Research Article

Curcumin (*Curcuma longa*): An Ecofriendly and Ayurvedic Antiproliferative Agent to Control Human Breast Cancer and Prostate Cancer

A. Anitha Joice[a],*, P. Senthilkumar[b], Dilla Jose [c], S. Pandiammal[d], J. Manju Bashini [e],*, Ettiappan Munuswamy[f], Dhurairaj Satheesh[g],*

[a] Department of Biotechnology, St. Joseph's Arts and Science College, Kovur, Chennai, Tamilnadu, India.

[b] PG and Research Department of Zoology, Sir Theagaraya College, Chennai, Tamilnadu, India.

[c] PG and Research Department of Zoology, Christ College, Irinjalakuda, Kerala, India.

[d] DMI College of Education, Palanchur, Chennai, Tamilnadu, India.

[e] Department of Zoology, Loganatha Narayanasamy Government College (Autonomous), Ponneri, Tamilnadu, India.

[f] Department of Plant-Biology and Plant-Biotechnology, Loganatha Narayanasamy Government College (Autonomous), Ponneri, Tamilnadu, India.

[g] PG and Research Department of Chemistry, Loganatha Narayanasamy Government College (Autonomous), Ponneri, Tamilnadu, India.

***Corresponding author's E-mail:** anitha4321@gmail.com

Received: 29-09-2021; **Revised:** 08-12-2021; **Accepted:** 13-12-2021; **Published on:** 15-01-2022.

ABSTRACT

Curcumin, a strong anti-cancer ingredient that is found in turmeric and curry, is a prominent yellow pigment and spice. Curcumin's anti-tumour properties include inhibition of tumour proliferation, angiogenesis, invasion, and metastasis, induction of tumour apoptosis, increased chemotherapy sensitivity, and cell cycle and cancer stem cell regulation, suggesting that curcumin has a strong therapeutic potential of modulating cancer progression. Curcumin's anticancer impact has been demonstrated in human breast cancer cell lines and human prostate cancer cell lines using the Proliferating Cell Nuclear Antigen (PCNA) assay and colony formation test, respectively. Curcumin was employed at various concentrations (1, 5, 10, 25, 50, and 100 g/ml). Curcumin inhibits cancer cell growth in the PCNA assay, and colonies were stained with crystal violet and the number of clones in a particular region was counted for each condition in the colony forming study. Higher concentrations of curcumin were found to be effective against cancer cell lines in this investigation.

Keywords: Breast cancer cell lines, human prostate cancer cell lines, curcumin, PCNA assay, colony formation.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v72i01.001

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v72i01.001

INTRODUCTION

Curcumin (diferuloyl methane), the primary polyphenol in dietary spice, is a powerful chemopreventive agent that stops cancer cells from multiplying by stopping them at different stages of the cell cycle depending on the type of cell. It is a non-nutritive food chemical obtained from the rhizome of the turmeric plant (Curcuma longa) and has been proven to have potent antioxidant, anticancer, and anti-inflammatory activities in vitro and in vivo. Curcumin's anti-proliferative activity in various cancer cell lines, cytoprotective effect on oxidative damage, and activation of anti-cancer pathways were scanned in the prior study literature and are presented in the following sections. Curcumin's anticancer properties are mediated by a complex molecular signalling network that includes the proliferation, estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2) pathways. Curcumin also regulates apoptosis, cell phase–related genes, and microRNA in breast cancer cells, according to research. The recent research efforts in understanding the molecular targets and anticancer mechanisms of curcumin in breast and prostate cancer were reviewed by Yiwei Wang et al[1].

Breast cancer is one of the most common cancers among women in both industrialized and developing countries. Breast cancer is becoming more common in developing countries as people live longer lives, urbanize more, and adopt western lifestyles. Although prevention can reduce risk, it cannot eliminate the majority of breast cancers that arise in low- and middle-income nations, where breast cancer is discovered at advanced stages, according to the World Health Organization (WHO). Breast cancer cell lines have made remarkable advances in our understanding of the biology of breast cancer over the last few decades. With this data in hand, the study must now move beyond previous "one marker, one cell line" studies and apply what it has learned to more effectively use cell lines or cell line panels as experimental models to study specific subgroups of breast cancer, as this is expected to have the greatest impact on improving patient outcomes. Curcumin has an inhibitory effect on cancer progression by regulating the expressions of multiple mRNAs, according to Siying Zhou et al.[2], and curcumin could provide new insight into the molecular targeting treatment of malignant tumors. Jiao Zou et al.[3] proposed new mechanisms for cisplatin resistance study and curcumin as a cisplatin-sensitizing drug in breast cancer cells. FEN1 could be a

therapeutic target for breast cancer patients who are resistant to cisplatin. The current study's overarching goals and objectives are to observe the following end results:

Anti-proliferation studies

1. Using breast and prostate cancer cell lines and the Proliferating Cell Nuclear Antigen (PCNA) test, the anti-proliferative impact of curcumin was investigated.
2. Curcumin's anti-proliferative impact was investigated utilizing breast and prostate cancer cell lines as well as a colony formation test.

MATERIALS AND METHODS

Curcumin preparation

Curcumin was received from Unilever R&D with a purity of 95%. (Sanjivani Phytopharma Pvt. Ltd, India). Curcumin was dissolved in DMSO to a stock concentration of 20 mM in a dark-colored bottle and kept at 10°C, where it was diluted to the desired concentration with medium when needed (Figure 1 and Figure 2).

Figure 1: Turmeric plant *(Curcuma longa)*

Figure 2: Turmeric rhizome and Powder

Cancer cell lines

The American Type Culture Collection provided MCF-7 and MDA-MB-231 human breast cancer cell lines, as well as CRL-714 (normal breast cell) (ATCC, Rockville, MD). The National Center for Cell Sciences provided prostate cancer cell lines (PC-3, DU145, and LNCap) as well as PrEC (normal prostate cell) (Pune, India). Mycoplasma identification, DNA fingerprinting, isoenzyme analysis, and cell vitality detection were all used to characterize these cell lines, according to the literature that came with them. These cells were kept in cell culture media and conditions according to the American Type Culture Collection's instructions. MCF-7 cells were cultured at 37°C in a humidified environment containing 95 percent air and 5 percent CO_2 in DMEM medium containing 10% (V/V) FBS without antibiotics. MDA-MB-231 cells were cultured at 37°C in L-15 media with 10% (V/V) FBS and no antibiotics.

Cultured cells and cells

MCF-7 human breast carcinoma cells were incubated at 37°C in 5% CO2 in RPMI1640 media supplemented with 0.22 percent sodium bicarbonate, 10% foetal bovine serum (FBS), 100 U/ml penicillin, and 100 g/ml streptomycin. Curcumin was dissolved in 5 mM dimethylsulfoxide (DMSO) and promptly diluted to the appropriate concentration with RPM I1640medium. Control cells were cultured in media with an equivalent final amount of DMSO (final concentration 0.01%, V:V).

Curcumin therapy

The test cell lines were grown to a density of around 75% before being treated with the test ingredient curcumin at various doses for the durations stated. The control cell lines were incubated in the same final concentration of DMSO without curcumin. As a control, the cells were treated with an equivalent dose of DMSO without curcumin. The cells were grown for seven or twelve days, with the media being changed every two days. Crystal violet was used to tint the colonies (Sigma Chemical Co, St. Louis, MO). Each condition had its quantity of clones counted in a specific location.

Western Blotting

The cells were handled according to the legends in the figures. The cells were placed on ice after the treatments, washed in cold PBS, and lysed in RIPA lysis buffer supplemented with 1 mM PMSF, a protease inhibitor cocktail, and a phosphatase inhibitor cocktail. The lysates were centrifuged for 5 minutes at 14,000 g at 4°C. A BCA kit was used to determine protein concentrations. A total of 40–50 g of cellular protein was loaded onto 8% or 12% SDS-polyacrylamide gels and electro transferred to nitrocellulose (NC) membranes (0.22/0.45 m) from each sample. Different primary antibodies were used to incubate the blotted membranes, followed by secondary antibody incubations.

Co-immuno precipitation and Western Blotting

Cells were lysed in lysis buffer (50 mM Hepes, pH 7.6, 200 mM NaCl, 1 mM EDTA, 5 % Nonidet P-40) for direct Western blot examination. phosphatase (5 µM each of o-phosphoserine, o-phosphotyrosine, o-phosphothreonine, o-glycerophosphate, p-nitrophenylphosphate, and sodium vanadate inhibitor cocktails) and protease (10 µg/ml each of benzamidine, trypsin inhibitor, and bacitracin, 5 µg/ml each of leupeptin, pepstatin A, anti SDS-polyacrylamide gel was used to separate the lysate containing 50 µg of protein. The gel was transferred to a nitrocellulose membrane after electrophoresis. Chemiluminescence was used to visualize the protein of interest. To ensure equivalent protein loading, the blots were re-probed with anti-actin antibody (Santa Cruz Biotechnology).

