.



A B

Figure 5. Relative quantification of P53 (a) and Bcl-2 (b) gene expression.

(C: control with 10µl ethanol, T1: Treatment 1 with concentration 25 µg/ml, T2: Treatment 2 with concentration 50 µg/ml, T3: Treatment 3 concentration 100 µg/ml). According to Tukey's multiple range comparisons, means indicated by different letters are significantly different, meanwhile those indicated by the same letters are not. P-value < 0.001

According to data revealed from this study, treatment 3 (100 µg/ml) is the best concentration to achieve our target.

Studies showed that apoptosis-related protein expression is regulated by ginger extract. The MCF-7 cells were exposed to ethanolic the ginger extract at the specified doses for 24 hrs. It was found that up-regulation to Bax occurred while down-regulation to Bcl-2 balancing the apoptotic event (ElKady *et al*., 2012). Moreover, Ginger displays its anticancer capabilities, by terminating cell cycle and apoptosis in transformed or malignant cells through the p53 signal pathway (Nigam *et al*., 2010). Moreover, similar research found that 6-gingerol could cause cell cycle arrest in the G1 phase and overcome medication resistance caused on by p53 gene mutation (Park *et al*., 2006). Furthermore, Ginger extract raised the P53 expression in female Swiss albino mice, according to other in vivo investigations (El-Ashmawy *et al*., 2018). These studies agree with our findings.

As a result, novel treatments with controlled toxicity are required. Previous research has shown that entire plant extracts are more efficient than their individual, purified components (Seeram *et al*., 2004). Therefore, this investigation focused on how (MCF-7) breast cancer cell line responded to crude ginger extracts in terms of cytotoxicity and apoptosis.

Additionally, a human study found that consuming two gram of ginger supplement per day for four weeks can lower the incidence of colon tumors by causing apoptosis and reducing gene expression of Bax (Citronberg *et al*., 2013).

CONCLUSION

So, according to this study and based on all the available information, the ethanolic ginger extract prohibited, in a dose- and time-dependent manner, the reproduction of the MCF-7 breast cancer cell line. The ginger anti-proliferative ability could be referred its induction of apoptosis by raising the P53 expression and decreasing the expression of the Bcl-2 gene. Therefore, ginger extract might validate to be an optimal chemo-preventive or natural drug for breast cancer. Further investigations and analysis should be conducted to provide more information.

ACKNOWLEDGEMENT

The authors like to thank the Animal & Human Tissue Culture Lab at college of biotechnology, Misr university for Science and Technology, for providing needed facilities to implement the work.

REFERENCES

ACS (2014). Major risk factors of cancer in world. American Cancer Society. https :// www. cancer. Org /research /cancer-facts-statistics/all-cancer-facts-figures /cancer -facts-figures-2014.html

ANWER M., MOHAMED A. F. and ABD EL AZIZ H. (2020). Effect of Electromagnetic Field and Allicin as Natural Extract on Hepatocellular Carcinoma (HepG2). Med. J. Cairo Univ. 88 (2): 815-825.

Babasheikhali R., S., Rahgozar S., and Mohammadi M. (2019). Ginger extract has anti-leukemia and anti-drug resistant effects on malignant cells. J Cancer Res Clin Oncol, 145(8): p. 1987-1998

Bauml, J. M., *et al*., (2015). Do attitudes and beliefs regarding complementary and alternative medicine impact its use among patients with cancer? A cross-sectional survey. Cancer, 121: (14), 2431-2438.

Citronberg, J., *et al*., (2013). Effects of ginger supplementation on cell-cycle biomarkers in the normal-appearing colonic mucosa of patients at increased risk for colorectal cancer: results from a pilot, randomized, and controlled trial. Cancer Prev Res (Phila), 6(4): p. 271-81.

El-Ashmawy, N.E., *et al*., (2018). Ginger extract adjuvant to doxorubicin in mammary carcinoma: study of some molecular mechanisms. Eur J Nutr,. 57(3): p. 981-989.

Elkady A. I., Abuzinadah O. A., Baeshen N. A., and Rahmy T. R. (2012). Differential Control of Growth, Apoptotic Activity, and Gene Expression in Human Breast Cancer Cells by Extracts Derived from Medicinal Herbs Zingiber officinale. Journal of Biomedicine and Biotechnology, Volume 2012, Article ID 614356, 14 pages doi: 10. 1155 /2012 /614356

Fresco P., Borges F., Diniz C. and Marques M. P. M. (2006). New insights on the anticancer properties of dietary polyphenols, Med. Res. Rev., 26 (6): 747-766

Hanahan D. and Weinberg R.A. (2011). "Hallmarks of cancer: the next generation," Cell, 144 (5): 646–674.

Joo, J.H., Hong S.S., Cho Y.R. and Seo D.W. (2016). 10-gingerol inhibits proliferation and invasion of MDA-MB-231 breast cancer cells through suppression of Akt and P38 MAPK activity. Oncology Reports, 35: 779-784.

