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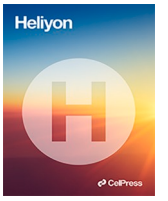

Research article

Inducing breast cancer cell death: The impact of taxodone on proliferation through apoptosis

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ABSTRACT

Breast cancer is the most prevalent form of cancer in women and a major contributor to cancer-related fatalities worldwide. Several factors play a role in the development of breast cancer, encompassing age, hormone levels, etc. Taxodone has shown significant anti-tumor properties in both laboratory experiments and living organisms. However, its impact on the human MCF-7 breast cancer cell line has not been researched. This investigation explores the chemo-preventive potential of taxodone in the MCF-7 breast cancer cells. The anticancer potential of taxodone against MCF-7 cells was determined by MTT assay. Further, the induction of apoptosis in MCF-7 breast cancer cells was confirmed via ELISA, which indicated the increased incidences of chromatin condensation and ssDNA breakage in the MCF-7 apoptotic cells upon 24 h of taxodone treatment. The intracellular reactive oxygen species (ROS) level was evaluated using H_2DCFDA fluorescent dye to elucidate the mechanism of action triggered upon taxodone treatment. The increasing intercellular ROS level sequentially activated the caspase-mediated apoptosis pathway. Consequently, the outcomes revealed that taxodone decreased the cell viability of MCF-7 dose-dependently. Taxodone triggers apoptosis in MCF-7 cells by increasing intracellular ROS levels and activating the caspase cascade through the mitochondrial apoptosis-induced channel, an early marker of apoptosis onset. Our results indicate that taxodone exhibits anti-proliferative and apoptotic properties against human MCF-7 breast cancer cells, suggesting it to be a natural anticancer agent.

1. Introduction

Among various types of cancers, breast cancer associated with women's health is known to be the most common type of cancer. It is one of the prominent reasons for cancer-related casualties among women worldwide [1]. Breast cancer is a cancer of tissue involving the inner layers of milk glands, lobules, and ducts (a form of tiny tubes involved in carrying the milk) [2]. Numerous factors are implicated in causing breast cancer, which include the age of the patient [3], high levels of hormones [4], iodine deficiency in their diets [5], economic status, and race, etc [6]. The other factors involved in increasing the risk of breast cancers include aging, gene mutations, history of long menstrual cycle, conceiving problems or mature age first pregnancy, drug practices such as a replacement of postmenopausal hormone therapy, climacteric overweight, lethargy and use of alcohol [7]. Currently, 21 distinct types of breast

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cancers are determined histologically, and there are four variations; each subtype differs [8]. As per the statistics, most of the women are at a high alarming risk of developing breast cancer, and only 5–10 % of women with breast cancer have genetic mutations in the breast cancer genes such as BRCA1 and BRCA2. Around 90 % of the breast cancer occurring in the general women population does not involve these hereditary mutations of breast cancer genes [9].

According to the National Breast Cancer Coalition statistics for Breast Cancer 2020, there have been an estimated 276,480 new cases of invasive breast cancer diagnosis in women, out of which only 2620 cases were diagnosed in men. Additionally, 1 in 8 women has breast cancer, and there is a reported death of a woman every 13 min suffering from breast cancer worldwide [10]. Around 20–30 % of women diagnosed with breast cancer were well treated employing anticancer therapies with no further symptoms of cancer. Later, these patients were found to have frequent recurrence rates [11].

Taxodone is an abietane-type diterpenoid found in plants such as *Metasequoia glyptostroboides* [12] and *S. chorassanica* [13]. Taxodone has been shown to possess numerous multi-therapeutic properties such as antibacterial [12], antifungal [13], and anticancer [14] activities. Previously, some researchers have isolated an abietane-type diterpenoid taxodone from the cone of Metasequoia glyptostroboides, which showed a potential effect on inhibiting a panel of foodborne pathogenic bacteria [15]. Also, the taxodone exhibited an antifungal effect against two wood decay fungi, *Trametes versicolor* and *Fomitopsis palustris* [16]. Taxodone has been demonstrated to possess significant anti-tumor properties *in vitro* and *in vivo.* However, its role in the human MCF-7 breast cancer cell line has not been studied. In this study, we present the evaluation of taxodone against the human MCF-7 breast cancer cell lines and explore the mechanistic pathway through which it induces cell death.

2. Materials and methods

2.1. Chemicals and reagents

Taxodone of highly pure quality, penicillin-streptomycin, fetal bovine serum (Gibco, USA), trypsin, dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide regent (MTT) were purchased from Sigma-Aldrich. Eagle's Minimum Essential Medium (EMEM) cell culture medium was also purchased from Sigma-Aldrich. Except for those listed above, the chemicals used in this study for molecular studies were of the highest quality. The chemical structure of taxodone is shown in Fig. 1.

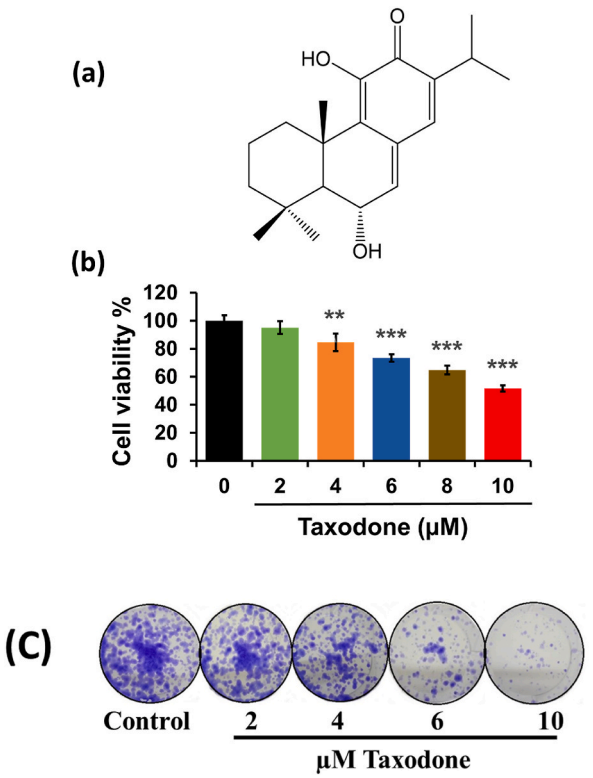

Fig. 1. The effect of taxodone treatment against the human breast cancer cells. (a) The 2D chemical structure of taxodone. (b) The percentage of cell viability was determined using MTT assay. The experiments were performed in triplicate and results are presented as mean ± SD values; the *p*-values are represented as follows: * <0.05, ** <0.01, *** <0.001 in comparison with the control untreated group. (c) Clonogenic assay, MCF-7 cells were cultured in the presence and absence of taxodone over 7 days, followed by crystal violet staining. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.2. Cell culture and maintenance

The MCF-7 cell line (human breast cancer cells) was obtained from American Type Culture Collection (ATCC) and allowed to grow in EMEM medium supplied with 10 % heat-inactivated fetal bovine serum and stocked under 5 % CO_2 at 37 °C.

