Original Article

Cytotoxic Effects Of Gallic Acid In Human Breast Cancer Cell Mda-Mb-231

Safa A. Yahya[1]*, Firas A. Hassan[2]

*[1,2]Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq.

***Corresponding author:** Safa A. Yahya

*Department of Chemistry, College of Science, Al-Nahrain University, Baghdad, Iraq. E-mail: safaammer91@gmail.com, Tel: +9647513863683

Date Of Publication 22 October, 2022

DOI: 10.47750/pnr.2022.13.S08.166

Abstract

Background: Gallic acid is a naturally occurring phenolic molecule with antitumor effects on most forms of cancer. The impact of Gallic acid on MDA-MB-231 breast cancer cells was investigated in the current study.

Methods: The cytotoxic effects of Gallic acid on MDA-MB-231 cells were investigated using caspase-8&9 activity and high content screening assays. Apoptosis mechanisms following Gallic acid treatment were also investigated.

Results: Gallic acid have had greatest cytotoxic impact on MDA-MB-231 cells at a half-maximal inhibitory concentration (IC50) of 43.86 μg/mL. When MDA-MB-231 cells were treated with 200 μg/mL of Gallic acid, a substantial increase in caspase-8&9 activity was also detected. In addition, Gallic acid (200 μg/mL) increased cell membrane permeability, cytochrome c level, and nuclear intensity compared to untreated cells. Gallic acid (200 μg/mL) treatment resulted in a significant reduction in cell viability and mitochondrial membrane permeability compared to untreated cells serving as the negative control.

Conclusions: High-content screening and caspase-8&9 activity tests indicated that Gallic acid was cytotoxic to MDA-MB-231 cells i*n vitro*. Gallic acid has shown as a potential chemopreventive agent for triple-negative breast cancer.

Keywords: Gallic acid, breast cancer, cytotoxic effect, MDA-MB-231.

INTRODUCTION:

Breast cancer is a significant life-threatening disease for women in the majority of Asian nations, and the prevalence is rising more rapidly than in western countries, possibly due to changes in lifestyle and food [1]. Despite the enormous progress that has been achieved in surgery, radiotherapy, and targeted therapy, drug resistance and metastases in this kind of cancer continue to pose significant challenges. Therefore, there is a significant demand for new effective breast cancer treatments [2]. Many effective anti-cancer medications today are derived from natural substances or their analogues, and many more are undergoing clinical studies [3]. Gallic acid (3, 4, 5-trihydroxyl-benzoic acid) is a polyhydroxyl phenolic substance that is widely distributed in fruits, gallnuts, green tea, and oak bark [4–6]. Gallic acid has been shown to have a wide range of biological actions, including antibacterial properties [7] and anti-inflammatory activities [8]. Various biological actions of Gallic acid, including antiviral, antibacterial, and anti-inflammatory properties, have been described to date. However, the primary interest in Gallic acid is to its anti-tumor activity, which has been found in a range of cancer cells [9, 10]. Gallic acid has been studied as a possible anticancer agent in several human cancer cell lines, including MKN-28 (gastric cancer), TE-2 (esophageal cancer), HT-29 and Colo201 (colon cancer), CaSki (lung cancer) (cervix cancer), and MCF-7 (breast cancer), [11]. Programmed cell death, also known as apoptosis, is an essential evolutionarily conserved process for the formation and preservation of cellular homeostasis in multicellular animals that is disrupted in cancer cells [12]. Apoptosis can result in alterations to cell properties. These modifications include caspase activation, mitochondrial depolarization, cell volume reduction, and DNA breakage [13]. Caspases may cleave a variety of proteins in different cell types [14]. These pathways control the on/off state of other proteins. In addition to their role in apoptotic cell death, caspases have a role in biochemical processes such as nuclear fragmentation, chromatin condensation, the production of active signaling molecules, and cellular contraction [15, 17]. Caspases are characterized principally according to their involvement in apoptosis and their method of action. Caspases-8 and -9 are initiator caspases, whereas caspases-3, -6, and -7 are executioner caspases [18]. Here, we examined the cytotoxic effects of Gallic acid on MDA-MB-231 breast cancer cells and the anticancer activity of Gallic acid on triple-negative breast cancer cells.

MATERIALS AND METHODS:

Cell culture

From the American Type Culture Collection, we obtained the MDA-MB-231 breast cancer cell line. At 37 degrees Celsius in a 5% CO2 atmosphere. Cells were grown in Dulbecco's Modified Eagle medium (Life Technologies, Inc., Rockville, MD, USA) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), 1% each of streptomycin and penicillin, and 2 mmol/L of glutamine [19].