Proliferation assay

The uptake method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was used to measure cell growth. Curcumin (0–70 M) was added to the cells and grown for another 24 hours after they were seeded (5103/well) in 200 l of DMEM media onto 96-well plates and grown overnight. The MTT reagent (5 mg/ml) was then added, and the incubation was extended for another 4 hours. 150 mL dimethyl sulfoxide (DMSO, Sigma) per well was used to finish the reaction. An MRX Revelation 96-well multiscanner was used to determine absorbance values (Dynex Technologies, Chantilly, VA, USA). The control group consisted of cells grown in DMEM. The cell viability index was computed by multiplying the experimental OD value by the control OD value. The experiments were carried out three times.

Western blot

Five micrograms of total proteins were extracted with Pierce's M-Permammalian protein extraction reagent or 1 percent SDS and run on a 4 percent acrylamide concentration gel and a 10 percent acrylamide separation gel before being transferred to a Hybond P membrane (AP-Biotech). Membranes were pre-hybridized in PBS containing 0.1 percent (v/v) Tween 20 and 5% fat free milk (PBS-T). After that, the primary antibody was used to hybridize in PBS-T containing 5% fat free milk. Membranes were washed three times in PBS-T for five minutes each time and twice for ten minutes each time before being hybridized with secondary antibody in PBS-T containing 5% fat free milk.

Membranes were washed three times in PBS-T for five minutes each time and twice for ten minutes each time. ECL-plus Western blot detection reagent was used to visualize proteins of interest (AP-Biotech). Secondary anti-rabbit and anti-mouse antibodies (Santa Cruz) were used in conjunction with polyclonal rabbit anti-pro-caspase 9 and anti-pro-caspase 8 antibodies (Pharmingen), a polyclonal mouse antiGSTP1-1 antibody (Transduction Laboratories), a monoclonal mouse anti-b-actin (Sigma), a monoclonal mouse anti-PARP (Pharmingen), and secondary anti-rabbit and antimouse antibodies (Santa Cruz) were used.

Clonogenic Assay for measuring Cell survivability

In a nutshell, medium from a stock culture of cells actively developing in monolayer was removed, followed by two washes with 1x PBS to eliminate dead cells. The cells were then detached using 500l of trypsin incubated for 5-10 minutes. With the addition of 500l medium, trypsin was neutralized. The cells were then suspended in a solution. A hemocytometer is used to count the number of cells per milliliter in this suspension. Approximately 600 cells were planted into each well of a six-well plate from this stock culture. For around two weeks, the plate was incubated. After 2 weeks, 1ml of 1x ice cold PBS was added and left for 10 minutes, followed by 10-15 minutes in 100% ice cold methanol. Then it was stained with 0.05 percent crystal violet and left undisturbed for 10-15 minutes. It was destined in normal water four times to avoid excessive discoloration. The plates were examined using an Epi-fluorescent Microscope with a UV filter (Nikon TE 2000E). All of the cells in the colony are the offspring of a single cell. The number of colonies counted should be at least 50 for the 600 cells planted into the dish.

Soft agar colony forming assay

One of the characteristics of malignant cell transformation is anchorage-independent growth. The Soft Agar Test for Colony Formation is an anchorage-independent growth assay that is considered the most stringent assay for evaluating cell proliferation and sensitivity to carcinogenic inhibitors in semisolid culture mediums. The soft agar plate creates a three-dimensional structure that closely resembles our biological environment. In standard soft agar experiments, cells pretreated with carcinogenic inhibitors or medicines are cultivated on soft agar medium for 14-21 days with suitable controls in 35mm dishes. Following this incubation period, formed colonies can be morphologically examined using cell stain and the number of colonies formed per well can be calculated. In a microwave, 0.7 percent agar was melted.

Preparation of Base Agar

2X DMEM with 10% FBS and 0.7 percent agar were carefully mixed in equal amounts. Then 1.5ml of this mixture was poured into each 35mm petri plate and allowed to harden for 5 minutes.

Preparation of Top Agarose

The number of cells per ml was counted after cells were pretreated with medicines and trypsinized. For each plate, 5000 cells were harvested. In the microwave, 0.8 percent agarose was melted. In a 10ml tube, 3ml of 2X DMEM+10% FBS and 3ml of 0.8 percent agarose were added to the cell suspension. For proper mixing, the mixture was gently stirred. 1.5ml of this mixture was added to each of the previously prepared agar dishes. To be safe, three to four replicas were made. There were no cells in the negative control and no therapy in the positive control. For 14-21 days, the plates were cultured at 37°C in a humidified incubator. The cells were fed DMEM 1-2 times each week. To avoid microbiological contamination, all steps were carried out aseptically.

Staining

After 21 days, the plates were dyed for 30-60 minutes with 0.5 mL of 0.005 percent crystal violet. Washing with regular water for 2-3 times removed the excessive stains. A dissecting microscope was used to count the colonies

RESULTS

Cell proliferation

Proliferation by PCNA

Curcumin from Curcuma longa was tested for its inhibitory effect on the PCNA assay after 3 hours of exposure in a 40 g/ml concentration. PCNA indicates the capacity of cancer

cells to grow in the body. The percent of cells with PCNA was represented in Table 1 and Figure 3. PCNA measurement result with respect to MDA-MB231 Breast Cancer cell line was explained in Table 2 and Figure 4. The proliferation of cells with PCNA CRL-714 normal breast cancer cell line was represented in Table 3 and Figure 5.

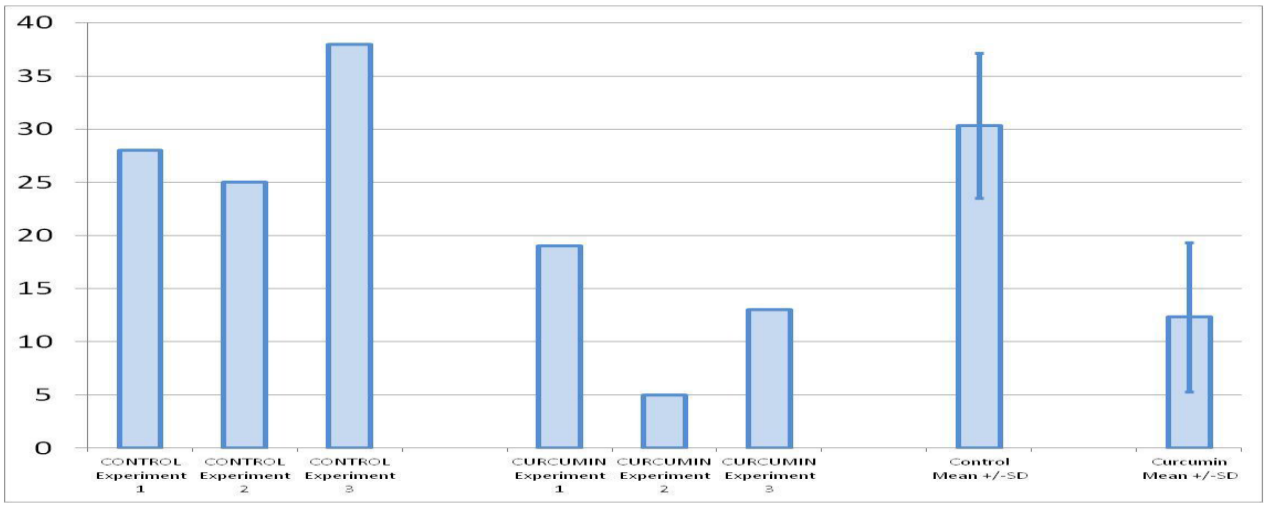

Figure 3: Graphical representations of PCNA measurements of MCF7 breast cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