2.3. Cell viability analysis

To evaluate the anticancer potential of taxodone, we treated taxodone to MCF-7 cells for 24 h (h) dose-dependently. In brief, MCF-7 breast cancer cells were seeded at a density of 2×10^4 in a 96-well plate and granted proliferation until reaching their confluence of 80 %. Once the cells had 80 % confluent, they were treated with different concentrations (0, 2, 4, 6, 8, 10, μM) of taxodone for evaluating its anticancer study against breast cancer cells MCF-7. The cell culture medium was separated from the wells after 24 h, and the fresh EMEM culture medium (100 μL) was added into each well supplemented with 10 μL of MTT (5 mg/mL) per well, followed by further incubation of plates at 37 °C for 4 h. After that, the cell culture medium was re-aspirated followed by formation of insoluble formazan crystals which were then dissolved in DMSO (50 μL) followed by incubation of plates in a plate shaker (Tecan, LabTech, Sorisole, Italy) for 30 min. A TECAN Infinite-PRO microplate reader was then employed to measure the absorbance at 540 nm, as previously mentioned [15]. The viable cells produced a dark blue formazan product, whereas no such staining was formed in the dead cells. The percentage calculation of viable cells was estimated using the following equation: (%) = [100 × (sample absorbance)/(control absorbance)].

As a confirmation of dead cell counts, a Clonogenic assay was also performed [38]. The MCF-7 cells were seeded in a 12-well culture plate at a density of 5×10^4 cells/well. The cells were then allowed to attach for 24 h, followed by treatment of cells with taxodone (0, 2, 4, 6, 10, μM), and the plate containing the treatment cells was incubated at 37 °C for seven days under a CO_2 atmosphere. Eventually, the cells were subjected to PBS washing and stained with 0.5 % crystal violet.

Scanning electron microscopy (SEM) studies observing conformational morphological changes, in brief, MCF-7 cells were grown on coated thin glass slides; after cell growth, all the slides were treated with different concentrations of taxodone (0, 2, 6, and 10 μM) followed by incubation of 24 h. After which, all treated and control slides were washed with PBS and fixed with 1 % glutaraldehyde, followed by dehydration using gradient ethanol. The glass slides were then dried in a desiccator, placed on the SEM stage, super-coated with gold, and visualized under SEM.

2.4. H_2DCHFDA staining

For the staying analysis of MCF-7 breast cancer cells, the cells were cultured in EMEM culture medium and enriched with a mixture of penicillin-streptomycin (1 %) and heat-inactivated fetal bovine serum (10 %) followed by incubation under 5 % CO_2 environment at 37 °C. For endogenous ROS generation, we treated the human MCF-7 breast cancer cells with varied concentrations of taxodone (0, 2, 6, 10 μM) for 24 h. After the treatment, cells were then utilized for the study of fluorescence analysis according to the protocol of Lee et al. [16]. In brief, after treatment with taxodone for 24 h, the cells were subjected to phosphate-buffered saline (PBS) washing and were ice-fixed on 4 % paraformaldehyde for 10 min. Further, the ice-fixed cells were subjected to re-wash with PBS followed by treatment with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) (20 μM) and further incubated at 37 °C for 30 min. The cells were then re-washed with PBS, and the cell fluorescence intensity was measured using a fluorescence microscope (Olympus BX50, Tokyo, Japan).

2.5. Caspase-3 activity

A colorimetric kit caspase-3 assay was used to determine the events of early apoptosis as per the instructions of the kit manual [17]. To this aim, a 96-well plate was used to seed the MCF-7 breast cancer cells (1×10^5 cells/mL), followed by CO_2 (5 %) incubation at 37 °C. Cells were treated with taxodone for 24 h while reaching the confluency of 70 %, as reported by Dey and Kang (2020) [17]. Treated cells were washed with 1× ice-cold PBS followed by lysis with cell lysis buffer and then centrifuged (16,000×*g* for 15 min at 4 °C) to get the cell lysate. Then, the cell lysate was mixed with reaction buffer (85 μL) and Caspase-3 substrate (Ac-DEVD-pNA (10 μL) as per the information provided in the kit manual (Sigma, MO, USA). Following the protocol of the kit manual, plates containing the reaction mixture were incubated at 37 °C overnight. After that, the absorbance of treated and untreated reaction mixtures was measured at 405 nm using an ELISA microplate reader (TECAN 200 infinite PRO).

2.6. Quantitative reveres transcription polymerase chain reaction (RT-PCR)

For quantitative polymerase chain reaction (PCR), total Ribonucleic acid (RNA) was isolated from the MCF-7 breast cancer cells using the Tri-Reagent as per the published paper [18]. Briefly, 2 Units of Deoxyribonuclease (DNAse) (Invitrogen, USA) were used to treat 2 μg of total RNA to initiate the RT-PCR reaction. A synthesis complementary DNA (cDNA) kit (Bio-Rad, USA) was employed to perform the RT-PCR following the instructions of the kit manual. The Go Taq qPCR Mastermix (Promega, USA) was used while performing the q-PCR using a Real-Time PCR thermocycler (Model: CFX96 - Bio-Rad, USA). A housekeeping gene, β-actin, was used to compare the fold-change mRNA expression levels. The $2^{-\Delta\Delta Ct}$ method was used to analyze the experimental data; the heat-map approach was used to visualize expression data. Table 1 provides information on the primers used in this study.

2.7. Apoptosis assay

To confirm the apoptosis activation upon taxodone treatment against breast cancer cells MCF-7, we carried out the apoptosis analysis as previously described by Dey et al. [19]. Briefly, after the denaturation process, a monoclonal antibody against single-stranded (ss) DNA was used to detect the denatured DNA using the Apo-Strand (Plymouth Meeting, PA, USA). Experiments were performed following the instructions in the kit manual and according to the standard protocol.

2.8. Statistical analysis

All the experiments in this study were performed to determine the quantitative data, and the expression of experimental data values was plotted as a mean ± standard deviation (SD). For calculating the significance of statistical analysis and group experimental differences, one-way analysis of variance (ANOVA) coupled with Tukey's test and Dunnett's post-comparison test (multiple groups) were employed ($p < 0.05$, $^{**}p < 0.01$, *** and $p < 0.001$).

