

SELECTIVE CYTOTOXICITY AND APOPTOSIS INDUCTION BY SAHA IN MCF-7 CELL LINE

WAEEL S. ABD EL AAL¹, OSAMA HANI¹, GHADA M. NASR^{1*}, MAHMOUD MOAWAD²

¹ Department of Molecular Diagnostics, Genetic Engineering, and Biotechnology Research Institute, University of Sadat City, Egypt.

² Department of Pathology, National Cancer Institute, Cairo University, Egypt. mahmoud.moawad@nci.cu.edu.eg ORCID 0000-0003-3250-7925.

*Corresponding author: Ghada M. Nasr (nasr_mi@yahoo.com)

Breast cancer (BC) is the most frequent malignancy in women and has a complex genetic makeup. The worldwide incidence of breast cancer is constantly increasing. BC remains the most common malignancy among women globally, representing 11.7% of all cancer cases and causing 685,000 deaths annually Sung *et al.*, (2021); Altrawy *et al.*, (2025). This is why, even though detection and treatment have improved, resulting in decreasing mortality rates, it is still critical to discover new methods to treat it and predict how well it will perform Smolarz *et al.*, (2022).

Superpolyamide hydroxamic acid (SAHA), a second-generation hybrid polar molecule, is a newly developed prototype. It is a novel of histone deacetylase (HDAC) inhibitor that efficiently stimulates cell differentiation in mouse erythroleukemia cells. SAHA, a potent HDAC inhibitor, exhibits significant anticancer properties by

modulating gene expression through epigenetic mechanisms. The majority of SAHA research employed hematopoietic tumor cells. HDAC-inhibitors, such as SAHA, serve a variety of important functions in cancer therapy, including angiogenesis prevention, apoptosis or differentiation induction, and cell cycle arrest Micheletti *et al.*, (2024).

In this study, we investigated the cytotoxic and apoptotic effects of SAHA on MCF-7 breast cancer cells compared to WI-38 normal fibroblasts. Both cell lines were treated with varying concentrations of SAHA (1–15 μ M) over 24, 48, and 72 hours.

MATERIAL AND METHODS

Material:

The breast cell lines were purchased from VACSERA and were maintained under standard lab conditions.

Methods:

1. Cell Culture and Treatment

Breast cancer cell lines were cultured in DMEM and/or RPMI 1640 medium supplemented with 10% FBS, 2% L-glutamine, and 1% penicillin-streptomycin. Subsequent to thawing in a water bath at 37°C, the cells were combined with full media and then transferred to T-flasks containing 10 mL of pre-warmed fluid. The cultures were extracted after 12 hours of incubation at 37°C in 5% CO₂. The cells were harvested with 0.05% trypsin-EDTA when reaching about 60% confluency after 24 hours. Subsequently, they were neutralized with serum-containing media, washed twice with phosphate-buffered saline (PBS), and resuspended for further analysis.

2. Cell Viability Assays

2.1. Trypan Blue Exclusion Test:

Hemocytometer counts were obtained by mixing cells in a 0.4% trypan blue solution at a 1:1 ratio. The number of viable (unstained) and non-viable (blue-stained) cells were evaluated both before and after treatment with superpolyamide hydroxamic acid (SAHA).

2.2. MTT Assay:

A measurement of the cells' metabolic activity was taken using the MTT assay. The 96-well plates were seeded with cells and then treated with chemicals. MTT solution (0.45 mg/mL

final concentration) was added and incubated for 1–4 hours. Formazan crystals were solubilized using a solution containing 40% dimethyl formamide, 2% glacial acetic acid, and 16% SDS (pH 4.7). Absorbance was measured at 570 nm.

3. RNA Extraction and cDNA Synthesis

The DiazoL RNA Miniprep Kit (Zymo Research, R2050) was used for total RNA extraction in accordance with the manufacturer's procedure. After being homogenized, cells were subjected to processing via spin columns after being lysed in RLT buffer that included β -mercaptothion. We used RNase-free water to elute the RNA. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, 4368814). Each 20 μ L reaction contained random primers, dNTPs, reverse transcriptase, and RNase inhibitor. The thermal cycling conditions were 10 minutes at 25°C, 120 minutes at 37°C, and 5 minutes at 85°C.

4. Quantitative Real-Time PCR

The qPCR technique using SYBR Green (QuantiTect SYBR Green PCR Master Mix, Qiagen) was used to assess the expression levels of the MMP9, Caspase 3, and P21 genes. Each reaction used 1000 ng of cDNA and gene-specific primers in a 15 μ L volume. After a 15-minute denaturation at 95°C, the thermal profile underwent 35–45 cycles including denaturation (94°C, 15 s), annealing (54°C, 30 s), and extension (72°C, 30 s).

5. Flow Cytometry for Cell Cycle and Apoptosis

5.1. Cell Cycle Analysis:

Before being stained with propidium iodide (PI), the cells were fixed and treated with RNase A. PI fluorescence intensity was used to determine cell cycle distribution, and DNA content was measured by flow cytometry (Ex: 488 nm, Em: FL2).

