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RESEARCH ARTICLE

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A STUDY TO EVALUATE EFFECTIVENESS OF THE ANTICANCER PROPERTY OF ASPIRIN AND IBUPROFEN IN MCF-7 BREAST CANCER CELL LINES.

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ABSTRACT

Background: Breast cancer is a leading cause of morbidity and mortality among women worldwide. Insights employing MCF-7 cell lines for breast cancer research have continued unabatedly in the last five decades. The MCF-7 cell line has radically changed the trajectory of breast cancer research and helped to improve patient outcomes, despite the drawbacks of research on established cancer cell lines maintained in tissue culture and xenografts. NSAIDs, including Aspirin and Ibuprofen, have been associated with a reduced risk of breast cancer.

Objectives: The present study was undertaken as a pre-clinical cross-sectional study to evaluate and compare the anti-cancer properties of Aspirin and Ibuprofen in MCF 7 breast cancer cell lines.

Methodology: 3-(4,5- dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, DAPI (4,6 Diamidino-2-phenylindole dihydrochloride) staining, Annexin assay and DNA fragmentation by Gel electrophoresis were employed in the study.

Results: IC50 values for Ibuprofen (28 µg/ml) were higher than Aspirin (21.18 µg/ml). No significant difference in the percentage of cancer cell death between Aspirin and Ibuprofen was noted. When combined, Aspirin and Ibuprofen showed significantly higher anti-cancer properties (IC50-14.93 µg/ml) on breast cancer cell lines compared to their standalone samples. Apoptosis level by annexin assay was increased at 15µg of Aspirin and Ibuprofen. DAPI staining also showed that a greater number of cancer cells were stained at 15µg of Aspirin and Ibuprofen. Fragmentation of the combination drugs of 10µg shows the highest apoptosis from control through DNA ladder Assay.

Conclusion: Thus, Aspirin and Ibuprofen, exclusively and in combination, have been found to have anti-cancer properties against breast cancer cell lines through various laboratory methods.

Keywords: Aspirin, Ibuprofen, Anti-cancer property, MCF 7 cell line, Breast cancer.

INTRODUCTION

In 2020, an estimated 2.3 million new cases of breast cancer were reported, surpassing the combined incidence of the second-third-most frequent cancers in women, colorectal and cervix uterine cancer. According to data on diagnosed women, 685,000 people worldwide died from breast cancer in 2020. Breast cancer is responsible for approximately 411,000 deaths annually, representing 15% of all cancer-related deaths, and is the most common malignancy among women.1 Breast cancer is neither an infectious nor a contagious disease. Unlike several cancers with infection-related origins, such as human papillomavirus (HPV) infection and cervical cancer, there are no known viral or bacterial infections linked to the development of breast cancer.

In the glandular tissue of the breast, breast cancer develops in the lining cells (epithelium) of the ducts (85%) or lobules (15%). Initially, the malignant development is contained within the duct or lobule ("in situ"), where it often exhibits no symptoms and has a low risk of spreading (metastasis). This in situ (stage 0) tumor may develop over time and infect the breast tissue around them (invasive breast cancer), then disseminate to neighboring lymph nodes (regional metastasis), or other body organs (distant metastasis). Breast cancer deaths in women are often due to widespread metastases. Treatment for breast cancer can be successful, especially if the disease is discovered early. A combination of surgical removal, radiation therapy, and medication (hormonal therapy, chemotherapy, and targeted biological therapy) is frequently used to treat breast cancer. Such treatment can stop the growth and spread of cancer, saving lives in the process.

Around 50 years ago, George Gey created the first human cell line in a lab in Baltimore. Henrietta Lacks, who suffered from cervical cancer and served as the cell line's source, is remembered by the name HeLa for this cell line. Gey's theory led to the expansion of cell culture internationally, and today it is a crucial experimental tool in cancer research. One of the key advantages of using developed cell lines in cancer research is that they provide an endless source of relatively homogeneous cell populations that can self-replicate in a typical cell culture medium. The first breast cancer cell line, BT-20, was created in 1958.

However, it took another 20 years for breast cancer cell lines to be used more extensively, including the MD Anderson series and MCF-7, the most commonly used breast cancer cell line in the world today. MCF-7, created in 1973 at the Michigan Cancer Foundation, is highly popular because it expresses the estrogen receptor (ER), which gives it excellent hormone sensitivity and makes it an ideal model for studying hormone response. Despite these early successes, only a few breast cancer cell lines have been developed more recently. This is mainly due to the difficulty in producing homogeneous populations free of significant stromal contamination. Still, it is also due, at least in the United Kingdom, to the stringent ethical guidelines that must be followed when obtaining human tissue for research. One success story is the SUM series of 10 cell lines, created from breast primary tumors, pleural effusions, or other metastatic sites in certain patients. These cell lines are now widely accessible to the general public through commercial cell banks.2

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently prescribed medication classes globally. They are used to treat fever, pain, and inflammation and have antipyretic, analgesic, and anti-inflammatory properties.3 NSAIDs include a wide range of drugs, such as ibuprofen, mefenamic acid, celecoxib, Aspirin, and Diclofenac. A growing body of research has focused on using NSAIDs to prevent and treat cancer, which is linked to chronic inflammation. There are numerous publications on the anti-cancer effects of NSAIDs, and several epidemiological studies demonstrate that these drugs are associated with a reduced risk of developing cancer in various cancer types, such as breast, prostate, colorectal, ovarian, and head and neck cancers. However, how NSAIDs prevent cancer is not yet clear, as the evidence is contradictory and inconclusive.4 Despite having different chemical structures, all of these drugs can inhibit the enzymes prostaglandin endoperoxide H synthase (PGHS) or cyclooxygenase (COX). The link between COX, prostaglandin production, and inflammation provides the clearest picture of how NSAIDs affect cancer.