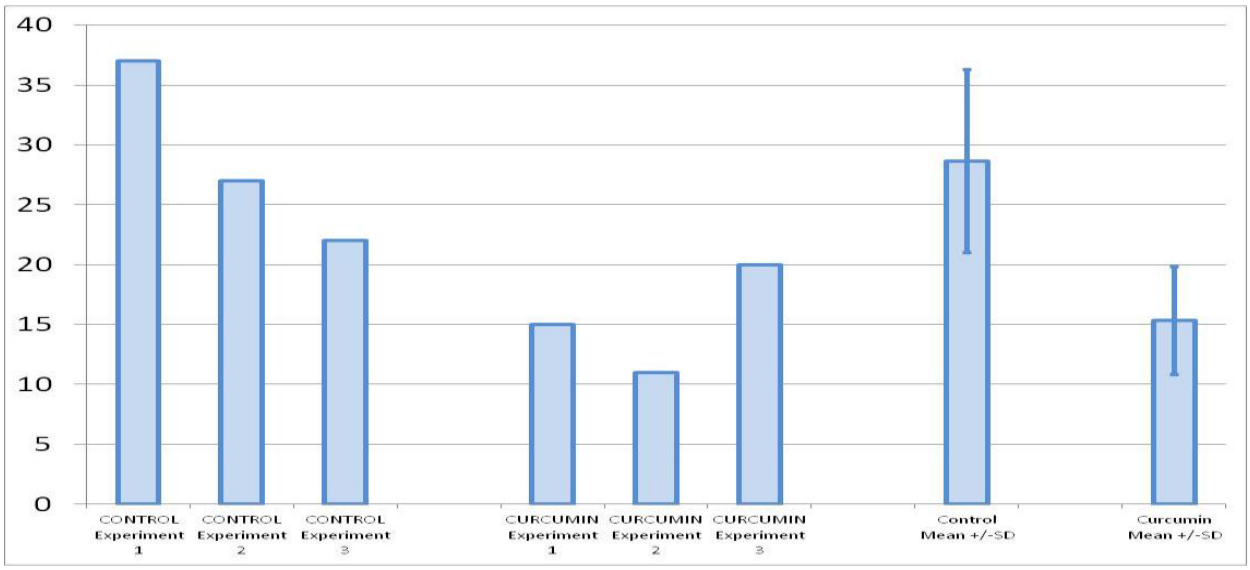

Figure 4: Graphical representations of PCNA measurements of MDA-MB231 breast cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

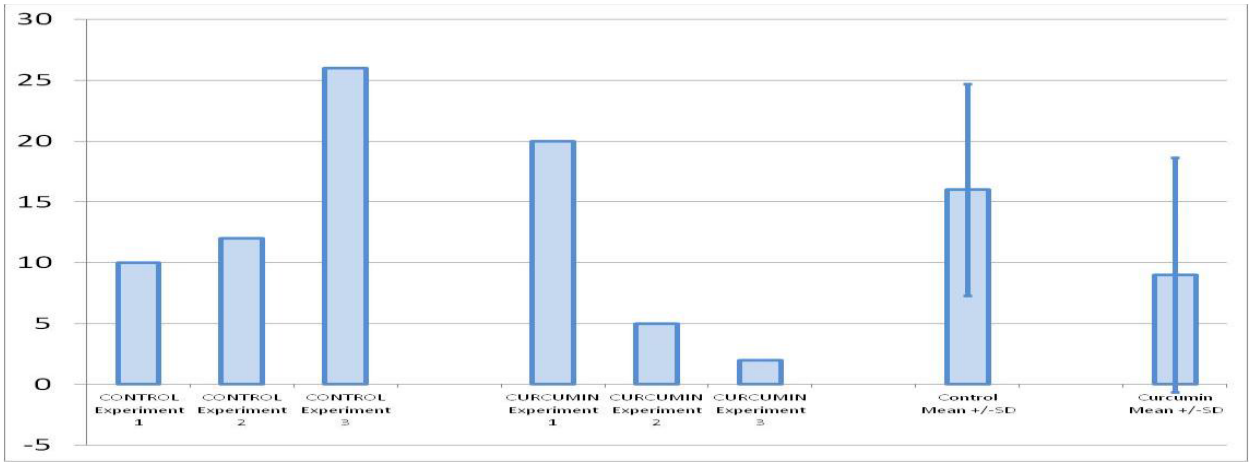

Figure 5: Graphical representations of PCNA measurements of CRL-714 normal breast cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

Table 1: PCNA measurement results of MCF7 breast cancer cell line.

		Percent cells with PCNA
Control	Experiment 1	28
	Experiment 2	25
	Experiment 3	38
	Mean	30.33
	SD	6.81
Curcumin (40 µM; 3 hours)	Experiment 1	19
	Experiment 2	5
	Experiment 3	13
	Mean	12.33
	SD	7.02

Table 2: PCNA measurement results of MDA-MB231 breast cancer cell line

		Percent cells with PCNA
Control	Experiment 1	37
	Experiment 2	27
	Experiment 3	22
	Mean	28.67
	SD	7.64
Curcumin (40 µM; 3 hours)	Experiment 1	15
	Experiment 2	11
	Experiment 3	20
	Mean	15.33
	SD	4.51

Table 3: PCNA measurement results of CRL-714 normal breast cancer cell line

		Percent cells with PCNA
Control	Experiment 1	10
	Experiment 2	12
	Experiment 3	26
	Mean	16.00
	SD	8.72
Curcumin (40 µM; 3 hours)	Experiment 1	20
	Experiment 2	5
	Experiment 3	2
	Mean	9.00
	SD	9.64

The higher the PCNA percentage, the faster the tumour can develop and spread. PCNA levels in all cancer cell lines ranged from 16 to 33%, showing that all cancer cell lines are actively multiplying. The most growing prostate and breast cancer cell lines were PC3 and MCF 7. Because of its near-normal karyotype, CRL-741 had the least proliferating breast cancer cell lines. The prostate cancer cell line was treated with curcumin and the percent cells was monitored and recorded. The percent cells with PCNA treatment for PC3 prostate cancer cell line, DU145 prostate cancer cell line and LNCaP prostate cancer cell line were tabulated in Table 4-6 and represented in Figure 6-8. Apart from this the normal prostate cancer cell line PrEC was also being treated with Curcumin and PCNA were tabulated in Table 7 (Figure 9).

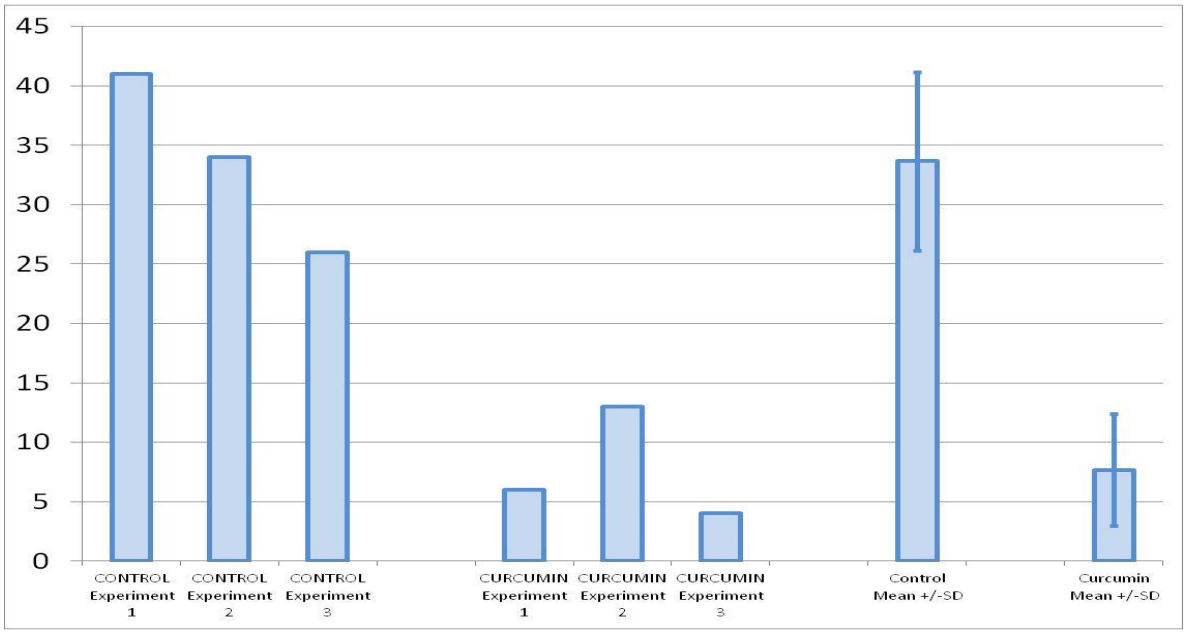

Figure 6: Graphical representation of PCNA measurements of PC3 prostate cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