5.2. Apoptosis Assay:

The Annexin V-FITC/PI Apoptosis Detection Kit (Abcam, K101) was used to detect cellular mortality. After labelling with Annexin V-FITC and PI, the cells were incubated at room temperature in the dark for 5 minutes. The FL1 and FL2 channels in flow cytometry were used to quantify apoptotic and necrotic cells.

6. Data Analysis

Flow cytometry data were analyzed using standard gating strategies to exclude cell debris and aggregates according to the characteristics of forward scatter (FSC) and side scatter (SSC). Propidium iodide (PI) fluorescence was acquired using a 488 nm excitation laser and detected in the FL2 channel. DNA content histograms were plotted using a linear scale, where distinct peaks corresponding to 2N (G0/G1 phase) and 4N (G2/M phase) DNA content were expected, with the 4N peak approximately twice the fluorescence intensity of the 2N peak. Sub-G1 (<2N), S-phase (2N–4N),

and hyperploid (>4N) populations were also marked to assess cell cycle distribution. To ensure accurate comparison between samples, minor voltage adjustments were applied where necessary to align the 2N peaks across histograms.

RESULTS AND DISCUSSION

1. SAHA Exhibits Selective Cytotoxicity in MCF-7 Breast Cancer Cells

Regulating the acetylation state of nucleosomal histones is a dual process involving histone acetyltransferases and histone deacetylases. An intriguing new approach to fighting several solid and blood malignancies is epigenetic modification with small-molecule HDAC inhibitors Rangwala *et al.*, (2012). One second-generation hybrid polar molecule that has recently gained clinical approval for the treatment of cutaneous T-cell lymphoma (CTCL) is SAHA. It is a powerful pan-HDAC inhibitor Marks, (2007). Since histone deacetylases (HDACs) have a wide range of physiological and pathological substrates and target proteins, SAHA is a pan-HDAC inhibitor that may exhibit a wide variety of anticancer effects in a wide variety of tumor types New *et al.*, (2012) and Zhao *et al.*, (2014).

SAHA is a powerful anticancer drug that has anti-neoplastic effects in a variety of BC cell types in both laboratory and animal studies by inhibiting proliferation, migration, invasion, induction of differentiation and apoptosis,

and cell-cycle arrest. It can be used alone or in conjunction with other traditional chemotherapeutics Wawruszak *et al.*, (2021).

The present study explored the impact of SAHA on the viability, cell cycle progression, apoptosis, and gene expression of MCF-7 BC cells and WI-38 normal fibroblasts. The data suggest that SAHA exhibits selective cytotoxicity towards breast cancer cells, while showing a relatively lower effect on normal fibroblasts, aligning with its expected mechanism as an epigenetic regulator and anticancer agent.

Cell proliferation and viability in response to different treatments may be assessed using the MTT test, a colorimetric approach that has gained widespread acceptance. Active mitochondrial respiration causes the transformation of the yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into the purple formazan crystals. Once formazan is dissolved, its concentration may be measured by spectrophotometry at 570 nm; this value is directly proportional to the quantity of viable cells.

This assay enables the assessment of cellular metabolic activity, indirectly reflecting cell proliferation and cytotoxicity. Optimization of the protocol has minimized processing steps, improving assay efficiency. Under cell-free conditions, MTT yields minimal background absorbance, ensuring reliable detection of treatment-induced changes in

cell viability. Figure (1) illustrates the impact of SAHA treatment on cell viability.

2. Dose- and Time-Dependent Inhibition of Cell Viability

MTT assay was performed on MCF-7 BC cells and WI-38 normal fibroblasts to determine the cytotoxic potential of SAHA, an HDAC inhibitor. Cells were treated to increasing dosages of SAHA (1, 2.5, 5, 10, and 15 μ M) for 24-48-72 hours as shown in Table (1).

SAHA treatment decreased cell viability in MCF-7 cells, as shown by the dose- and time-dependent MTT assay. The IC₅₀ values for MCF-7 cells were progressively lower with increased exposure time: 6 μ M at 24 hours, 4 μ M at 48 hours, and 2 μ M at 72 hours. This suggests that MCF-7 cells became more sensitive to SAHA over time, which is consistent with the well-documented effects of HDAC inhibitors on epigenetic reprogramming and gene expression changes over prolonged exposure. These results agree with previous study that demonstrated that SAHA dose-dependently halted the growth of TAMR/MCF-7 cells Lee *et al.*, (2012).

In contrast, WI-38 normal fibroblasts exhibited higher IC₅₀ values compared to MCF-7 cells, suggesting greater resistance to SAHA. At 24, 48, and 72 hours, the IC₅₀ values for WI-38 cells were approximately 15 μ M, 12 μ M, and 8 μ M, respectively, showing a slower decrease over time compared to MCF-7

cells. These findings align with previous studies demonstrating that HDAC inhibitors often show selective cytotoxicity towards tumor cells due to their epigenetic dysregulation, while normal cells are generally more resistant due to stable epigenetic control mechanisms Min *et al.*, (2015); Karaj *et al.*, (2022).

