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Unlocking synergistic potential: enhancing paclitaxel efficacy in combination with silibinin in breast cancer cell line through H19 LncRNA and P53/Bax/Bcl2 axis

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Abstract

Breast cancer is a major global health concern and the second most frequently diagnosed malignancy in women. Paclitaxel, a key chemotherapeutic agent, shows strong antineoplastic activity but is limited by toxicity and resistance. While combining paclitaxel with natural compounds is promising, the role of H19 lncRNA in mediating paclitaxel-silibinin synergy is not yet known. This study evaluates their combined efficacy via modulation of H19 lncRNA and the P53/Bax/Bcl2 apoptotic pathway. We investigated the chemosensitizing effect of silibinin, a flavonoid, in combination with paclitaxel in breast cancer cells. Drug interactions were analyzed using the Chou-Talalay combination index and cytotoxicity assessed by MTT assays. Apoptosis was measured through caspase-3/7 activity, and transcriptional changes in H19 lncRNA, P53, Bax, and Bcl-2 were quantified by real-time PCR. Silibinin and paclitaxel exhibited a synergistic effect, reducing paclitaxel IC50 and increasing cytotoxicity. Co-treatment enhanced caspase-3/7 activation, upregulated pro-apoptotic P53 and Bax, and downregulated anti-apoptotic Bcl-2 and oncogenic H19 lncRNA. Silibinin alone caused a threefold reduction in H19 expression, whereas paclitaxel alone had minimal effect. Silibinin potentiates paclitaxel cytotoxicity by suppressing H19 transcription, offering a potential strategy to overcome paclitaxel resistance. The combination promotes apoptosis via caspase activation, highlighting a novel synergistic therapeutic approach in breast cancer treatment.

Keywords: apoptosis, breast cancer, combination therapy, lncRNA H19, P53, paclitaxel, silibinin

Introduction

Breast cancer continues to be one of the most serious health issues affecting women today. In 2020, 2.3 million new breast cancer diagnoses were recorded and 685 000 breast-cancer-related deaths happened[1]. Based on these statistics, despite years of fruitful research, more research is still needed to better understand the disease and develop more effective treatments[2].

HIGHLIGHTS

- Silibinin potentiates paclitaxel efficacy in breast cancer cells.
- Combination treatment of silibinin and paclitaxel reduces IC50 and enhances apoptotic cell death.
- Co-treatment of silibinin and paclitaxel regulates apoptotic genes: ↑P53, ↑Bax, ↓Bcl-2.
- Silibinin downregulates H19, an oncogenic lncRNA linked to resistance.
- H19 suppression underlies the synergistic pro-apoptotic effect of silibinin.

Cancer treatment is often a combination of surgery, radiation therapy, chemotherapy, and targeted therapy[3,4]. Among chemotherapy agents, paclitaxel has become one of the common treatment choices for breast, ovarian, and lung cancer. It belongs to taxanes family and is a microtubule stabilizer that disrupts cancer cell division, which leads to cell apoptosis[5]. However, its effectiveness is limited by two major issues: significant side effects at therapeutic doses and the frequent development of drug resistance in cancer cells[6]. Various methods have been used to address these issues, one being the use of combination therapy with other chemotherapy agents like famitinib, camrelizumab, doxorubicin, trastuzumab, vinorelbine, and afatinib. This method, despite effective in eradicating cancer cells, causes more significant adverse effects and requires close monitoring

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the patients[7,8]. Administration of dexamethasone, diphenhydramine, and H2antagonists prior to paclitaxel treatment is another clinically used method to counter paclitaxel issues. Compared to combination therapy, this method is effective in reducing side effects; however, it is associated with reduced effectiveness of paclitaxel and higher risk of metastasis[9].

This has driven researchers to investigate combination therapies that could enhance paclitaxel's anticancer effects while reducing its adverse effects, and when it comes to reduced side effects, herbal ingredients are usually the first choice of researchers. Based on previous studies, herbal ingredients can affect different pathways, reduce the toxic side effects of chemotherapy, and inhibit the efflux of anticancer drugs while having anticancer effects through inducing apoptosis and autophagy[10,11]. For instance, a study investigated the impact of combination therapy of silibinin with paclitaxel and cisplatin in MCF-7 breast cancer cells, demonstrating enhanced early apoptosis and increased anti-proliferative effects of the single agents[11]. Combined with other agents, they can improve efficacy and introduce these compounds as valuable candidates for clinical research[12]. These findings highlight the multifaceted ways in which herbal compounds can enhance the efficacy of chemotherapy in cancer treatment.