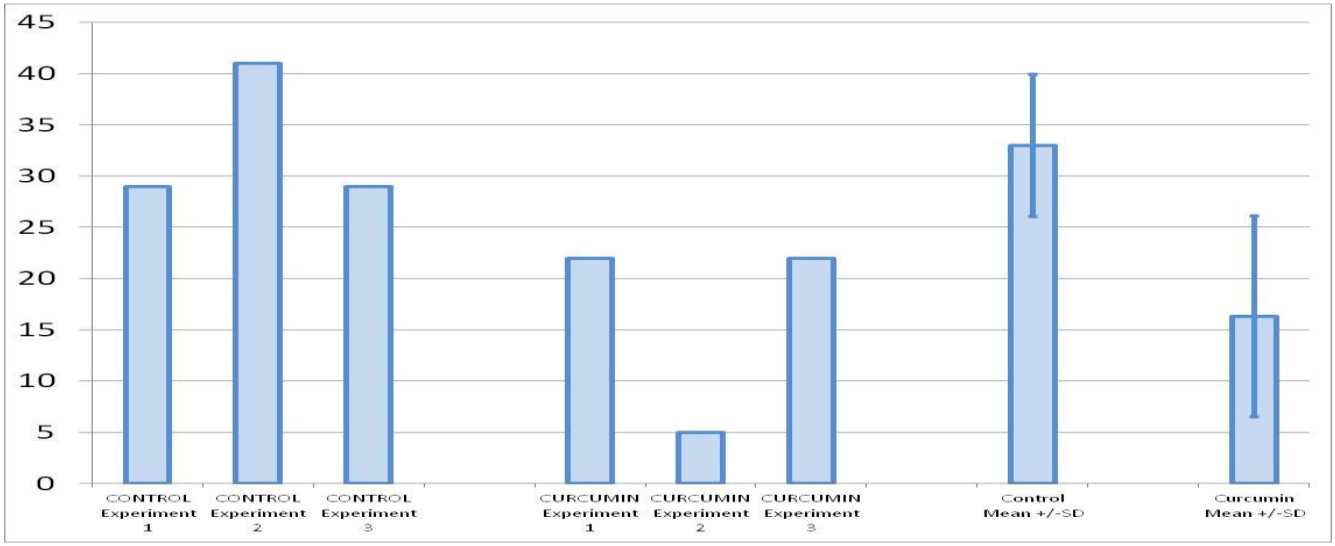

Figure 7: Graphical representation of PCNA measurements of DU145 prostate cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

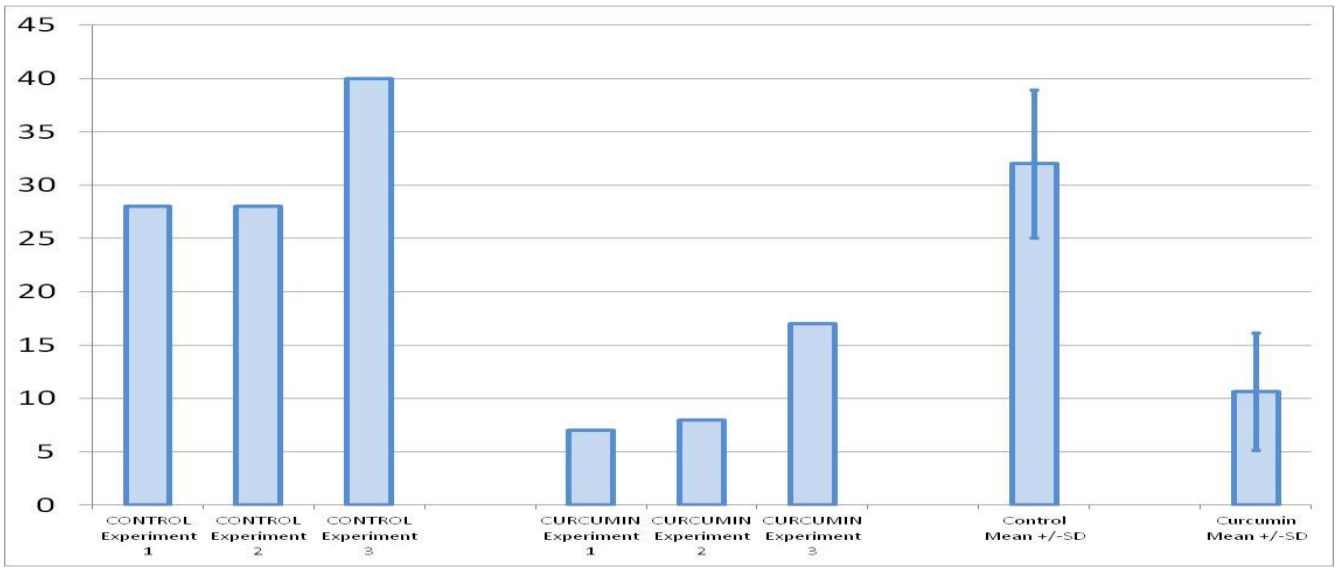

Figure 8: Graphical representation of PCNA measurements of LNCaP prostate cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

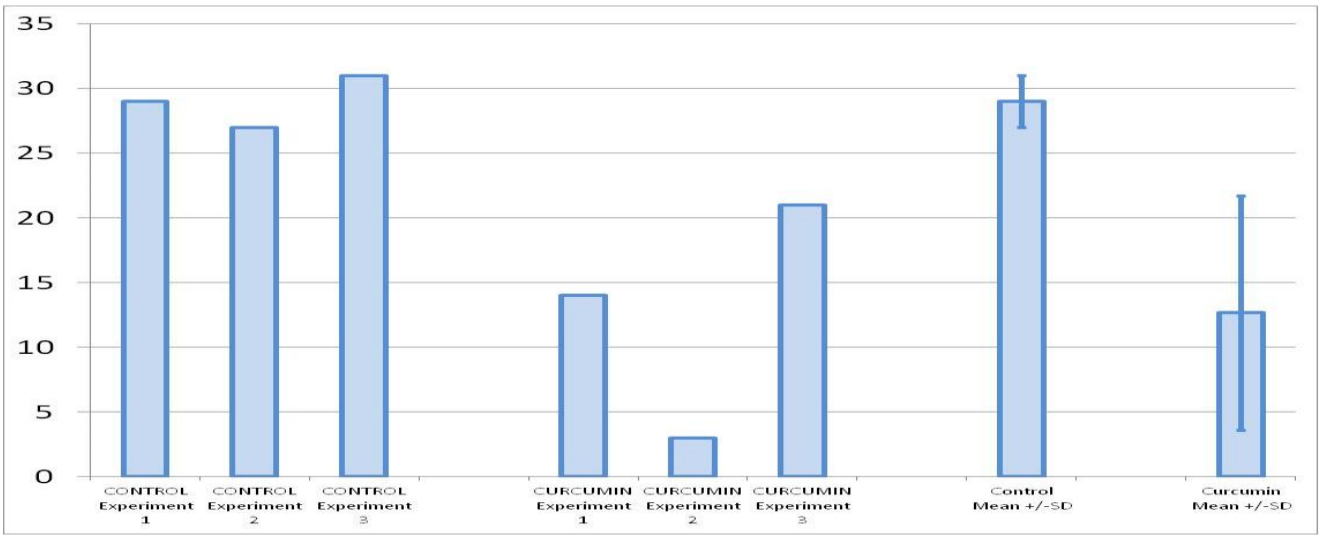

Figure 9: Graphical representation of PCNA measurements of PrEC normal prostate cancer cell line (Y axis: Percent cells with PCNA; X axis: Treatment groups)

Table 4: PCNA measurement results of PC3 prostate cancer cell line

		Percent cells with PCNA
Control	Experiment 1	41
	Experiment 2	34
	Experiment 3	26
	Mean	33.67
	SD	7.51
Curcumin (40 µM; 3 hours)	Experiment 1	6
	Experiment 2	13
	Experiment 3	4
	mean	7.67
	SD	4.73

Table 5: PCNA measurement results of DU145 prostate cancer cell line

		Percent cells with PCNA
Control	Experiment 1	29
	Experiment 2	41
	Experiment 3	29
	Mean	33.00
	SD	6.93
Curcumin (40 µM; 3 hours)	Experiment 1	22
	Experiment 2	5
	Experiment 3	22
	mean	16.33
	SD	9.81

Table 6: PCNA measurement results of LNCaP prostate cancer cell line

		Percent cells with PCNA
Control	Experiment 1	28
	Experiment 2	28
	Experiment 3	40
	Mean	32.00
	SD	6.93
Curcumin (40 µM; 3 hours)	Experiment 1	7
	Experiment 2	8
	Experiment 3	17
	mean	10.67
	SD	5.51

Table 7: PCNA measurement results of PrEC normal prostate cancer cell line

		Percent cells with PCNA
Control	Experiment 1	29
	Experiment 2	27
	Experiment 3	31
	Mean	29.00
	SD	2.00
Curcumin (40 µM; 3 hours)	Experiment 1	14
	Experiment 2	3
	Experiment 3	21
	mean	12.67
	SD	9.07

Curcumin treatment of the 5 cell lines resulted in a significant reduction in cell proliferation, implying that curcumin may limit cancer cell proliferation. PC3 showed the greatest reduction in PCNA after treatment with curcumin. Curcumin inhibits the multiplication of cancer cells in general, but especially in prostate tumours (Figure 10).

