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Research Article

Chemotherapeutic Efficacy of Rutin in Triple Negative Breast Cancer

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Abstract

Cancer is a complex group of diseases characterized by the uncontrolled growth and spread of abnormal cells in the body. It causes millions of deaths each year and remaining a significant global health concern for both men and women. Effective treatment strategies are crucial for improving patient outcomes in breast cancer, particularly in the case of triple-negative breast cancer (TNBC), characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Chemotherapy, like paclitaxel and docetaxel, is the standard treatment for TNBC due to the lack of targeted therapies for this subtype. Paclitaxel (PTX) is a widely used chemotherapeutic medication that is particularly effective against lung, ovarian, and other cancers; nevertheless, its clinical use is limited due to its multi-organ toxicity. As a result, the current study aims to improve treatment efficacy and reduce PTX-induced toxicity through the concurrent use of the natural polyphenolic substance Rutin. Rutin hydrate (purity > 94%) and paclitaxel were utilized in in vitro studies with 4T1 and MDA MB-231 cell lines. In the proliferation assay, cells were treated with rutin and paclitaxel at varying concentrations. Cytochrome-c release and cell cycle analysis were conducted, and flow cytometry assessed apoptosis. According to the findings of this investigation, rutin in combination with PTX considerably ($P<0.05$) lowers the growth and proliferation of breast cancer cell lines in vitro. Furthermore, flow cytometry research revealed that combining rutin with PTX triggered GO/Gl cell cycle arrest and apoptosis in a breast cancer cell line. Furthermore, after co-administration of rutin and PTX, mitochondrial depolarization increased significantly ($P<0.05$). Thus, the current study convincingly established rutin's sensitizing activity and suggests it could be a potential adjuvant in cancer chemotherapy.

Keywords

Triple Negative Breast Cancer, Rutin, Paclitaxel, Chemotherapy

1. Introduction

Breast cancer is a cancer that develops due to uncontrollable changes in the function of growth of the cells forming breast tissue. These changes transform these cells into cancerous cells that have the ability to spread. Breast cancer can occur in both men and women, but it is more common in women.

About 1 percent of all breast cancers diagnosed in the United States are in men, In 2019, about 2,700 men are projected to develop breast cancer, according to the American Cancer Society, compared to 270,000 women. Breast cancer

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in women is the most common cancer diagnosed in the United States [1]. In 2020, there were 2.3 million women diagnosed with breast cancer and 685,000 deaths globally. As of the end of 2020, there were 7.8 million women alive who were diagnosed with breast cancer in the past 5 years, making it the world's most prevalent cancer [2]. Each year, nearly 42,000 women die of breast cancer, making it the second-leading cause of cancer deaths among US women after lung cancer. The lifetime risk of dying of breast cancer is approximately 2.6%.

In men, the 2019 estimate is 2,670 new cases of breast cancer, accounting for less than 1% of all new cancer cases among men. Breast cancer in men is rare, accounting for less than 1% of breast cancer cases in the US [3, 4]. Risk factors include radiation exposure, BRCA1/2 gene mutations, family history of breast cancer. However, research indicates that there are several risk factors that may increase your chances of developing breast cancer. These include: Smoking-Tobacco use, Alcohol use, Hormone replacement therapy (HRT)-People who use hormone replacement therapy have a higher risk of being diagnosed with breast cancer. [5]

There are several different types of breast cancer, including:- Infiltrating (invasive) ductal carcinoma (IDC), Ductal carcinoma in situ (DCIS), Infiltrating (invasive) lobular carcinoma (ILC), Lobular carcinoma in situ (LCIS), Inflammatory breast cancer Paget's disease of the breast and Triple negative breast cancer (TNBC). Triple-negative breast cancer (TNBC) accounts for about 10-15% of all breast cancers. The term triple-negative breast cancer refers to the fact that the cancer cells don't have estrogen or progesterone receptors (ER or PR) and also don't make any or too much of the protein called HER2. (The cells test "negative" on all 3 tests.)

These cancers tend to be more common in women younger than age 40, who are Black, or who have BRCA1 mutation. TNBC differs from other types of invasive breast cancer in that it tends to grow and spread faster, has fewer treatment options, and tends to have worse prognosis [6, 7]. Treatment of Breast cancer depending on the diagnosis (type of tumor, stage, size) and the health status of the patient: Surgery (Surgery involves the physical removal of the tumor typically along with some of the surrounding tissue), Radiation therapy (Radiation therapy is the use of high-energy X-rays or other particles to destroy cancer cells).

Chemotherapy is the use of drugs to destroy cancer cells, usually by keeping the cancer cells from growing, dividing and making more cells. It may be given before surgery to Shrink a large tumor, make surgery easier, and/or reduce the risk of recurrence. When it is given before surgery, it is called neoadjuvant chemotherapy. It may also be given after surgery to reduce the risk of recurrence, called adjuvant chemotherapy. A neoadjuvant or adjuvant chemotherapy regimen, or schedule, usually consists of a combination of drugs given in a specific number of cycles over a set period of time. Chemotherapy may be given on many different schedules depending on what worked best in clinical trials for that spevitie (type of regimen,. It may be given once a week, once every 2 weeks, or once every 3 weeks. There are many types of chemotherapy used to treat breast cancer. Common drugs include Docetaxel (Taxotere), Paclitaxel (Taxol). Doxorubicin (available as a generic drug), Epirubiein (Ellence), Cyclophosphamide (available as a generic drug), Fluorouracil (S-FU), Protein-bound-Paclitaxel (Abraxane). [8]

Chemotherapy medications travel throughout the body. Main limitation of chemotherapy is the toxicity of the drug in our body and the side effects of the drug. Side effects depend on the drugs we receive and our reaction to them. Most side effects are temporary and subside once treatment is finished. Sometimes chemotherapy can have long-term or permanent effects. Common short-term side effects include: Hair loss, Fatigue, Loss of appetite. Nausea and vomiting. Constipation or diarrhea, Mouth sores, Skin and nail changes, Increased risk of developing infection (due to fewer white blood cells that help fight infection), Nerve damage (neuropathy) and certain chemotherapy drugs for breast cancer can cause long-term side effects, including: Infertility, Bone thinning, Heart damage, Leukemia [9].