Silibinin, an active component extracted from *Silybum marianum L.* (milk thistle), has shown promising anticancer and antiangiogenic and antimetastatic effects in previous studies[13–15]. Several studies, including *in vitro, in vivo*, and clinical trials, stated that combination therapy with silibinin mitigates different types paclitaxel-induced toxicities while acting as a chemosensitizer and inducing apoptosis through p53/Bax/Bcl2[16,17]. Previous studies have reported that silibinin exhibits minimal cytotoxicity toward normal breast epithelial cells, suggesting a favorable safety profile[18].

When talking about chemoresistance, long non-coding RNAs are pivotal factors that are dysregulated in cancer, especially in those with poor prognosis[19]. It has been shown that paclitaxel resistance is related to genes such as p53, Bax, and Bcl2 and interestingly, H19 has been associated with tumorigenesis and drug resistance through various mechanisms, including its interactions with regulatory proteins such as p53 and its effects on apoptotic pathways[20–23]. Naturally, H19 is a lncRNA that is mainly produced in cardiac and skeletal muscles after birth but is highly expressed in nearly 70% of breast cancer patients. Studies showed that H19 may influence p53 through miR-675 and thus effecting Bax and Bcl2 consequently[24,25].

Recent research has explored the potential of herbal compounds in cancer therapy, demonstrating their ability to enhance tumor cell sensitization to the action of chemotherapy, hormonal therapy, and gene therapy through different mechanisms[26]. Moreover, phytochemicals such as resveratrol, curcumin, genistein, quercetin, and others have been shown to modulate the expression of lncRNAs, in different types of cancers[27]. While silibinin demonstrates anticancer activity through multiple pathways, its ability to synergize with paclitaxel via H19 lncRNA suppression and subsequent P53/Bax/Bcl2 pathway activation has not been investigated. This study bridges this gap by evaluating: (1) the synergistic cytotoxicity of silibinin-paclitaxel cotreatment, (2) H19 lncRNA's role in mediating this synergy, and (3) mechanistic links between H19 suppression and apoptotic rebalancing

Material and method

Cell culture and toxicity assay

MCF-7 cells were chosen due to their intact p53 signaling and previously reported involvement of H19 lncRNA in apoptosis and chemoresistance, making them a suitable model for mechanistic investigation. The MCF-7 human breast cancer cell line was obtained from the Pasteur Institute of Iran. The cells were cultured in T25 cell culture flasks using DMEM-high glucose medium supplemented with 10% FBS, 100 mg/ml of streptomycin, and 100 U/ml of penicillin. The culture flasks were kept in a humidified incubator at 37 °C with 5% CO_2.

To assess the cytotoxic effect of paclitaxel and silibinin on the MCF-7 cells, the standard protocol of the MTT assay was performed. In this assay, 5×10^3 cells were seeded in each well of 96-well plates and incubated for 24 hours. The old media was then discarded, and 200 µl of fresh medium containing different concentrations of paclitaxel (ranging from 1.25 to 80 µM) and silibinin (ranging from 10 to 640 µM) was added to the wells. The plates were further incubated for 48 hours in a humidified incubator. After the incubation period, 10 µl of MTT solution (5 mg/ml) was added to each well and incubated for an additional 4 hours. The medium was then replaced with 200 µl of DMSO and gently shaken for 20 minutes. The absorbance of each well was measured at 570 nm using a 3200 StatFax microplate reader from the USA.

Drug–drug interaction and combination index calculation

The interaction between paclitaxel and silibinin was assessed using the Chou and Talalay method[28]. MCF-7 cells were exposed to varying concentrations of paclitaxel in combination with silibinin at a fixed ratio concentration (1:25), which was determined based on the IC50 values of each compound. The concentration ranges tested were as follows: paclitaxel: 0.24–3.92 µM; silibinin: 12.25–196.07 µM. After 48 hours, the inhibition of cell growth was evaluated using the MTT assay. The combination index (CI) was calculated using CompuSyn software version 1.0 (ComboSyn, Paramus, NJ, USA) to determine the nature of the interaction. The fraction affected (Fa) value was defined as the percentage of inhibition divided by 100, and CI was computed for each Fa value. CI > 1 shows antagonism, CI = 1 is a sign of additive effects, and CI < 1 counts as synergism. Additionally, the dose reduction index (DRI) was calculated to compare the individual drug doses with the reduced dose when used in combination, also utilizing the same software.

