Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43A, No.1,
January-June 2024: P.132-143

Print version ISSN 0970 0765
Online version ISSN 2320 3188
DOI: https://doi.org/10.52710/bpas-zoo.32

Original Research Article *Content Available online at: https://www.bpasjournals.com/zoology*

Evaluation of the chemo-preventive potential of Curcumin against Bisphenol- A exposed MCF-7 and RL95-2 Cancer Cells

[1]Reena Tomer and [2]Indu Sharma*

Author's Affiliation:
[1]Department of Zoology, Panjab University, Chandigarh, 160014, India
E-mail: reenatomer1992@gmail.com
ORCID: 0000-0003-1133-763X
[2]Department of Zoology, Panjab University, Chandigarh, 160014, India
E-mail: indu2702@pu.ac.in; indusharma@puchd.ac.in; indupgi.9@gmail.com
ORCID: 0000-0001-9846-9787

***Corresponding author:**
Dr. Indu Sharma,
Department of Zoology, Panjab University, Chandigarh-160014, India
E-mail: indu2702@pu.ac.in; indusharma@puchd.ac.in; indupgi.9@gmail.com

Received on 12.01.2024
Revised on 28.03.2024
Accepted on 22.04.2024

ABSTRACT:
Curcumin (CUR) has been reported to exhibit cytotoxic effects on various human cancer cell lines *in vitro*, with limited reports on its mechanism of action in MCF-7 and RL95-2 cells. Over the years, Bisphenol A (BPA), an industrial compound has received heightened attention owing to its endocrine-disrupting and carcinogenic properties. Therefore, the impact of CUR (5µM) and BPA (1µM) alone and also in combination in both cancer cell lines, for 5 days. This evaluation aimed to examine cell cytotoxicity (MTT), and apoptosis (AO/EtBr dual staining) along with transcriptional gene expression to further substantiate the observations. Results revealed that BPA enhanced the proliferation, while CUR alone and in combination inhibited the proliferation and induced apoptosis. qRT-PCR results showed significantly down-regulated expression of the *ERa, ERβ*, and *c-MYC* genes after treatment with CUR in BPA-exposed in both cell lines. Overall, our findings showed that CUR inhibited BPA-induced upregulation of genes related to cell proliferation and survival. These findings underscore CUR's potential to counteract the harmful effects of BPA exposure, paving the way for novel strategies to prevent and treat endocrine disruption and possible carcinogenesis caused by environmental agents.

Keywords:
Apoptosis, Bisphenol-A, Breast Cancer, Curcumin, Endometrial Cancer, *c-MYC*

How to cite this article: Tomer R. and Sharma I. (2024). Evaluation of the chemo-preventive potential of Curcumin against Bisphenol-A Exposed MCF-7 and RL95-2 Cancer Cells. *Bulletin of Pure and Applied Sciences-Zoology*, 43A (1), 132-143

INTRODUCTION

Breast cancer ranks as the second most common disease afflicting women in the United States, and substantial advances in detection and treatment have resulted in a 90% five-year relative overall survival rate after diagnosis (Giaquinto et al., 2022). However, breast cancer survivors have a 30% greater risk of acquiring secondary malignancies, with endometrial cancer being the most common. Endometrial adenocarcinomas account for the bulk of uterine malignancies, with a higher frequency among breast cancer survivors than in the general

population (Dörk et al., 2020). Obesity, underlying genetics, and hormonal effects all contribute to an increased risk of uterine cancer among breast cancer survivors (Wijayabahu et al., 2020). Furthermore, hormonal therapy for breast cancer may increase the chance of developing hormone-dependent tumors such as endometrial cancer (Paleari et al., 2021).

Endocrine disruptors can further interfere with hormone signaling pathways and promote aberrant cell proliferation in the hormone-responsive tissues, hence it has been proposed that exposure to these compounds could be a risk factor for breast and endometrial cancer development (Kowalczyk et al., 2022; Stephens et al., 2022). The chemical bisphenol A (BPA), which is used to make epoxy resins and polycarbonate plastics, has been identified as a significant environmental endocrine disruptor (Kawa et al., 2021). The presence of BPA has been established across a range of human tissues., including blood, follicular fluid, amniotic fluid, breast milk, saliva, adipose tissue, and urine, at concentrations between 0.2 ng/ml and 10 ng/ml (0.5-40 nM) (Simić et al., 2023). Although there have been studies associating BPA exposure with PCOS, repeated miscarriages, obesity, and endometrial hyperplasia, the effect of BPA exposure on human health is still debatable. Through epidemiological studies, a positive association has been found between elevated environmental BPA levels and the occurrence of cancer among humans. (Mercogliano & Santonicola, 2018; Murata & Kang, 2018; Yang & Zhao, 2023). Breast and endometrial cancers, as well as other hormone-dependent cancers, have been related to BPA estrogenic activity (Vom Saal et al., 2007; Diamanti-Kandarakis et al., 2009). It causes epigenetic alterations that lead to cancers of the breast, prostate, and ovary (Doherty et al., 2010; Qu et al., 2018). Exposure to BPA raises inflammation and oxidative stress, which are linked to the development and spread of cancer (Rezg et al., 2014; Shi et al., 2021). According to *in vitro* research, BPA stimulates the migration and multiplication of cancer cells, which helps with tumor growth and metastasis (Pupo et al., 2012; Xu et al., 2017).

Estrogen, which is essential for breast and endometrial cancer development and progression, activates via estrogen receptors (ERs), which are members of the nuclear receptor superfamily (Chimento et al., 2022; Pu et al., 2023). Despite the lack of consensus on estrogen-response elements (ERE) in their promoters, many estrogen-regulated genes are impacted by estrogen via alternative pathways (Miziak et al., 2023). *c-MYC* is a well-known estrogen-regulated oncogene and its activation promotes cell cycle progression and proliferation. *c-MYC* has also been demonstrated to interact with the ER and regulate estrogen-mediated signaling (Schulze et al., 2020). Numerous target genes are regulated by the MYC protein, which also controls apoptosis, cellular transformation, and the course of the cell cycle (Duffy et al., 2021). Between 20% to 30% of breast tumors have *c-MYC* overexpression. Reducing the expression of *c-MYC* in breast cancer cells may considerably impede the formation of mammary tumors (Liu et al., 2021).