Phytochemicals like Rutin are used to overcome this limitation of chemotherapeutic drugs, Rutin is a unique antioxidant flavonoid that is mainly found in fruits, vegetables, cereals, and many other plant-based human diets. Rutin has anticancer properties. Rutin is demonstrated to inhibit the proliferation of breast, colon, lung, and prostate cancers and other tumors. Furthermore, Rutin alone or in combination with other therapeutic agents has been shown to regulate several signaling pathways involving the Ras/Raf and PI3K/Akt, MAPK, and TGF β2Smad2/3 Akt/PTEN, etc., which are related to the processes of carcinogenesis and induction of apoptosis. The combination of rutin with other chemotherapy drugs may benefit on prevention of tumor cells by decreasing drug resistance and chemotherapy side effects. Moreover, rutin induces apoptosis synergistically with the therapeutic agent [10]. This combining effect of drug and rutin like compound has much lower toxicity and higher effectiveness in our body than a single of chemotherapeutic-drugs.

2. Materials and Methods

In Vitro Studies:

1) Compounds: Rutin hydrate (purity > 94) was purchased from Sigma-Aldrich (chemical private limited, Bangalore, India) and Paclitaxel was obtained from (Medsign Biotech Pvt. Ltd).
2) Cell culture: In this study two triple negative breast cancer cells, 4T1 a mammary gland epithelial adherent cell line of Mus musculus maintained in DMEM media supplemented with 10% fetal bovine serum. Human breast can- cer cell line MDA MB-231 is an epithelial

mammary gland cell line maintained in RPMI-1640 supplemented with 10 % fetal bovine serum. Both the cells were maintained at 37 ℃ with 5% CO_2.

3) In vitro proliferation assay: Cell viability analysis was performed in both 4TI and MDA MB 231 cells using MTT (3-(4, 5-dimethylthiazol-2-yl), 2, 5 diphenyltetrazolium bromide) reduction assay method. This assay principally determines cell viability by mitochondrial enzyme activity such as succinate dehydrogenase which reduces the MTT dye into a purple insoluble form or formazan which is then dissolved by DMSO and optical density was measured by a spectrophotometer at 570 nm. Briefly 4T1 and MDA MB-231 cells were grown (1 x 104 cells/well) in the 96-well plates in a final volume of 100 ul culture medium containing DMEM and RPMI-1640 respectively after achieving 80% confluency, the cells were incubated for 24h. Different concentrations of rutin (1-100 uM) and Paclitaxel (10-1000nM) were added to the cells and incubated for 48 h. Then 10 ul of the MTT solution (5 mg/ml) was added to each well and incubated at 37 ℃ for 4 h. For terminating the reaction, 180 ul of DMS0 was added to each well and incubated for 10 min. After complete solubilization of the purple formazan crystals, the 96-Well plates were subjected to agitation, and the OD was measured at 570 nm in 96-well plate reader (Tecan Plate reader, Infinite pro 200). The average of triplicate readings was considered.

4) Cytochrome-c release assay: Both 4TI and MDA MB-231 cells were seeded in 35 mm culture dish over a glass coverslip in 10% serum containing DMEM and RPMI-1640 respectively for 24hr. After reaching 80% confluency cells were treated with IC50 dose of rutin and paclitaxel as monotherapy and combination of Rutin and Paclitaxel (1C25 of rutin and IC25 of Paclitaxel) and incu- bated at 37 ℃ for 4 h. After all the groups were stained with Mito tracker red (CMXRos, Cat. MS712) for staining mitochondria and for Immuno-cytochemistry stained with cytochrome-c Ab (Santa Cruz, sc-13156) for detection of cytochrome-c released from mitochondria to cytosol.

5) Cell cycle analysis: Flow cytometry analysis can be done with the measurements of increased DNA content in proliferating cells going through cell cycle phases. 4TI cells were seeded at a concentration of Ix106 cells/60 mm cell culture dish in 2ml complete DMEM culture medium and after 24 h of incubation these cells were treated with various concentrations of Rutin, paclitaxel and both in combination for 6h. After this incubation period media was removed from the culture and the cells were collected by scraping and prepared for cell cycle analysis by FACS. Flow cytometry analysis: Flow cytometry was conducted on single suspension of both 4Tl and MDA MB-231 cells seeded in 60 mm dish (1× 106 cells/ml) overnight with DMEM and RPMI 1640 respectively and 10% FBS under the condition of 5% co2 and 37 ℃. After cells reaches 80% confluency, Rutin and paclitaxel were added in their corresponding 1C30 values alone and in combination (IC25). Incubated for 48 hrs after which cells were harvested. Thereafter apoptotic and necrotic cell death was determined by using FITC Annexin V Apoptosis Detection Kit l, BD Pharmingen TM, Briefly, Ix106 cells were suspended in 100 ul binding buffer. After that Annexin-V-FITC and propidium iodide (PI) was added in a 1:1 ratio and incubated at room temperature for 15 min in dark condition. 400 ul of binding buffer was then added and percentage of apoptotic and necrotic cells were analyzed with a flow cytometer.