Figure (2) shows the Cytotoxicity due to the treatment with SAHA. SAHA has a toxic effect on BC (MCF7) while showing zero toxic effect on normal cells (Wi). This differential response highlights SAHA's selective cytotoxicity toward cancer cells, likely attributable to the greater epigenetic instability of tumor cells compared to normal cells. These findings support SAHA's potential as a targeted therapeutic agent with reduced toxicity to non-cancerous tissue.

This selectivity for cancer cells is particularly significant as HDAC inhibitors like SAHA have been proposed to target cancer cells more effectively by inducing cell cycle arrest, apoptosis, and altered gene expression while having a lesser impact on normal cells.

3. SAHA Induces G₂/M Cell Cycle Arrest in MCF-7 Cells

Flow cytometry has been instrumental in advancing cell cycle research through the quantification of DNA content. This method employs DNA-binding fluorescent dyes, which bind stoichiometrically meaning fluorescence intensity directly reflects

DNA quantity. As cells progress from the G₁ phase to G₂/M, their DNA content doubles, resulting in a corresponding two-fold increase in fluorescence signal. This allows precise discrimination between G₁, S, and G₂/M phases, making flow cytometry a powerful tool for assessing cell cycle distribution across diverse cell types, including mammalian, yeast, plant, and bacterial cells. Figures (3 and (4) illustrate the percentage of the cell cycle each cell line controls and treated in detail.

Flow cytometric analysis revealed that SAHA treatment altered cell cycle distribution in MCF-7 breast cancer cells. Compared to the control group (G₀/G₁: 44.39%, G₂/M: 4.32%), treatment with SAHA resulted in a reduction in the G₀/G₁ phase (36.71% and 34.52% in treatment 1 and treatment 2, respectively) and a marked increase in the G₂/M population (18% and 26.26%, respectively). as shown in Table (2) These results indicate that SAHA induces G₂/M phase arrest in a dose-dependent manner. Notably, treatment 2 demonstrated a more pronounced effect, suggesting a greater efficacy in halting cell cycle progression at the G₂/M checkpoint.

Cell cycle analysis of treated and untreated cells revealed that SAHA treatment significantly altered cell cycle distribution, particularly in MCF-7 cells. The increased proportion of cells in the G₂/M phase suggests cell cycle arrest at the G₂/M checkpoint, which is a common

mechanism through which HDAC inhibitors induce apoptosis in cancer cells Salanci *et al.*, (2024).

In contrast, WI-38 cells showed a less pronounced shift in the cell cycle, indicating that SAHA treatment did not significantly disrupt the normal cell cycle in these fibroblasts. This result is in line with previous studies showing that normal cells exhibit greater resistance to cell cycle arrest induced by HDAC inhibitors, likely due to the more stable and regulated nature of their epigenetic landscape Grabarska *et al.*, (2017).

Flow cytometric analysis was used to quantify the extent of apoptosis and necrosis in MCF-7 breast cancer cells and WI-38 normal fibroblasts, both untreated (control) and treated with SAHA.

4. SAHA Promotes Apoptosis in MCF-7 Cells

Apoptosis was evaluated by flow cytometry to assess cell death. Fiuger (5) illustrates the apoptotic response of BC cell lines to varying concentrations of Treatment 1 and Treatment 2. Treatment 1 demonstrated a higher efficacy, inducing apoptosis in 22.46% of cells, compared to 15.24% with Treatment 2, and 2.61% in the untreated control group. Based on these findings, Treatment 1 seems to have a greater impact in inducing programmed cell death in breast cancer cells. in Table (3). This is consistent with studies demonstrating that HDAC inhibitors like SAHA promote apoptosis in cancer cells by inducing cell cycle arrest and

modulating pro-apoptotic proteins, such as p21, p53, and caspase-3 (Bhagat *et al.*, 2018). This result further supports the idea that SAHA preferentially targets tumor cells, while normal cells are less affected due to their more stable epigenetic environment.

On the other hand, the WI-38 cells showed relatively lower levels of apoptosis, indicating the selectivity of SAHA for cancer cells as shown in Fig. (6) This difference is important as it highlights the potential for using SAHA as a therapeutic agent in targeting tumor cells while minimizing effects on normal cells, a key goal in cancer treatment.

5. Gene Expression Modulation by SAHA

Gene expression analysis revealed significant modulation of key regulatory genes involved in cell cycle regulation and apoptosis SAHA's effect on gene expression in MCF-7 breast cancer cells and WI-38 normal fibroblasts was evaluated by real-time PCR. as shown in Table (4) and Fig. (7).The tumour suppressor gene p21 was markedly upregulated in MCF-7 cells after SAHA therapy, indicating enhanced cell cycle arrest. Additionally, there was a notable downregulation of MMP-9, suggesting a reduction in the metastatic potential of these cancer cells. An increase in caspase-3 expression was also observed, signifying the induction of apoptosis. Conversely, WI-38 cells exhibited minimal changes in the expression levels of p21, MMP-9, and caspase-3 upon

SAHA treatment, indicating a lower impact on normal cells. This study provides new evidence that SAHA may be a useful targeted therapeutic drug for the treatment of breast cancer. SAHA seems to promote apoptosis and reduce proliferation in malignant cells with no impact on normal fibroblasts. The upregulation of p21 is consistent with the cell cycle arrest observed in the G2/M phase, as p21 is a known inhibitor of cyclin-dependent kinases (CDKs) and is involved in cell cycle regulation Salanci *et al.*, (2024); Pellarin *et al.*, (2025).