Biological activity

Gallic acid was purchased from Sigma (St Louis, Missouri, USA). Tests using the MTT method were carried out at the Biotechnology Research Centre Al-Nahrain University in Baghdad/Iraq, during the months of October 2021 and January 2022. At the Natural Product Research and Drug Discovery Centre, located in the Department of Pharmacology, Faculty of Medicine, University of Malaya in Kuala Lumpur, Malaysia, tests for caspase-8 and 9 as well as high content screening (HCS) were carried out between the months of November 2021 and February 2022. The MTT and HCS conducted as previously reported [20].

Cytotoxicity assay (MTT assay)

Comply with the manufacturer's directions for installation [21]. 1 x 10^4 to 1 x 10^6 cells mL-1 were cultured to a final volume of 200 mL well-1 in 96-well plates. Plates were covered with sterile parafilm, mixed gently, and incubated for 24 hours at 37 ° C, 5% CO 2 with gentle stirring. Following incubation, the medium was withdrawn and 200 mL of a 2-fold serial dilution of Gallic acid (25, 50, 100, 200 µg/mL) was added to each well. At each concentration and control, triplicate tests were conducted. 48 hours were spent incubating the plates at 37 °C and 5% carbon dioxide. Following extract exposure, 10 mL of MTT solution was applied to each well. Plates were incubated for a further 4 hours at 37 °C and 5% CO2. The medium was then removed with caution, 100 mL of the dissolving solution was added to each well, and the plates were incubated for five minutes. Using an ELISA reader (Bio-Rad, Germany) and a wavelength of 575 nm, absorbance was recorded. To compute the IC50, a statistical analysis of the optical density values was done. As indicated by the following equation:

Viability (%) = (optical density of sample/optical density of control) ×100.

High-content screening

After MDA-MB-231 cells were treated in vitro to Gallic acid, five characteristics of the cells' health were measured using a multiparametric cytotoxicity assay [22]. The variables were cell viability, total nuclear intensity, cell membrane permeability, mitochondrial membrane permeability, and cytochrome c release. MDA-MB-231 Cells were stained with Hoechst 33342 dye which enables monitoring of cell loss, nuclear morphology changes and DNA content, which is proportional to the total Hoechst intensity per nucleus. Cells were also stained with permeability dye (Excitation 491/Emission 509), which enables monitoring of membrane permeability, and cells were stained with MMP dye (Excitation 552/Emission 576) for mitochondrial membrane potential changes, and with goat antimouse secondary antibody conjugated with DyLight TM for Cytochrome C releasing. An ArrayScan HCS Analyzer was used to look at the plates (Thermo Scientific, USA).

Caspase-8 and -9 Activity Bioluminescent Assays

On a white 96-well microplate, Caspase-GloH 8 and 9 assay kits were used to conduct a time-dependent analysis of caspase-8 and -9 activity. Cells were seeded at a concentration of 1 x 10^4 cells/well and treated with 100 µL of Gallic acid (25, 50, 100, and 200 µg/mL) for 24 hours. Caspase activities were examined in accordance with the manufacturing process. Briefly, 100 µL of caspase-Glo reagent was added and incubated for 30 minutes at room temperature. Apoptotic cells with active caspases cleaved the aminoluciferin-labeled synthetic tetrapeptide, therefore releasing substrate for the luciferase enzyme. The luminescence of each sample was measured using ELISA reader at 405 nm.

RESULTS

Cytotoxic effects of Gallic acid on MDA-MB-231 cell viability

Gallic acid's cytotoxic effects on the MDA-MB-231 cell line were determined using the MTT assay [23]. MDA-MB-231 cells were treated with Gallic acid at various doses (25, 50, 100, and 200 µg/mL) for 24 hours at 37 °C. The viability of MDA-MB-231 cells treated with Gallic acid was resolved to be 33.91%, 38.33%, 49.68%, and 63.16 %, respectively, as shown in Figure (1). The maximum cytotoxic activity (33.91%) was obtained at 200 µg/mL with an IC50 of 43.86 µg/mL.

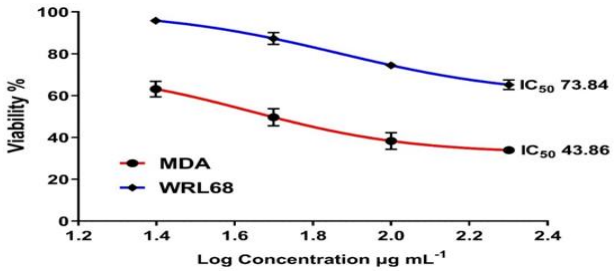


Figure (1): Dose-dependent cytotoxic effect of Gallic acid on MDA-MB-231 and WRL68 cells

Cytotoxic effects of Gallic acid on MDA-MB-231 cells using HCS

HCS was used to investigate the cytotoxicity of Gallic acid on MDA-MB-231 cells during 24 hours. Gallic acid was employed at four different doses (200, 100, 50, and 25 µg/mL) to identify changes in MDA-MB-231 cell viability, nuclear intensity, cell membrane permeability, mitochondrial membrane permeability, and cytochrome c. Table (1) displays the differences in these five parameters. The largest significant changes in viable cell count, nuclear intensity, MMP, and cytochrome c were seen at a Gallic acid concentration of 200 µg/mL when compared to untreated cells as the negative control ($P < 0.01$).