Evaluation of caspase 3,7 activation through colorimetric assay

The MCF-7 cell lysate was prepared by incubating the cells with silibinin, paclitaxel, and their combination for 48 hours, following the instructions provided in the Kiazist Kit manual (Hamadan, Iran). To perform the assay, 55.5 µL of a solution containing caspase buffer, DTT, and caspase substrate, along with 50 µL of the cell lysate, were transferred to a 96-well plate. The plate was then incubated at 37 °C for 1.5 hours. The absorption at 405 nm was measured using an ELISA plate reader. It is worth mentioning that the activity of caspase 3,7 was determined based on a standard curve using p-nitroaniline (pNA) as the standard. The results were reported as mU/mg protein after normalizing them using a Bradford assay.

Table 1

Primer sequences

Gene name	Primer sequence	Product length
H19	Forward: 5'-ATCGGTGCCTCAGCGTTCGG-3' Reverse: 5'- CTGTCCTCGCCGTCACACCG-3'	141
P53	Forward: 5'-AGGCCTTGGAACTCAAGGAT-3' Reverse: 5'-TGAGTCAGGCCCTTCTGTCT-3'	140
BAX	Forward: 5'-CGTGGTTGCCCTCTTCTACTTT-3' Reverse: 5'-GATCAGCTCGGGCACTTTAGTG-3'	73
BCL2	Forward: 5'-GTCATCCACAGAGAGCGATGTT-3' Reverse: 5'-GATGACTTCTCTCGTCGCTA-3'	229
β -actin	Forward: 5'-TCCCTGGAGAAGAGCTACG-3' Reverse: 5'-GTAGTTTCGTGGATGCCACA-3'	131

RNA isolation and quantitative reverse transcription-PCR (qRT-PCR)

The control and treated MCF-7 cells were subjected to RNA extraction using the BehGene extraction kit, following the manufacturer's instructions. The concentration of RNA was determined by measuring the absorbance at 260/280 nm using a NanoDrop UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Subsequently, the RNA samples were converted to cDNA using the ParsToos^{TM} RT Series cDNA synthesis kit, and quantitative real-time PCR (qRT-PCR) was performed on a LightCycler® 96 instrument (Roche, Germany).

For qRT-PCR, SYBR Green (ParsToos, Iran) was utilized along with specific primers for H19, P53, Bax, Bcl-2, and β-actin, as listed in Table 1. The relative expression level was determined using the $2^{-\Delta\Delta Ct}$ method, where the expression of the target gene was normalized to the expression level of β-actin, serving as an internal control.

Statistical analysis

Statistical significance between groups was assessed using version 9.5.1 of the GraphPad Prism software, which conducted the Shapiro–Wilk test to evaluate normality. Subsequently, the one-way analysis of variance was performed, followed by the Tukey post hoc test. A significance level of $P < 0.05$ was employed to determine statistical significance, while the mean and standard deviation (SD) were utilized to present the quantitative data. All experiments were performed in triplicate and repeated at least three independent times.

Declaration of artificial intelligence use (TITAN 2025 compliance statement)

In the preparation of this manuscript, ChatGPT (OpenAI, GPT-5 model) was used solely for language refinement and grammar correction purposes after the scientific content had been fully developed by the research team.

All stages of study design, experimental procedures, data analysis, interpretation of results, and preparation of the initial draft were entirely conducted by the authors without the use of AI tools. The outputs from ChatGPT were carefully reviewed, edited, and verified by all authors to ensure scientific accuracy, integrity, and originality.

The authors affirm that no AI tool was involved in generating, analyzing, or interpreting scientific data, nor in the conceptual development of the manuscript. The final version has been thoroughly reviewed and approved by all co-authors, who take full responsibility for the content.

This declaration follows the TITAN Guidelines 2025 on the transparent reporting and use of artificial intelligence in scientific writing[29].