3. Observation and Results

The growth inhibition in 4T1 and MDA MB-231 were analyzed by MTT assay to determine the in vitro therapeutic efficacy of rutin in combination with Paclitaxel (Figure 1). Primarily the IC50 values of Rutin in 4T1 and MDA MB-231 were found to be 46.18 µM and 145 µM respectively (Figure 1 A, B). IC50 values Paclitaxel in 4T1 and MDA MB-231 were 265.33 nM and 404.75 nM respectively (Figure 1 B, D).

For both 4T1 and MDA MB-231; all the results are represented in graphical form, (A) effect of Rutin over 4T1 cells, (B) effect of Paclitaxel over 4T1 cells, (C) effect of Rutin over MDA MB-231 cells, (D) effect of Paclitaxel over MDA MB-231 cells; all results were measured after 48 hrs. of drug treatment; r2 - values of each graphical representation displayed on the top right side of each graph which denotes the reliability of the data fits with the trendline, closer the r2 – value to 1 better the trendline fits the data. All data were represented from three independent experiments.

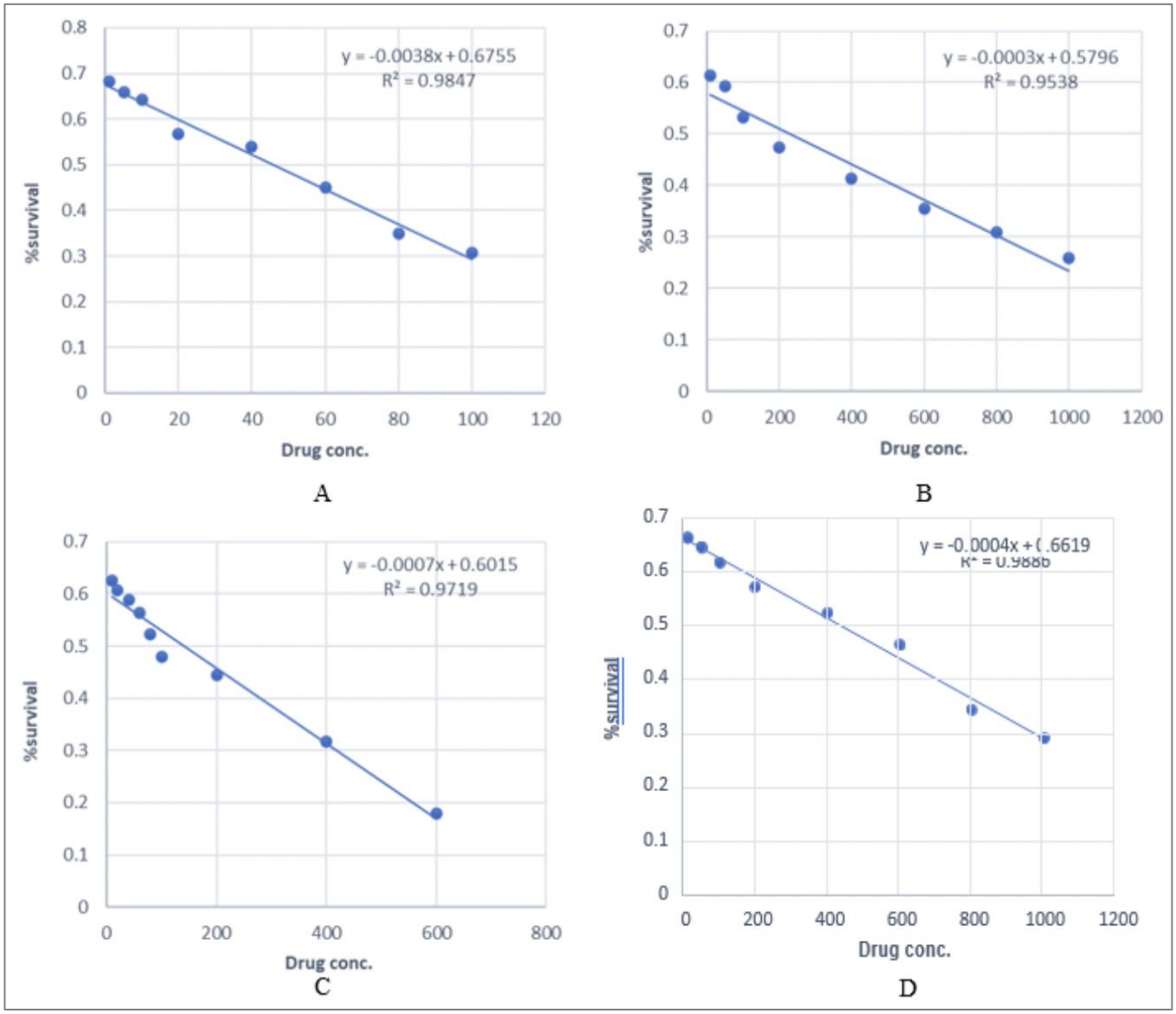


Figure 1. *In vitro proliferation assay was done to determine the IC50 (inhibitory concentration) value of both rutin and paclitaxel; various concentration of Rutin ranging from 1–100 µM and 10–600 µM was used for 4T1 and MDA MB-231 respectively and various concentration of Paclitaxel ranging from 10-1000nM used.*

Treatment with Rutin and paclitaxel showed externalization of cytochrome C from the mitochondria into the cytosol when compared to untreated control cells (Figure 2). Combination with Rutin and Paclitaxel showed greater externalization of cytochrome-C out of mitochondria which is an indicator of induction of apoptosis (Figure 2) and cause nucleus shrinkage which is evident by DAPI staining.