3. Results

3.1. Taxodone exhibits potent anticancer activity against human breast cancer cells

We performed a preliminary evaluation of diterpenoid taxodone's anticancer role (Fig. 1A) in inhibiting the cell proliferation of this primary tumor, which has invasive breast ductal carcinoma characteristics. After the MCF-7 cells had reached 70 % confluence, we treated the MCF-7 breast cell line with taxodone at varying concentrations of (2–10 μM) for 24 h. To evaluate the inhibition of cell proliferation and cell death by the compound, we performed an MTT cell viability assay, which measures the cell metabolic activity and is the basis of evaluating live and dead cells [20]. As observed from the results shown in Fig. 1B, the cell viability of MCF-7 cells at 2, 4, 6, 8, and 10 μM of taxodone was found to be 95.08 ± 4.61, 84.5 ± 6.24, 73.46 ± 2.6, 64.78 ± 3.2, and 51.69 ± 2.24 %, respectively. Taxodone treatment for 24 h in MCF-7 cells indicated decreased cell viability, with an IC_{50} value of 6 μM (Fig. 1C). The IC_{50} of taxodone against the MCF-7 cell line was identified as ≈6 μM, indicating a potent dose-dependent inhibition of cell proliferation at a lower concentration than other anticancer compounds and similar to FDA-approved chemotherapeutic drugs [21]. Collectively, from the results obtained, we selected a taxodone concentration dose of 2, 6, and 10 μM for the rest of our study, which also showed us a direction that taxodone treatment has the potential to inhibit the growth of human breast cancer cells.

3.2. Treatment with taxodone elevates the production of intracellular generation of ROS

Natural compound-induced generation of intracellular ROS has been extensively studied and reported [22]. To illustrate and substantiate the mechanistic role of taxodone, we performed a commonly used H_2DCFDA staining technique to analyze the production of intracellular ROS in MCF-7 breast cancer cells. H_2DCFDA is a fluorogenic dye that measures ROS, hydroxyl, peroxyl, and other free radical activity within cells or tissue. Reports from researchers have shown an imbalance in the redox reaction arising from excessive amounts of intracellular ROS, which directly damages important biomolecules, DNA, RNA, proteins, and lipids [23]. Concisely, H_2DCFDA staining was used to treat MCF-7 cells after 24 h of taxodone treatment. The observation from our staining images revealed that cells had high expression green fluorescence, which was increased in a dose-dependent manner in taxodone-treated groups of 2, 6, and 10 μM in comparison to a control group that showed fewer green fluorescent intensity in MCF-7 breast cancer cells (Fig. 2A). We also quantified the green fluorescence intensity in MCF-7 cells, which was calculated through the images from ImageJ software. The percentage of ROS production for 2, 6, and 10 μM of taxodone was found to be 18.56 ± 2.41, 39.52 ± 1.64 and 56.46 ± 2.66 %, respectively (Fig. 2B). These results confirmed our assumption that taxodone is directly involved in the generation of ROS in MCF-7 cancer cells, a preceding event that introduced us to the next step of our molecular study in exploring the apoptotic pathway as it was inconsistent with the studies performed by other researchers for the induction of ROS mediated apoptosis [23].

Table 1
List of primers used in the study.

Gene name	Sequence
B-cell lymphoma 2 (***Bcl-2)***	Forward, 5′-CTCGTCGCTACCGTCGTGACTTGG-3′ Reverse, 5′-CAGATGCCGGTTCAGGTACTCAGTC-3′
BCL2 associated X ***(BAX)***	Forward, 5′-AAGCTGAGCGAGTGTCTCCGGCG-3′ Reverse, 5′-CAGATGCCGGTTCAGGTACTCAGTC-3′
Caspase-9	Forward, 5′-GGCCCTTCCTCGCTTCATCTC-3′ Reverse, 5′-GGTCCTTGGGCCTTCCTGGTAT-3′
Caspase-3	Forward, 5′-CAAACTTTTTCAGAGGGGATCG-3′ Reverse, 5′-GCATACTGTTTCAGCATGGCA-3′
β-actin	Forward, 5′-GAG CTA CGA GCT GCC TGA CG-3′ Reverse, 5′-GTA GTT TCG TGG ATG CCA CAG-3′

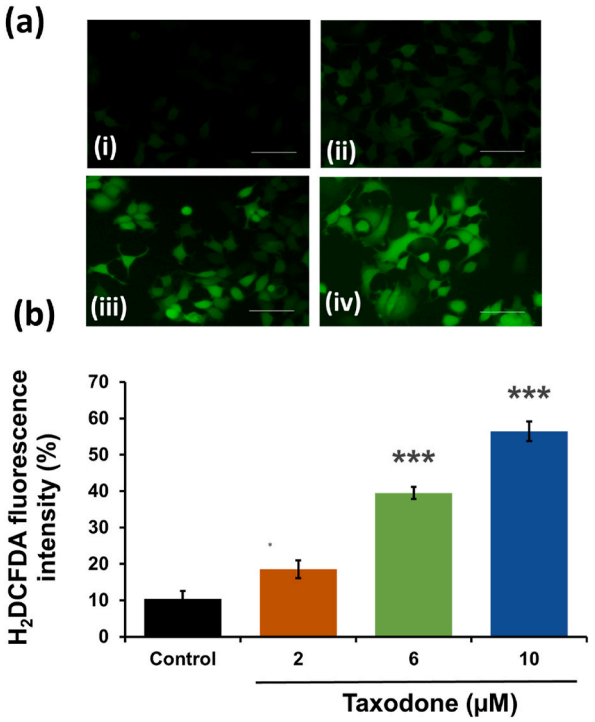

Fig. 2. The effect of taxodone treatment on ROS generation was estimated. (a) The representative fluorescent images indicating the effect of taxodone treatment against the human breast MCF-7 cancer cells (i): Control; (ii): treated with 2 μM; (iii): treated with 6 μM; (iv) treated with 10 μM taxodone. The scale bar of the images is 100 μm. (b) The fluorescent intensity was represented, and the significance was estimated when compared to control untreated group. The experiments were performed in triplicate and results are presented as mean ± SD values; the *p*-values are represented as follows: * <0.05, ** <0.01, *** <0.001.