Results

Anti-proliferative activity and drug–drug interaction of silibinin and paclitaxel

The efficacy of silibinin and/or paclitaxel-treated MCF-7 cells was evaluated using the MTT method to determine if there was a synergistic impact of silibinin and paclitaxel. Initially, MCF-7 cells were exposed to varying concentrations of silibinin (ranging from 18.75 to 600 μM) or paclitaxel (ranging from 1.25 to 80 μM) for 48 hours. This time period was carefully chosen based on previous studies that found phytochemicals like silibinin have are most effective after 48 hours (30–32). The inhibitory effect of silibinin and paclitaxel on breast cancer cells decreased significantly in a dose-dependent manner. The IC50 values for silibinin and paclitaxel were 123.40 μM and 12.88 μM, respectively, after 48 hours of treatment with each agent (Fig. 1A–C). Subsequently, the impact of combining silibinin and paclitaxel for 48 hours on the viability of MCF-7 cells was assessed through MTT assays. The CI for various inhibition fractions (Fa) of MCF-7 cell growth was calculated using the Chou-Talalay method and Compusyn 1.0 software. CI values ranged from 0.21 to 0.41 across different fractional effect levels, confirming strong synergism. The CI results and isobologram diagrams were presented as a function of the effect level (Fig. 1D–F). The findings confirmed that silibinin synergistically increased the cytotoxicity of paclitaxel against MCF-7 breast cancer cells. The most potent synergy between the two agents (0.21) was observed at 0.86 fractional inhibition of MCF-7 growth, while the DRI values suggested that the effective paclitaxel dose in synergistic combination with silibinin could be significantly reduced (Table 2).

Combination of silibinin and paclitaxel on caspase 3,7 activation

Caspase-3,7 activity was influenced by silibinin and paclitaxel and their co-treatment. Caspase-3,7 activity was visibly higher in co-treatment groups compared to control cells ($P < 0.01$). Caspase-3,7 activity in MCF-7 cells was stimulated by paclitaxel in the IC50 group significantly more than cells treated with silibinin in the IC50 group ($P < 0.01$). This activity was boosted in the 29 μM concentration of drug combination group treated cells ($P < 0.001$) and in the 60 μM group ($P < 0.01$). All together, these data show that paclitaxel is more effective in caspase-3,7

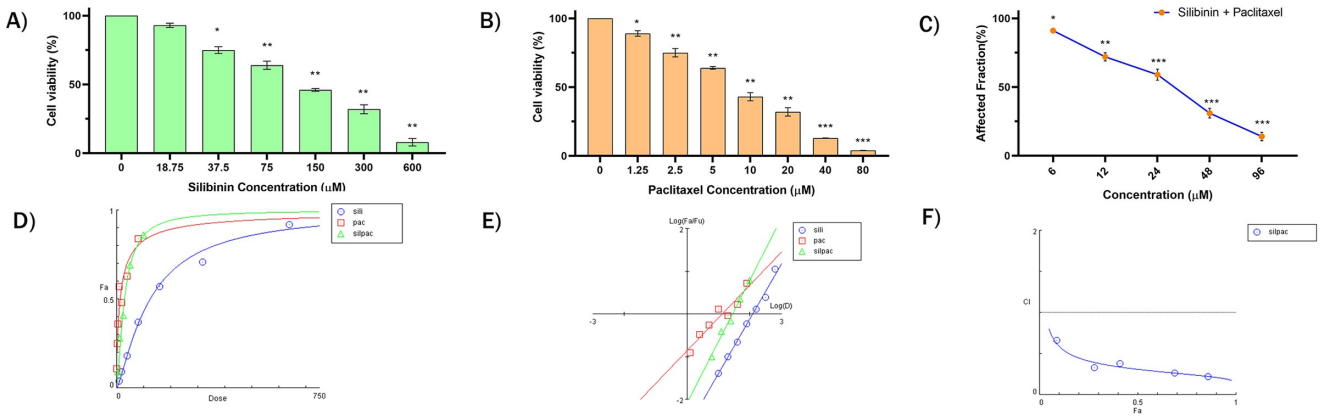

Figure 1. Concentration–response effects of silibinin and paclitaxel on MCF-7 cells using (A, B) MTT assay and (C) MTT assay following 48-hours treatment with both agents in combination. (D–F) Analysis of synergy between silibinin and paclitaxel combination after 48 hours post-treatment against MCF-7 cells represent dose effect plot, median-effect plot, and combination index plot, respectively (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control).

activation than silibinin alone, but the combination of two agents effectively synergizes this effect.