Aspirin and other NSAIDs have attracted a lot of attention as potential chemopreventive agents. There is a negative correlation between NSAIDs and the risk of gastrointestinal malignancies, including colorectal cancer (such as stomach and esophageal cancer). The protective effect of NSAIDs against cancer has motivated studies on preventing breast cancer by NSAIDs. Although multiple meta-analyses have pointed to an Aspirin or NSAID-based chemo-preventive effect against breast cancer, the results of epidemiological studies are still inconsistent. However, additional cohort and case-control studies did not find any evidence that taking Aspirin or other NSAIDs, other than aspirin, reduces the risk of breast cancer. The conflicting results may be caused by several factors, including poor accuracy and random fluctuation, low response rates with possible selection bias, short follow-up periods, insufficient exposure data, or an inability to distinguish between various NSAID subclasses.

Inflammation is the body's defensive mechanism to combat illnesses, toxins, and tissue damage. The pathophysiology of pain involves the release of inflammatory mediators that lead to symptoms like swelling and pain. Steroids are effective in reducing inflammation and associated pain, but side effects and the need for gradual discontinuation complicate their use. NSAIDs like indomethacin, ibuprofen, and diclofenac have a generally safe response profile but long-term use can lead to severe gastrointestinal and renal side effects.

Recent studies on cyclooxygenase isozymes (COX-1/2) have advanced our understanding of inflammatory pathways. Selective COX-1 inhibitors, known as "Coxibs," were developed to minimize the negative gastrointestinal and renal effects associated with NSAIDs. COX-2 inhibitors were initially thought to affect cardiovascular health negatively, but these are now considered drug-dependent rather than class-dependent. Additionally, COX-2 is overexpressed in several types of cancer, and inhibiting it is associated with a reduced risk of cancer development.5

In particular, previous research on breast cancer cell lines is limited in addressing and identifying the anti-cancer properties of the combination of Aspirin and Ibuprofen in the MCF 7 cell line. Hence, the present study was conducted to evaluate the effectiveness of Aspirin and Ibuprofen in MCF 7 cell lines.

MATERIALS AND METHODS

The study was conducted in Aaranya bioscience laboratory at SIPCOT, Siruseri, Chennai. This is an In vitro type of study by purchasing the cell line from the National Centre for Cell Science, Pune, India (NCSS). A temperature of less than -130°C is required to completely stabilize cell preparations. This is achieved by storage in liquid nitrogen (-196°C), liquid nitrogen vapor, or in a cryogenic freezer (- 150°C). The most important element of safety in handling breast cancer cell line culture laboratory is the strict adherence to standard microbiological practices and techniques. Safety equipment in a cell culture laboratory includes primary barriers such as biosafety cabinets, enclosed containers, and other engineering controls designed to remove or minimize exposure to hazardous materials, as well as personal protective equipment that is often used in conjunction with the primary barriers.

MTT Assay was experimented with aspirin and ibuprofen as individual compounds and also a study on a combination of ibuprofen and aspirin was simultaneously carried out. Whereas, DAPI staining, annexin staining, and DNA fragmentation study were done on a combination of ibuprofen and aspirin only. In all of the above studies, the control was carried out without the addition of any compounds or test doses.

1. ASPIRIN MTT Assay

Materials required: Cell line, Dulbecco Minimum Essential Medium, Trypsin-EDTA, Foetal calf serum, Compounds-Aspirin, Phosphate buffered saline, Antimycotic solution (antibiotic solution), Dimethyl sulfoxide, MTT-(3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide, MTT Stock: MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide) stock solution 5mg/ml

Methodology: This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol-2-yl)- 2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent(eg. DMSO) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The MTT assay measures cell viability based on the generation of reducing equivalents in metabolic active cells. The higher the absorbance measured the higher is the cell viability. The percentage cell viability is calculated.

2. IBUPROFEN MTT ASSAY

This is a colorimetric assay that measures the reduction of yellow 3-(4,5- dimethythiazol-2-yl)- 2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent(eg. DMSO) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The MTT assay measures cell viability based on the generation of reducing equivalents in metabolic active cells. The higher the absorbance measured the higher the cell viability. The percentage of cell viability is calculated.

Materials Required: Cell line, Dulbecco Minimum Essential Medium, Trypsin-EDTA, Foetal calf serum, Compounds-Ibuprofen, Phosphate buffered saline, Antimycotic solution (antibiotic solution), Dimethyl sulfoxide, MTT-(3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide, MTT Stock: MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide) stock solution 5mg/ml.

3. COMBINATION OF ASPIRIN AND IBUPROFEN MTT ASSAY

This colorimetric assay measures the reduction of yellow 3-(4,5- dimethythiazol- 2-yl)- 2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (eg. DMSO) and the released, solubilized formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The MTT assay measures cell viability based on the generation of reducing equivalents in metabolic active cells. The higher the absorbance measured the higher the cell viability. The percentage of cell viability is calculated.