A naturally occurring substance called CUR is extracted from the rhizome of the spice *Curcuma longa*, commonly found in India. CUR has received a lot of attention as a potential cancer chemotherapeutic agent during the past three decades, showing promise in treating several cancers, including colorectal, pancreatic, breast, and hematological malignancies (Kong et al., 2022; Pathak et al., 2022). CUR exhibits various biological activities, including anti-inflammatory, antioxidant, and anticancer properties. It modulates signaling pathways involved in cancer progression like cell proliferation, apoptosis, angiogenesis, and metastasis (Tomeh et al., 2019).

These anti-cancer benefits are mostly related to the down-regulation of several oncogenic molecules and pathways within cancer cells. These include Activator protein 1, Wnt/β-catenin, Peroxisome proliferator-associated receptor γ, Nuclear factor-kB, Signal transducer and activator of transcription, tumor necrosis factor-α interleukins, inducible nitric oxide synthase, lipooxygenase, cyclooxygenase-2, c-Jun N-terminal kinase, p38 MAPK, extracellular signal-regulated kinase, cyclin D1, p53, and intracellular adhesion molecule-1. These regulatory effects play a crucial role in suppressing tumor growth and promoting cancer

cell death, making them potential targets for anti-cancer therapies (Farghadani et al., 2021; Wong et al., 2021).

The current work aimed to assess CUR's efficacy against BPA-exposed MCF-7 and RL95-2 cells by investigating its interactions with the *c-MYC* and ER signaling pathways. *c-MYC* and ERs have been linked to the development and progression of both breast and endometrial cancers. Understanding how CUR impacts these pathways in the context of BPA exposure may reveal its mode of action in reducing BPA-mediated cancer-promoting effects.

MATERIAL AND METHODS

Chemicals and Reagents

BPA (Cat. #229658) and Dimethyl sulfoxide (DMSO) (Cat. #D8418), Insulin (Cat. #16634), and KAPA SYBR® Fast qPCR Kit Master Mix (2x) (Cat. #KK4600), and MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide) (Cat. #475989) were procured from Sigma-Aldrich. CUR (Cat. #RM 1449), Dulbecco's Modified Eagle Media/Nutrient Mixture F12 (DMEM/F12) with phenol red (Cat. #AL139A), DMEM/F12 phenol red-free (Cat. #AL215A) and Fetal Bovine Serum (FBS) (Cat. #RM9955) was obtained from HiMedia Laboratories Pvt. Ltd., India. Roswell Park Memorial Institute (RPMI-1640) with phenol red (Cat. #12-702F) and without phenol red (Cat. #12-918F), and Dulbecco's Phosphate Buffered Saline (DPBS) (Cat. #17-512F) were sourced from Lonza™ BioWhittaker™. The charcoal-stripped fetal bovine serum (Cat. #SH30068.03) was obtained from HyClone Cytiva. Trypsin–EDTA (Cat. #15400054) was supplied by Gibco, Revert Aid First-strand cDNA synthesis kit (Cat. #K1621) was acquired by Thermo Fisher Scientific. TRIzol reagent (Cat. #15596018) from Ambion, Life Technologies.

Cell line maintenance and treatment

The MCF-7, human breast cancer epithelial cells was obtained from the National Centre for Cell Science (NCCS) in Pune, India. These cells were grown in T-25 cm2 culture flasks in RPMI-1640 media containing phenol red. The culture medium was supplemented with 10% heat-inactivated FBS and 1% Penicillin/Streptomycin/Amphotericin. The RL95-2, human endometrial carcinoma epithelial cells (ATCC®CRL-1671™) were obtained from the American Type Culture Collection (USA). These cells were grown in DMEM-F12 media containing phenol red, 10% heat-inactivated FBS, and 1% antibiotics. In addition, RL95-2 cells were fed with insulin at a dosage of 2-3 µg/ml. Every three days, the cell culture media was changed. When the cells achieved 80% confluence, they were trypsinized using 0.05% trypsin-EDTA and moved to other culture flasks to continue the subculture process. BPA and CUR were both dissolved in DMSO to make stock solutions, with BPA at 100 mM and CUR at 50 mM, respectively for entire experimental studies.

Steroid deprivation and treatment of CUR and BPA in RL-95-2 cells

MCF-7 and RL95-2 cells were initially seeded in a 6-well plate and exposed to steroid deprivation before being treated with varying concentrations of CUR and BPA. For two days, the cells were cultured in a media devoid of phenol red and supplemented with 10% charcoal-stripped bovine serum to eliminate all steroidal components present in the regular culture conditions. Following that, the cells were treated with BPA and CUR alone and in combination for 5 days. Throughout the experiment, the media was replenished every 3rd day.

Cell toxicity assay

MCF-7 and RL95-2 cells were plated onto a 96-well culture plate with a density of 5000 cells per well and left to adhere at 37°C within the CO_2 incubator for 24 hours. The cells were then starved for 2 days before being reintroduced with fresh media that was phenol red-free and supplemented with 10% charcoal-stripped FBS to eliminate steroid hormones. Following starvation, the cells were treated with various doses of CUR (1-80 µM) and BPA (0.1-1000 µM) for 5 days. An inverted microscope was utilized to conduct the cell morphology assessment. To assess cell toxicity, 10µl of a stock solution of MTT solution (5 mg/ml in PBS) was added into each well, and followed by incubation at 37°C for 2-4 hours. After incubation, the solution in each well was aspirated and replaced with 100µL of DMSO to dissolve the formed formazan crystals. Finally, the optical density at 570nm was measured using

an ELISA microplate reader (Agilent BioTek Epoch, Santa Clara, California, USA). An inhibition curve was used to determine the IC50, which was reported as the mean ± standard deviation based on the data from three independent experiments (Kamiloglu et al., 2020).