Table (1): Cytotoxic effects of Gallic acid on cellular parameters by HCS.

HCS parameter	Cytotoxic effect (mean±SD)					
	Untreated	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	P value at 200 µg/ml
CV	2144±250.4	1957±613.7	926.0±4.000	926.0±4.000	906.7±21.83	0.0016
CP	85.33±8.083	76.33±6.110	70.33±6.506	77.33±2.517	112.7±9.292	0.0023
NI	448.7±31.97	479.3±52.00	479.7±18.77	691.0±691.06	863.0±52.56	<0.0001
MMP	413.0±24.98	494.0±58.95	482.7±66.21	370.3±11.59	245.3±19.04	0.0023
CC	215.3±23.03	236.3±24.44	278.0±52.94	465.7 ±12.90	526.3±40.08	<0.0001

MMP: mitochondrial membrane potential; CV: cell viability; NI: nuclear intensity; CP: cell permeability; CC: cytochrome c. Untreated cell is the negative control.
*Significant differences ($P < 0.01$).

Cell viability

As seen in (Figure 2A), the viability of MDA-MB-231 cells decreased significantly with increasing Gallic acid concentration over 24 hours. The percentage cell viability was 57.8% (200 µg/mL), 56.9% (100 µg/mL), 56.9% (50 µg/mL), and 8.8% (25 µg/mL) for cells treated with Gallic acid. (Table 3) demonstrates that the drop in cell count was dose-dependent, with the greatest decline occurring after exposure to high concentrations 200 µg/ml (P 0.0016), 100 µg/ml (P 0.0018), and 50 µg/ml (P 0.0018), which agrees with the MTT results. As with other concentrations of 25 µg/ml, the experiment reveals no significant suppression of cell count ($P = 0.8535$). Gallic acid at 200 µg/mL produced the greatest decrease in cell number (906.7) compared to the negative control (2144) ($P = 0.0016$).

Cell membrane permeability

Compared to the negative control, treatment with 200 µg/mL Gallic acid substantially improved the permeability of MDA-MB-231 cell membranes by 32% ($P = 0.0023$; Figure 2B). The permeability of MDA-MB-231 cell membranes at different Gallic acid concentrations (100, 50, and 25 µg/mL) didn't vary significantly from the negative control.

Nuclear intensity

MDA-MB-231 cell nuclear intensity (Figure 2C) was significantly increased (92.3%; P= <0.0001with 200 µg/mL and (54%; P= 0.0002 with100 µg/mL) of Gallic acid compared with negative control. Nuclear intensities following treatment with other Gallic acid concentrations (50, and 25 µg/mL) showed no significant difference compared with the control ($P < 0.0001$).

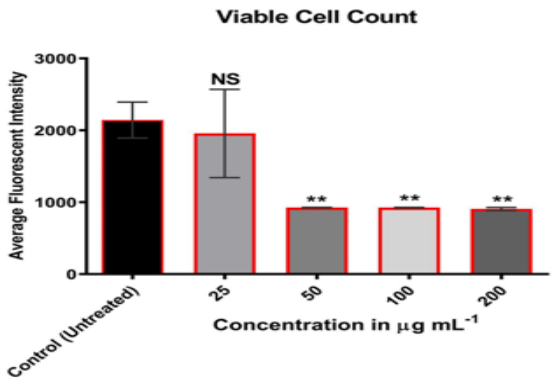
Mitochondrial membrane permeability

MDA-MB-231 mitochondrial membrane potential decreased with increasing Gallic acid concentration (Figure 2D). Only the higher concentration of Gallic acid 200 µg/ml induced a significant decrease ($P =0.0023$) in MMP (40.7%) compared to that of the negative control ($P =0.0002$).

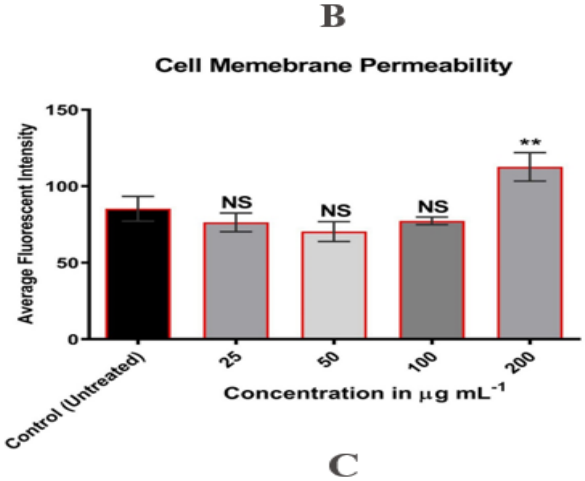
Cytochrome c

Gallic acid treatment of MDA-MB-231 cells resulted in a dose-dependent increase in cytochrome C release (Figure 2E). The release of cytochrome C was likewise considerably higher at 200 µg/mL ($P <0.0001$) and 100 µg/mL ($P <0.0001$) of Gallic acid when compared to the intensity observed with the negative control. There were no significant differences between the other Gallic acid concentrations.