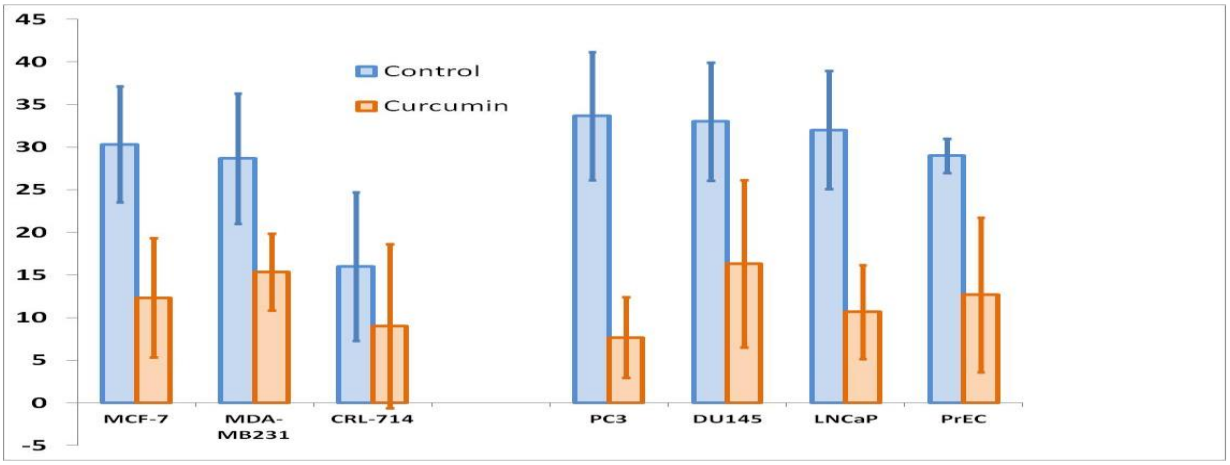

Figure 10: Graphical comparison of PCNA measurements of various breast cancer and prostate cancer cell lines (Y axis: Percent cells with PCNA; X axis: Cell lines)

Colony forming assay

The goal of the study was to see if curcumin from Curcuma longa might reduce colony formation in human breast cancer cell lines (MCF-7 and MDA-MB231) and human prostate cancer cell lines (PC3, DU145, and LNCaP) compared to normal cell CRL-714 (Breast cell) and PrEC (Prostate cell). The colony formation test revealed that all breast and prostate cancer cell lines generated 60-70 colonies on a plate in this investigation. The results of Colony formed when MCF-7 breast cancer cell line, MDA-MB231 and normal breast cancer cell line CRL-714 was treated with Curcumin was represented in Table 8-10 (Figure 11-13) respectively.

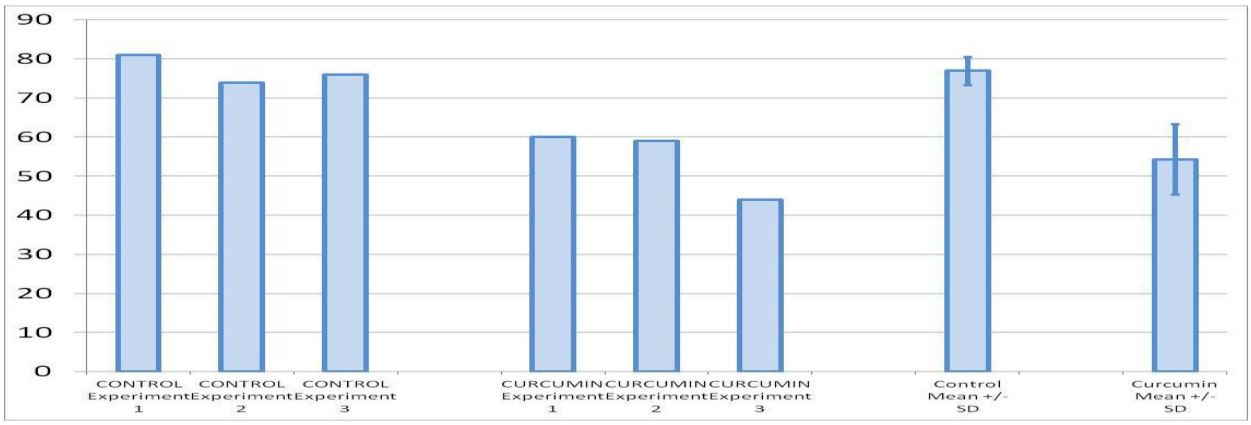

Figure 11: Graphical representation of Colony formation assay of MCF7 breast cancer cell line (Y axis: Number of colonies; X axis: Treatment group)

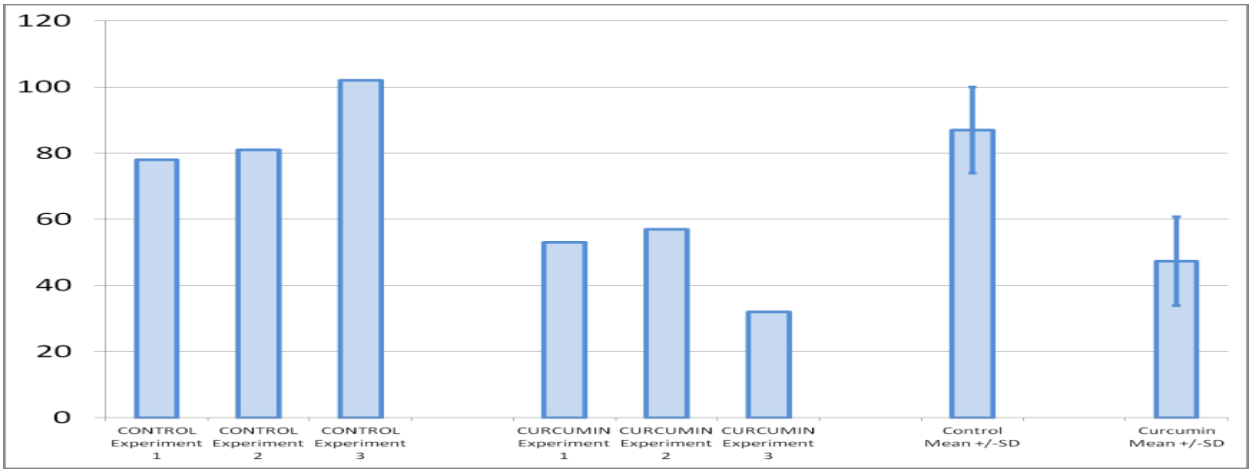

Figure 12: Graphical representation of Colony formation assay of MDA-MB231 breast cancer cell line (Y axis: Number of colonies; X axis: Treatment groups)

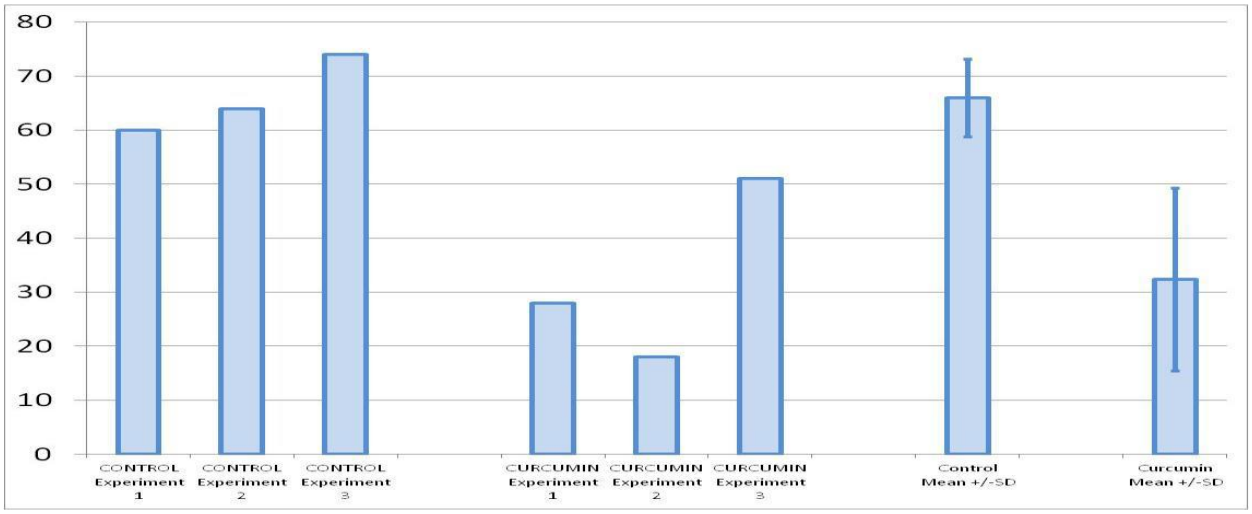

Figure 13: Graphical representation of Colony formation assay of CRL-714 normal breast cancer cell line (Y axis: Number of colonies; X axis: Treatment groups)

Table 8: Colony formation assay results of MCF7 breast cancer cell line.