Dual Acridine orange (AO)/Ethidium bromide (EtBr) staining

Based on the observations from the MTT assay, MCF-7, and RL95-2 cells were seeded in 12-well plates and subjected to treatment with BPA (1µM) and CUR (5µM) alone, as well as in combination, for 5 days. After the treatment, the cells were underwent PBS rinsing and fixed in 70% ethanol for 30 minutes. Subsequently, the cells were then stained with a dye mixture (100µg/mL of each AO and EtBr) for 20 minutes. The stained cells were then observed under an EVOS® FL Cell Imaging fluorescence microscope (ThermoFisher Scientific, Walthan, Massachusetts, United States) for morphological assessment of apoptosis (El-Garawani et al., 2016).

qPCR analysis

Total RNA was extracted manually with TRIzol reagent. Following that, 1µg of total RNA was reverse transcribed into a PCR machine (HiMedia Prima-96™ Thermal Cycler, HiMedia Laboratories, Mumbai, India) using the Revert Aid First-strand cDNA synthesis kit (ThermoFisher Scientific, Vilnius, Lithuania, United States). The primer sequences and optimal annealing temperature, as listed in **Table 1.** The relative mRNA expression levels of genes were quantified using qPCR on an ABI 7300 Step One Plus™ Real-Time PCR System (Applied Biosystems, Foster City, California, USA). Against β-actin as an invariant control. The KAPA SYBR® FAST qPCR Kit Master Mix (2x) (R&D Cape Town, South Africa) was used to analyze the expression of targeted genes. The PCR reaction mixture tube, with a total volume of 10µl, included the following components: RNase-free water (4.8 µl), SYBR Green PCR Master Mix (3 µl), primers (1µl), High ROX (0.2µl) and cDNA (1 µl). The fold changes in the expression of each gene were determined using the comparative threshold cycle (Ct) method, calculated by the formula 2^(-ΔΔCt).

Table 1: Sequences and annealing temperature of primers used for RT-qPCR analysis

Gene	Tm (°C)	Sequence (5′-3′)
β-Actin	57°C	F-GAATTGCTATGTGTCTGGGT R-CATCTTCAAACCTCCATGATG
ERa	59°C	F-AGGTGCCCTACTACCTGGAGAAC R-GGTGGCTGGACACATATAGTCGTT
ERβ	61°C	F-CATCTTACCCCTGGAGCACG R-CAAGTTAGTGACATTGCTGGGA
c-MYC	57°C	F-TACAACACCCGAGCAAGGAC R-GAGGCTGCTGGTTTTCCACT

Statistical analysis

The data were presented as mean ± standard deviation, obtained from independent experiments conducted in triplicate for each group. Statistical analysis was performed using GraphPad Prism 8 software, which included two-way analysis of variance (ANOVA) followed by post-hoc Tukey's multiple comparison tests. A significance level of $p<0.05$ was used for the analysis.

RESULTS

Effect of BPA and CUR on the viability of MCF-7 and RL95-2 cancer cells

MTT assay was used to assess the cytotoxicity of CUR and BPA on the MCF-7 and RL95-2 cell lines. Cells were treated with different concentrations of CUR (1 - 80 µM) and BPA (0.1-1000 µM) alone in triplicate for 5 days and cell viability was calculated. The results revealed that CUR significantly inhibited cell viability at 10 µM or higher concentrations ($p < 0.001$) **(Fig. 1b).** In the case of BPA, cell viability is concentration-dependent since BPA reduced cell proliferation at higher doses (100 -1000 µM) ($p < 0.001$), but increased proliferation at lower doses (1 µM &10 µM) **(Fig. 1a).** Based on these results, we further examined the effects of CUR (5 µM) and BPA (1µM) alone and in combination in both the cancer cell lines.

The proliferative capacity of BPA was countered by the antiproliferative potential of CUR in MCF-7 and RL95-2 cancer cells

To further evaluate the protective roles of CUR on BPA-exposed cancer cells, cell viability was observed. As shown in **(Fig. 1c)**, CUR decreased the proliferation of BPA-exposed both the cancer cell lines.