3.3. Taxodone treatment vehemently activates caspase-dependent execution of MCF-7 breast cancer cells

As discussed in the previous section, taxodone treatment maneuvered the generation of ROS, which aggravated DNA damage in the cells, spurring necrotic and apoptotic events of either caspase-independent or caspase-dependent pathways to initiate the cell death machinery [24]. Therefore, we evaluated the activity of caspase-3, the chief executioner of apoptotic cell death, by transforming the intact cells into a remnant to ingest cells through phagocytosis. This process happens through the caspases known as

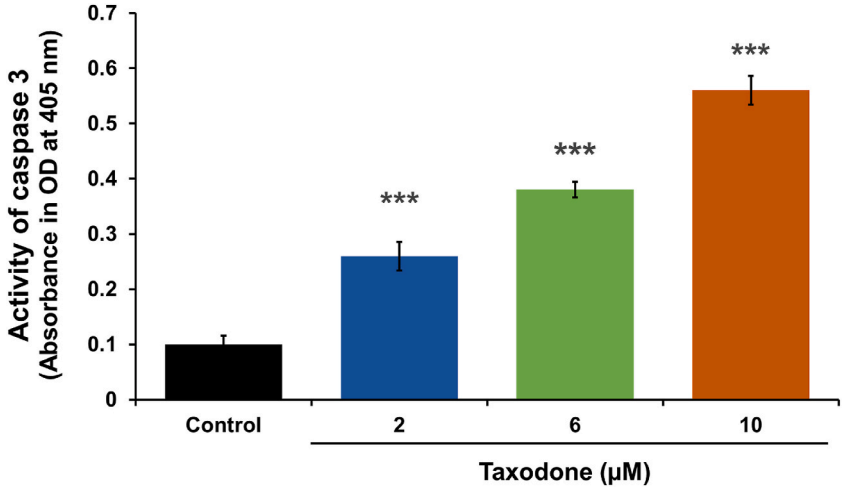

Fig. 3. Effect of taxodone treatment was estimated against caspase 3 activity. The representative graph indicates the statistical significance when compared to control untreated group. The experiments were performed in triplicate and results are presented as mean ± SD values; the *p*-values are represented as follows: * <0.05, ** <0.01, *** <0.001.

cysteine-dependent aspartate-directed proteases [25]. Accordingly, we evaluated the activity of caspase-3 through ELISA to determine whether treatment with taxodone could induce similar apoptotic events in MCF-7 cells by activating the caspases. The results depicted in Fig. 3 show MCF-7 cells with or without treatment of taxodone. As a result, we observed a significant induction and upregulation of caspase-3 activity in a dose-dependent manner at a dose of 2, 6, and 10 μM of taxodone. In contrast, the activity of caspase-3 in control cells was relatively lower compared to the drug treatment groups. This finding substantiated our claim to activate the caspase-mediated apoptotic pathway after 24 h of treatment with taxodone in human breast cancer cells.

3.4. Taxodone treatment triggers intrinsic apoptosis in MCF-7 human breast cancer cells

Since the activity of caspase-3 was found to be significantly upregulated in taxodone-treated MCF-7 cells, we confirmed the final execution of cell death by evaluating the activation of apoptosis. To this aim, we performed an ELISA assay for apoptosis detection using an ApoStrand™ ELISA apoptosis detection kit. This experiment identifies the specifically denatured DNA by formamide and the detection of denatured DNA with the use of a monoclonal antibody binding to the single-stranded DNA (ssDNA), specific to apoptotic cells and not for necrotic cells or cells undergoing DNA breakage in the absence of apoptosis [25,26]. The results depicted in Fig. 4 clearly showed apoptosis in MCF-7 cells treated with taxodone for 24 h. The percentage of apoptotic cells significantly increased with an increase in the concentration of taxodone in comparison to cells that were not treated with taxodone, which exhibited relatively lower apoptotic cells. In summary, from the obtained results, we confirmed the occurrence of taxodone-induced apoptosis in MCF-7 human breast cancer cells dose-dependently.

3.5. Taxodone treatment alters the mRNA level of apoptosis-associated genes in HCT-116 cells

After confirming apoptotic events to be highly upregulated in taxodone-treated MCF-7 cells, we further targeted to evaluate the expression of genes involved in regulating the intrinsic mediated apoptosis. To this aim, we treated MCF-7 cells with varying concentrations of taxodone (2, 6, 10 μM) for 24 h and extracted total RNA from the MCF-7 cells using the Tri-Reagent. Next, we carried out the experiment using quantitative PCR analysis. Our study showed differential expression in the mRNA level of the key genes involved in the intrinsic apoptosis pathway compared with the control cells not treated with taxodone. The relative gene expression was normalized using a housekeeping β-actin gene and using the specific primers of the target genes involved in the study.

The gene expression of Bcl-2 was higher in control groups than in taxodone-treated groups, which showed a relatively significant downregulation (Fig. 5A). Higher expression of the genes such as BAX, Caspase 3, and Caspase 9 was observed in all the concentrations of taxodone treatment groups when compared with the control groups, as shown in Fig. 5B, C, and D. These findings were consistent with the results of other research reports regarding intrinsic apoptotosis [27]. Similar results confirmed intrinsic cell death via scanning electron microscopy (SEM) analysis for deep morphological death and leakage (Fig. 6). The images were captured for MCF-7 cancer cells and used as a control for their interactions with different concentrations of taxodone, respectively, as illustrated in Fig. 6. The images obtained show that there was not much noticeable difference at the initial concentration of 2 μM. However, when the concentration of taxodone was increased to 6–10 μM, it significantly impacted the growth of cells, and this effect was dose-dependent. These results substantiated and confirmed the mechanistic role of taxodone in triggering the intrinsic apoptosis signaling pathway against human breast cancer cells and inhibiting cancer cell proliferation.

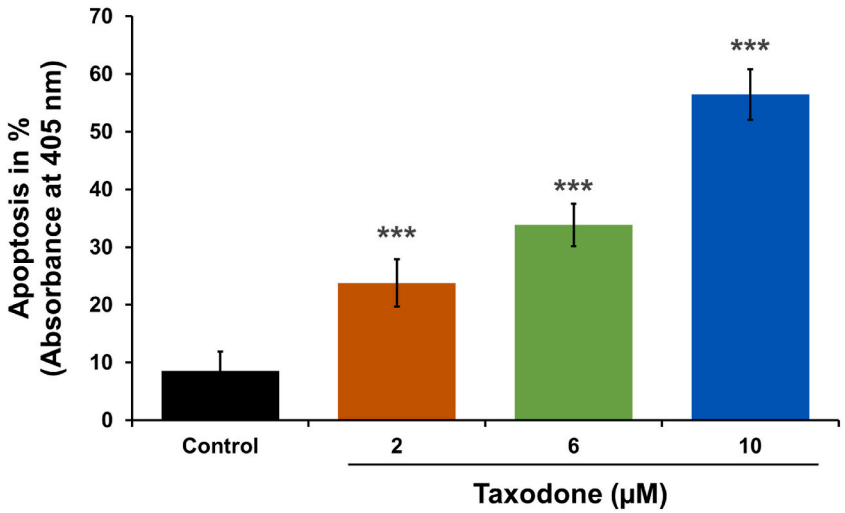

Fig. 4. Taxodone treatment activated apoptosis pathway. The representative graph indicates the statistical significance between the treated and untreated groups, indicating the increasing percentage of apoptotic cell population upon taxodone treatment in a dose-dependent manner. The experiments were performed in triplicate and results are presented as mean ± SD values; the *p*-values are represented as follows: * <0.05, ** <0.01, *** <0.001.