The downregulation of MMP-9 indicates the potential for SAHA to inhibit cancer cell migration and invasion, as MMP-9 is involved in the degradation of the extracellular matrix, a critical step in tumor metastasis Izdebska *et al.*, (2021).

In WI-38 normal fibroblasts, the gene expression changes were less pronounced, reinforcing the idea that SAHA exhibits selectivity for cancer cells. This is consistent with previous studies showing that HDAC inhibitors may cause less perturbation in normal cells, thus reducing the risk of side effects when used as cancer therapeutics Roy *et al.*, (2023).

6. Summary of Anticancer Activity and Selectivity

In Summary, our findings support the hypothesis that SAHA is a selective anticancer medication, since it has a profoundly detrimental impact on WI-38 normal fibroblasts but a significantly

more beneficial effect on MCF-7 breast cancer cells. Previous studies have shown that HDAC inhibitors, such as SAHA, have therapeutic promise as cancer therapies owing to their dose- and time-dependent decrease of cell viability, induction of apoptosis, and selective effects on gene expression in MCF-7 cells Lee *et al.*, (2012).

The selectivity of SAHA for cancer cells is a promising feature, as it suggests that SAHA may provide effective treatment with reduced toxicity to normal cells, a critical factor in the development of targeted cancer therapies.

The drug's effects are more pronounced in MCF-7 cells than in WI-38 normal fibroblasts, highlighting the selectivity of SAHA for cancer cells. These results support previous data demonstrating that HDAC inhibitors like SAHA can target epigenetically dysregulated cancer cells while sparing normal cells Karagiannis and Rampias, (2021).

The time-dependent IC₅₀ lowering that SAHA exhibits contributes to its promise as an effective anticancer treatment, notably for breast cancer. This is in addition to the fact that it induces apoptosis and stops the cell cycle in the G2/M phase.

The selectivity of SAHA for cancer cells, in conjunction with its low impact on normal fibroblasts, lends credence to the use of SAHA in therapeutic techniques that seek to target

tumour cells while maintaining the integrity of normal tissues.

SUMMARY

Specific cytotoxicity against MCF-7 BC cells is shown by SAHA, which causes dose- and time-dependent declines in the cells' viability levels, with IC₅₀ values decreasing from 6 μ M at 24 hours to 2 μ M at 72 hours. In contrast, WI-38 normal fibroblasts demonstrate higher IC₅₀ values and reduced sensitivity, indicating minimal impact on normal cells. SAHA treatment leads to G₂/M phase cell cycle arrest and promotes apoptosis in MCF-7 cells, accompanied by upregulation of p21 and downregulation of MMP-9, suggesting inhibition of proliferation and metastatic potential. These findings support SAHA's potential as a targeted epigenetic therapeutic agent for BC.

These findings suggest that SAHA selectively inhibits proliferation and induces apoptosis in MCF-7 breast cancer cells through mechanisms involving G₂/M cell cycle arrest and modulation of apoptosis-related genes, while sparing normal fibroblasts, highlighting its potential as a targeted epigenetic therapy for BC treatment.

Competing interests

No conflicts of interest were detected among the authors that may have affected the study's results.

Data availability

All generated data are presented in the current MS

Author contribution

OH: conducted the experimental work, MM, GN, WSA: conceptualized the idea and supervised the work, GN: wrote the MS.

REFERENCES

- Altrawy A., Talaat R. M., Nasr G. M., Badr E. A. E., Arneth, R., Arneth B. and Sabit H., (2025). Diagnostic and Prognostic Roles of miR-155 and miR-3173 in Breast and Ovarian Cancer: Implications for Early Detection and Personalized Treatment. 13, 1604.
- Bhagat S. D., Singh U., Mishra R. K. and Srivastava A., (2018). An Endogenous Reactive Oxygen Species (ROS)-Activated Histone Deacetylase Inhibitor Prodrug for Cancer Chemotherapy. *ChemMedChem*, 13, 2073-2079.
- Grabarska A., Łuszczki J. J., Nowosadzka E., Gumbarewicz E., Jeleniewicz W., Dmoszyńska-Graniczka M., Kowalczyk, K., Kupisz K., Polberg K. and Stepulak A., (2017). Histone Deacetylase Inhibitor SAHA as Potential Targeted Therapy Agent for Larynx Cancer Cells. *J Cancer*, 8, 19-28.