Rutin treatment cause G2/M blockage in cell cycle analysis: Treatment with Rutin in monotherapy (Figure 3B) over 4T1 cell show 15.01% increase in G2/M blockage whereas paclitaxel monotherapy group (Figure 3C) show only 0.63 % blockage compare to control group (Figure 3A). When rutin and paclitaxel were used in combination therapy (Figure 3D) there was 1.69 % blockage in G2/M checkpoint. Which signifies rutin increased the effect of paclitaxel by 1.06% compared with the paclitaxel monotherapy group.

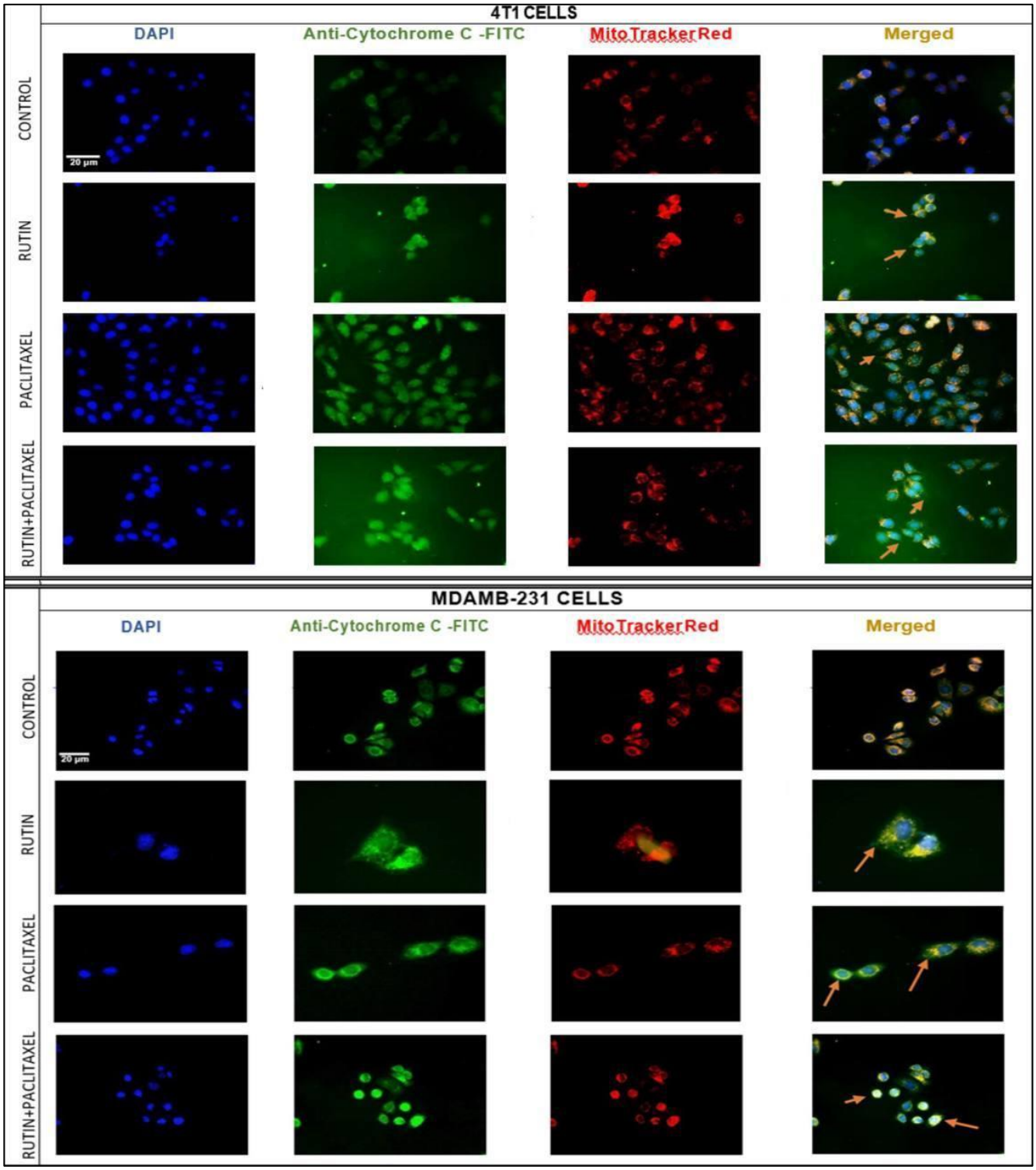
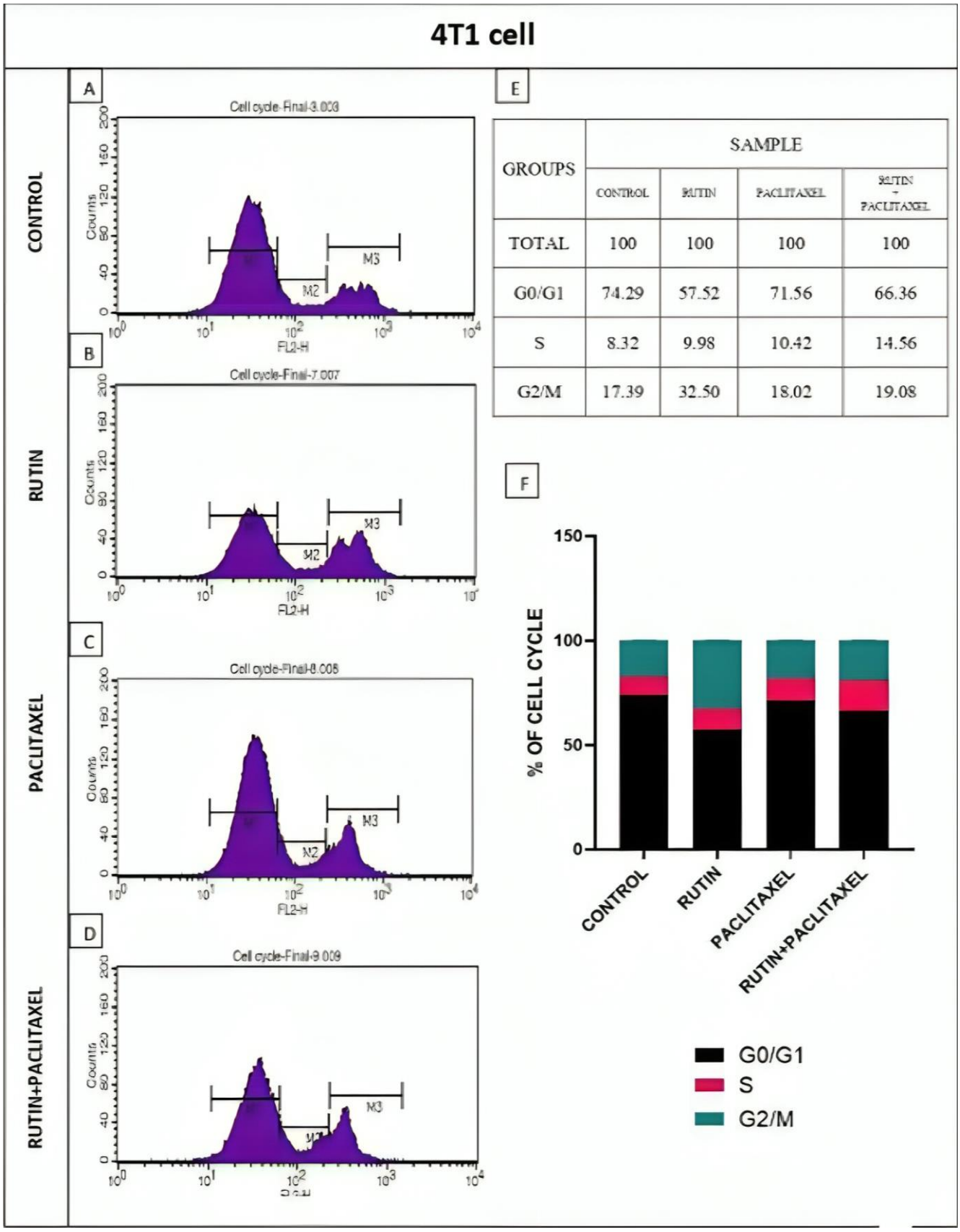