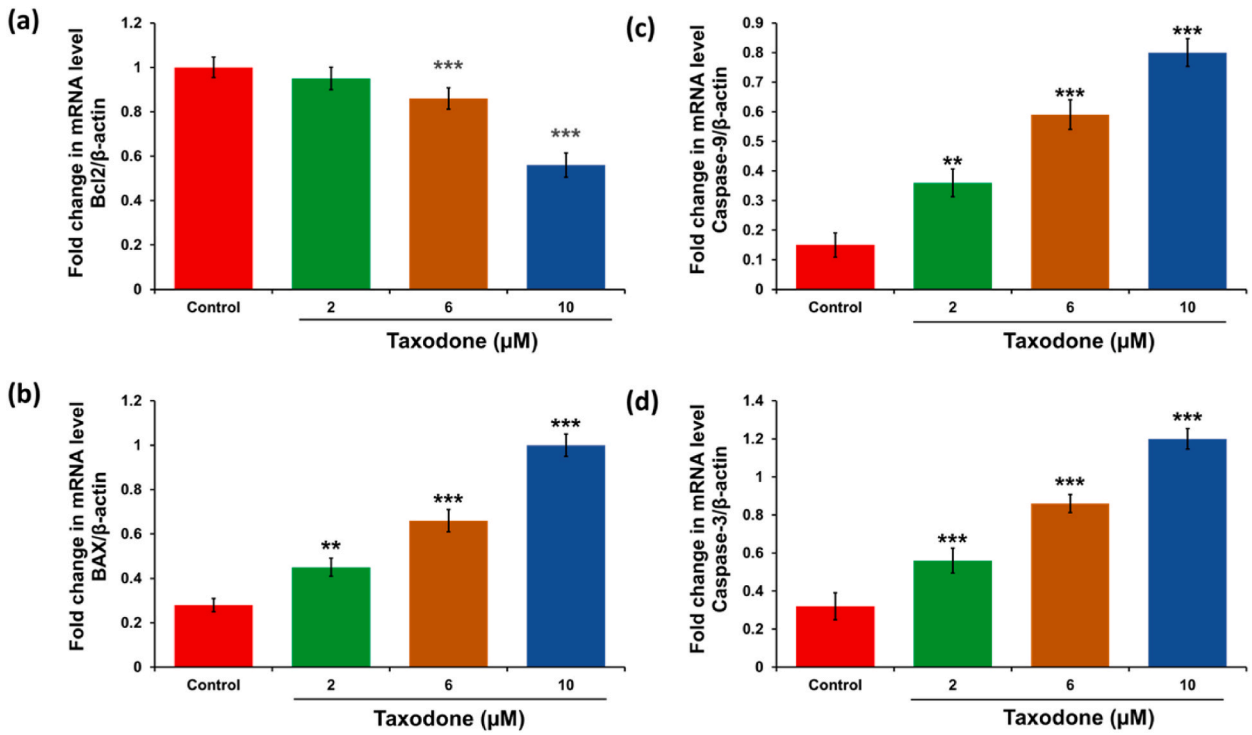

Fig. 5. The effect of taxodone treatment was analysis against the apoptosis signaling pathway. The representative graph indicates the statistical differences in terms of mRNA level between the treated and untreated groups against (a) Bcl2; (b) Caspase-9; (c) BAX; and (d) Caspase-3 gene. The experiments were performed in triplicate and results are presented as mean ± SD values; the *p*-values are represented as follows: * <0.05, ** <0.01, *** <0.001.

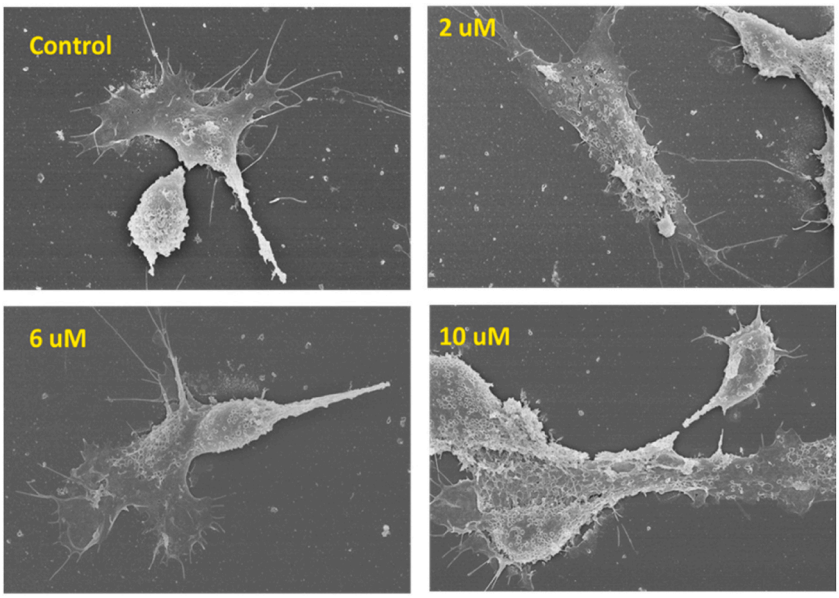

Fig. 6. Confirmation of breast cancer cell death via scanning electron microscopic analysis. Microscopic images obtained through scanning electron microscopy (SEM) from JEOL Inc. (Tokyo, Japan) show that taxodone causes damage to cells. At a low concentration of 2 μM, there is no visible change in cell morphology, but at concentrations of 6 and 10 μM, the cells are destroyed by forming apoptotic bodies.

4. Discussion

In this study, we have elucidated the intricate molecular mechanism underlying the apoptotic induction exerted by taxodone in breast cancer cells (MCF-7). Our findings demonstrate that the taxodone treatment effectively suppressed the growth of cancer cells and their colony-forming capability in a dose-dependent manner.