- Izdebska M., Zielińska W., Krajewski A., Hałas-Wiśniewska M., Mikołajczyk K., Gagat M. and Grzanka A., (2021). Downregulation of MMP-9 Enhances the Anti-Migratory Effect of Cyclophosphamide in MDA-MB-231 and MCF-7 Breast Cancer Cell Lines. *Int. J. Mol. Sci.*, 22.
- Karagiannis D. and Rampias T., (2021). HDAC Inhibitors: Dissecting Mechanisms of Action to Counter Tumor Heterogeneity. *Cancers (Basel)*, 13.
- Karaj E., Sindi S. H., Kuganesan N., Koranne R. A., Knoff J. R., James A. W., Fu Y., Kotsull L. N., Pflum M. K., Shah Z., Taylor W. R. and Tillekeratne L. M. V., (2022). First-in-Class Dual Mechanism Ferroptosis-HDAC Inhibitor Hybrids. *Journal of Medicinal Chemistry*, 65, 14764-14791.
- Lee Y. J., Won A. J., Lee J., Jung J. H., Yoon S., Lee B. M. and Kim H. S., (2012). Molecular Mechanism of SAHA on Regulation of Autophagic Cell Death in Tamoxifen-Resistant MCF-7 Breast Cancer Cells. *International Journal of Medical Sciences*, 9, 881-893.
- Marks P., (2007). Discovery and development of SAHA as an anticancer agent. *Oncogene*, 26, 1351-1356.
- Micheletti G., Boga C., Drius G., Bordoni S. and Calonghi N., (2024). Suberoylanilide Hydroxamic Acid Analogs with Heteroaryl Amide Group and Different Chain Length: Synthesis and Effect on Histone Deacetylase. *Molecules*, 29, 238.
- Min A., Im S.-A., Kim D. K., Song S.-H., Kim H.-J., Lee K.-H., Kim T.-Y., Han S.-W., Oh D.-Y., Kim T.-Y., O'connor M. J. and Bang Y.-J., (2015). Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), enhances anti-tumor effects of the poly (ADP-ribose) polymerase (PARP) inhibitor olaparib in triple-negative breast cancer cells. *Breast Cancer Research*, 17, 33.
- New M., Olzscha H. And La Thangue N. B., (2012). HDAC inhibitor-based therapies: can we interpret the code? *Molecular oncology*, 6, 637-656.
- Pellarin I., Dall'acqua A., Favero A., Segatto I., Rossi V., Crestan N., Karimbayli J., Belletti B. and Baldassarre G., (2025). Cyclin-dependent protein kinases and cell cycle regulation in biology and disease. *Signal Transduction and Targeted Therapy*, 10, 11.
- Rangwala S., Zhang C. and Duvic M. (2012). HDAC inhibitors for the treatment of cutaneous T-cell

- lymphomas. Future medicinal chemistry, 4, 471-486.
- Roy R., Ria T., Roymahapatra D. and Sk U. H., (2023). Single Inhibitors versus Dual Inhibitors: Role of HDAC in Cancer. ACS Omega, 8, 16532-16544.
- Salanci Š., Vilková M., Martinez L., Mirossay L., Michalková R. and Mojžiš J., (2024). The Induction of G2/M Phase Cell Cycle Arrest and Apoptosis by the Chalcone Derivative 1C in Sensitive and Resistant Ovarian Cancer Cells Is Associated with ROS Generation. International Journal of Molecular Sciences, 25, 7541.
- Smolarz B., Nowak A. Z. and Romanowicz H., (2022). Breast Cancer and Epidemiology, Classification, Pathogenesis and Treatment (Review of Literature). 14, 2569.
- Sung H., Ferlay J., Siegel R. L., Laversanne M., Soerjomataram I., Jemal A. and Bray F. J. C. A. C. J. F. C. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. 71, 209-249.
- Wawruszak A., Borkiewicz L., Okon E., Kukula-Koch W., Afshan S. and Halasa M., (2021). Vorinostat (SAHA) and Breast Cancer: An Overview. Cancers, 13, 4700.
- Zhao, Zhao Y. Yu D., Wu H., Liu H., Zhou H., Gu R., Zhang R., Zhang S. and Wu G., (2014). Anticancer activity of SAHA, a potent histone deacetylase inhibitor, in NCI-H460 human large-cell lung carcinoma cells in vitro and in vivo. International journal of oncology, 44, 451-458.

Table (1): Dose- and Time-Dependent Cytotoxic Effects of SAHA on MCF-7 Breast Cancer and WI-38 Normal Fibroblast Cell Lines.

cell Line	Conc. Of SAHA (μM)	24h IC50 (μM)	48h IC50 (μM)	72h IC50 (μM)
MCF-7 (Breast Cancer)	1	>15	>15	>15
	2.5	10±1.0	7–8±0.5	4–5±0.5
	5	6–7±0.5	4–5±0.3	2.5–3±0.2
	10	4–5±0.4	2.5–3±0.2	1.5–2±0.2
	15	2.5–3±0.3	1.5–2±0.3	1±0.1
WI-38 (Normal Fibroblasts)	1	>15	>15	>15
	2.5	>15	>15	10–15±1.0
	5	>15	12–15±1.5	8–10±1.0
	10	10–15±1.0	8–10±1.0	6–8±0.8
	15	8–10±0.8	6–8±0.7	5–6±0.5

Table (2): Percentage of the cell cycle in detail from G0 to G2/M in each cell line control and treatment. C: control, T: Treated. Mcf7: Breast cancer cells. Wi: Normal Cells.