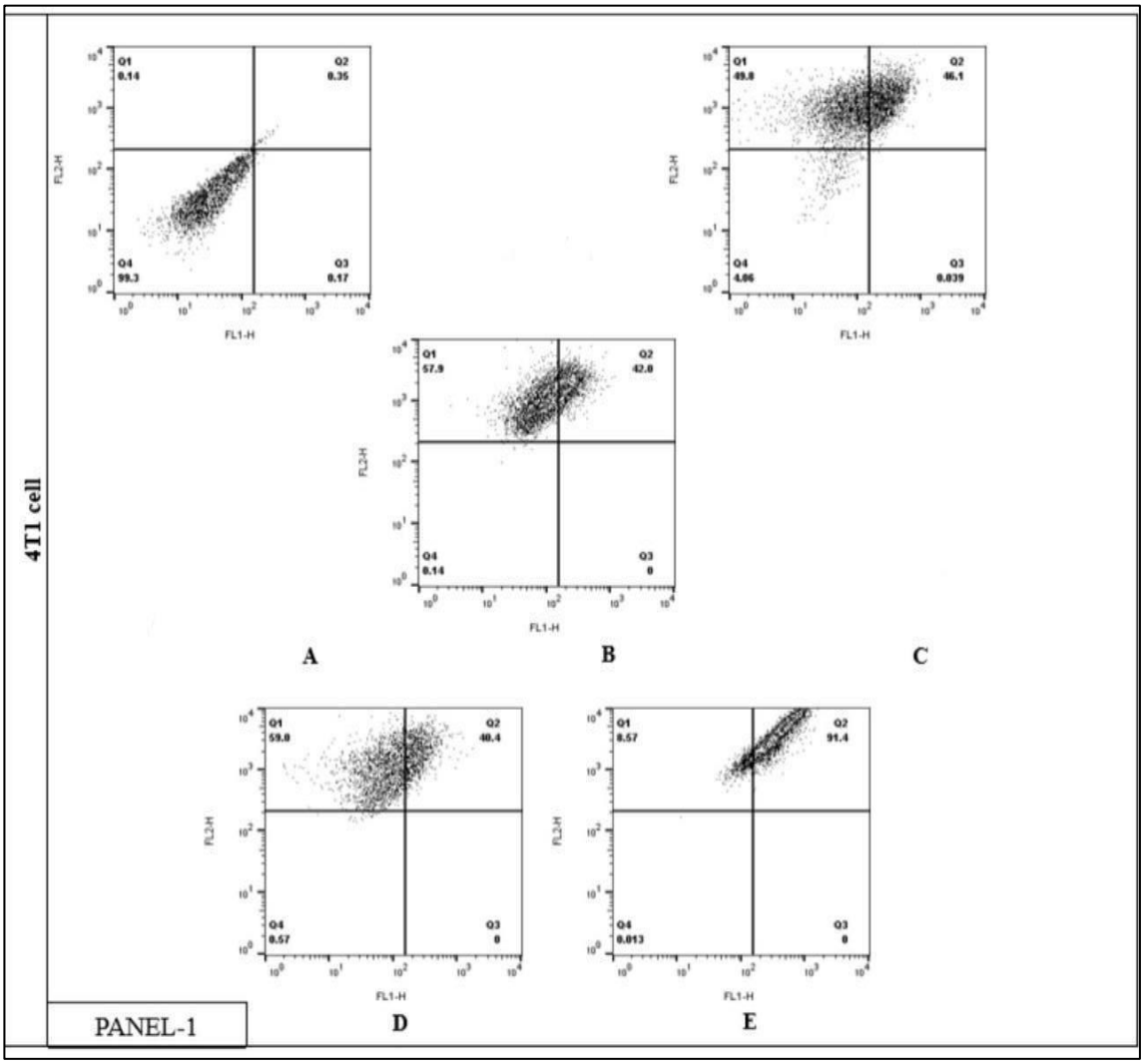
Figure 2. *4T1 and MDA MB-231 cells were stained with DAPI (blue) for nuclear staining and FITC-conjugated secondary antibody (green) for staining Cytochrome-C and Mito tracker red (Red) for mitochondrial staining, different filters were used to capture DAPI (Ex-358 nm, Em- 461 nm), FITC (Ex-495 nm, Em-519 nm) and Mito tracker (Ex- 490 nm, Em- 516 nm) and merged image was produced by LASx software of Leica. It showed that after treatment cytochrome-C came into cytosol from mitochondria as green color showed more prominently (marked by arrow) than the control cells in which cytochrome-C remain within the mitochondria and produce yellow color. Images scale bar 20 µm. All data were represented from three independent experiments.*