Materials Required: Cell line, Dulbecco Minimum Essential Medium, Trypsin-EDTA, Foetal calf serum, Compounds- Aspirin and Ibuprofen, Phosphate-buffered saline, Antimycotic solution (antibiotic solution), Dimethyl sulfoxide, MTT-(3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide, MTT Stock: MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyl tetrazolium bromide) stock solution 5mg/ml.

4. DAPI STAINING

Principle: DAPI (4,6 Diamidino-2-phenylindole dihydrochloride) is a dye that belongs to the group of indole dyes. It is mainly used for nuclear staining (DNA). DAPI selectively bind to DNA and forms strongly fluorescent DNA-DAPI complexes with high specificity on adding DAPI to culture cells, it readily takes up cellular DNA which yields in detection of highly fluorescent nuclei.

Reagents: PBS (phosphate buffered saline), Paraformaldehyde (3% in PBS), Triton-X 100 (0.2% in PBS), DAPI stock solution- 5mg/ml in Double distilled water and store it in -15 to -25°c. 5. DAPI working solution- 1µgm/ml in PBS and store it in 2 to 8°c.

Procedure:

1. The cells were fixed with 3% Paraformaldehyde (50µl) for 10 minutes at room temperature.
2. The fixed cells were then permeabilized with 0.2% Triton-X 100 (50µl) for 10 minutes at room temperature.
3. The cells were stained with DAPI and incubated for 15 minutes at RT.
4. The cells were washed with PBS and after drying they were observed under a microscope using a DAPI filter at 400X magnification.
   paraformaldehyde in PBS for 10 min at 37° C. The cells were washed three times with PBS for 10 min each. The nuclei were stained with 50 ul DAPI for 15 min. Cells were washed for 2 min with PBS, mounted with Vectashield, and observed under fluorescence microscope using proper magnification.

5. ANNEXIN ASSAY

Principle: The appearance of phosphatidylserine (PS) residues (normally hidden within the plasma membrane) on the surface of the cell is an early event in apoptosis, and can also be used to detect and measure apoptosis. During apoptosis, PS is translocated from the cytoplasmic face of the plasma membrane to the cell surface. Annexin V has a strong, Ca2+-dependent affinity for PS and therefore can be used as a probe for detecting apoptosis.

Incubation of cells with annexin V-FITC

1. Induce apoptosis by the desired method.
2. Collect 1–5 x 105 cells by centrifugation.
3. Resuspend cells in 500 µL of 1X binding buffer.
4. Add 5 µL of annexin V-FITC
5. Incubate at room temperature for 5 min in the dark.
6. Proceed to fluorescence microscopy for detection and analysis.

- Place the cell suspension on a glass slide. Cover the cells with a glass coverslip. The cells are washed and fixed in 2% formaldehyde before visualization.
- Cells must be incubated with annexin V-FITC before fixation since any cell membrane disruption can cause non-specific binding of annexin V to PS on the inner surface of the cell membrane

6. DNA fragmentation by Gel electrophoresis for Aspirin and Ibuprofen

Cell line was used to determine obtained from National Centre for Cell Science, Pune, India (NCSS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100µg/ml) in a 5% CO2 at 370 c. Cell lines were seeded in 96-well plates and both were incubated for a specified period. Various concentrations of the sample were added and incubated for 24h incubation. After the medium was removed it was washed with the phosphate saline solution. They were analyzed for DNA fragmentation assay.

DNA fragmentation by Gel electrophoresis:

1. The cells were incubated with testing concentration for 24h at 37 °C
2. After treatment, they were harvested by pipetting and rinsed with an ice-cold buffer. The cell pellets were resuspended in 200 µl of lysis buffer
3. DNA from the cells was released into the lysis buffer [0.2% Triton X-100, 10 mM Tris, and 1 mM EDTA (pH 8.0)] by rupturing the nucleus, incubated for 10sec at room temperature and centrifuged at 5000g for 5 min.

4. The DNA in the supernatant was extracted using a 25:24:1 (v/v/v) equal volume of phenol: chloroform: isoamyl alcohol.
5. The DNA was precipitated with ethanol, airdried, and dissolved in TE buffer [5 mM Tris-HCl (pH 8.0) and containing RNase A(0.1 mg/ml)
6. The samples were analyzed electrophoretically on 1% agarose gel containing 0.1 µg/ml ethidium bromide.
7. The compound has shown DNA fragmentation at the tested concentration.