Sample	%G0-G1	%S	%G2/M	%Pre-G1
C Mcf7	44.39	51.29	4.32	2.61
T Mcf7	36.71	45.29	18	15.24
C Wi	28.41	31.76	39.83	36.17
T Wi	34.52	39.22	26.26	22.46

Table (3): Percentages of apoptotic and necrotic cells in treated and control samples.

	Total	Early	Late	Necrosis
C Mcf7	2.61	0.27	0.41	1.93
T Mcf7	15.24	2.92	9.15	3.17
C Wi	36.17	8.72	23.34	4.11
T Wi	22.46	6.71	12.26	3.49

Table (4): Treatment with SAHA on Genes Expression in human BC cell lines.

Sample data			Results Fold Change		
Ser		Sample code	P21	MMP-9	Caspase3
1		MCF-7/SAHA	4.2	3.1	3.8
2		WI/SAHA	2.4	1.8	1.7
3		cont. MCF7	1	1	1
4	cont. WI	1	1	1	

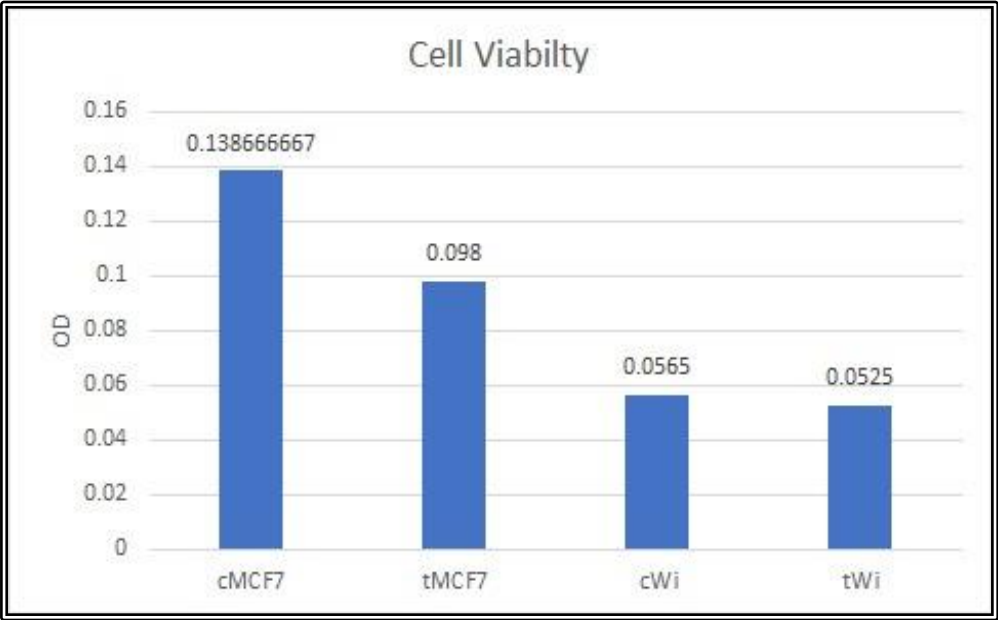


Fig. (1): Cell viability chart MDA: Breast Cancer. Wi: Normal Cells.

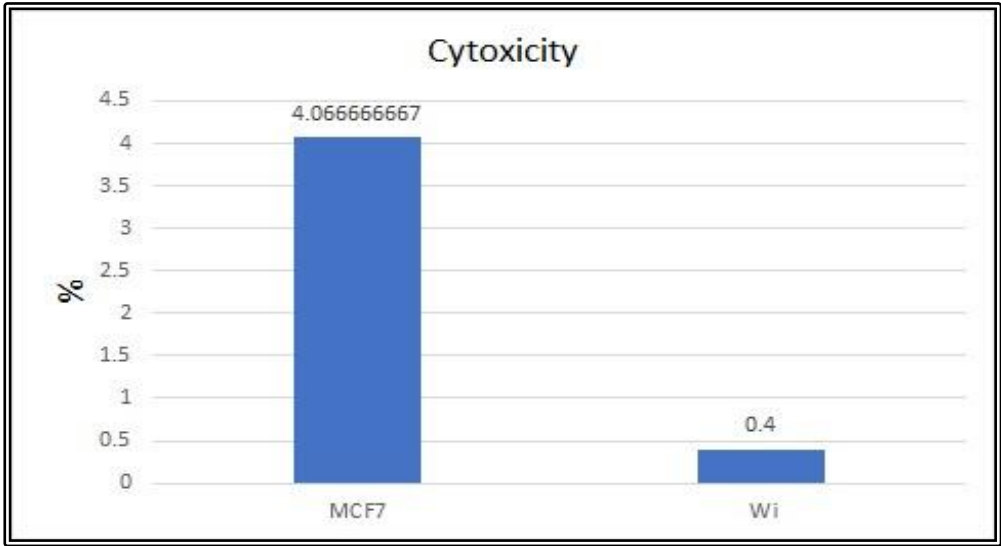


Fig. (2): Percentage of cytotoxicity chart Mcf7: Breast Cancer. Wi: Normal Cells.