		Number of Colonies
Control	Experiment 1	81
	Experiment 2	74
	Experiment 3	76
	Mean	77.00
	SD	3.61
Curcumin (40 µM; 3 hours)	Experiment 1	60
	Experiment 2	59
	Experiment 3	44
	Mean	54.33
	SD	8.96

Table 9: Colony formation assay results of MDA-MB231 breast cancer cell line**.**

		Number of Colonies
Control	Experiment 1	78
	Experiment 2	81
	Experiment 3	102
	Mean	87.00
	SD	13.08
Curcumin (40 µM; 3 hours)	Experiment 1	53
	Experiment 2	57
	Experiment 3	32
	Mean	47.33
	SD	13.43

Table 10: Colony formation assay results of CRL-714 normal breast cancer cell line

		Number of Colonies
Control	Experiment 1	60
	Experiment 2	64
	Experiment 3	74
	Mean	66.00
	SD	7.21
Curcumin (40 µM; 3 hours)	Experiment 1	28
	Experiment 2	18
	Experiment 3	51
	Mean	32.33
	SD	13.43

On a colony forming assay, PrEC, a prostate cancer cell line with minor chromosomal abnormalities, showed minimal colonies. In all five cell lines (Table 11 and Figure 14, 15), curcumin therapy reduced the number of colonies produced by about half. Following treatment with curcumin, PC3 demonstrated the greatest reduction in colony formation. (It's more effective in the case of prostate cancer). The results of the colony formation assay indicate the ability of cancer cells to survive in the body. Curcumin inhibits the growth of cancer cells (Figure 16).

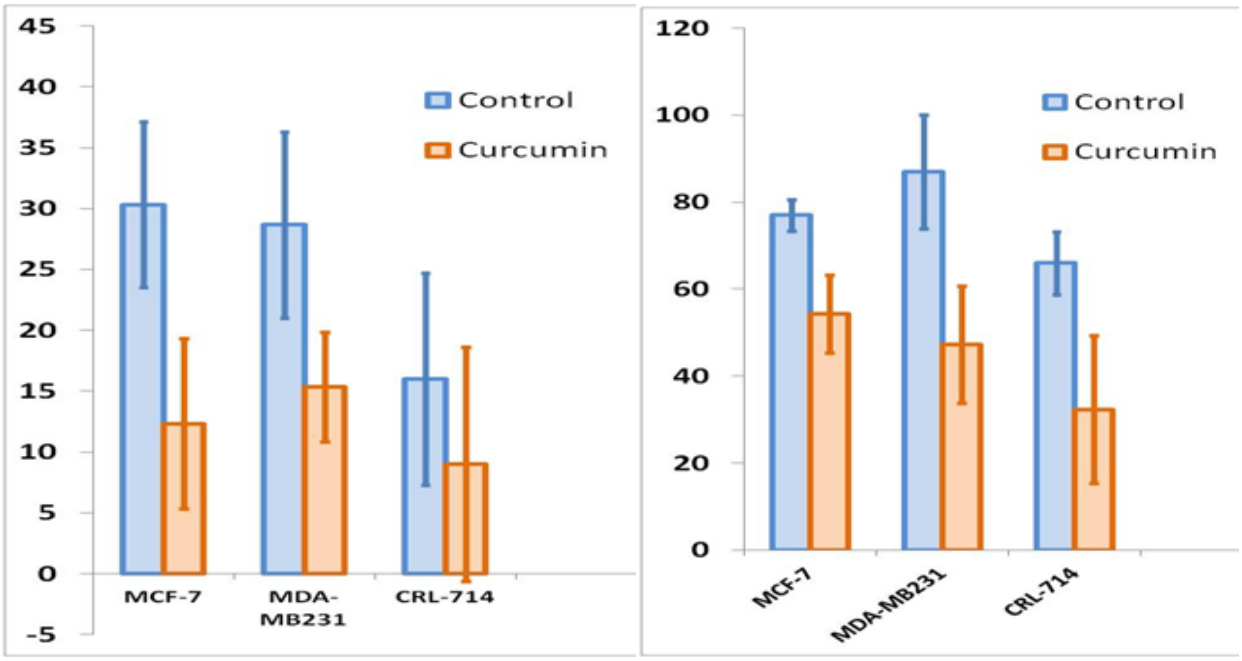

Figure 14: Graphical comparison of PCNA measurements (Y axis: Percent cells with PCNA; X axis: Cell lines) and Colony formation assay of breast cancer cell lines (Y axis: Number of colonies; X axis: Cell lines)

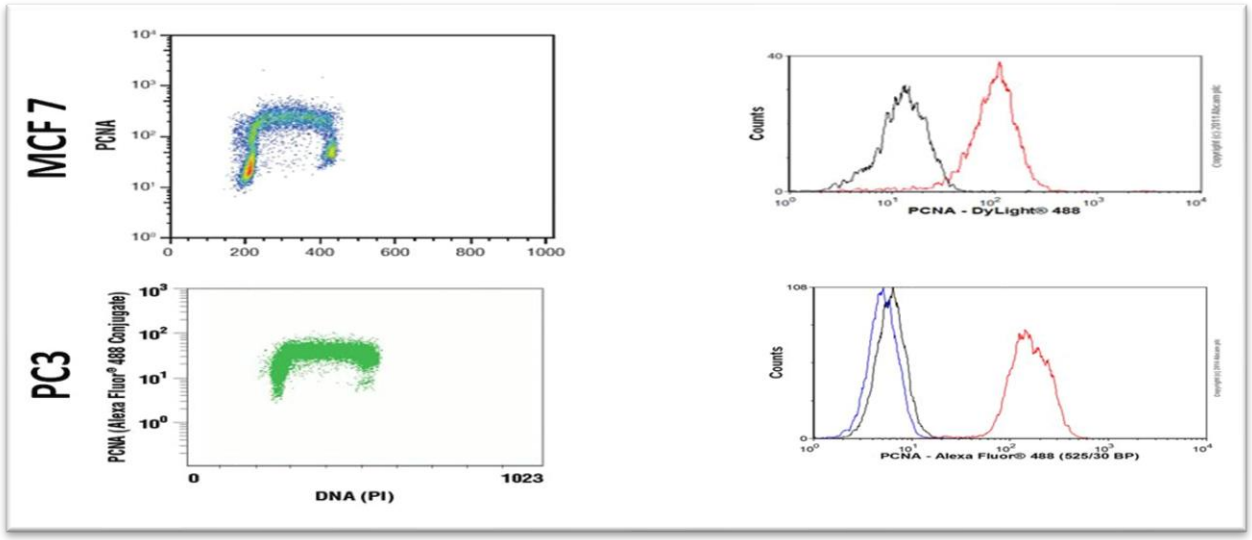

Figure 15: Representative PCNA measurements by Flow cytometry in Breast cancer and Prostate cancer cell lines

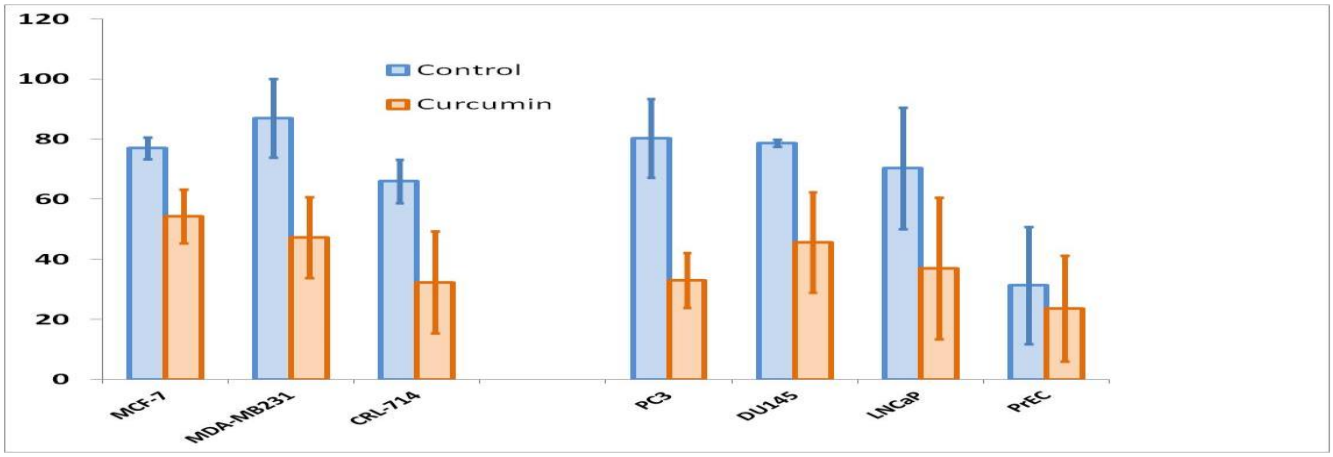

Figure 16: Graphical comparison of Colony formation assay of various breast cancer and prostate cancer cell lines (Y axis: Number of colonies; X axis: Cell lines)