RESULTS:

The MTT assay of the aspirin revealed mean percentage cell death of 77.4%, 65.61%, 46.26%, 34.39%, 27.35 and 15.39% of the MCF7 cell lines at the aspirin doses of 100, 50, 25, 12.5, 6.125 and 1.25 µg/mL, respectively. (Table 1)

Table 1: MCF-7 cell death percentage against the aspirin MTT Assay

Aspirin µg/mL	MCF7- % Cell Death					
	Mean	SD	SEM	Well 1	Well 2	Well 3
100	77.40	1.58	0.91	74.40633	80.21108	77.57256
50	65.61	1.41	0.81	69.65699	64.37995	62.79683
25	46.26	2.92	1.69	53.29815	40.63325	44.85488
12.5	34.39	2.56	1.48	37.73087	35.09235	30.34301
6.125	27.35	1.19	0.68	27.44063	26.12137	28.49604
1.25	15.39	0.94	0.54	17.1504	15.30343	13.72032

Table 2: MCF-7 live cells percentage against the aspirin MTT Assay

Aspirin µg/mL	MCF7- % Live Cells
100	22.60
50	34.39
25	53.73
12.5	65.61
6.125	72.65
1.25	84.61
Control	100

Figure 1: Aspirin MTT Assays at various doses

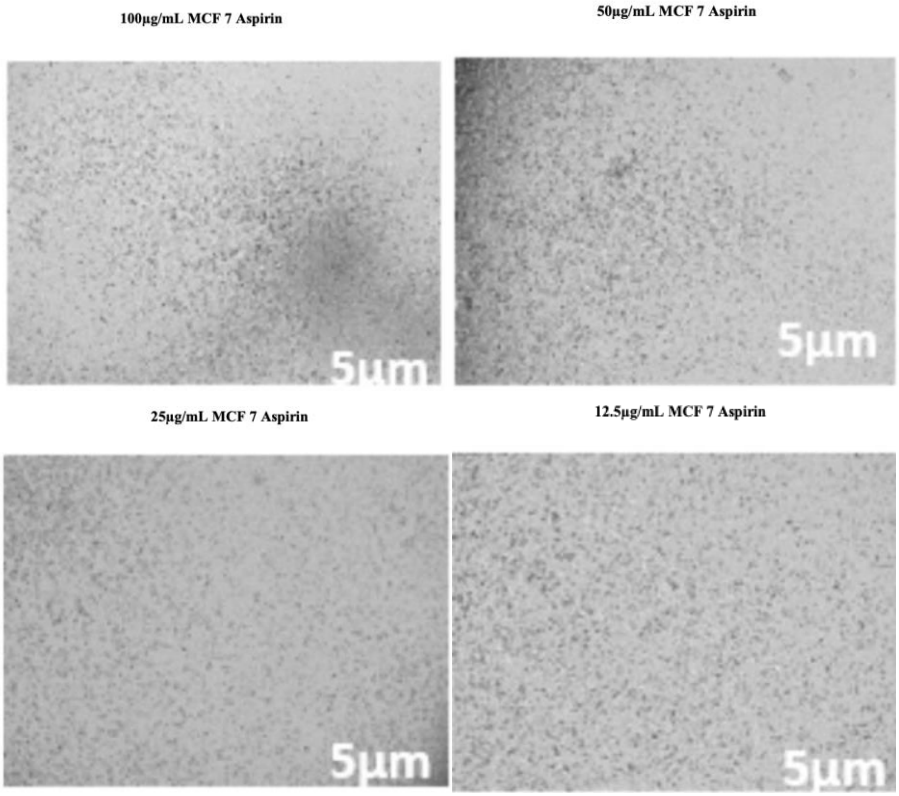


Table 3: Absorbance values against various doses of aspirin

Aspirin µg/mL	Absorbance value		
	OD1	OD2	OD3
100	0.097	0.075	0.085
50	0.115	0.135	0.141
25	0.177	0.225	0.209
12.5	0.236	0.246	0.264
6.125	0.275	0.28	0.271
1.25	0.314	0.321	0.327
Control	0.395	0.375	0.369
Control mean	0.379667		

The MTT assay of the ibuprofen revealed mean percentage cell death of 79.95%, 70.54%, 57.69%, 44.94%, 29.02% and 15.65% of the MCF7 cell lines at the ibuprofen doses of 100, 50, 25, 12.5, 6.125 and 1.25 µg/mL, respectively. (Table 4)

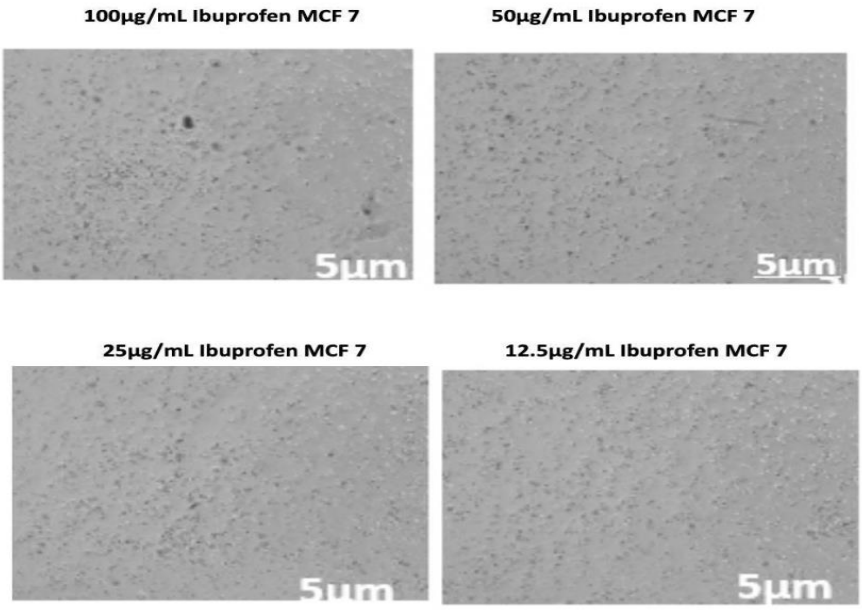
Table 4: MCF-7 cell death percentage against the Ibuprofen MTT Assay