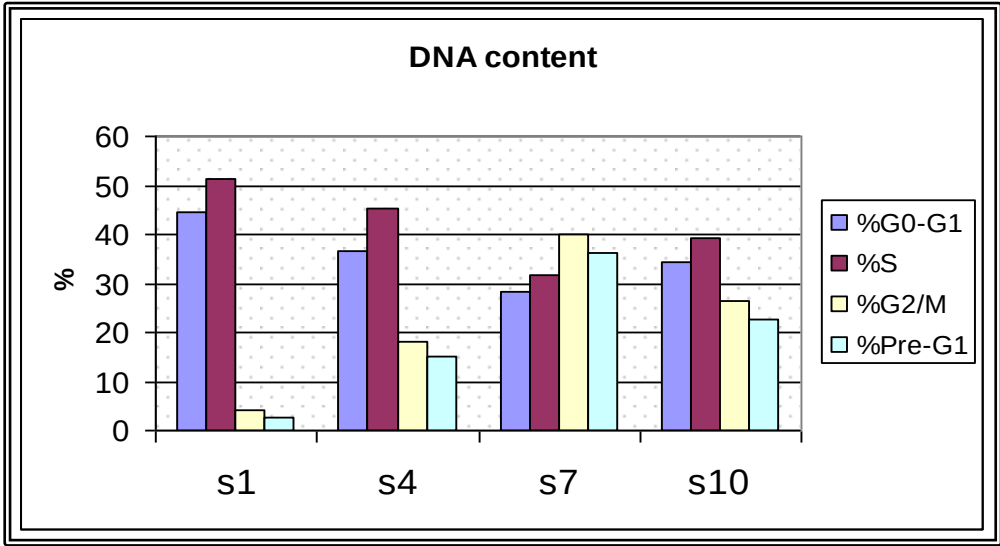


Fig. (3): Cell cycle analysis.

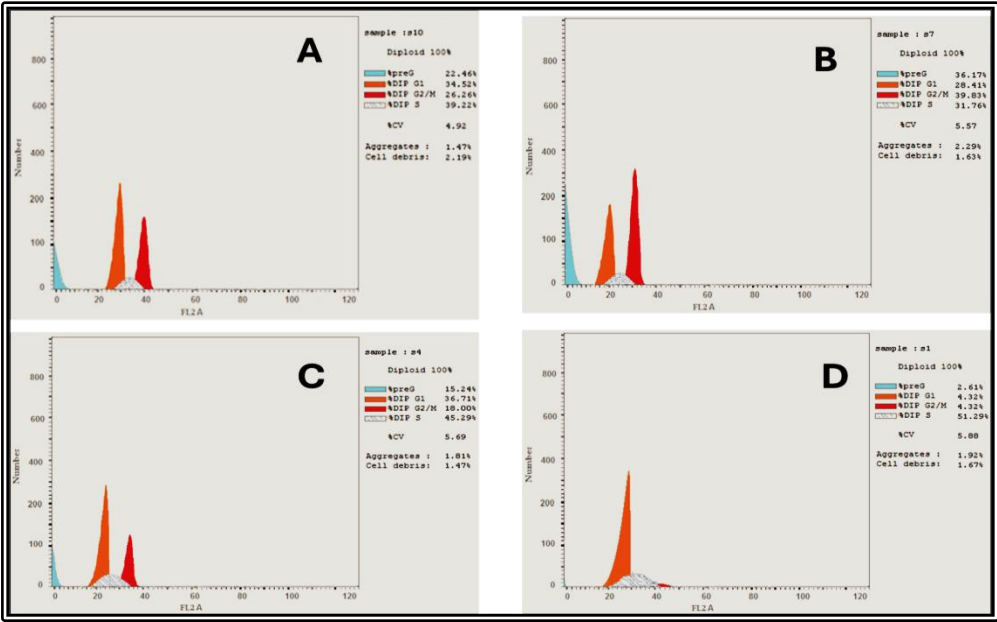


Fig. (4): Cell cycle analysis of each cell line controls and treated.