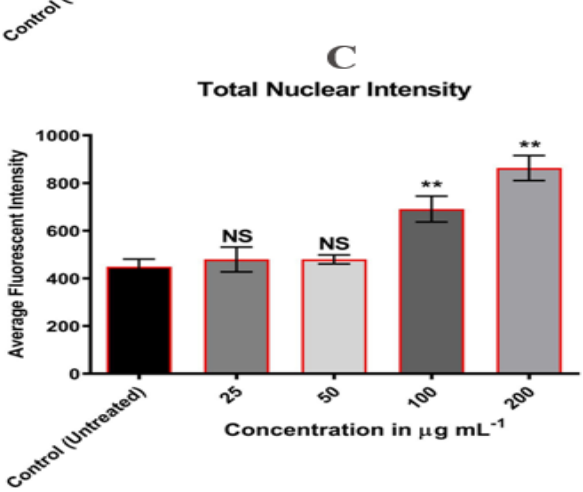
A


Viable Cell Count
Average Fluorescent Intensity
3000
2000
1000
0
NS
**
**
**
Control (Untreated)
25
50
100
200
Concentration in µg mL-1

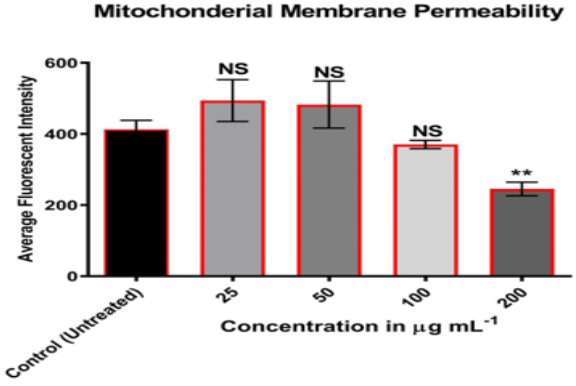
B


Cell Memebrane Permeability
Average Fluorescent Intensity
150
100
50
0
NS
NS
NS
**
Control (Untreated)
25
50
100
200
Concentration in µg mL-1

C


Total Nuclear Intensity
Average Fluorescent Intensity
1000
800
600
400
200
0
NS
NS
**
**
Control (Untreated)
25
50
100
200
Concentration in µg mL-1

D


Mitochonderial Membrane Permeability
Average Fluorescent Intensity
600
400
200
0
NS
NS
NS
**
Control (Untreated)
25
50
100
200
Concentration in µg mL-1

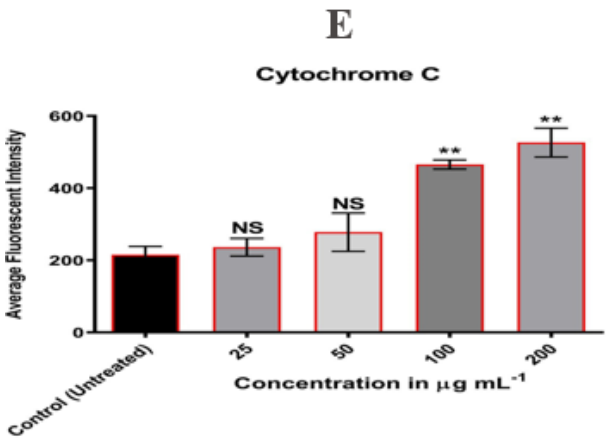


Figure (2): Effect of Gallic acid treatment on (A) cell viability, (B) cell membrane permeability, (C) nuclear intensity, (D) mitochondrial membrane potential, and (E) cytochrome c in MDA-MB-231: breast cancer cell line.

The effect of multi-parameter cytotoxicity (nuclear intensity, cell membrane permeability, mitochondrial membrane potential, and cytochrome C release) on the nucleus and mitochondria of MDA-MB-231 cells is showed in Figure (3).