Table 11: Comparison of PCNA measurements and Colony formation assay of breast cancer cell lines

Cell lines	Cell proliferation by PCNA and colony formation assay	
Breast cancer cells	**Control**	**Curcumin**
MCF-7	Normal	Decreased
MDA-MB231	Normal	Decreased
CRL-714 (normal breast cell)	Normal	Normal

MB-231 breast carcinoma cells, according to Di et al.,[21] who investigated the anti-proliferation impact of curcumin on human breast cancer cells and its mechanism. Curcumin's anti-proliferative effects in ER (oestrogen receptor)-positive MCF-7 cells are oestrogen dependent, with more apparent effects in estrogen-containing medium and in the presence of exogenous 17-beta estradiol, according to Shao et al.[22] Curcumin also reduces the expression of ER downstream genes in ER-positive MCF-7 cells, including pS2 and TGF-beta (transforming growth factor), and this suppression is estrogen-dependent. Curcumin suppressed Fen1-dependent proliferation of MCF-7 cells and dramatically increased Nrf2 protein expression while reducing Fen1 protein expression, according to Chen et al.[23] In a Nrf2-dependent mechanism, curcumin can suppress Fen1 gene expression. Curcumin could cause Nrf2 to translocate from the cytoplasm to the nucleus, lowering Fen1 promoter activity via reducing Nrf2 recruitment to the Fen1 promoter, according to further research. Curcumin and its derivatives have been shown to reduce the proliferation of MDA-MB-231 and MDA-MB-435 human breast cancer cells, which are triple negative breast cancer cell lines[24-26].

Cancer is characterized by abnormal cell growth and proliferation. In this case, cell proliferation inhibition experiments would be a better way to examine the test molecule's anti-proliferative properties. In the current in

vitro cancer investigations, cell proliferation as measured by PCNA and colony formation test was used. Compounds that stop or slow tumour cell proliferation could be used as anticancer treatments. Curcumin has been shown to induce apoptosis in a range of tumour cells in vitro, including B-cell and T-cell leukemia, colon carcinoma, breast carcinoma, and other cancer cell types[27]. Curcumin treatment of prostate cancer cell lines LNCaP and PC-3 was studied for signal transduction and expression of androgen receptor (AR) and AR-related cofactors by Nakamura et al.[28] Curcumin has a possible therapeutic effect on prostate cancer cells by down regulating AR and AR-related cofactors (AP-1, NF-B, and CBP), according to the findings of the study. Curcumin's effects on the survival and proliferation of many cell types have been investigated. It has been discovered to stop prostate cancer cells from multiplying[29]. Wu et al.[30] revealed that curcumin inhibits lymphoma cell proliferation in a dose- and time-dependent manner via exerting its impact.

Srivastava et al.[31] investigated the molecular mechanism of curcumin-induced cell cycle arrest in prostate cancer cells, as well as androgen-sensitive LNCaP and androgen-insensitive PC-3 cells. Curcumin treatment of both cell lines resulted in cell cycle arrest at the G1/S phase, according to the paper, and this cell cycle arrest was followed by apoptotic induction. Curcumin increased the expression of CDK inhibitors p16 (/INK4a), p21 (/WAF1/CIP1), and p27 (/KIP1), and inhibited the expression of cyclin E and cyclin D1, as well as hyperphosphorylation of the Rb protein. Curcumin's effects caused cell proliferation to stop and cell cycle control to be disrupted, resulting in apoptosis. Curcumin may be developed as a chemopreventive drug for human prostate cancer, according to the findings. Curcumin inhibits the constitutive activation of both NF-B and STAT3 in H-RS cells. Curcumin's potential to reduce H-RS cell viability is related to its ability to cause cell cycle arrest, which reduces cell proliferation. The observed results, together with curcumin's well-established pharmacological safety, support curcumin's prospective application as a new treatment treatment for Hodgkin's lymphoma patients[32]. Curcumin inhibits prostate cancer proliferative and metastatic development by down-regulating androgen receptor and epidermal growth factor receptor, as well as inducing cell cycle arrest. It inhibits pro-inflammatory mediators and the NF-B signalling pathway, which controls the inflammatory response. As a result, curcumin looks to be a safe option for prostate cancer prevention, therapy, or co-therapy[33]. Bangaru et al.[34] discovered that curcumin (Diferuloylmethane) inhibits cell proliferation and clonogenicity in human medulloblastoma cells, preventing them from migrating. Curcumin is a promising chemopreventive agent for prostate cancer in its early stages[35].

Curcumin stimulates PKD1, resulting in alterations in -catenin signalling by reducing nuclear-catenin transcription activity and increasing membrane-catenin levels in prostate cancer cells, according to Sundram et al.[36] In prostate cancer cells, curcumin modulation of these biological activities was linked to lower cell proliferation, colony formation, and cell motility, as well as increased cell-cell aggregation. Curcumin also inhibits cell motility by lowering the amounts of active cofilin, a PKD1 downstream target, according to the findings. Curcumin's powerful anti-cancer properties in vitro were replicated in a prostate cancer xenograft mice model. In vivo tumour growth inhibition was also linked to increased-catenin membrane localization. Overall, the findings have shown a novel molecular mechanism of curcumin activity in prostate cancer cells via PKD1 activation. Curcumin and Epigallocatechingallate (EGCG) were coupled to explore and evaluate their anticancer effects on PC3 prostate cancer cells by Eom et al.[37] EGCG had a lesser inhibitory effect on PC3 cell proliferation than LNCaP and DU145, two additional prostate cancer cell lines. Curcumin co-treatment increased EGCG's antiproliferative impact on PC3 cells. Extensive study has recently indicated that combining tumour chemotherapy with curcumin can improve the curative effect, block or reverse resistance responses, and lessen chemotherapy adverse effects[38]. In contrast to traditional curcumin, new technologies are being developed to deliver therapeutic compounds, particularly in tumours, and to boost anti-cancer effects while avoiding systemic and unpleasant side effects[39,40]. Despite the study's limitations, Siying Zhou et al.[2] concluded that curcumin could provide new insight into the molecular targeting of malignant tumours.

CONCLUSION

The effectiveness and toxicity profiles of *Curcuma longa* extracts on two human breast cancer cell lines (MCF7 and MDA-MB231) and three human prostate cancer cell lines (PC3, DU145 and LNCaP) were studied in the current study. This study used the in vitro tests to evaluate the anti-cancer and cytotoxicity capabilities of Curcuma longa extracts.

- The PCNA method
- Colony formation assay for cell proliferation research.

According to the findings,

1. Curcumin is protective in breast and prostate cancer cells,
2. Prostate cancer cells have a stronger effect.
3. Mechanism of action

a. Reduces cancer's ability to grow.
b. Reduce cancer cells' ability to multiply.

REFERENCES

1. Yiwei Wang, Jiayi Yu, Ran Cui, Jinjin Lin, Xianting Ding. Curcumin in Treating Breast Cancer: A Review. Journal of Laboratory Automation. 2016; 21(6): 723–731.
2. Siying Zhou, Sijie Zhang, Hongyu Shen, Wei Chen, Hanzi Xu, Xiu Chen, Dawei Sun, Shanliang Zhong, Jianhua Zhao, Jinhai Tang. Curcumin inhibits cancer progression through regulating expression of microRNAs. Tumor Biology. 2017; 1–12.