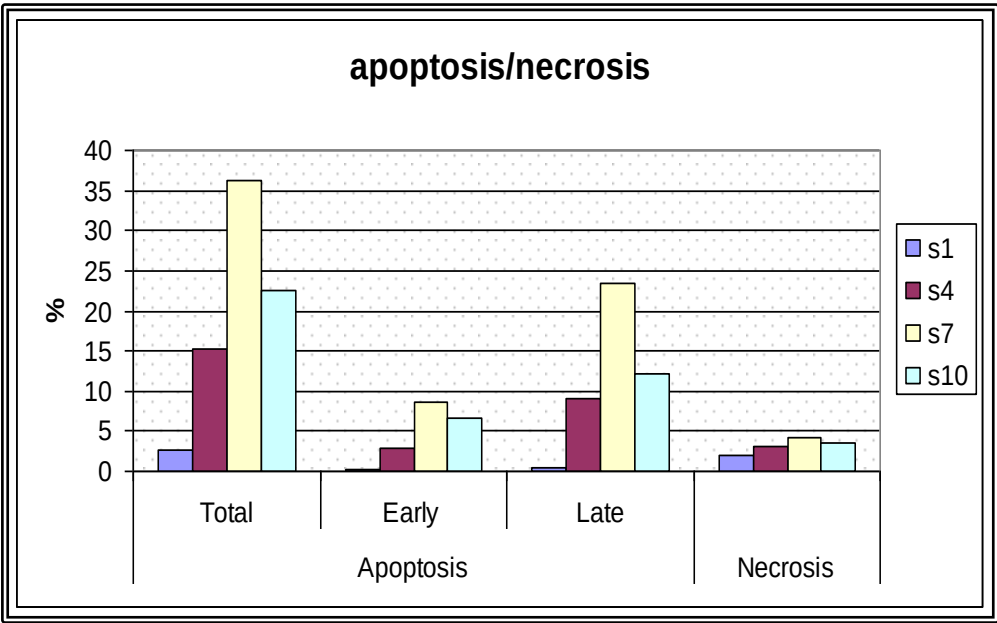
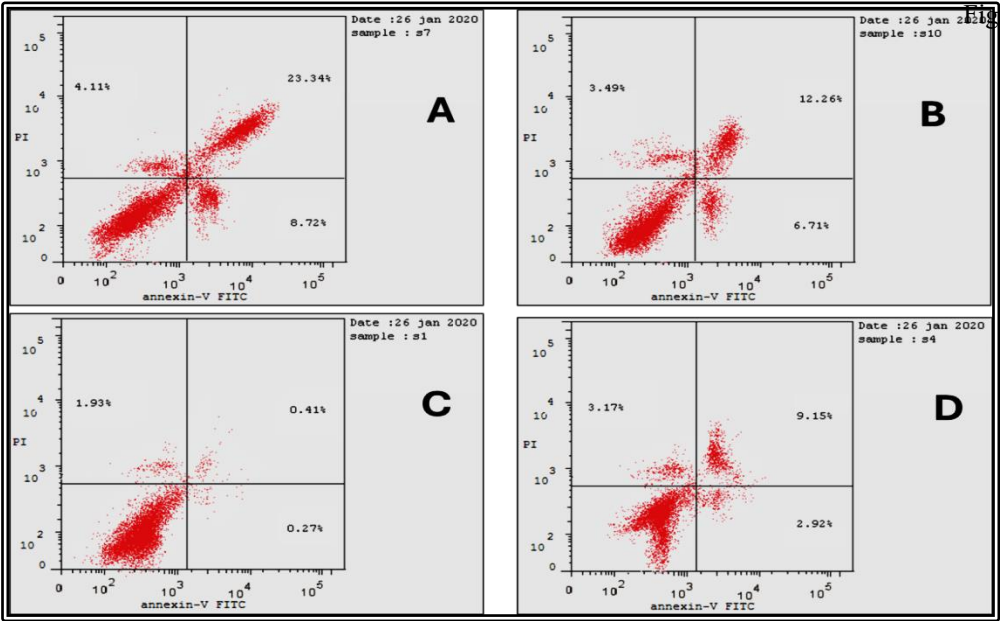


Fig. (5): Apoptosis in BC cell lines treated with varying doses of Treatment 1 and 2.



(6): Control Normal cells (Wi) Apoptosis/Necrosis shows low annexin-V & PI which indicates low apoptosis.

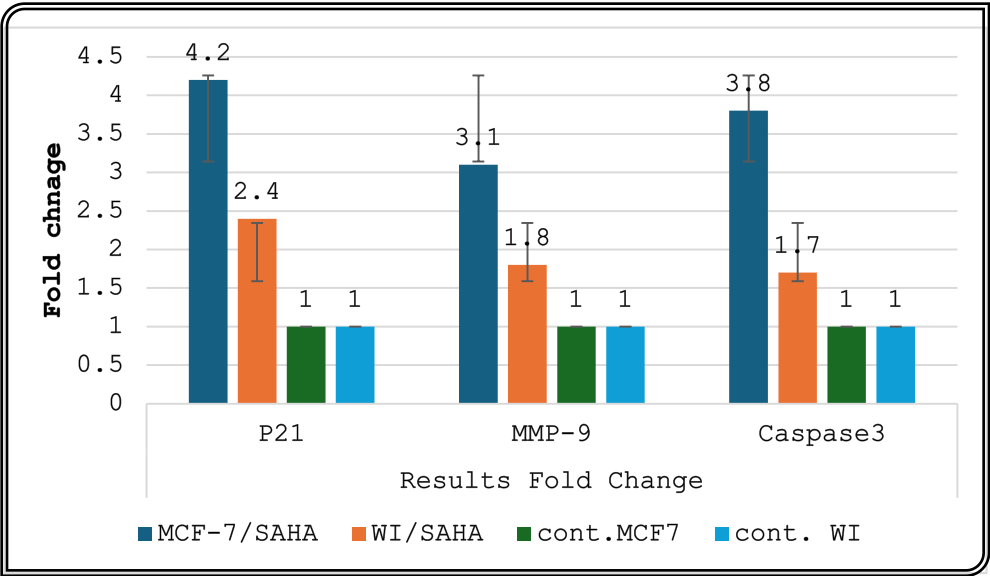


Fig. (7): Impact of SAHA on p21, MMP-9, and Caspase-3 Expression in MCF-7 and WI-38 Cells.