Ibuprofen µg/mL	MCF7- % Cell Death					
	Mean	SD	SEM	Well 1	Well 2	Well 3
100	79.95	0.85	0.49	77.04485	81.26649	81.53034
50	70.54	1.34	0.77	69.65699	72.29551	69.65699
25	57.69	3.4	1.98	58.5752	53.82586	60.68602
12.5	44.94	3.7	2.13	45.64644	48.28496	40.8971
6.125	29.02	1.5	0.89	32.45383	26.12137	28.49604
1.25	15.65	0.79	0.45	17.1504	14.24802	15.56728

Table 5: MCF-7 live cells percentage against the ibuprofen MTT Assay

Ibuprofen µg/mL	MCF7- % Live Cells
100	20.05
50	29.46
25	42.30
12.5	55.06
6.125	70.98
1.25	84.34
Control	100

Figure 2: Ibuprofen MTT Assay at various doses



The absorbance of the ibuprofen dosages of 100, 50, 25, 12.5, 6.125 and 1.25 µg/mL varied from 0.07 to 0.087, 0.105-0.115, 0.149-0.175, 0.196-0.224, 0.256-0.28 and 0.314-0.325 at the optical density of 570 nm. The mean absorbance of the control group was 0.380. (Table 6)

Table 6: Absorbance values against various doses of ibuprofen

Ibuprofen µg/mL	Absorbance value		
	OD1	OD2	OD3
100	0.087	0.071	0.07
50	0.115	0.105	0.115
25	0.157	0.175	0.149
12.5	0.206	0.196	0.224
6.125	0.256	0.28	0.271
1.25	0.314	0.325	0.32
Control	0.395	0.375	0.369
Control mean	0.379667		

There was no statistically significant difference in the % cell deaths between the aspirin and ibuprofen, at all doses of the drugs ($p>0.05$).

Table 7: MCF-7 cell death percentage against Aspirin and Ibuprofen MTT Assay

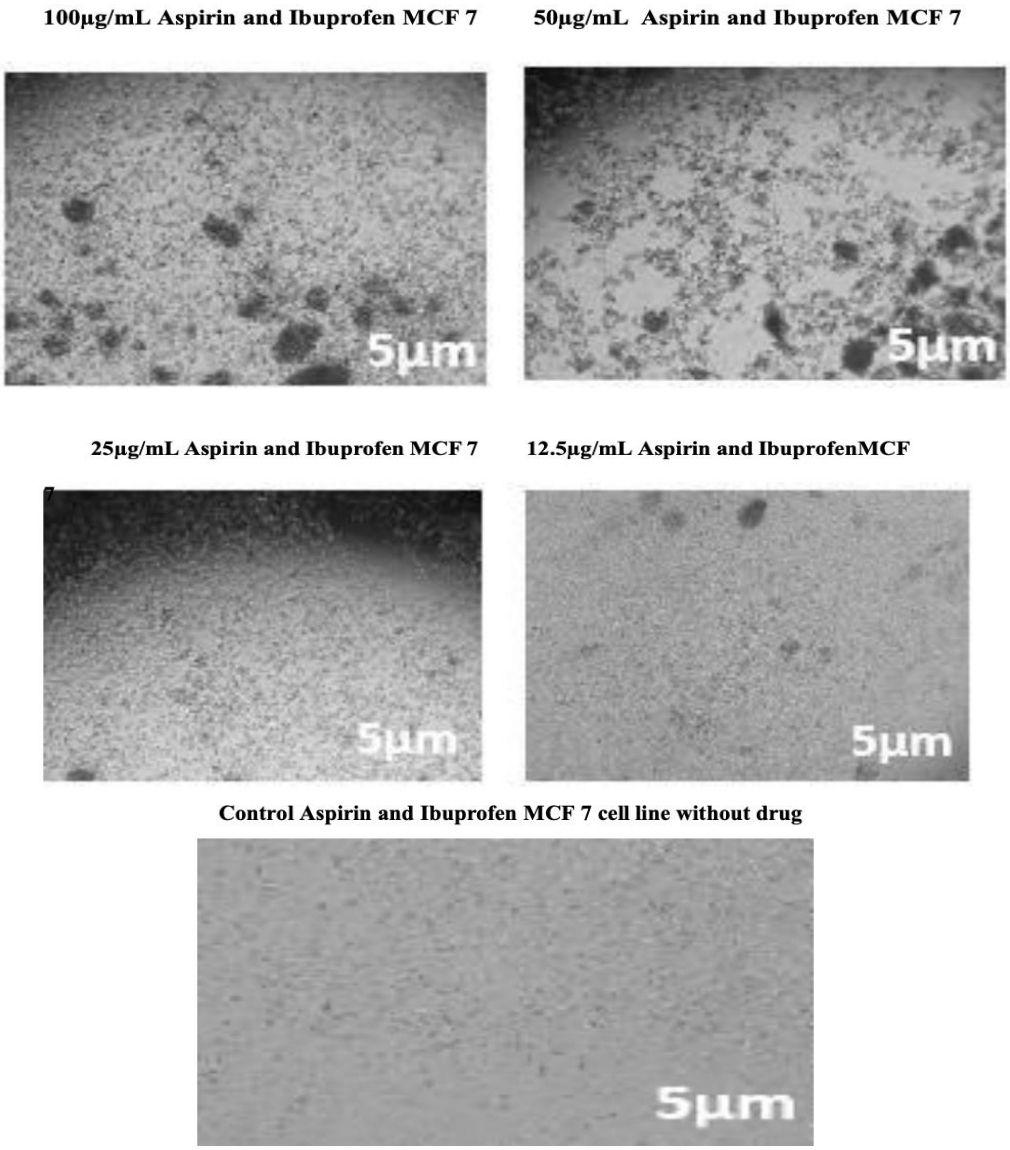
Aspirin and Ibuprofen µg/mL	MCF7- % Cell Death					
	Mean	SD	SEM	Well 1	Well 2	Well 3
100	74.23	0.97	0.56	77.04485	73.35092	72.29551
50	58.22	1.83	1.05	53.82586	59.1029	61.74142
25	42.74	2.11	1.22	42.74406	40.63325	44.85488
12.5	37.91	2.37	1.37	37.73087	40.36939	35.62005
6.125	29.02	1.55	0.89	32.45383	26.12137	28.49604
1.25	24.19	1.18	0.68	21.63588	24.53826	26.38522