Effect of silibinin and paclitaxel on H19, P53, Bcl-2, and Bax expression in MCF-7 cells

MCF-7 cells were treated with silibinin and paclitaxel alone and in combination, and the results showed that the expression of H19 was significantly decreased by ~ 3-fold in the silibinin-treated group ($P < 0.001$). Co-treatment with 60 μM concentration showed ~ 2-fold H19 expression reduction ($P < 0.01$) (Fig. 2D–F). Furthermore, P53 and Bax were significantly upregulated, while Bcl-2 was significantly downregulated in a dose-dependent manner in silibinin and co-treatment groups.

Discussion

Breast cancer remains the most prevalent cancer among women globally and is the leading cause of cancer-related deaths in this population[30]. Despite significant advancements in its management, chemotherapy continues to be a cornerstone of treatment, with paclitaxel playing a critical role as a first-line agent[31]. For instance, a 2022 study evaluated a novel combination therapy involving famitinib, camrelizumab, and nab-paclitaxel for advanced immunomodulatory triple-negative breast cancer, demonstrating promising antitumor activity[32]. Another investigation highlighted the efficacy of combining paclitaxel with trastuzumab in stage I HER2-positive breast cancer, showcasing its potential in targeted treatment approaches[7]. Furthermore, paclitaxel has been paired with agents like vinorelbine and afatinib in HER2-positive breast cancer patients, particularly those who have progressed after trastuzumab and/or lapatinib therapy[33]. These studies collectively emphasize the ongoing evolution of paclitaxel-based combination regimens aimed at improving therapeutic outcomes.

Despite its clinical benefits, paclitaxel is associated with significant side effects and the development of drug resistance, especially in patients receiving prolonged treatment courses[34]. In response to these challenges, our study explored the use of medicinal plant-derived compounds to enhance the efficacy of paclitaxel while minimizing its required dose. Silibinin was chosen for this investigation due to two primary reasons. First, our earlier work demonstrated that silibinin, when used in combination with vinblastine, effectively induces apoptosis in cancer cells, underscoring its potential in managing TNBC-related pathologies[35]. Second, silibinin is known to inhibit CYP3A4 and P-glycoprotein, two key factors involved in drug metabolism and efflux, thereby potentially increasing intracellular paclitaxel accumulation[36]. Supporting this, previous studies by Pashaei-Asl *et al* and Zhou *et al* confirmed the effectiveness of silibininpaclitaxel combinations in ovarian cancer cells[37,38]. Additionally, Ho *et al* showed that silibinin with paclitaxel reduces proliferation and migration in 4T1 breast cancer cells[39]. Previous studies have reported that silibinin is safe in normal cells; however, direct assessment in normal breast epithelial cells was not performed in the current study and

Table 2

Dose–response association of paclitaxel and silibinin alone or in combination

			CI values			DRI		
Compound	**IC50(μM)**	**r**	**Fa 0.25**	**Fa 0.5**	**Fa 0.75**	**Fa 0.25**	**Fa 0.5**	**Fa 0.75**
Paclitaxel	12.88	0.95				6.02	11.65	22.54
Silibinin	123.40	0.99				4.08	4.46	4.88
Sili + Pac	28.74	0.99	0.41	0.030	0.24			

DRI, dose reduction index; r, correlation coefficient.