GROUPS	SAMPLE			
	CONTROL	RUTIN	PACLITAXEL	RUTIN + PACLITAXEL
TOTAL	100	100	100	100
G0/G1	74.29	57.52	71.56	66.36
S	8.32	9.98	10.42	14.56
G2/M	17.39	32.50	18.02	19.08

Figure 3. *Cell cycle analysis, 4T1 cells were treated with Rutin and paclitaxel monotherapy and a combination therapy; A) Control group, B) Rutin treated group, C) Paclitaxel treated group, D) Rutin and paclitaxel treated group, E) A tabular representation of various % of cells in G0/G1 phase, S-phase and G2/M-phase in different treatment group and control group, F) A graphical representation of cell cycle analysis of different group prepared by GraphPad prism 8.4.2 where G0/G1-phase depicted by black color, S-phase by magenta color and G2/M-phase by green color.*

Treatment with rutin only, 4T1 cells became more apoptotic as the percentage of late- apoptotic and necrotic cells were increased to 46.1% (Q2) and 49.8% (Q1) (Figure 4, Panel-1; B) receptively. However, treatment with Paclitaxel only (according to its IC50 dose) the percentage of late-apoptotic and necrotic cells were increased to 42.0% (Q2) and 57.9% (Q1) (Figure 4, Panel-1; C) in compare with untreated control cells (Figure 3, Panel-1; A) where percentage of late- apoptotic and necrotic cells were 0.35% and 0.14% respectively. After treatment with both rutin and paclitaxel (Figure 3, Panel-1; D) (in combination dose contain both rutin and paclitaxel in half of their ic50 dose) percentage of late apoptosis is 40.4% (Q2) and necrotic death is 59.0% (Q1) also a high dose of paclitaxel used as positive control showed high percentage of death found in the late apoptotic region 91.4% (Q2) and 8.57% in the necrotic region (Q1) (Figure 4, Panel-1; E). In MDA MB-231 cells after treatment with Rutin only the percentage of late-apoptotic and necrotic cells were increased to 2.33 (Q2) and 42.8% (Q1) (Figure 4, Panel-2; B) and in Paclitaxel only (according to its IC50 dose) the percentage of late-apoptotic and necrotic cells were increased to 1.29% (Q2) and 72.7% (Q1) (Figure 4, Panel-2; C) respectively in compare with untreated cells (Figure 4, Panel-2; A) where percentage of late-apoptotic and necrotic cells were 0.35% (Q2) and 0.14% (Q1) respectively. In combination treatment with both rutin and paclitaxel (Figure 4, Panel-2; D) percentage of late-apoptotic and necrotic cells were 11.0% (Q2) and 88.3% (Q1) and also a high dose of Doxorubicin used as positive control showed high percentage of death found in the late apoptotic region 99.7% (Q2) and 0.33% (Q1) in the necrotic region (Q1) (Figure 4, Panel-2; E).