Complementary to the percentage of cell death, the mean percentage of live cells were 25.77%, 41.77%, 57.25%, 62.09%, 70.98%, and 75.81% among the MCF7 cell lines at the aspirin and ibuprofen doses of 100, 50, 25, 12.5, 6.125 and 1.25 µg/mL, respectively. The control showed 100% live cells (Table 8)

Table 8: MCF-7 live cells percentage against the aspirin and ibuprofen MTT Assay

Aspirin and Ibuprofen µg/mL	MCF7- % Live Cells
100	25.77
50	41.77
25	57.25
12.5	62.09
6.125	70.98
1.25	75.81
Control	100

Figure 3: Aspirin+Ibuprofen MTT Assays at various doses



The absorbance of the aspirin and ibuprofen dosages of 100, 50, 25, 12.5, 6.125 and 1.25 µg/mL varied from 0.087-0.105, 0.145-0.175,0.209-0.225,0.226-0.244, 0.256-0.28 and 0.279-0.297, respectively, at the optical density of 570 nm. The mean absorbance of the control group was 0.380. (Table 9)

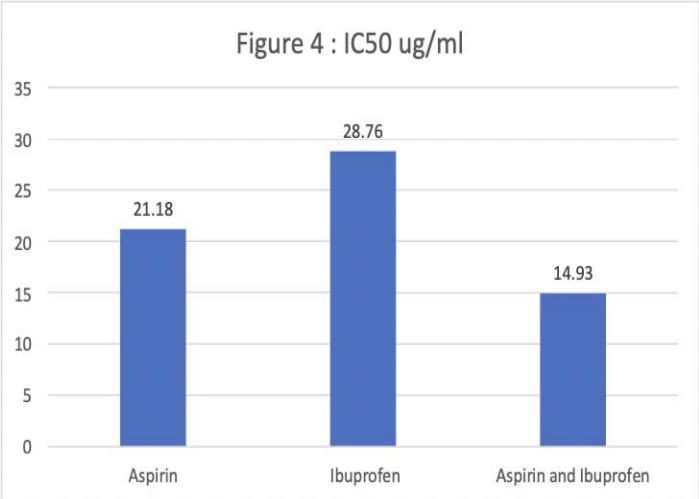
Table 9: Absorbance values against various doses of aspirin+ibuprofen

Aspirin and Ibuprofen µg/mL	Absorbance value		
	OD1	OD2	OD3
100	0.087	0.101	0.105
50	0.175	0.155	0.145
25	0.217	0.225	0.209
12.5	0.236	0.226	0.244
6.125	0.256	0.28	0.271
1.25	0.297	0.286	0.279
Control	0.395	0.375	0.369
Control mean	0.379667		

The half-maximal inhibitory concentration (IC50) of aspirin, ibuprofen and aspirin+ibuprofen were 21.18 ug/ml, 28.76 ug/ml and 14.93 ug/ml, respectively. (Table 10)