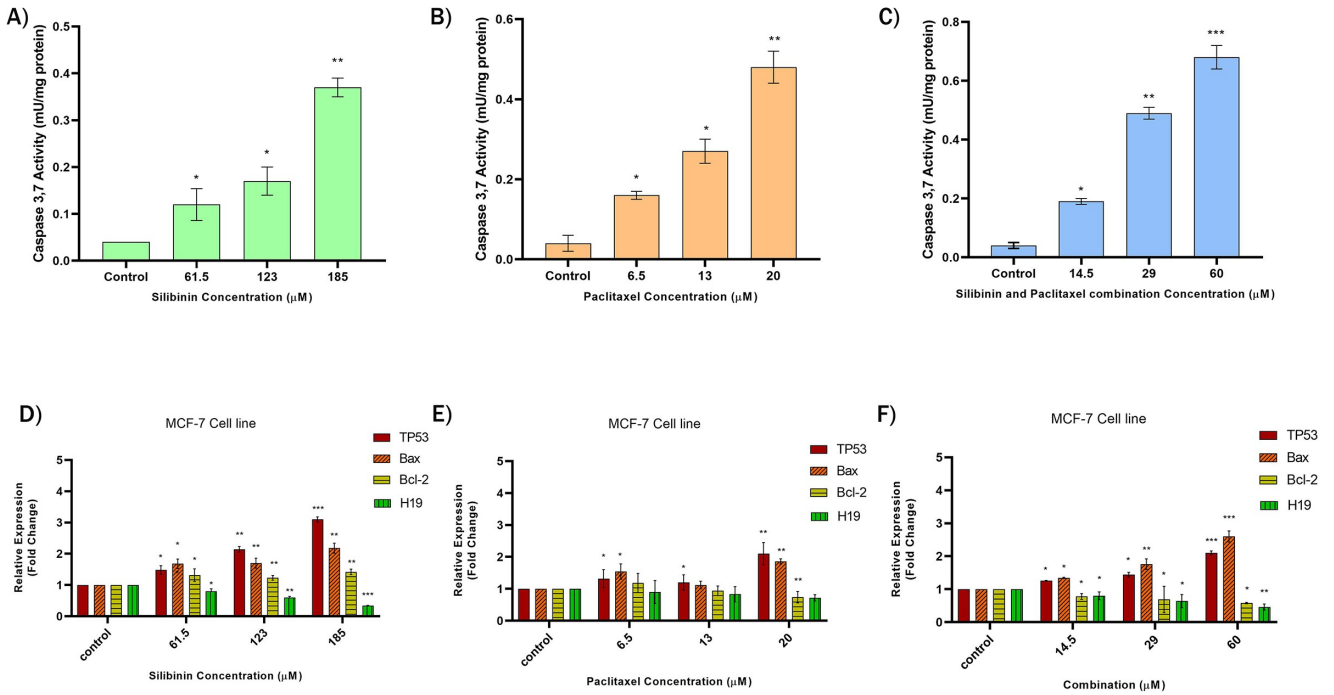

Figure 2. Effects of silibinin, paclitaxel, and their combination on apoptotic response and related gene expression. Effect of (A) silibinin, (B) paclitaxel, and (C) combination on caspase-3,7 activity in breast cancer MCF-7 cells. (D) Silibinin, (E) paclitaxel, and (F) combination on the messenger. RNA (mRNA) expression of TP53, Bax, Bcl-2, and H19 in MCF-7 cell line (mean ± SD) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs untreated control).

remains an important area for future investigation. In our study, this combination also led to a significant reduction in the required paclitaxel dose in breast cancer cell lines (Table 1). The synergistic interaction was quantitatively confirmed using the Chou–Talalay method, with CI values below 1 across multiple fractional effect levels (Fa) and visualized using isobologram and CI plots.

Paclitaxel's antitumor activity is primarily attributed to its ability to stabilize microtubules and inhibit their dynamic behavior, leading to mitotic arrest and subsequent apoptosis[40]. Central to the apoptotic response are proteins such as p53, Bax, Bcl-2, and caspase-3[41]. The tumor suppressor protein p53 plays a pivotal role in regulating pro-apoptotic Bax and anti-apoptotic Bcl2, thereby orchestrating the cellular response to stress and ensuring genomic integrity[42]. While Bcl-2 inhibits apoptosis by preventing cytochrome c release from mitochondria, Bax promotes this process, tipping the balance towards programmed cell death[43]. Caspase-3, a key executioner of apoptosis, is activated downstream and ensures irreversible cell death. Paclitaxel has been shown to activate caspase-3 in various cancers, including ovarian, non-small cell lung cancer, and leukemia[44]. In line with these findings, our real-time RT-PCR data showed that the combination of silibinin and paclitaxel significantly upregulated p53 and Bax, downregulated Bcl-2, and increased caspase-3/7 activity, confirming enhanced apoptotic signaling. Given that MCF-7 cells are deficient in caspase-3, caspase-3/7 activity primarily reflects caspase-7 activation. While apoptosis was assessed through caspase-3/7 activity assays and transcriptional analysis of key apoptotic regulators, we acknowledge that additional validation methods such as Annexin V/PI staining or protein-level assessment by Western blotting would further strengthen these findings. These approaches were beyond the scope of the current study and are recommended for future investigations.