3. Jiao Zou, Linlin Zhu, Xiaomei Jiang, Yang Wang, Yue Wang, Xiangwei Wang, Bin Chen. Curcumin increases breast cancer cell sensitivity to cisplatin by decreasing FEN1 expression. Oncotarget., 2018; 1-11.
4. Li K, Deng Wu, Xi Chen, Ting Zhang, Lu Zhang, Ying Yi, Zhengqiang Miao, Nana Jin, Xiaoman Bi, Hongwei Wang, Jianzhen Xu, Dong Wang. Current and Emerging Biomarkers of Cell Death in Human Disease A review article. Hindawi Publishing Corporation Bio. Med Research International. 2012; 1-10.
5. Jia T, Li Zhang, Yale Duan, Min Zhang, Gang Wang, Jun Zhang, Zheng Zhao. The differential susceptibilities of MCF-7 and MDA-MB-231 cells to the cytotoxic effects of curcumin are associated with the PI3K/Akt-SKP2-Cip/Kips pathway. Cancer Cell International. 2014; 14: 126.
6. Agarwal BB, Anushree Kumar, Manoj Aggarwal S, Shishir Shishodia. Curcumin Derived from Turmeric (Curcuma longa): A Spice for All Seasons. CRC Press LLC, 2005; 340-380.
7. Bhaumik S, Anjum R, Rangaraj N, Pardhasaradhi BV, Khar A. Curcumin mediated apoptosis in AK-5 tumor cells involves the production of reactive oxygen intermediates. FEBS Lett. 1999; 456: 311-314.
8. Elattar TM, Virji AS. The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells in vitro. Anticancer Res. 2000; 20: 1733-1738.
9. Bielak Zmijewska A, Koronkiewicz M, Skierski J, Piwocka K, Radziszewska E, Sikora E. Effect of curcumin on the apoptosis of rodent and human non-proliferating and proliferating lymphoid cells. Nature Cancer. 2000; 38: 131-138.
10. Cipriani B, Borsellino G, Knowles H, Tramonti D, Cavaliere F, Bernardi G, Battistini L, Brosnan CF. Curcumin inhibits activation of V gamma 9 Vdelta 2 T cells by phosphor antigens and induces apoptosis involving apoptosis-inducing factor and large-scale DNA fragmentation. J. Immunol. 2001; 167: 3454-3462.
11. Pan MH, Chang WL, Lin Shiau SY, Ho CT, Lin JK. Induction of apoptosis by garcinol and curcumin through cytochrome c release and activation of caspases in human leukemia HL-60 cells. J. Agric. Food Chem. 2001; 49: 1464-1474.
12. Morin D, Barthelemy S, Zini R, Labidalle S, Tillement JP. Curcumin induces the mitochondrial permeability transition poremediated by membrane protein thiol oxidation. FEBS Lett. 2001; 495: 131-136.
13. Moragoda L, Jaszewski R, Majumdar AP. Curcumin induced modulation of cell cycle and apoptosis in gastric and colon cancer cells. Anticancer Res. 2001; 21: 873-878.
14. Mukhopadhyay A, Ramos BC, Chatterjee D, Pantazis P, Agarwal BB. Curcumin down regulates cell survival mechanisms in human prostate cancer cell lines. Oncogene., 2001; 20(52): 7597-7609.
15. Pal S, Choudhuri T, Chattopadhyay S, Bhattacharya A, Datta GK, Das T, Sa G. Mechanisms of curcumin-induced apoptosis of Ehrlich's as cites carcinoma cells. Biochem Biophys. Res. Commun. 2001; 288: 658-665.
16. Gomez Lechon MJ, OConnor E, Castell JV, Jover R. Sensitive markers used to identify compounds that trigger apoptosis in cultured hepatocytes. Toxicol. Sci. 2002; 65: 299-308.
17. Khafif A, Schantz SP, Chou TC, Edelstein D, Sacks PG. Quantitation of chemopreventive synergism between (-)-epigallocatechin-3-gallateand curcumin in normal, premalignant and malignant human oral epithelial cells. Carcinogenesis. 1998; 19: 419-424.
18. Jing Han, Xue-Yang Pan, Yan Xu, Yuan Xiao, Yu An, Lu Tie, Yan Pan & Xue-Jun Li Curcumin induces autophagy to protect vascular endothelial cell survival from oxidative stress damage. Autophagy. 2012; 8(5): 812-825.
19. Ozaki K, Kawata Y, Amano S, Hanazawa S. Stimulatory effect of curcumin on osteoclast apoptosis. Biochem Pharmacol. 2000; 59: 1577-1581.
20. Kim JY, Tae Jin Cho, Bok Hee Woo, Kyung Un Choi, Chang Hun Lee, Mi Heon Ryu and Hae Ryoun Park. Curcumin-induced autophagy contributes to the decreased survival of oral cancer cells. Archives of Oral Biology. 2012; 57: 1018–1025.
21. Di GH, Li HC, Shen ZZ, Shao ZM. Analysis of anti-proliferation of curcumin on human breast cancer cells and its mechanism. 2003; 83(20): 1764-1768.
22. Shao ZM, Shen ZZ, Liu CH, Sartippour MR, Go VL, Heber D, Nguyen M. Curcumin exerts multiple suppressive effects on human breast carcinoma cells. Int J. Cancer. 2002; 98(2): 234-240.
23. Chen B, Youzhi Zhang, Yang Wang, Jun Rao, Xiaomei Jiang, Zihui Xu. Curcumin inhibits proliferation of breast cancer cells through Nrf2-mediated down-regulation of Fen1 expression. The Journal of Steroid Biochemistry and Molecular Biology. 2014; 143: 11–18.
24. Rowe DL, Ozbay T, ORegan RM, Nahta R. Modulation of the BRCA1 protein and induction of apoptosis in triple negative breast cancer cell lines by the polyphenolic compound curcumin. Breast Cancer. 2009; 3: 61-75.
25. Yodkeeree S, Ampasavate C, Sung B, Aggarwal BB, Limtrakul P. Demethoxycurcumin suppresses migration and invasion of MDA-MB-231 human breast cancer cell line. Eur. J. Pharmacol. 2010; 627: 8-15.
26. Hua WF, Fu YS, Liao YJ. Curcumin induces down-regulation of EZH2 expression through the MAPK pathway in MDA-MB-435 human breast cancer cells. Eur. J. Pharmacol. 2010; 637: 16-21.
27. Anto RJ, Mukhopadhyay A, Denning K, Aggarwal BB. Carcinogenesis. Curcumin (diferuloylmethane) induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release: its suppression by ectopic expression of Bcl-2 and Bcl-xl. 2002; 23(1): 143-50.
28. Nakamura K, Yutaka Yasunaga, Takehiko Segawa, Daejin Ko, Judd W. Moul, Shiv Srivastava, Johng S. Rhim. Curcumin down-regulates AR gene expression and activation in prostate cancer cell lines. International Journal of Oncology.2002; 21: 825-830.
29. Agarwal BB, Anushree Kumar, Bharti AC. Anticancer Potential of Curcumin: Preclinical and Clinical Studies. Anticancer Research. 2003; 23: 363-398.

30. Wu Q, Chen Y, Li XG. Effect of curcumin on caspase 8- and caspase 9- induced apoptosis of lymphoma Rajicell. Article in Chinese. 2005; 13(4): 624-627.

31. Srivastava RK, Chen Q, Siddiqui I, Sarva K, Shankar S. Linkage of curcumin-induced cell cycle arrest and apoptosis by cyclin-dependent kinase inhibitor p21 (/WAF1/CIP1). Cell Cycle. 2007; 6(23): 2953-2961.

32. Mackenzie GG, Nina Queisser, Manuel L. Wolfson, Cesar G. Fraga, Ana M. Adamo, Patricia I. Oteiza. Curcumin induces cell-arrest and apoptosis in association with the inhibition of constitutively active NF-jB and STAT3 pathways in Hodgkin's lymphoma cells. Int. J. Cancer, 2008; 123: 56–65.

33. Teiten MH, Serge Eifes, Mario Dicato, Marc Diederich. Curcumin—The Paradigm of a Multi-Target Natural Compound with Applications in Cancer Prevention and Treatment. Toxins. 2010; 2: 128-162.

34. Bangaru MY, Shenglin Chen, Jeffrey Woodliff, Sanjay Kansra. Curcumin (Diferuloylmethane) Induces Apoptosis and Blocks Migration of Human Medulloblastoma Cells. Anticancer Research. 2010; 30(2): 499-450.

35. Teiten MH, Gaascht F, Cronauer M, Henry E, Dicato M, Diederich M. Anti-proliferative potential of curcumin in androgen-dependent prostate cancer cells occurs through modulation of the Wingless signaling pathway. Int J Oncol. 2011; 38(3): 603-611.

36. Sundram V, Chauhan SC, Ebeling M, Jaggi M. Curcumin Attenuates β-catenin Signaling in Prostate Cancer Cells through Activation of Protein Kinase D1. PLoS ONE. 2012; 7(4): e35368.

37. Eom DW, Lee JH, Kim YJ, Hwang GS, Kim SN, Kwak JH, Cheon GJ, Kim KH, Jang HJ, Ham J, Kang KS, Yamabe N. BMB Synergistic effect of curcumin on epigallo cateching allate-induced anticancer action in PC3 prostate cancer cells. *BMB Rep*. 2015; 48(8): 461-466.

38. Yang C, Su X, Liu A. Advances in clinical study of curcumin. Curr Pharm Des. 2013; 19(11): 1966–1973.

39. Zaman MS, Chauhan N, Yallapu MM. Curcumin nanoformulation for cervical cancer treatment. Sci Rep. 2016; 6: 20051.

40. Momtazi AA, Sahebkar A. Difluorinated curcumin: a promising curcumin analogue with improved anti-tumor activity and pharmacokinetic profile. Curr Pharm Des., 2016; 22(28): 4386–4397.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.