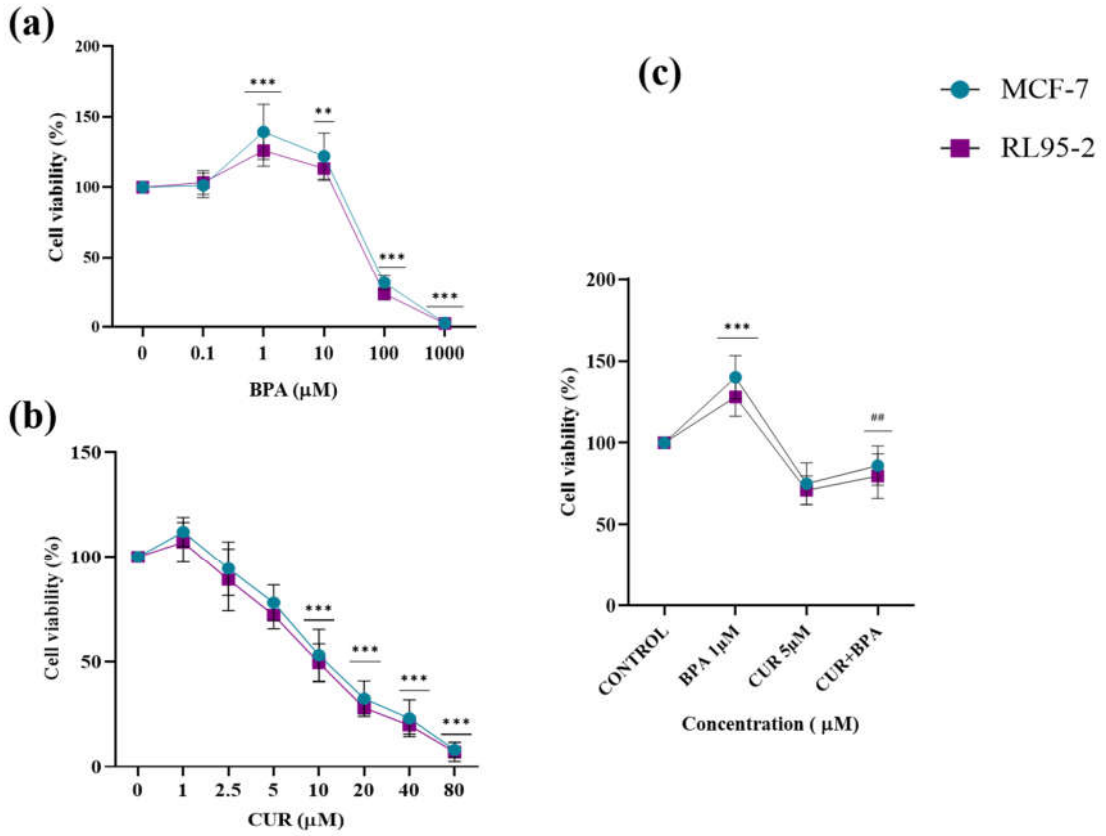

Figure 1: Bar diagrams showing percent cell viability was assessed by MTT assay after treatment with BPA and CUR at various concentrations for 5 days. a) MCF-7 Cells were treated with various concentrations of a) BPA (0.1 -1000µM), b) CUR (1-80µM), and c) a combination of CUR and BPA for 5 days. Data are expressed as mean ± SD of 3 independent experiments **p<.01, ***p<.001(compared with control group); ##p < .01 (compared with BPA exposed group).

CUR counteracted the anti-apoptotic effect of BPA through the induction of apoptosis in cancer cells.

To determine whether CUR-induced growth suppression was associated with apoptosis, we used an AO/EtBr dual staining experiment to measure the degree of apoptosis. Fluorescence microscopy of both the cell lines indicated the early and late apoptotic markers such as chromatin condensation, formation of apoptotic body, blebbing of the membrane, and fragmented nuclei by CUR treatment in contrast to the control undamaged nuclear structure. CUR increased apoptotic bodies by 18% in BPA-exposed MCF-7 cancer cells and by 19% in RL95-2 cancer cells compared to both control and BPA-exposed cells, respectively ($p<0.01$) **(Fig. 2).**

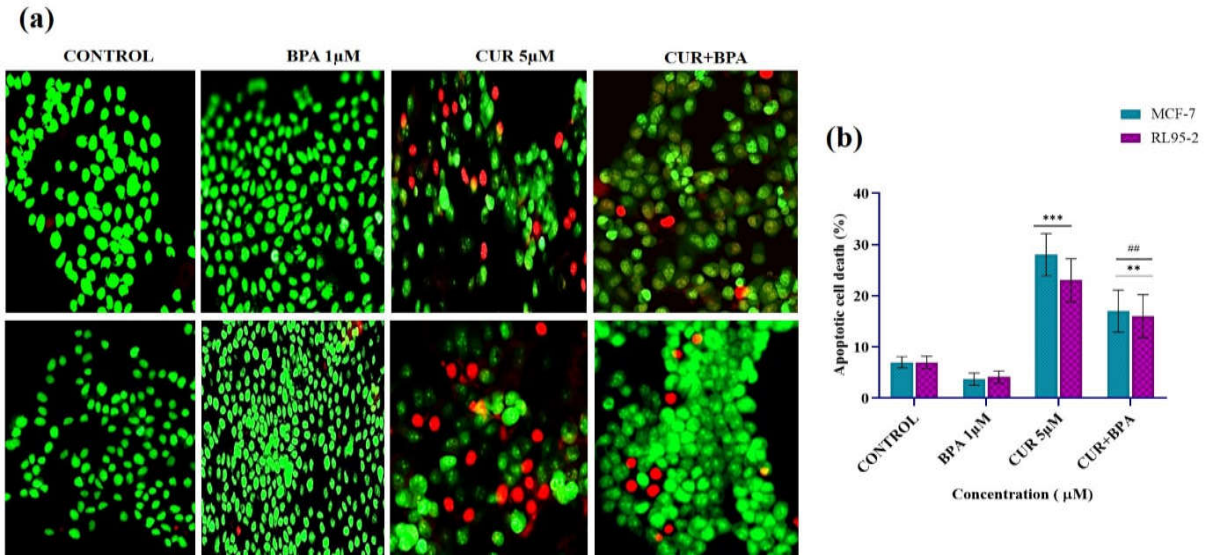

Figure 2: Acridine Orange/Ethidium Bromide (AO/EtBr) dual staining assay, MCF-7 and RL95-2 cells were treated with different concentrations of BPA (1µM) and CUR (5µM) alone and in combination (CUR 5µM: BPA 1µM for 5 days, a) Microscopic pictures with a fluorescent microscope (magnification: 200x) after 5 days of exposure. Scale bar represents (200µm) b) Bar diagrams showing apoptosis rate (%). Data are expressed as mean ± SD of 3 independent experiments, **p<.01, ***p<.001 (compared with control group); ##p < .01 (compared with BPA exposed group).

CUR downregulated the BPA-induced overexpression of genes related to cell proliferation and survival of MCF-7 and RL95-2 cancer cells

The differences in gene expression were observed when MCF-7 and RL95-2 cells were seeded in 12-well plates and treated with BPA (1µM) and CUR (5µM) alone, as well as in combination, for 5 days. The expression of *ERa*, *ERβ*, and *c-MYC* genes were up-regulated (*P<0.01*) in BPA 1µM exposed MCF-7 and RL95-2 cancer cells. After CUR treatment, we observed statistically significant downregulation of genes *ERa*, *ERβ*, and *c-MYC* (*p<0.05)* in both the breast and endometrial cancer cell lines. **(Fig. 3).**