In recent years, long non-coding RNAs (lncRNAs) have emerged as critical regulators of cellular functions, with their dysregulation being linked to tumorigenesis. H19, one of the earliest identified lncRNAs, is now recognized for its oncogenic, proliferative, and anti-apoptotic roles across various *in vitro* and *in vivo* cancer models[45]. However, its specific role and mechanisms in breast cancer remain poorly defined. Previous studies have shown that the oncogene c-Myc can directly activate H19, enhancing clonogenicity in breast and lung cancer cells[46,47]. Lottin *et al* demonstrated that H19 overexpression in MDA-MB-231 cells accelerates tumor growth in xenograft models, while Sun *et al* found that H19 overexpression boosts cell proliferation in MCF7 cells, with its silencing reducing cell viability[48]. Additionally, H19 was shown to promote cell migration in tamoxifen-resistant breast cancer cells[49].

Our findings reveal that silibinin significantly reduces H19 lncRNA expression (3-fold decrease, $P < 0.001$), a novel observation that provides insight into its chemosensitizing effects. H19 lncRNA is known to promote chemoresistance by inhibiting apoptosis and interfering with tumor suppressor pathways. Its suppression by silibinin likely disrupts these protective mechanisms, rendering cancer cells more susceptible to paclitaxel-induced cytotoxicity. This is further supported by the observed upregulation of P53 and Bax, alongside Bcl-2 downregulation, indicating a shift toward pro-apoptotic signaling[50]. The combination treatment also amplified caspase-3/7 activation, confirming enhanced apoptotic cell death (Fig. 3). The association between H19 and p53 has also been reported in

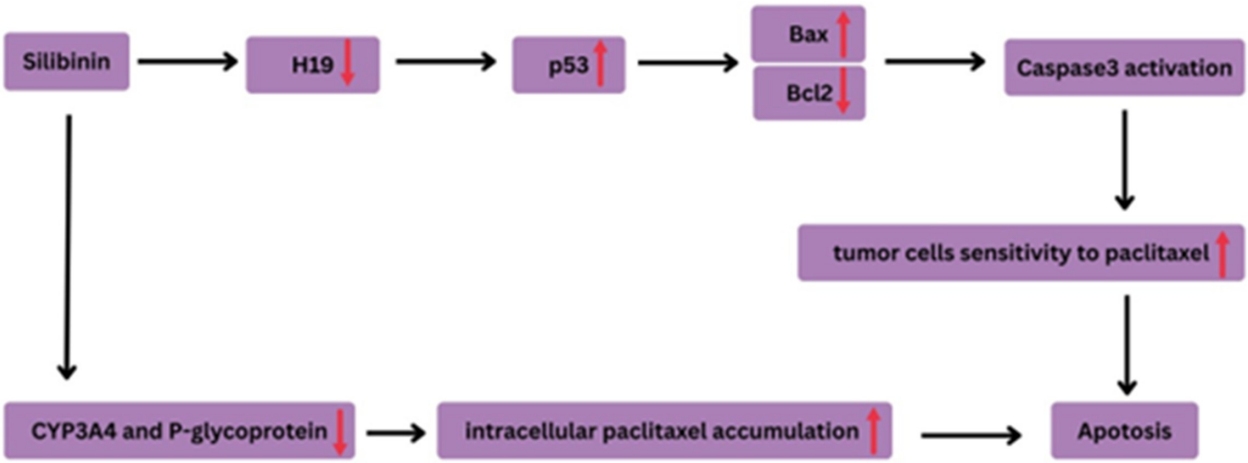

Figure 3. Schematic summary illustrating how silibinin and paclitaxel induce apoptosis in breast cancer cells.

various other types of cancer. In gastric cancer cells, H19 directly interacts with p53, leading to the inactivation of p53[51,52].

Although our data demonstrate a strong association between H19 lncRNA suppression and enhanced chemosensitivity to paclitaxel, it should be noted that the present study does not establish a direct causal relationship. Genetic manipulation approaches such as H19 knockdown or overexpression would be required to definitively confirm its mechanistic role. Nevertheless, our findings are biologically plausible and supported by previous studies showing that H19 silencing restores chemosensitivity and promotes apoptosis in breast cancer cells.

Moreover, Bai and Tang found a positive correlation between H19 and Bcl-2, and an inverse correlation with Bax. Silencing H19 resulted in decreased Bcl-2 and increased Bax expression at both transcript and protein levels[53]. Zhu *et al* identified H19 as a mediator of doxorubicin resistance via the cullin4A-MDR1 pathway in breast cancer cells[54], suggesting that H19 suppression may also reduce P-gp levels and facilitate paclitaxel accumulation in MCF-7 cells.