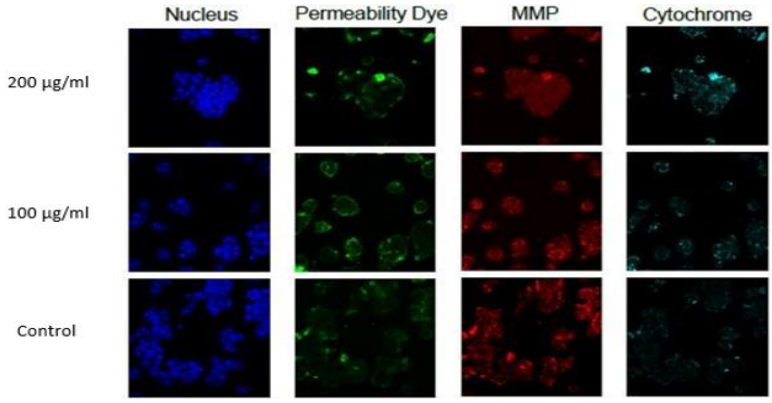


Figure (3): Multi-parameter cytotoxicity on the nuclear and mitochondria of MDA-MB-231 cells.

Effects of Gallic acid on MDA-MB-231 cell caspase-8&9 activity

A significant increase in the mean activity of caspase-8&9 (23062±719.8) and (53753±2939) was observed in MDA-MB-231 cells treated with Gallic acid (200 µg/mL) with an IC50 of 33.91 µg/mL figure (4, 5). Result shows a significant increase in activity of caspase -8 in MDA-MB-231 cell line at 100 and 200 µg/ml compared to negative control (DMSO) with evaluated p values 0.0074 and 0.0036 for mentioned concentrations respectively with using of doxorubicin as a reference drug (positive control) the obtained data are listed in Table (2).

Table (2): Effect of Gallic acid on caspase 8 activity at (50, 100 and 200µg/ml)

Treatments (µg/ml)	Mean ± SD	Sig.	P value
Control (DMSO)	11944±671.8	----	----
50	11529±1415	NS	0.9966
100	21402±1825	**	0.0074
200	23062±719.8	**	0.0036
doxorubicin	61943±2817	**	<0.0001

: significant (p<0.1)

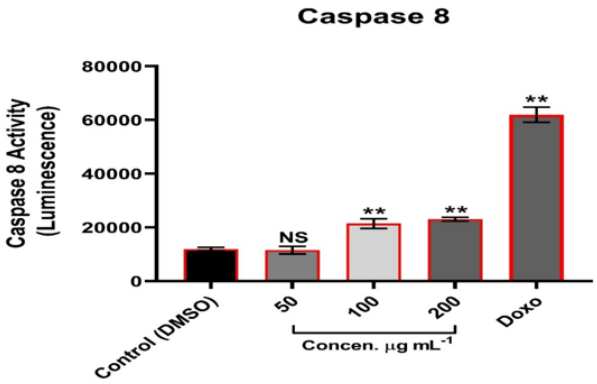


Figure (4): Effect of Gallic acid on caspase 8 activity at (50, 100 and 200 µg/ml) compared with negative and positive control

Considerable increase in caspase-9 appears at a treated concentration of 100µg/ml and 200µg/ml with 0.0046 and 0.0001 p values respectively, all of the concentration values have been compared with the MDA-MB-231 cells that have been treated by doxorubicin as positive control and (DMSO) as negative control.

Table (3): Effect of Gallic acid on caspase 9 activity at (50, 100 and 200µg/ml)

Treatments µg/ml	Mean ± SD	Sig.	P value
Control (DMSO)	14404±1317	--	----
50	13954±3291	NS	0.9992
100	32179±3649	**	0.0046
200	53753±2939	**	0.0001
doxorubicin	77171±2464	**	<0.0001

: significant (p<0.1)

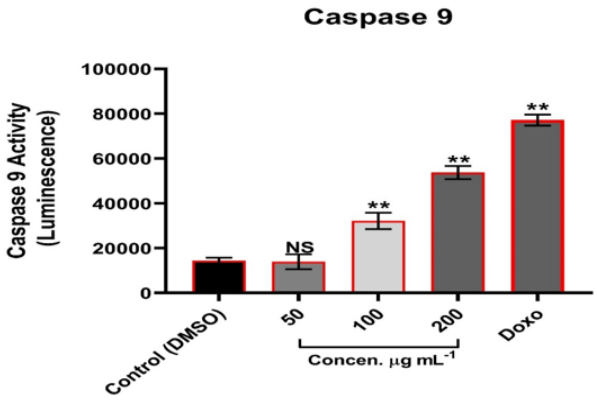


Figure (5): Effect of Gallic acid on caspase 9 activity at (50, 100 and 200 µg/ml) compared with negative and positive control

DISCUSSION

We utilized the MTT colorimetric test to determine the effect of Gallic acid on the proliferation of MDA-MB-231 breast cancer cells. To determine the viability of the cells, they were treated with various doses of Gallic acid for 48 hours. The results demonstrated that Gallic acid dose-dependently suppressed cell proliferation (Figure 1). The maximum cytotoxic activity (33.91%) was seen at a concentration of 200 µg/mL with an IC50 of 43.86 µg/mL, and there is a relatively less effect on normal cells WRL68 with an IC50 of 73.8 µg/mL.