Table 10: IC50 values of aspirin, ibuprofen and aspirin+ibuprofen

Drugs	IC50 ug/ml
Aspirin	21.18
Ibuprofen	28.76
Aspirin and Ibuprofen	14.93

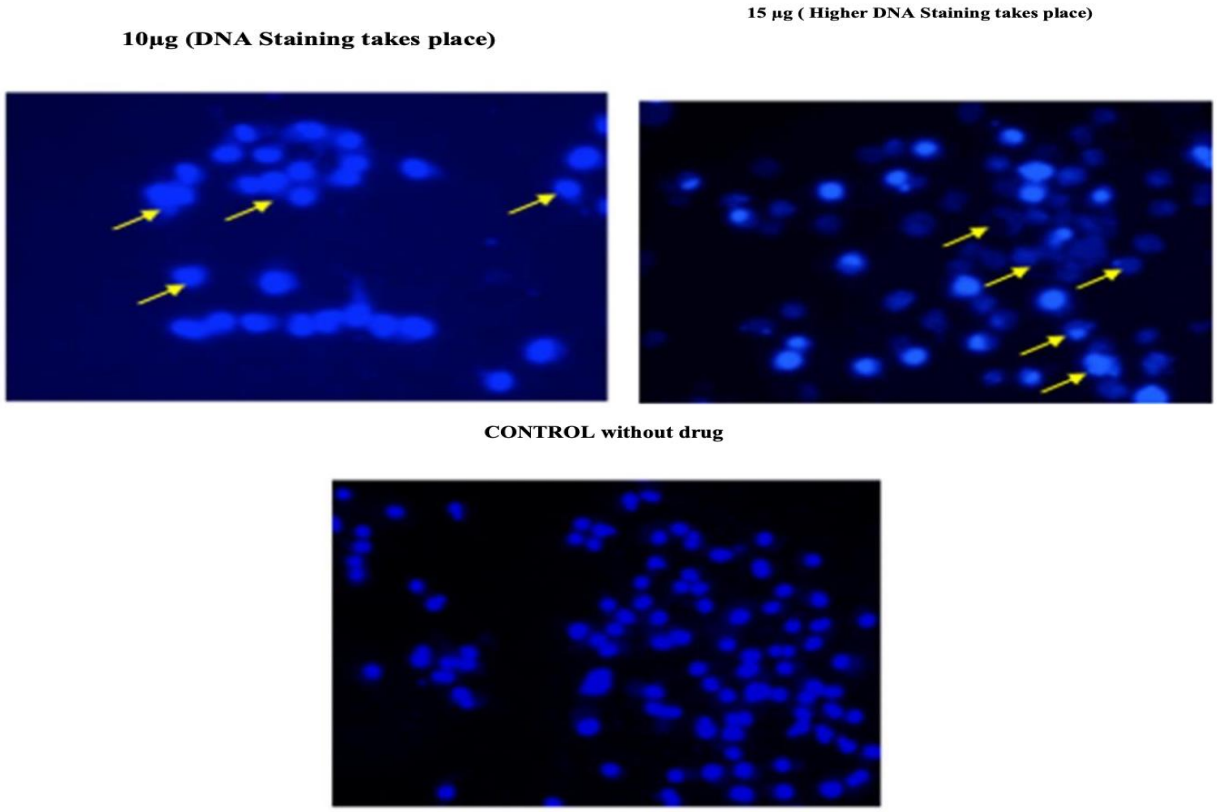


Control mean value of DAPI Staining samples is 1.45. The mean value among the samples of Aspirin+Ibuprofen was 41.97 for 10µg and 62.10 for 15µg doses. (Table 11)

Table 11: DAPI staining results of the control and the aspirin+ibuprofen.

DAPI STAINING						
	Well 1	Well 2	Well 3	MEAN	STD	SEM
CONTROL	1.25	1.45	1.63	1.45	0.20	0.11
10	42.32	41.25	42.32	41.97	0.62	0.36
15	60.52	63.25	62.5	62.10	1.42	0.82

Figure 5: DAPI staining of the control and the aspirin+ibuprofen



5. ANNEXIN ASSAY

Annexin assay revealed a mean control value of 2.65. The mean value of the Annexin assay among the samples of Aspirin+Ibuprofen was 38.31 for 10µg and 54.81 for 15µg doses. (Table 12)