An *in-silico* study has confirmed that taxodone can bind at the isopentenyl diphosphate site of farnesyl diphosphate synthase (FDPS), which possibly inhibits its activity and could be an interesting target to explore its anticancer activity [28]. FDPS is a key enzyme that plays an important role in maintaining cancer stemness [29]. Additionally, taxodone treatment induced a significant generation of reactive oxygen species (ROS), stimulating the activation of caspase-mediated cell death pathways and controlling cellular proliferation and associated signaling pathways. Consistent with these observations, similar results have been reported in BCR-ABL-positive myelogenous leukemia cells [30]. Conversely, research has indicated the potential of taxodone to inhibit ROS levels in serum/glucose-deprived neuronal cells, consequently regulating apoptosis rates [31]. This effect is postulated to be attributable to the antioxidant properties of taxodone [32]. ROS is well-known to serve as a pivotal signaling bio-molecule implicated in diverse cellular activities, including cell proliferation, DNA damage, and apoptosis activation. The interplay of ROS levels often serves as a determinant factor governing the balance between cell survival and death [33]. Recent studies have revealed the significant modulation in ROS levels, potentially inducing DNA mutations and activating distinct pro-oncogenic signaling pathways, which further fosters cancer progression and angiogenesis [34–36]. Our results and a comprehensive literature review suggest that taxodone treatment modulates its activity based on cell line specificity and diverse genetic backgrounds. This selective and specific activity of taxodone makes it a unique and interesting candidate for studying its anti-cancerous activity in detail. Moreover, an abnormal accumulation of intercellular ROS levels in a cellular environment induces cytotoxic effects, making it an interesting target for stimulating various signaling pathways in cancer cells compared to normal healthy cells.

The data obtained from this study will help us better understand how taxodone regulates miRNAs, determines therapeutic targets, and thus potentially consider taxodone as a promising alternative anticancer compound for breast cancer treatment. Based on the results mentioned here in Fig. 5, the effect of taxodone treatment was analyzed against the apoptosis signaling pathway, supporting the fact that taxodone has been reported to increase the activation of proapoptotic proteins, such as caspase-9, caspase-3, and Bax, and inhibited Bcl-2 expression, suggesting that taxodone could induce apoptosis in MCF-7 cells via the caspase-dependent pathway. Similar findings have also been observed for cordycepin, a major compound of *Cordyceps sinensis* (Wang et al., 2016) [37].

These findings strongly follow a recent study in which a plant-derived secondary metabolite has been shown to induce cell cycle assay and consequent apoptosis in various cells [38]. Similarly, taxanes, such as paclitaxel and docetaxel, are widely prescribed chemotherapeutic drugs. They have been used to treat many forms of cancer, including breast, ovarian, and lung cancers [39,40]. In recent times, it has been noted that a few plant-originated bioactive compounds have limitations in clinical evaluations. Therefore, an approach of combined effects with usually utilized chemotherapeutics is knocking on the door to get synergistic outcomes with increased toxicity levels in cancer cells to the particular drug, thereby reducing the drug dose and toxicity of bioavailability concerns. Along these lines, several types of research have been carried out for these combinational effects evaluations [41]. For example, resveratrol has been used as an adjuvant of several existing chemotherapeutic drugs, such as temozolomide, doxorubicin, and paclitaxel on in-vitro assays and mice models [42]. A few other compounds commonly used as a chemosensitizing agent is curcumin. When curcumin is combined with docetaxel, a decrease in drug resistance is observed in breast cancer cells [43]. Similar results were also observed when combined with vincristine, paclitaxel, irinotecan, and cisplatin, enhancing the efficacy of these drugs, compared to a single treatment [44–47]. As a detailed survey, we have observed that Olga et al. (2013) also reported the cytotoxicity of diterpenoids containing an abietane skeleton, including taxodone and taxodione, which were found to be the most cytotoxic compounds for human leukemia cells16. Overexpression of the protective mitochondrial proteins Bcl-2 and Bcl-xL did not confer resistance to abietane diterpene-Taxodone-induced cytotoxicity. Similarly, the earlier findings of Kupchan et al. (1968) were also in the same coordination with the anticancer effects of texadone [48]. Furthermore, Ahmed and Emary also isolated four diterpenes, taxodone, taxodione, 11-11-hydroxymontbretol, and ferruginol, from *Taxodium distichum* (L.) rich seeds. Importantly, taxodone and taxodione have been reported to possess antitumor activity [49].

5. Conclusion

Studies from different researchers have shown the anticancer activity of taxodone through cell death pathways. However, cell death can be activated through various pathways, such as necrosis, intrinsic and extrinsic apoptosis, caspase-independent apoptosis, and autophagic cell death. Our findings from this study elucidate the role of taxodone in inducing the excessive generation of endogenous ROS at a relatively low concentration of taxodone (2–10 μM). The result obtained was the execution of human breast cancer cell death through ROS-mediated apoptosis. Taxodone significantly inhibited the proliferation of the cancer cells, elevated the oxidative stress, and avoided the neutralization of the ROS through the antioxidant enzymes in these cancer cells, subsequently triggering the caspase-mediated intrinsic apoptotic cell death of MCF-7 cells. In summary, our study paves the way for the role of taxodone as a potential anticancer drug development. Conclusively, our study provides new insights into the mechanical action of taxodone in human breast cancer cells. The major limiting point of this study might be in large-scale clinical trials and standardization procedures due to lesser hydrophilic nature and less bioavailability or absorption. However, there is a necessity for more studies to be conducted using taxodone before approving it for clinical trials by utilizing the strategies of nano-based formulations to improve solubility or chemical modification to reduce toxicity. Therefore, all limitations of this study could be coordinated with work of interdisciplinary areas like medicinal chemistry, pharmacology, biochemistry and biology to deeply understand the availability,

efficacy, safety, mechanism of action and synergistic effects of these new drugs.

Data availability

All relevant data are within the paper.