A relatively large dosage (800 µg/l) of Gallic acid has been shown to inhibit the proliferation of lung cancer cells A549 and HeLa [24, 25]. A549 and HeLa are both kinds of cervical cancer. Intriguingly, earlier research indicates that very modest doses of Gallic acid (170 g/ml) can also inhibit the development of the human breast cancer cell line MCF-7 [26]. In this work, Gallic acid at various doses (25, 50, 100, and 200 µg/ml) dramatically reduced the viability of MDA-MB-231 TNBC (triple-negative breast cancer) cells. Different types of cell death and/or cell-cycle arrest can reduce the viability of cells. It has been shown that Gallic acid therapies trigger cell death by many mechanisms, including cell-cycle arrest, apoptosis, and necrosis [27].

In HCS, MDA-MB-231 cells were used to evaluate the multiparametric cytotoxicity of Gallic acid. In this experiment, five distinct parameters (cell count viability, nuclear intensity, cell membrane permeability, mitochondrial membrane potential, and cytochrome C release) were identified, and images of untreated MDA-MB-231 cells were also processed. Figure (2A) shows that when comparing untreated and treated MDA-MB-231 cells, the concentration of Gallic acid required to significantly reduce the viable cell count was 200 µg mL-1. The drop in cell count at 200, 100, and 50 µg /mL of Gallic acid compared to the control is statistically significant, but the reduction at 25 µg /mL concentration is not. Gallic acid was found by MTT test to be cytotoxic to MDA-MB-231 cells, providing strong evidence for this conclusion.

Cell viability is a key toxicity assessment parameter [28]. Cell density and Gallic acid concentration affected the reduction in viability. This impact may be caused by cytostatic and cytotoxic actions, both of which have the potential to inhibit the functioning of telomerase [29]. When MDA-MB-231 cells were treated with increasing doses of Gallic acid, the outcome was an increase in nuclear size. This was caused by enhanced nuclear swelling as well as increased permeability of the cell membrane. The morphology of the nucleus, which was stained with Hoechst Blue, demonstrated nuclear concentration, which is a phenomenon that generally takes place in the presence of high concentrations of Gallic acid (200 µg mL-1). The effects was significantly different from untreated cells (Figure 2B) ($p < 0.0023$) and no such events were induced at lower concentrations of Gallic acid. Changes in cell membrane permeability are often associated with toxic or apoptotic responses. In addition to this, cytotoxicity can result in a disruption of the integrity of the cell membrane [30]. It is possible for Gallic acid to change the composition of lipids in cells as well as their physical arrangement in the plasma membrane. The continual activation of the second messenger and protein kinase C through the phospholipase pathway is what causes this impact, which in turn causes a change in the permeability of the plasma membrane. Increased plasma membrane permeability and sustained protein kinase C activity can affect binding outside the rod. On the other hand, Figure (3) shows that the intensity of MDA-MB-231 cell membrane permeability (green) gradually increased in a dose-dependent manner, and a significant increase was observed after exposure to Gallic acid 200 µg mL-1.

Results shows that the nuclear intensity increased significantly after exposure to 200 µg/mL ($P <0.0001$), and 100 µg/mL (P 0.0002) of Gallic acid. The fluorescence intensity of Gallic acid is gradually increased dose dependence especially at 200 µg/ml as shown in Figure (2C)

The mitochondrial membrane potential of the cell, and the release of cytochrome C were also examined. As seen in Figures (2D, 2E), only the higher dose of Gallic acid 200 µg/ml ($P = 0.0023$) significantly decreased the mitochondrial membrane potential by 40.7%. The assessment of the mitochondrial membrane potential is dependent on the average fluorescence intensity of the MMP dye, which permeates the mitochondria in each nucleus cytoplasmic area; the lower the fluorescence intensity, the greater the influence on the mitochondria. Alternatively, the release of cytochrome C was considerably higher at 200 µg/mL ($P <0.0001$) and 100 µg/mL (P 0.0001) of Gallic acid. Cytochrome C plays a crucial part in the start of apoptosis, according to the literature, and can be released from mitochondria into the cytoplasm [31]. The treatment of MDA-MB-231 cells with Gallic acid produced a significant nuclear staining of cytochrome C, which led to the conclusion that Gallic acid mimics cytochrome C from mitochondria to cytoplasm [32].

Caspases are a class of cysteine proteases that are subdivided into executioner caspases like caspase-3 or -7 and initiator caspases like caspase-8 and -9. Caspase-8 is known to be triggered by an extrinsic mechanism, whereas caspase-9 is activated by cytochrome c leakage from mitochondria. Both initiator caspases can activate caspase-3 or -7, which commit cells to apoptosis [33].