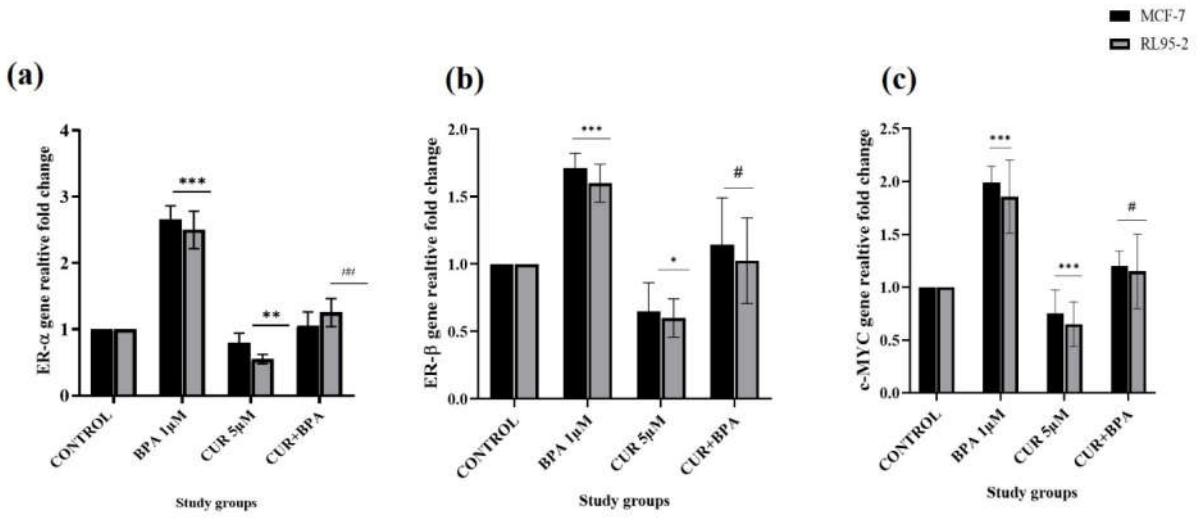

Figure 3: Expression of ERα, ERβ, and MYC genes in MCF-7 and RL95-2 cells using real-time PCR (qPCR) machine: cells were treated with different concentrations of BPA (1μM) and CUR (5μM) alone and in combination (CUR 5μM: BPA 1μM for 5 days. Bar diagrams showing relative fold change of a) *ERa*, b) *ERβ*, and c) *c-MYC*. Data are expressed as mean ± SD of three independent experiments, **p*<.05, ***p*<.01, ****p*<.001 (compared with control group); #*p* < .05 and ##*p* < .01 (compared with BPA exposed group).

DISCUSSION

High endogenous estrogen levels are recognized as a risk factor for breast and endometrial cancer in women. Increased estrogen levels can accelerate cell growth, potentially increasing the risk of acquiring random genetic diseases (Mohanty et al., 2021; Bukato et al 2024). Furthermore, estrogen metabolism in the body can cause the formation and accumulation of reactive oxygen species and nitric oxide molecules. These molecules can affect normal cellular processes such as apoptosis and DNA repair via ER-mediated signaling, causing oxidative damage to DNA within cells (Pourbagher-Shahri et al., 2021; Xiang et al., 2021). Endocrine-disrupting chemicals (EDCs), which have structural similarities to E2, can mimic estrogenic effects and disrupt estrogen signaling, potentially leading to cancer in estrogen-responsive tissues (Eve et al., 2020; Hall et al., 2021).

BPA, a hormone-like industrial chemical compound is believed to be a potential contributor to the development of cancer. It is a potent endocrine disrupter that mimics estrogen and leads to adverse health effects. It binds with androgen, estrogen, and thyroid hormone receptors. BPA is frequently used to produce resin linings for food and beverage containers, as well as polycarbonate plastics. Huge quantities of BPA are produced annually and leach into food and water supplies making humans vulnerable to it (Vandenberg et al., 2007; Rathee et al., 2012).

A convincing strategy for combating cancer using natural compounds entails targeting pathways and enzymes that contribute to anti-proliferation and cytotoxicity. CUR (diferuloylmethane), the main curcuminoid of turmeric has a wide range of uses and is frequently used as a flavoring and food coloring. In addition, numerous *in vivo* and *in vitro* studies inferred that CUR and its derivative exhibit extensive biological activity as an antioxidant, neuroprotective, anticancer, and anti-inflammatory (Sidhar et al., 2017; Semlali et al., 2021). It is well known that CUR has cytotoxic properties against certain cancer cell types, and numerous studies have also identified CUR's ability to inhibit cell proliferation (Tomeh et al., 2019). The present *in vitro* study revealed that

CUR is a potent inhibitor of breast and endometrial cancer cells with an IC50 value of only a few micromolar units. Similarly, previous studies also reported that CUR drastically reduced cell proliferation, and promoted apoptosis in prostate cancer DU-145 cells, pancreatic cancer cells, liver cancer HepG2 cells, and leukemia cells at higher concentrations (Iqbal et al., 2016; Sha et al., 2016; Zhou et al., 2016). Additionally, it also inhibited the proliferation of human cancer cell lines, including HL60, k562, T47D, and HeLa cells (Khazaei et al., 2015).

To evaluate the cytotoxicity of CUR at doses of 1, 2.5, 5, 10, 20, 40, and 80µM and BPA at doses of 0.1, 1, 10, 100, and 1000 µM on the MCF-7 and RL95-2 cell line, an MTT assay was performed. Our results showed that BPA causes extreme cell death in concentrations of above 100 µM, while at doses ranging from 1 to 10 µM, it significantly increased proliferation in both epithelial cancer cell lines. This cell proliferation-inducing action of BPA in ER-positive cancer cells is consistent with our prior research, which found BPA to be xenoestrogen as an EDC. These findings revealed that BPA, like estradiol (E2), can promote breast, endometrial, and ovarian cancer cell proliferation via an ER-dependent pathway (Shi et al., 2017; Stillwater et al., 2020). These findings emphasize EDC's inherent estrogenic behavior in estrogen-dependent malignancies (Pan et al., 2024). Further study demonstrated that CUR exhibited toxicity at greater levels at concentrations above (10 -80 µM) in both cancer cell lines, in a dose-dependent manner ($p < 0.05$). When CUR at a concentration of 5µM was combined with BPA (1µM), it statistically inhibited cell proliferation ($p < 0.05$). This shows that CUR has chemo-preventive qualities against the cancer-causing effects of endocrine-disrupting chemicals (EDCs) like BPA.

The result of AO/EtBr staining inferred CUR increased apoptosis. Results showed that the apoptosis rate of the BPA 1µM was not significantly different from that of the control group. CUR induced apoptosis significantly in both cancer cell lines at 5 µM concentrations ($p < 0.01$). In the HCT-116 cells, CUR (10 - 30µM) caused apoptosis in a dose-dependent manner. With increasing drug concentration, early and late apoptotic cell proportions gradually increased (Xiang et al., 2020). Kumar et al. showed that CUR causes DNA damage and apoptosis in the HeLa cervical cell line (Kumar et al., 2016).