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Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

CRediT authorship contribution statement

Tahani Bakhsh: Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nouf M. Alyami:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. (2018), https://doi.org/10.3322/caac.21492.
[2] P.D. Subhedar, S.A. McLaughlin, Breast cancer in the young patient: review of therapy and treatment considerations, Breast Cancer Management (2020), https://doi.org/10.2217/bmt-2020-0008.
[3] E. Steiner, D. Klubert, D. Knutson, Assessing Breast Cancer Risk in Women, American Family Physician, 2008.
[4] J.D. Yager, N.E. Davidson, Estrogen carcinogenesis in breast cancer, N. Engl. J. Med. (2006), https://doi.org/10.1056/nejmra050776.
[5] F.R. Stoddard, A.D. Brooks, B.A. Eskin, G.J. Johannes, Iodine alters gene expression in the MCF7 breast cancer cell line: evidence for an anti-estrogen effect of iodine, Int. J. Med. Sci. (2008), https://doi.org/10.7150/ijms.5.189.
[6] S. Shariff-Marco, J. Yang, E.M. John, A.W. Kurian, I. Cheng, R. Leung, J. Koo, K.R. Monroe, B.E. Henderson, L. Bernstein, Y. Lu, M.L. Kwan, R. Sposto, C.L. P. Vigen, A.H. Wu, T.H.M. Keegan, S.L. Gomez, Intersection of race/ethnicity and socioeconomic status in mortality after breast cancer, J. Community Health (2015), https://doi.org/10.1007/s10900-015-0052-y.
[7] M. Escala-Garcia, A. Morra, S. Canisius, J. Chang-Claude, S. Kar, W. Zheng, S.E. Bojesen, D. Easton, P.D.P. Pharoah, M.K. Schmidt, Breast cancer risk factors and their effects on survival: a Mendelian randomisation study, BMC Med. (2020), https://doi.org/10.1186/s12916-020-01797-2.
[8] American Cancer Society: Breast Cancer Facts & Figures. In. Atlanta, Ga.: The Society: v.
[9] D. Verhoeven, C.S. Kaufman, S. Siesling, R. Mansel (Eds.), Breast Cancer: Global Quality Care, Oxford University Press, USA, 2019.
[10] Risk L, Age IB: FACTS & FIGURES.
[11] M. Colleoni, Z. Sun, K.N. Price, P. Karlsson, J.F. Forbes, B. Thürlimann, L. Gianni, M. Castiglione, R.D. Gelber, A.S. Coates, A. Goldhirsch, Annual hazard rates of recurrence for breast cancer during 24 years of follow-up: results from the international breast cancer study group trials I to V, J. Clin. Oncol. (2016), https://doi.org/10.1200/JCO.2015.62.3504.
[12] V.K. Bajpai, Y.H. Park, S.C. Kang, A diterpenoid taxodone from Metasequoia glyptostroboides with antimycotic potential against clinical isolates of Candida species, J. Mycol. Med. (2015), https://doi.org/10.1016/j.mycmed.2014.10.017.
[13] N. Kusumoto, T. Ashitani, T. Murayama, K. Ogiyama, K. Takahashi, Antifungal abietane-type diterpenes from the cones of Taxodium distichum rich, J. Chem. Ecol. (2010), https://doi.org/10.1007/s10886-010-9875-2.
[14] S. Morris Kupchan, A. Karim, C. Marcks, Tumor inhibitors. XLVIII. Taxodione and taxodone, two novel diterpenoid quinone methide tumor inhibitors from Taxodium distichum, J. Org. Chem. (1969), https://doi.org/10.1021/jo01264a036.
[15] V.K. Bajpai, S.C. Kang, Antibacterial abietane-type diterpenoid, taxodone from 22 Metasequoia glyptostroboides Miki ex Hu, J Biosci 35 (2010) 533–538.
[16] O. Burmistrova, M.F. Simões, P. Rijo, J. Quintana, J. Bermejo, F. Estévez, Antiproliferative activity of abietane diterpenoids against human tumor cells, J. Nat. Prod. 76 (8) (2013) 1413–1423, https://doi.org/10.1021/np400172k.
[17] D.K. Dey, S.C. Kang, CopA3 peptide induces permanent cell-cycle arrest in colorectal cancer cells, Mech. Ageing Dev. 196 (2021) 111497, https://doi.org/10.1016/j.mad.2021.111497.
[18] H. Lee, C. Oh, S. Kim, D.K. Dey, H.K. Kim, V.K. Bajpai, Y.K. Han, Y.S. Huh, Metasequoia glyptostroboides potentiates anticancer effect against cervical cancer via intrinsic apoptosis pathway, Sci. Rep. 11 (2021) e894, https://doi.org/10.1038/s41598-020-79573-8.