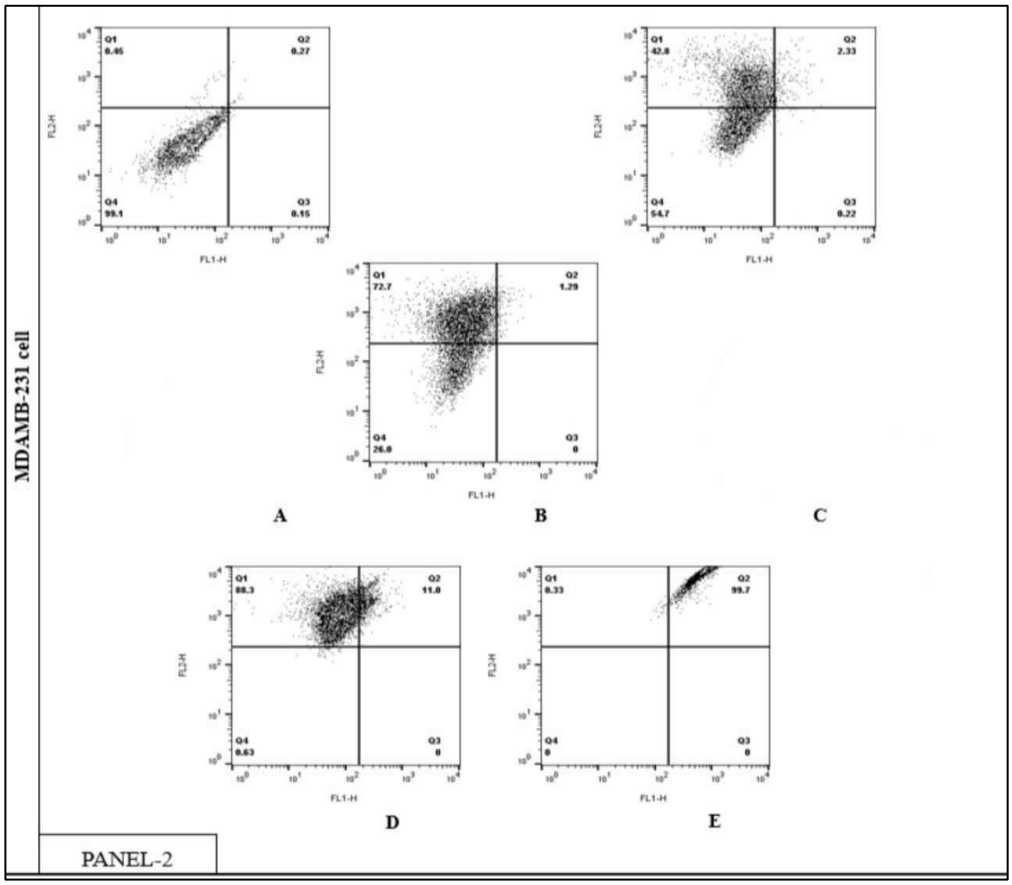


Figure 4. *Flowcytometry analysis by Annexin V/PI, in PANEL-1; (A) untreated cell, (B) treatment with IC50 dose of Rutin, (C) treatment with IC50 dose of Paclitaxel, (D) treatment with both Rutin & Paclitaxel (combination dose contain both rutin and paclitaxel in half of their IC50 dose), in PANEL-2; (A) untreated cell, (B) treatment with IC50 dose of Rutin, (C) treatment with IC50 dose of Paclitaxel, (D) treatment with both Rutin & Paclitaxel (combination dose contain both rutin and paclitaxel in half of their IC50 dose); all drug treatment were done for 48 hrs. FL1-H for Annexin V and FL2-H for PI. All data were represented from, three, independent-experiment.*

4. Discussion

Due to the absence of ER (estrogen receptor), PR (progesterone receptor) and HER2 (Human epidermal growth factor receptor 2), there are very limited options for treatment of Triple Negative Breast Cancer (TNBC) [11]. Endocrine (hormone) therapy or other targeted modalities are hence not helpful. Therefore, chemotherapy is the main treatment option [12]. And even though there is response to the treatment, it tends to recur more frequently than other breast cancers. A significant challenge associated with Paclitaxel therapy in TNBC is the development of chemoresistance over time [13]. TNBC tumors can exhibit intrinsic or acquired resistance, limiting the effectiveness of Paclitaxel and contributing to treatment failure. Dose-limiting toxicities, such as myelosuppression and peripheral neuropathy [14], often necessitate dose reductions or treatment interruptions.

Despite initial responses, long-term remissions with paclitaxel monotherapy in TNBC are often limited. Hence, there is a critical need for novel combination therapies or the development of adjunctive agents to extend the duration of response and improve overall survival outcomes. The aim of this study is to highlight the use of Rutin as an adjuvant therapy to enhance treatment efficacy and improve patient outcomes. Currently there are very few studies that study the role of Rutin as an adjuvant for breast cancers. This study is of particular significance in understanding cellular responses to this combination therapy and to highlight its synergistic effect on paclitaxel, a routine chemotherapeutic agent.

In vitro proliferation assay demonstrates the ability of Rutin to decrease cell proliferation in both the cell lines 4T1 and MDA MB 231. The proliferation of both the cell lines was significantly inhibited by Rutin in a dose dependent manner, with IC50 values being 46.18 µm and 145 µm respectively. The results have also been replicated in various studies, including Sghaier et al. in 2016 [15], which highlights Rutin's action in inhibiting proliferation in human lung (A549) and colon (HT29 and CACO-2) cancer cell lines. However, those cell lines seem to be more resistant to Rutin therapy, with a threefold higher IC50 concentration. Suganya et al [16] demonstrates the activity of Rutin on the same MDA MB 231 cell line, which reproduces the same result, with IC50 being 40 µm.

Induction of apoptosis was studied by cytochrome c release assay. Greater externalization of cytochrome c was seen in cells treated with combination of Rutin and Paclitaxel, indicating a higher apoptotic potential of combination therapy. Studies have individually confirmed the induction of apoptosis of Paclitaxel therapy alone and Rutin therapy along. Khing et al [17] demonstrates the effect of Paclitaxel on AGS (gastric adenocarcinoma cells), while Pandey et al [18] shows fragmented nuclei on DAPI staining of SiHa (cervical cancer cell line). These studies show that the pathway likely to be followed by both Rutin and Paclitaxel for mediating cell toxicity is apoptosis. However, no studies were found to depict the combined effect of both Rutin and Paclitaxel making our study one of the first steps to identifying and describing this novel combination therapy.