Our qPCR results further support our findings, showing that exposure to BPA leads to the upregulation of *ERa*, *ERβ,* and *c-MYC* genes in both cell lines. Conversely, treatment with CUR alone downregulates the expression of *ERa*, *ERβ*, and *c-MYC* genes. Interestingly, when cells are treated with both CUR and BPA simultaneously, CUR attenuates the augmenting effect of BPA on gene expression, indicating a potential reversal of BPA's impact. Moreover, our findings are consistent with previous studies, revealing a positive correlation between *c-MYC* expression and *ER-a*. This correlation suggests that *c-MYC* may play a role in the development of endometrial carcinoma through ER signaling pathways (Bircan et al., 2005). Additionally, it was previously demonstrated that *c-MYC* plays a role in promoting tumor growth and inhibiting apoptosis, emphasizing its significance in endometrial cancer pathogenesis (Konopka et al., 2004). Previous studies have shown that estrogen promotes the increased production of *c-MYC* in ER-positive breast cancer cells breast cancer cells. This suggests that high levels of estradiol, a powerful estrogen, may induce *c-MYC* expression in breast cancer (Dadiani et al., 2009). In breast cancer, estrogen triggers *c-MYC* is an enhancer activated by both ERs and AP-1 (Wang et al., 2011).

In summary, we investigated the impact of BPA, also known as xenoestrogen endocrine-disrupting chemicals (EDCs), on breast and endometrial cancer progression. BPA has been shown to stimulate cell proliferation and inhibit apoptosis via increasing the expression of the genes, *ERa, ERβ*, and *c-MYC*. CUR, on the other hand, significantly inhibited BPA-induced breast and endometrial cancer cell proliferation and induced apoptotic effects by modulating the expression of genes, *ERa*, *ERβ*, and *c-MYC*. CUR, therefore, appears as a potent chemo-preventive chemical capable of triggering apoptosis in breast and endometrial cancer cells. These findings point to the possible use of CUR to prevent tumor aggressiveness and as an alternative to conventional treatment drugs in situations that

are consistently exposed to a variety of EDCs, including BPA.

CONCLUSION

The results of the study highlight the anticancer characteristics of CUR in preventing the progression of endometrial and breast cancers triggered by BPA. CUR effectively Impeded the growth of BPA -BPA-stimulated cells and caused cell death by influencing crucial genes involved in estrogen signaling and the development of cancer. These outcomes have implications, for practice as CUR shows potential as a natural remedy that can counteract the carcinogenic effects of BPA and chemicals that alter the hormone signaling. Additionally, CUR's excellent safety profile makes it an appealing choice for supplementary therapy in cancers associated with BPA and similar substances. Further research should delve into the mechanisms of CUR to assess its effectiveness in living organisms and investigate synergies, with existing cancer therapies to enhance outcomes in hormone-disrupting chemical-related malignancies

Funding
The authors are grateful to the Council of Scientific and Industrial Research (CSIR), Government of India, for providing financial support in the form of a Junior Research Fellowship (09/135(0789)2017-EMR-1). The authors are also thankful to the Department of Science and Technology (DST)-FIST-I and DST-PURSE for their financial support to the Department of Zoology, Panjab University, Chandigarh.

Ethical Clearance
Not Applicable

Author contributions
Both authors contributed to the study's conception and design. Reena Tomer conducted the content preparation, data collection, and analysis. The manuscript was reviewed and edited by Dr. Indu Sharma

Declaration of competing interest
The authors declare that they have no competing financial interests or personal relationships that may have influenced the work described in this document.

Acknowledgments
The authors would like to thank the Department of Zoology, Panjab University, Chandigarh for providing the required infrastructure.

Abbreviations
BPA: Bisphenol A
CUR: Curcumin
DMSO: Dimethyl sulfoxide
ER: Estrogen receptor;
c-MYC: Myelocytomatosis oncogene

REFERENCES

1. Bircan, S., Ensari, A., Ozturk, S., Erdogan, N., Dundar, I., & Ortac, F. (2005). Immunohistochemical analysis of c-myc, c-jun and estrogen receptor in normal, hyperplastic and neoplastic endometrium. *Pathology & Oncology Research*, *11*, 32-39.
2. Bukato, K., Kostrzewa, T., Gammazza, A. M., Gorska-Ponikowska, M., & Sawicki, S. (2024). Endogenous estrogen metabolites as oxidative stress mediators and endometrial cancer biomarkers. *Cell Communication and Signaling*, *22*(1), 205.
3. Chimento, A., De Luca, A., Avena, P., De Amicis, F., Casaburi, I., Sirianni, R., & Pezzi, V. (2022). Estrogen receptors-mediated apoptosis in hormone-dependent cancers. *International Journal of Molecular Sciences*, *23*(3), 1242.
4. Dadiani, M., Seger, D., Kreizman, T., Badikhi, D., Margalit, R., Eilam, R., & Degani, H. (2009). Estrogen regulation of vascular endothelial growth factor in breast cancer in vitro and in vivo: The role of estrogen receptor α and c-Myc. *Endocrine-Related Cancer*, *16*(3), 819-834.
5. Diamanti-Kandarakis, E., Bourguignon, J. P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., ... & Gore, A. C. (2009). Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine Reviews*, *30*(4), 293-342.
6. Doherty, L. F., Bromer, J. G., Zhou, Y., Aldad, T. S., & Taylor, H. S. (2010). In utero exposure

to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: an epigenetic mechanism linking endocrine disruptors to breast cancer. *Hormones and Cancer*, *1*, 146-155.

7. Dörk, T., Hillemanns, P., Tempfer, C., Breu, J., & Fleisch, M. C. (2020). Genetic susceptibility to endometrial cancer: Risk factors and clinical management. *Cancers*, *12*(9), 2407.

8. Duffy, M. J., O'Grady, S., Tang, M., & Crown, J. (2021). MYC as a target for cancer treatment. *Cancer Treatment Reviews*, *94*, 102154.

9. El-Garawani, I. M., & Hassab El-Nabi, S. E. (2016). Increased sensitivity of apoptosis detection using direct DNA staining method and integration of acridine orange as an alternative safer fluorescent dye in agarose gel electrophoresis and micronucleus test. *Cjpas*, *10*(2), 3865-3871.