[19] D.K. Dey, S.C. Kang, Aflatoxin B1 induces reactive oxygen species-dependent caspase-mediated apoptosis in normal human cells, inhibits Allium cepa root cell division, and triggers inflammatory response in zebrafish larvae, Sci. Total Environ. 737 (2020) 139704, https://doi.org/10.1016/j.scitotenv.2020.139704.
[20] S.N. Chang, D.K. Dey, S.T. Oh, W.H. Kong, K.H. Cho, E.M. Al-Olayan, B.S. Hwang, S.C. Kang, J.G. Park, Phorbol 12-myristate 13-acetate induced toxicity study and the role of tangeretin in abrogating hif-1α-nf-κb crosstalk in vitro and in vivo, Int. J. Mol. Sci. (2020), https://doi.org/10.3390/ijms21239261.
[21] D.K. Dey, S.N. Chang, S.C. Kang, The inflammation response and risk associated with aflatoxin B1 contamination was minimized by insect peptide CopA3 treatment and act towards the beneficial health outcomes, Environ Pollut 268 (2021) e115713.
[22] M.V. Berridge, A.S. Tan, Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT): subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction, Arch. Biochem. Biophys. (1993), https://doi.org/10.1006/abbi.1993.1311.
[23] J.O. Suberu, I. Romero-Canelón, N. Sullivan, A.A. Lapkin, G.C. Barker, Comparative cytotoxicity of artemisinin and cisplatin and their interactions with chlorogenic acids in MCF7 breast cancer cells, ChemMedChem (2014), https://doi.org/10.1002/cmdc.201402285.
[24] S.N. Chang, I. Khan, C.G. Kim, S.M. Park, D.K. Choi, H. Lee, B.S. Hwang, S.C. Kang, J.G. Park, Decursinol angelate arrest melanoma cell proliferation by initiating cell death and tumor shrinkage via induction of apoptosis, Int. J. Mol. Sci. (2021), https://doi.org/10.3390/ijms22084096.
[25] D.K. Dey, S.N. Chang, Y. Vadlamudi, J.G. Park, S.C. Kang, Synergistic therapy with tangeretin and 5-fluorouracil accelerates the ROS/JNK mediated apoptotic pathway in human colorectal cancer cell, Food Chem. Toxicol. (2020), https://doi.org/10.1016/j.fct.2020.111529.
[26] S.N. Chang, S.H. Kim, D.K. Dey, S.M. Park, O. Nasif, V.K. Bajpai, S.C. Kang, J.T. Lee, J.G. Park, 5-o-Demethylnobiletin alleviates ccl4-induced acute liver injury by equilibrating ros-mediated apoptosis and autophagy induction, Int. J. Mol. Sci. (2021), https://doi.org/10.3390/ijms22031083.
[27] C.E. Johnson, S. Kornbluth, Caspase cleavage is not for everyone, Cell (2008), https://doi.org/10.1016/j.cell.2008.08.019.
[28] O.S. Frankfurt, A. Krishan, Identification of apoptotic cells by formamide-induced DNA denaturation in condensed chromatin, J. Histochem. Cytochem. (2001), https://doi.org/10.1177/002215540104900311.
[29] S. Márquez-Jurado, J. Díaz-Colunga, R.P. Das Neves, A. Martinez-Lorente, F. Almazán, R. Guantes, F.J. Iborra, Mitochondrial levels determine variability in cell death by modulating apoptotic gene expression, Nat. Commun. (2018), https://doi.org/10.1038/s41467-017-02787-4.
[30] Y.L. Liu, S. Lindert, W. Zhu, K. Wang, J.A. McCammon, E. Oldfield, Taxodione and arenarone inhibit farnesyl diphosphate synthase by binding to the isopentenyl diphosphate site, Proc Natl Acad Sci U S A (2014), https://doi.org/10.1073/pnas.1409061111.
[31] H.Y. Kim, D.K. Kim, S.H. Bae, H. Gwak, J.H. Jeon, J.K. Kim, B.I. Lee, H.J. You, D.H. Shin, Y.H. Kim, S.Y. Kim, S.S. Han, J.K. Shim, J.H. Lee, S.G. Kang, H. Jang, Farnesyl diphosphate synthase is important for the maintenance of glioblastoma stemness, Exp. Mol. Med. (2018), https://doi.org/10.1038/s12276-018-0166-2.
[32] Y. Uchihara, K. Tago, H. Taguchi, Y. Narukawa, F. Kiuchi, H. Tamura, M. Funakoshi-Tago, Taxodione induces apoptosis in BCR-ABL-positive cells through ROS generation, Biochem. Pharmacol. (2018), https://doi.org/10.1016/j.bcp.2018.05.018.
[33] N. Shafaei-Bajestani, S.A. Emami, J. Asili, Z. Tayarani-Najaran, Anti-apoptotic effect of taxodione on serum/glucose deprivation-induced PC12 cells death, Cell. Mol. Neurobiol. (2014), https://doi.org/10.1007/s10571-014-0085-2.
[34] U. Kolak, A. Kabouche, M. Oztürk, Z. Kabouche, G. Topçu, A. Ulubelen, Antioxidant diterpenoids from the roots of *Salvia barrelieri*, Phytochem. Anal. (2009), https://doi.org/10.1002/pca.1130.
[35] G.E. Villalpando-Rodriguez, S.B. Gibson, Reactive oxygen species (ROS) regulate different types of cell death by acting as a Rheostat, Oxid. Med. Cell. Longev. (2021), https://doi.org/10.1155/2021/9912436.
[36] A. Sakai, M. Nakanishi, K. Yoshiyama, H. Maki, Impact of reactive oxygen species on spontaneous mutagenesis in *Escherichia coli*, Gene Cell. (2006), https://doi.org/10.1111/j.1365-2443.2006.00982.x.
[37] D. Wang, Y. Zhang, J. Lu, Y. Wang, J. Wang, Q. Meng, R.J. Lee, D. Wang, L. Teng, Cordycepin, a natural antineoplastic agent, induces apoptosis of breast cancer cells via caspase-dependent pathways, Nat. Prod. Commun. 11 (1) (2016 Jan) 63–68.
[38] S. Kumari, A.K. Badana, G. MM, G. S, R. Malla, Reactive oxygen species: a key constituent in cancer survival, Biomark. Insights (2018), https://doi.org/10.1177/1177271918755391.
[39] D.K. Dey, C. Sharma, Y. Vadlamudi, S.C. Kang, CopA3 peptide inhibits MDM2-p53 complex stability in colorectal cancers and activates p53 mediated cell death machinery, Life Sci. (2023), https://doi.org/10.1016/j.lfs.2023.12147.
[40] Connied Oshiro, Sharona Marsh, Howardb McLeod, Michelle Whirlc Carrillo, Terid Klein, Russd e. Altman, Taxane pathway, Pharmacogenetics Genom. 19 (12) (December 2009) 979–983, https://doi.org/10.1097/FPC.0b013e3283335277.
[41] M.P. Shanthi, et al., In vitro anticancer activity of Biophytum sensitivum whole plant extracts against cervical and liver cancer cell lines, Int. J. Pharm. Sci. Res. 7 (2016) 5128–5135 [Google Scholar].
[42] U. Amna, Evaluation of cytotoxic activity from Temurui (Murraya koenigii [Linn.] Spreng) leaf extracts against HeLa cell line using MTT assay, J. Adv. Pharm. Technol. Res. (2019), https://doi.org/10.4103/japtr.JAPTR.
[43] R. Lagoa, J. Silva, J. Rui, A. Bishayee, Advances in phytochemical delivery systems for improved anticancer activity, Biotechnol. Adv. 38 (2020) 107382.
[44] G. Rai, S. Mishra, S. Suman, Y. Shukla, Resveratrol improves the anticancer effects of doxorubicin in vitro and in vivo models: a mechanistic insight, Phytomedicine 23 (2016) 233–242.
[45] T.N. Aung, Z. Qu, R.D. Kortschak, D.L. Adelson, Understanding the effectiveness of natural compound mixtures in cancer through their molecular mode of action, Int. J. Mol. Sci. 18 (2017) 656.
[46] G.S. Sen, S. Mohanty, D.M.S. Hossain, S. Bhattacharyya, S. Banerjee, J. Chakraborty, S. Saha, P. Ray, P. Bhattacharjee, D. Mandal, et al., Curcumin enhances the efficacy of chemotherapy by tailoring p65NFκB-p300 cross-talk in favor of p53-p300 in breast cancer, J. Biol. Chem. 286 (2011) 42232–42247, https://doi.org/10.1074/jbc.M111.262295.
[47] P. Su, Y. Yang, G. Wang, X. Chen, Y. Ju, Curcumin attenuates resistance to irinotecan via induction of apoptosis of cancer stem cells in chemoresistant colon cancer cells, Int. J. Oncol. 53 (2018) 1343–1353.
[48] S.M. Kupchan, A. Karim, C. Marcks, Taxodione and taxodone, two novel diterpenoid quinone methide tumor inhibitors from Taxodium distichum, J. Am. Chem. Soc. 90 (21) (1968 Oct 9) 5923–5924, https://doi.org/10.1021/ja01023a061. PMID: 5679178.
[49] A. Ahmed, N. Ei-Emary, Biologically active abietane diterpenes from Taxodium distichum seeds, Bulletin of Pharmaceutical Sciences Assiut University 22 (1) (1999) 21–25, https://doi.org/10.21608/bfsa.1999.65968.