Cell cycle analysis was conducted using flow cytometry, to check for G2M phase arrest. Paclitaxel monotherapy showed only 0.63% blockage compared to the control group. Studies have shown mixed results with regards to the mechanism by which Paclitaxel mediates differential growth inhibition. Han et al. [19] has shown G2M arrest on glioma cell lines. However, Dziadyk et al. [20] study has demonstrated that Paclitaxel induced apoptosis may occur without G2/M arrest on BCap37 human breast cancer cell lines. Rakovitch et al. [21] further suggests a p53 independent apoptosis pathway.

Rutin monotherapy showed a high (15.01%) increase in G2/M blockade. Chen et al. has demonstrated a similar effect in LAN-5 cells (human neuroblastoma cells), showing a dose-dependent increase in the number of cells arrested in G2/M phase. Multiple studies hence show that the dominant mechanism mediated by flavonoids is via G2/M phase cell cycle arrest [22].

Combination of Rutin and Paclitaxel on the cell lines demonstrated a 1.69% blockage in G2/M checkpoint, an increase of 1.06% from paclitaxel monotherapy. Synergistic action of flavonoids on paclitaxel therapy on G2/M cell arrest has been demonstrated. Guo Y et al [23]. showed FV – 429, a flavonoid compound, makes cancer cells more sensitive to the drug paclitaxel by deactivating Wnt pathway. However, no studies were found of using Rutin as a potential combination therapy. The potentiating effect demonstrated in our study proves beneficial for treatment modalities, harboring both, a potential for increased efficacy, as well as overcoming drug resistance.

FITC Annexin V is used to quantify the percentage of cells undergoing apoptosis and confirm apoptotic pathway of drug in cell death. In 4T1 cells, Paclitaxel monotherapy led to the percentage of late apoptotic and necrotic cells being 42% and 57.9% respectively. Study of Khing et al [17]. on AGS cells (gastric adenocarcinoma) has confirmed similar findings on paclitaxel treated cells. In the study, Rutin monotherapy showed the percentage of late apoptotic and necrotic cells to be 46.1% and 49.8%. Hasani et al [24] has demonstrated the apoptotic effects of rutin on MCF-7 (ER positive breast cancer cells). On combined therapy of both rutin and paclitaxel on 4T1 cells, (doses half of their IC50 dose), there was an increase in necrotic cell death to 59%. On usage of a higher dose of paclitaxel in combination therapy, a significantly high number of cells were in the late apoptotic region (91.4%). The MDA MB-231 cells also showed an increased percentage of cells in late apoptotic and necrotic stage when treated with rutin and paclitaxel, as opposed to monotherapy. While synergistic effects of paclitaxel and apigenin (flavonoid compound) have been demonstrated by Xu Y et al [25], no conclusive studies were found of Paclitaxel and Rutin as a combination. This makes the current study unique in demonstrating the synergistic effects of rutin, allowing a better understanding of its agonistic action on cell death via apoptosis.

5. Conclusion

This study represents a significant stride toward enhancing TNBC therapeutic strategies. The observed potentiating effects of Rutin on cell death and apoptosis make the synergism of rutin and paclitaxel a compelling candidate for further clinical investigation. As the need for newer therapeutic modalities emerge due to increasing drug reactions, adverse effects, and lack of efficacy, combination therapy of a natural compound like Rutin with a conventional chemotherapeutic agent (paclitaxel) holds promising therapeutic implications.

TNBC is challenging to treat and treatment is crucial for patients and clinicians due to its grim prognosis and limited treatment options available. Rutin being anticarcinogenic can play a pivotal role in the treatment. The findings validate that rutin has a lot of pharmaceutical properties and its role and potential mechanisms in triple negative breast cancer should be investigated further.

Abbreviations

ER	Estrogen Receptor
PR	Progesterone Receptor
HER2	Human Epidermal Growth Factor Receptor 2
TNBC	Triple Negative Breast Cancer
IDC	Infiltrating (Invasive) Ductal Carcinoma
DCIS	Ductal Carcinoma In Situ
ILC	Infiltrating (Invasive) Lobular Carcinoma
LCIS	Lobular Carcinoma in Situ
HRT	Hormone Replacement Therapy
MTT	3-(4,5-dimethylthiazol-2-yl),2,5diphenyltetra zolium Bromide
RPMI	Roswell Park Memorial Institute Medium
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
DMSO	Dimethyl Sulfoxide
OD	Optical Density
PI	Propidium Iodide
FITC	Fluorescein Isothiocyanate
IgG	Immunoglobulin G

CMXRos	MitoTracker Red
Ab	Antibody
FACS	Fluorescence-Activated Cell Sorting
FL	Fluorescence
LASx	Leica Application Suite X
IC50	Half-Maximal Inhibitory Concentration
Wnt	Wingless Related Integrated Site

Author Contributions

The authors confirm contribution to the paper as follows:

Niragh Sikdar: study conception and design, data collection, analysis and interpretation of results, draft manuscript preparation

Shree Rath: analysis and interpretation of results, draft manuscript preparation

All authors reviewed the results and approved the final version of the manuscript.

Ethical Approval

Waived as commercially available stem cells were used and no human participants were involved

Availability of Data and Material

On request from authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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