10. Eve, L., Fervers, B., Le Romancer, M., & Etienne-Selloum, N. (2020). Exposure to endocrine disrupting chemicals and risk of breast cancer. *International Journal of Molecular Sciences*, *21*(23), 9139.

11. Farghadani, R., & Naidu, R. (2021). Curcumin: modulator of key molecular signaling pathways in hormone-independent breast cancer. *Cancers*, *13*(14), 3427.

12. Giaquinto, A. N., Sung, H., Miller, K. D., Kramer, J. L., Newman, L. A., Minihan, A., ... & Siegel, R. L. (2022). Breast cancer statistics, 2022. *CA: a Cancer Journal for Clinicians*, *72*(6), 524-541.

13. Hall, J. M., & Korach, K. S. (2021). Endocrine disrupting chemicals (EDCs) and sex steroid receptors. *Advances in Pharmacology (San Diego, Calif.)*, *92*, 191–235.

14. Iqbal, B., Ghildiyal, A., Singh, S., Arshad, M., Mahdi, A. A., & Tiwari, S. (2016). Antiproliferative and apoptotic effect of curcumin and TRAIL (TNF related apoptosis inducing ligand) in chronic myeloid leukaemic cells. *Journal of Clinical and Diagnostic Research: JCDR*, *10*(4), XC01.

15. Kamiloglu, S., Sari, G., Ozdal, T., & Capanoglu, E. (2020). Guidelines for cell viability assays. *Food Frontiers*, *1*(3), 332-349.

16. Kawa, I. A., Fatima, Q., Mir, S. A., Jeelani, H., Manzoor, S., & Rashid, F. (2021). Endocrine disrupting chemical Bisphenol A and its potential effects on female health. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *15*(3), 803-811.

17. Khazaei Koohpar, Z., Entezari, M., Movafagh, A., & Hashemi, M. (2015). Anticancer Activity of Curcumin on Human Breast Adenocarcinoma: Role of Mcl-1 Gene. *Iranian Journal of Cancer Prevention*, *8*(3), e2331.

18. Kong, W. Y., Ngai, S. C., Goh, B. H., Lee, L. H., Htar, T. T., & Chuah, L. H. (2021). Is curcumin the answer to future chemotherapy cocktail?. *Molecules*, *26*(14), 4329.

19. Konopka, B., Janiec-Jankowska, A., Paszko, Z., & Goluda, M. (2004). The coexistence of ERBB2, INT2, and CMYC oncogene amplifications and PTEN gene mutations in endometrial carcinoma. *Journal of Cancer Research and Clinical Oncology*, *130*, 114-121.

20. Kowalczyk, A., Wrzecińska, M., Czerniawska-Piątkowska, E., Araújo, J. P., & Cwynar, P. (2022). Molecular consequences of the exposure to toxic substances for the endocrine system of females. *Biomedicine & Pharmacotherapy*, *155*, 113730.

21. Kumar, D., Basu, S., Parija, L., Rout, D., Manna, S., Dandapat, J., & Debata, P. R. (2016). Curcumin and Ellagic acid synergistically induce ROS generation, DNA damage, p53 accumulation and apoptosis in HeLa cervical carcinoma cells. *Biomedicine & pharmacotherapy*, *81*, 31-37.

22. Liu, Y., Zhu, C., Tang, L., Chen, Q., Guan, N., Xu, K., & Guan, X. (2021). MYC dysfunction modulates stemness and tumorigenesis in breast cancer. *International Journal of Biological Sciences*, *17*(1), 178.

23. Mercogliano, R., & Santonicola, S. (2018). Investigation on bisphenol A levels in human milk and dairy supply chain: A review. *Food and Chemical Toxicology*, *114*, 98-107.

24. Miziak, P., Baran, M., Błaszczak, E., Przybyszewska-Podstawka, A., Kałafut, J., Smok-Kalwat, J., ... & Stepulak, A. (2023). Estrogen Receptor Signaling in Breast Cancer. *Cancers*, *15*(19), 4689.

25. Mohanty, S. S., & Mohanty, P. K. (2021). Obesity as potential breast cancer risk factor for postmenopausal women. *Genes & Diseases*, *8*(2), 117-123.

26. Murata, M., & Kang, J. H. (2018). Bisphenol A (BPA) and cell signaling

pathways. *Biotechnology Advances*, *36*(1), 311-327.

27. Paleari, L., Pesce, S., Rutigliani, M., Greppi, M., Obino, V., Gorlero, F., ... & Marcenaro, E. (2021). New insights into endometrial cancer. *Cancers*, *13*(7), 1496.

28. Pan, J., Liu, P., Yu, X., Zhang, Z., & Liu, J. (2024). The adverse role of endocrine disrupting chemicals in the reproductive system. *Frontiers in Endocrinology*, *14*, 1324993.

29. Pathak, K., Pathak, M. P., Saikia, R., Gogoi, U., Sahariah, J. J., Zothantluanga, J. H., ... & Das, A. (2022). Cancer chemotherapy via natural bioactive compounds. *Current Drug Discovery Technologies*, *19*(4), 4-23.

30. Pourbagher-Shahri, A. M., Farkhondeh, T., Talebi, M., Kopustinskiene, D. M., Samarghandian, S., & Bernatoniene, J. (2021). An overview of NO signaling pathways in aging. *Molecules*, *26*(15), 4533.

31. Pu, H., Wen, X., Luo, D., & Guo, Z. (2023). Regulation of progesterone receptor expression in endometriosis, endometrial cancer, and breast cancer by estrogen, polymorphisms, transcription factors, epigenetic alterations, and ubiquitin-proteasome system. *The Journal of Steroid Biochemistry and Molecular Biology*, *227*, 106199.

32. Pupo, M., Pisano, A., Lappano, R., Santolla, M. F., De Francesco, E. M., Abonante, S., ... & Maggiolini, M. (2012). Bisphenol A induces gene expression changes and proliferative effects through GPER in breast cancer cells and cancer-associated fibroblasts. *Environmental Health Perspectives*, *120*(8), 1177-1182.