Figure (4) shows that the activity of caspase-8 in the MDA-MB-231 cell line increased significantly at 100 and 200 µg/ml compared to the negative control (DMSO). The p values for these concentrations were 0.0074 and 0.0036, respectively, when doxorubicin was used as a reference drug (positive control). After 24 hours, the MDA-MB-231 cells treated with Gallic acid demonstrated a dose-dependent increase in the activation of caspase-9. All of the concentration values have been compared to the MDA-MB-231 cells that have been treated with the negative control (DMSO) and doxorubicin as a positive control figure (5). A significant increase is observed at concentrations of 100 µg/ml and 200 µg/ml, with p values of 0.0046 and 0.0001, respectively. Obtained data are indication of the fact that the MDA-MB-231 cells that have been treated with Gallic acid solution undergo the apoptosis through the disruption of the cytochrome C and cell membrane release, due to the fact that those two events are highly correlated with activating the Caspases [34].

CONCLUSION

Gallic acid therapy significantly lowers the cell viability of the malignant human breast cancer cell line MDA-MB-231 by the activation of apoptosis, as indicated by an increase in caspase-9 activity at a concentration of 200 µg/mL, as reported in the present study. The HCS experiment demonstrated that Gallic acid had dose-dependently toxic effects on MDA-MB-231 cells at 200 µg/mL, with increased nuclear intensity, membrane permeability, and cytochrome c levels. In addition, there was a reduction in cell viability and mitochondrial membrane potential. These results suggest that Gallic acid may have powerful anti-tumor actions against malignant TNBCs in human.

Conflicts of Interest: None.

REFERENCES

1. Van Hemelrijck M, Folkvaljon Y, Adolfsson J, Akre O, Holmberg L, Garmo H, and Stattin P. Causes of death in men with localized prostate cancer: a nationwide, population-based study. BJU Int 2016; 117:507-14.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66:7-30.
3. Kozłowski J, Kozłowska A, Kocki J. Breast cancer metastasis-insight into selected molecular mechanisms of the phenomenon. Postepy Hig Med Dosw (Online) 2015; 69:447-51.
4. Niemetz R, Gross GG. Enzymology of gallotannin and ellagitannin biosynthesis. Phytochemistry 2005; 66:2001–2011.
5. Fernandes FH, Salgado HR. Gallic Acid: Review of the Methods of Determination and Quantification. Crit Rev Anal Chem. 2016 May 3; 46(3):257-65.
6. Sales MS, Roy A, Antony L, Banu SK, Jeyaraman S, Manikkam R. Octyl gallate and gallic acid isolated from Terminalia bellarica regulates normal cell cycle in human breast cancer cell lines. Biomed Pharmacother. 2018 Jul; 103:1577-1584.
7. Kang MS, Oh JS, Kang IC, Hong SJ, Choi CH. Inhibitory effect of methyl gallate and gallic acid on oral bacteria. J Microbiol 2008; 46:744–750.
8. Liu W, Li M, Wang G, Ma H, Mu Y, Zheng D, Huang X, Li L. New Monoterpene Acid and Gallic Acid Glucose Esters with Anti-Inflammatory Activity from Blue Gum (Eucalyptus globulus) Leaves. J Agric Food Chem. 2022 Apr 27; 70(16):4981-4994.
9. Zhang T, Ma L, Wu P, Li W, Li T, Gu R, Dan X, Li Z, Fan X, Xiao Z. Gallic acid has anticancer activity and enhances the anticancer effects of cisplatin in non-small cell lung cancer A549 cells via the JAK/STAT3 signaling pathway. Oncol Rep. 2019 Mar; 41(3):1779-1788.
10. Maurya DK, Nandakumar N, Devasagayam TP. Anticancer property of gallic acid in A549, a human lung adenocarcinoma cell line, and possible mechanisms. J Clin Biochem Nutr. 2011 Jan; 48(1):85-90.

Wang K, Zhu X, Zhang K, Zhu L, and Zhou F. Investigation of gallic acid induced anticancer effect in human breast carcinoma MCF-7 cells. J Biochem Mol Toxicol. 2014 Sep; 28(9):387-93.

11. Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep. 2019 Jan 18; 39(1).
12. Koçak N, Dönmez H, Yildirim İH. Effects of melatonin on apoptosis and cell differentiation in MCF-7 derived cancer stem cells. Cell Mol Biol (Noisy-le-grand). 2018 Sep 30; 64(12):56-61.
13. Vogeler S, Carboni S, Li X, Joyce A. Phylogenetic analysis of the caspase family in bivalves: implications for programmed cell death, immune response and development. BMC Genomics. 2021 Jan 25; 22(1):80.
14. Denning DP, Hatch V, Horvitz HR. Both the caspase CSP-1 and a caspases-independent pathway promote programmed cell death in parallel to the canonical pathway for apoptosis in Caenorhabditis elegans. PLoS Genet 2013; 9:e1003341.
15. McIlwain DR, Berger T, Mak TW. Caspase functions in cell death and disease. Cold Spring Harb Perspect Biol 2013; 5:a008656.
16. Würstle ML, Laussmann MA, Rehm M. The central role of initiator caspase-9 in apoptosis signal transduction and the regulation of its activation and activity on the apoptosome. Exp Cell Res 2012; 318:1213-20.
17. Kesavardhana S, Malireddi RKS, Kanneganti TD. Caspases in Cell Death, Inflammation, and Pyroptosis. Annu Rev Immunol. 2020 Apr 26; 38:567-595.
18. Hsu JD, Kao SH, Ou TT, Chen YJ, Li YJ, Wang CJ. Gallic acid induces G2/Mphase arrest of breast cancer cell MCF-7 through stabilization of p27 (Kip1) attributed to disruption of p27 (Kip1)/Skp2 complex. J Agric Food Chem2011; 59:1996–2003.
19. Duellman SJ, Zhou W, Meisenheimer P, Vidugiris G, Cali JJ, Gautam P, Wennerberg K, Vidugiriene J. Bioluminescent, nonlytic, realtime cell viability assay and use in inhibitor screening. Assay Drug Dev Tech 2015; 13:456-65.
20. Rashid H. Umamaheswari G. EVALUATION OF THE CYTOTOXIC EFFECT OF GINGEREXTRACT AGAINST PROSTATE CANCER MODEL USING IN VITRO STUDY. WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES. 2017; 6 (12): 1044-1053.
21. Ali Z. Al-Saffar, Fatimah A. Sabry, Shymaa L. Al-Brazanchi, Ahmed F. Al-Shanon, Firas Hassan and Noora A. Hadi,. Phytochemical analysis, antioxidant and cytotoxic potentials of Pelargonium graveolens extract in human breast adenocarcinoma (MCF-7) cell line. Asian J. Biochem. 2017; 12: 16-26.
22. Hassan F, Abdul-Hameed A, Alshanon A, Abdullah MB, Huri HZ, Hairunisa N, Yousif E. Antitumor activity for gold (III) complex by high content screening technique (HCS) and cell viability assay. Asian J Biochem 2015; 10:252-66.
23. Ohno Y, Fukuda K, Takemura G, Toyota M, Watanabe M, Yasuda N, et al.
24. Induction of apoptosis by gallic acid in lung cancer cells. Anticancer Drugs 1999; 10:845–851.
25. You BR, Moon HJ, Han YH, Park WH. Gallic acid inhibits the growth of HeLa cervical cancer cells via apoptosis and/or necrosis. Food Chem Toxicol 2010; 48:1334–1340.
26. Aborehab NM, Elnagar MR, Waly NE. Gallic acid potentiates the apoptotic effect of paclitaxel and carboplatin via overexpression of Bax and P53 on the MCF-7 human breast cancer cell line. J Biochem Mol Toxicol. 2021 Feb; 35(2).
27. Ji BC, Hsu WH, Yang JS, Hsia TC, Lu CC, Chiang JH, et al. Gallic acid induces apoptosis via caspase-3 and mitochondrion-dependent pathways in vitro and suppresses lung xenograft tumor growth in vivo. J Agric FoodChem 2009; 57:7596–7604.
28. Adan A, Kiraz Y, Baran Y. Cell Proliferation and Cytotoxicity Assays. Curr Pharm Biotechnol. 2016; 17(14):1213-1221.
29. Al-Jailawi MH, Nasir HM, Aziz GM. Cytotoxic effect ofbiosurfactants produced by novel thermophillic Geobacillusthermoleovorans (JQ 912239). Int J Adv Res. 2015; 3:632-7.
30. Ajabnoor GM, Crook T, Coley HM. Paclitaxel resistance isassociated with switch from apoptotic to autophagic cell death inMCF-7breast cancer cells. Cell Death Dis 2012; 3:e260.
31. Hassan F, Mohammed G, El-Hiti GA, Alshanon A, Yousif E. Cytotoxic effects of tamoxifen in breast cancer cells. J Unexplored Med Data 2018; 3:3.
32. Chang WT, Huang SC, Cheng HL, Chen SC, Hsu CL. Rutin and Gallic Acid Regulates Mitochondrial Functions via the SIRT1 Pathway in C2C12 Myotubes. Antioxidants (Basel). 2021 Feb 13; 10(2):286.
33. Parsons MJ, Fassio SR, Bouchier-Hayes L. Detection of Initiator Caspase Induced Proximity in Single Cells by Caspase Bimolecular Fluorescence Complementation. Methods Mol Biol. 2016; 1419:41-56.
34. Tsai CL, Chiu YM, Ho TY, Hsieh CT, Shieh DC, Lee YJ, Tsay GJ, Wu YY. Gallic Acid Induces Apoptosis in Human Gastric Adenocarcinoma Cells. Anticancer Res. 2018 Apr; 38(4):2057-2067.