Figure 6: Apoptosis staining results of the control and the aspirin+ibuprofen

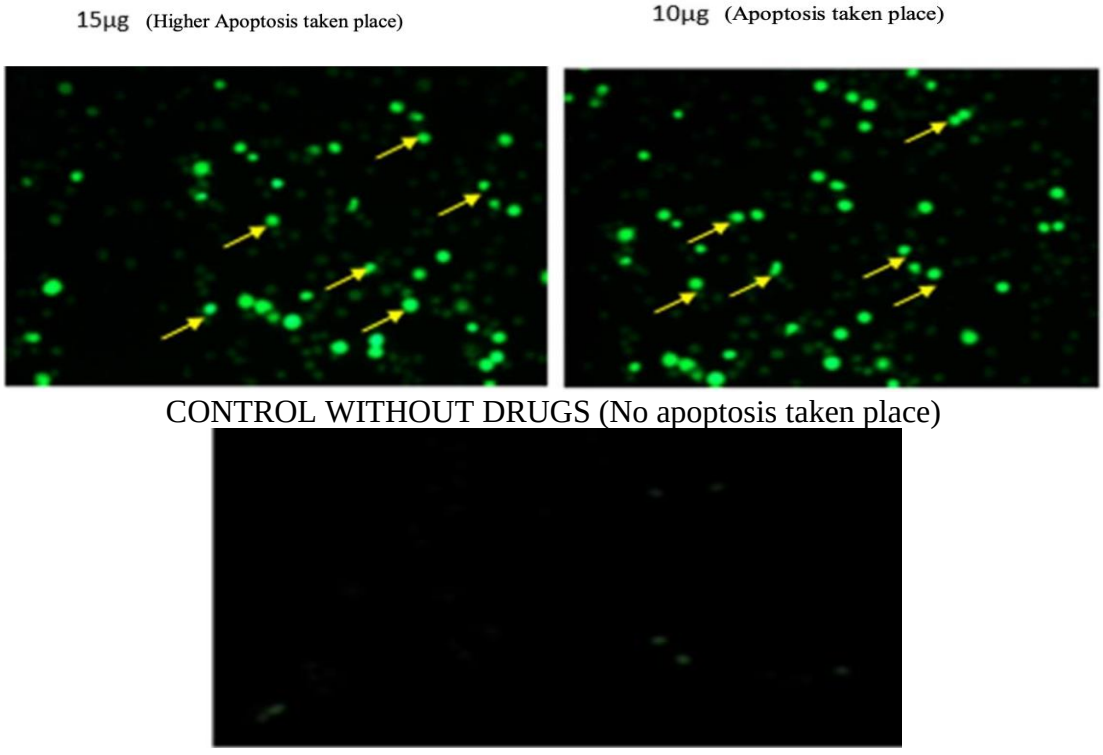


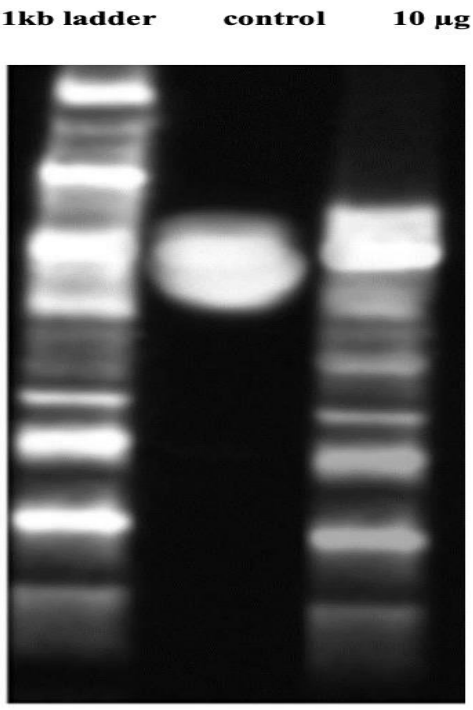
Table 12: Apoptosis staining results of the control and the aspirin+ibuprofen.

Apoptosis staining						
	Well 1	**Well 2**	**Well 3**	**MEAN**	**STD**	**SEM**
CONTROL	2.8	2.63	2.51	2.65	0.15	0.085
10	36.69	38.58	39.65	38.31	1.50	0.87
15	52.56	55.54	56.32	54.81	1.99	1.15

DNA fragmentation by Gel electrophoresis for Aspirin and Ibuprofen

Fragmentation of the combination drugs of 10µg shows the highest apoptosis from control through DNA ladder Assay.

Figure 7: **DNA Fragmentation**



DISCUSSION:

This research evaluates the anti-cancer properties of Aspirin and Ibuprofen in the MCF7 cell line. NSAIDs, particularly Aspirin and Ibuprofen, have been shown to reduce breast cancer risk by 39%. Administering Ibuprofen during tumor promotion can improve immune characteristics and reduce tumor growth without causing harmful autoimmune reactions.

1. **MTT Assay:** The study found that aspirin and Ibuprofen showed varying levels of cancer cell death in MCF-7 cells. Aspirin reduced cell viability with a 10 mM dose, while Ibuprofen caused 15.65% to 79.95% cell death. However, there was no significant difference in cancer cell death percentage between the two. The combination of aspirin and Ibuprofen showed a higher percentage of cancer cell death in the MCF-7 cell line.

2. **IC50:** The half-maximal inhibitory concentration (IC50) is a measure of a drug's efficacy, indicating the amount needed to inhibit a biological process by half. In a cell viability study, Ibuprofen had higher IC50 values than Aspirin, suggesting it may have more potent anti-cancer effects. However, when combined, Aspirin and Ibuprofen showed significantly higher anti-cancer properties on breast cancer cell lines, demonstrating their effectiveness in providing anti-cancer properties.

3. **Annexin Assay**: The study analyzes the apoptosis of MCF 7 cell lines using the Annexin Assay. Results show that the combination of Aspirin and Ibuprofen increases apoptosis levels, suggesting potential anti-cancer properties. Derivatives of Ibuprofen, including triazole containing ketone and oximes, have been reported as potential adjuvants.

4. **DAPI Staining Method:** DAPI labeling was employed to evaluate the overall cell morphology and count the number of nuclei. The control group's mean value of the DAPI-stained samples, according to the results, was 1.45. However, the results were 41.97 and 62.10, respectively, when aspirin and ibuprofen were combined at 10μg and 15μg. This suggests that there were more cancer cells stained. Consequently, it may be said that the anti-cancer effects of aspirin plus ibuprofen are more potent.

5. **DNA Fragmentation:** The study found that a 10μg combination of drugs, aspirin+ibuprofen, produced the highest level of apoptosis compared to the control group, confirming previous findings.

6. CONCLUSION:

Overall, Aspirin and Ibuprofen, exclusively and in combination, have been found to have anti-cancer properties against breast cancer cell lines, through various laboratory methods. A combination of Aspirin and Ibuprofen was found to have more antiproliferative and apoptotic properties than the individual drugs. Future studies are required to confirm and validate the findings of the current research using additional systems that combine more suitable growth circumstances, such as growth in three dimensions, tension, and coculture with different cell types, as well as growth in normoxia and hypoxia. When taken together, these findings will contribute to the further advancement of in vivo research and eventually to the treatment of breast cancer patients.

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