33. Qu, W., Zhao, Z., Chen, S., Zhang, L., Wu, D., & Chen, Z. (2018). Bisphenol A suppresses proliferation and induces apoptosis in colonic epithelial cells through mitochondrial and MAPK/AKT pathways. *Life Sciences*, *208*, 167-174.

34. Rathee, M., Malik, P., & Singh, J., 2012. Bisphenol A in dental sealants and its estrogen like effect. Indian J. Endocrinol. Metab. 16(3), 339-342. https://doi.org/10.4103/2230-8210.95660

35. Rezg, R., El-Fazaa, S., Gharbi, N., & Mornagui, B. (2014). Bisphenol A and human chronic diseases: current evidences, possible mechanisms, and future perspectives. *Environment International*, *64*, 83-90.

36. Schulze, A., Oshi, M., Endo, I., & Takabe, K. (2020). MYC targets scores are associated with cancer aggressiveness and poor survival in ER-positive primary and metastatic breast cancer. *International Journal of Molecular Sciences*, *21*(21), 8127.

37. Semlali, A., Contant, C., Al-Otaibi, B., Al-Jammaz, I., & Chandad, F. (2021). The curcumin analog (PAC) suppressed cell survival and induced apoptosis and autophagy in oral cancer cells. *Scientific Reports*, *11*(1), 11701.

38. Sha, J., Li, J., Wang, W., Pan, L., Cheng, J., Li, L., ... & Lin, W. (2016). Curcumin induces G0/G1 arrest and apoptosis in hormone-independent prostate cancer DU-145 cells by down-regulating Notch signaling. *Biomedicine & Pharmacotherapy*, *84*, 177-184.

39. Shi, J., Liu, C., Chen, M., Yan, J., Wang, C., Zuo, Z., & He, C. (2021). The interference effects of bisphenol A on the synthesis of steroid hormones in human ovarian granulosa cells. *Environmental Toxicology*, *36*(4), 665-674.

40. Shi, X. Y., Wang, Z., Liu, L., Feng, L. M., Li, N., Liu, S., & Gao, H. (2017). Low concentrations of bisphenol A promote human ovarian cancer cell proliferation and glycolysis-based metabolism through the estrogen receptor-α pathway. *Chemosphere*, *185*, 361-367.

41. Sidhar, H., & Giri, R. K. (2017). Induction of Bex genes by curcumin is associated with apoptosis and activation of p53 in N2a neuroblastoma cells. *Scientific Reports*, *7*(1), 41420.

42. Simić, M. D., Savić, B. G., Ognjanović, M. R., Stanković, D. M., Relić, D. J., Aćimović, D. D., & Brdarić, T. P. (2023). Degradation of bisphenol A on SnO2-MWCNT electrode using electrochemical oxidation. *Journal of Water Process Engineering*, *51*, 103416.

43. Stephens, V. R., Rumph, J. T., Ameli, S., Bruner-Tran, K. L., & Osteen, K. G. (2022). The potential relationship between environmental endocrine disruptor exposure and the development of endometriosis and

adenomyosis. *Frontiers in Physiology*, *12*, 807685.

44. Stillwater, B. J., Bull, A. C., Romagnolo, D. F., Neumayer, L. A., Donovan, M. G., & Selmin, O. I. (2020). Bisphenols and Risk of Breast Cancer: A Narrative Review of the Impact of Diet and Bioactive Food Components. *Frontiers in Nutrition*, *7*, 581388.

45. Tomeh, M. A., Hadianamrei, R., & Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. *International Journal of Molecular Sciences*, *20*(5), 1033.

46. Tomeh, M. A., Hadianamrei, R., & Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. *International Journal of Molecular Sciences*, *20*(5), 1033.

47. Vandenberg, L. N., Hauser, R., Marcus, M., Olea, N., & Welshons, W. V. (2007). Human exposure to bisphenol A (BPA). *Reproductive Toxicology*, *24*(2), 139-177.

48. Vom Saal, F. S., Akingbemi, B. T., Belcher, S. M., Birnbaum, L. S., Crain, D. A., Eriksen, M., ... & Zoeller, R. T. (2007). Chapel Hill bisphenol A expert panel consensus statement: integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. *Reproductive Toxicology*, *24*(2), 131-138.

49. Wang, C., Mayer, J. A., Mazumdar, A., Fertuck, K., Kim, H., Brown, M., & Brown, P. H. (2011). Estrogen induces c-myc gene expression via an upstream enhancer activated by the estrogen receptor and the AP-1 transcription factor. *Molecular Endocrinology*, *25*(9), 1527-1538.

50. Wijayabahu, A. T., Egan, K. M., & Yaghjyan, L. (2020). Uterine cancer in breast cancer survivors: a systematic review. *Breast Cancer Research and Treatment*, *180*, 1-19.

51. Wong, S. C., Kamarudin, M. N. A., & Naidu, R. (2021). Anticancer mechanism of curcumin on human glioblastoma. *Nutrients*, *13*(3), 950.

52. Xiang, D., Liu, Y., Zhou, S., Zhou, E., & Wang, Y. (2021). Protective effects of estrogen on cardiovascular disease mediated by oxidative stress. *Oxidative Medicine and Cellular Longevity*, *2021*, 1-15.

53. Xiang, L., He, B., Liu, Q., Hu, D., Liao, W., Li, R., ... & Zhao, G. (2020). Antitumor effects of curcumin on the proliferation, migration and apoptosis of human colorectal carcinoma HCT-116 cells. *Oncology Reports*, *44*(5), 1997-2008.

54. Xu, F., Wang, X., Wu, N., He, S., Yi, W., Xiang, S., ... & Ying, C. (2017). Bisphenol A induces proliferative effects on both breast cancer cells and vascular endothelial cells through a shared GPER-dependent pathway in hypoxia. *Environmental Pollution*, *231*, 1609-1620.

55. Yang, F., & Zhao, F. (2023). Mechanism of visible light enhances microbial degradation of Bisphenol A. *Journal of Hazardous Materials*, *443*, 130214.

56. Zhou, X., Su, J., Feng, S., Wang, L., Yin, X., Yan, J., & Wang, Z. (2016). Antitumor activity of curcumin is involved in down-regulation of YAP/TAZ expression in pancreatic cancer cells. *Oncotarget*, *7*(48), 79076–79088.

****