Kwan Y.P., *et al*., (2016). Evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by Euphorbia hirta in MCF-7 breast cancer cells. Pharm Biol; 54(7): 1223–1236

Livak K.J. and Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-DDCT method. Methods 25:402–408

Murray A.W. (2004). Recycling the cell cycle: Cyclins revisited. Cell, 116: 221 – 34.

Nigam, N., *et al*., (2010). Induction of apoptosis by [6]-gingerol associated with the modulation of p53 and involvement of mitochondrial signaling pathway in B[a]P-induced mouse skin tumorigenesis. Cancer Chemother Pharmacol, 65(4): p. 687-96.

Park, Y.J., Wen J, Bang S. , Park S. W., and Song S. Y. (2006). [6]-Gingerol induces cell cycle arrest and cell death of mutant p53-expressing pancreatic cancer cells. Yonsei Med J, 47(5): p. 688-97.

Sarami S., Dadmanesh M., Hassan Z. M., and Ghorban K. (2020). Study on the Effect of Ethanol Ginger Extract on Cell Viability And p53 Level in Breast and Pancreatic Cancer. Archives of Pharmacy Practice 11 (3): 115 – 121.

Seeram, N.P., Adams L. S., Hardy M.L. and Heber D. (2004). Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. J Agric Food Chem, 52(9): p. 2512-7.

Siegel, R.L., Miller, K.D., Fuchs, H.E. and Jemal, A. (2022). Cancer statistics, 2022. CA Cancer J. Clin., 72, 7–33. [CrossRef]

Tukey J. (1949). Comparing individual means in the analysis od variance. Biometrics 5 (2): 99 – 114.

Verma R. and Bisen P. S. (2022). Ginger–a potential source of therapeutic and pharmaceutical compounds. J. Food Bioact.;18:67–76

Wong R.S. (2011). "Apoptosis in cancer: from pathogenesis to treatment," Journal of Experimental Clinical Cancer Research, vol. 30, no. 1, article 87

Xin, Y., Yin M., Zhao L., Meng F., and Luo L. (2017). Recent progress on nanoparticle-based drug delivery systems for cancer therapy. Cancer Biol Med, 14 (3): p. 228- 241.

مستخلص الزنجبيل كمضاد فعال لسرطان الثدي

منى ابراهيم محمد ابراهيم[1] ، دنيا محمد وفا[2] و غادة حمدى محمود النادى[3]

[1]قسم التكنولوجيا الحيوية الزراعية - جامعة مصر للعلوم والتكنولوجيا
[2]قسم التكنولوجيا الحيوية البيئية – جامعة مصر للعلوم والتكنولوجيا
[3]مركز علاج وبحوث الامراض الوراثية – كلية الطب – جامعة عين شمس

الملخص

من المعروف أن طرق العلاج الكيميائى المضادة للسرطان غير قادرة على التمييز بين الخلايا السرطانية والخلايا السليمة ، مما قد يؤدي إلى نتائج علاجية دون المستوى وآثار جانبية خطيرة. في هذا الصدد ، قمنا باختبار مستخلص الزنجبيل كمضاد للسرطان فى خط خلايا MCF-7 الخاصة بسرطان الثدي. ولتحقيق هدفنا ، تم إجراء اختبار السمية MTT بتركيزات مختلفة من مستخلص الزنجبيل الإيثانولي (0، 12.5، 25، 50، 100، 200 ميكروجرام/مل) واضيف الى خط خلايا MCF-7 ووجد ان ال IC50 لمستخلص الزنجبيل الإيثانولي كان 49.7 ميكروجرام / مل. وبناءا عليه قد تم اختيار التركيزات (0، 25، 50، 100 ميكروجرام/ مل) لاستكمال الدراسة. وقد تم قياس التدفق الخلوي متبوعًا بـ Annexin V-FITC لدراسة تحليل دورة انقسام الخلية وتأكيد موت الخلايا المبرمج الناجم عن مستخلص الزنجبيل. وقد أظهرت نتائجه أن المعاملة 3 (100 ميكروجرام / مل) هي أفضل تركيز من مستخلص الزنجبيل الإيثانولي إلى زيادة نسبة التوقف عن الانقسام بنسبة في مرحلة G2 / M (37.72٪) مقارنة بالخلايا المقارنة (الكونترول) (8.4٪). بينما كشفت البيانات التي تم الحصول عليها من Annexin V-FITC أن هناك فرقًا معنويًا في موت الخلايا المبرمج في المرحلة المبكرة من موت الخلايا المبرمج بين الخلايا المعاملة والغير معاملة (الكونترول)، حيث يزيد الموت المبرمج فى الخلايا مع زيادة تركيز الزنجبيل. ولمناقشة الآلية الجزيئية التي تجعل خط خلية MCF-7 يخضع لموت الخلايا المبرمج ، تم تقييم التعبير الجينى للجينين P53 و Bcl-2. وقد أظهرت النتائج أنه مع زيادة جرعة الزنجبيل، ارفاع التعبير الجيني ل P53 بشكل كبير، وانخفاض التعبير الجيني ل Bcl-2. أظهرت البيانات ككل أن مستخلص الزنجبيل لديه القدرة على حث موت الخلايا المبرمج في خلايا MCF-7.