Understanding the relationship between silibinin, H19, and the p53/Bax/Bcl-2 axis offers several therapeutic advantages. First, it provides insight into how silibinin sensitizes breast cancer cells to paclitaxel, potentially guiding the development of more effective, lower-dose combination therapies. Second, it underscores the significance of lncRNA-mediated regulation in chemotherapy resistance, opening avenues for RNA-targeted treatments. Third, by modulating both pro-apoptotic and anti-apoptotic signaling pathways, silibinin may overcome resistance mechanisms and improve outcomes in patients with refractory or aggressive tumors. Overall, elucidating these molecular interactions could lead to the identification of novel biomarkers for drug response and resistance, ultimately contributing to more personalized and effective cancer therapies.

It is also important to consider that silibinin exerts pleiotropic anticancer effects through multiple signaling pathways. Previous studies have reported that silibinin modulates oxidative stress, inhibits P-glycoprotein activity, and suppresses CYP3A4-mediated drug metabolism, all of which may contribute to enhanced intracellular accumulation and efficacy of paclitaxel. Therefore, the observed synergistic interaction is likely mediated by a combination of H19-dependent and H19-independent mechanisms[55,56].

Nonetheless, this study has several limitations that should be acknowledged. First, the findings are based on *in vitro* experiments using the MCF-7 breast cancer cell line, which may not fully recapitulate the complexity of *in vivo* tumors. Validation in animal models, such as xenografts or patient-derived organoids, as well as in more clinically relevant systems including 3D spheroids or paclitaxel-resistant cell lines, will be necessary to further substantiate these results.

Second, although a strong association between H19 suppression and enhanced apoptotic response was observed, mechanistic studies involving H19 knockdown or overexpression are required to establish a direct causal relationship. In addition, the absence of protein-level validation represents a limitation of the current study, and future investigations incorporating Western blotting or other proteomic approaches would provide further mechanistic insight.

Finally, while the present data suggest a promising chemosensitizing effect of silibinin, clinical translation of these findings remains preliminary and warrants further investigation, particularly in the context of paclitaxel-resistant breast cancer. The potential involvement of miR-675, a downstream product of H19, also merits exploration to achieve a more comprehensive understanding of the underlying regulatory network.

Taken together, this study provides mechanistic insight into the chemosensitizing effects of silibinin while clearly acknowledging the experimental limitations inherent to *in vitro* models. Framing these findings within a realistic experimental scope enhances their scientific rigor and highlights clear directions for future research.

Conclusion

In summary, our study highlights the promising potential of silibinin as a chemosensitizing agent that enhances the efficacy

of paclitaxel in breast cancer treatment. By significantly downregulating H19 lncRNA and modulating key apoptotic regulators – including upregulation of p53 and Bax and downregulation of Bcl-2 – silibinin promotes a shift toward apoptosis and reduces the required dose of paclitaxel. These findings not only provide novel insights into the molecular mechanisms underlying the combined anticancer effects of silibinin and paclitaxel but also emphasize the therapeutic value of targeting lncRNAs such as H19 to overcome chemoresistance.

Although the present findings provide mechanistic insight at the cellular level, *in vivo* validation and clinical correlation are necessary to determine the therapeutic relevance of silibinin–paclitaxel combination therapy, our results pave the way for the development of more effective, low-toxicity treatment strategies for breast cancer, especially in cases where standard chemotherapy is limited by resistance or adverse effects.

Ethical approval

This research was approved by The Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1402.273).

Consent

Not applicable.

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Conflicts of interest disclosure

The authors declare no conflict of interest.

Author contributions

H.D. and V.Gh. contributed to the study concept and design. E. H. developed the methodology. H.D. drafted, reviewed, and revised the manuscript. H.D. and E.H. contributed to data acquisition, analysis, interpretation, and statistical analysis. T. A. and A.M. provided technical and material support and prepared the figures. All authors read and approved the final version of the manuscript.

Research registration unique identifying number (UIN)

This study did not involve human participants and therefore was not eligible for registration in a clinical or systematic review registry.

Guarantor

H.D. accepts full responsibility for the integrity of the work, had full access to the data, and controlled the decision to publish.

Provenance and peer review

Not commissioned; externally peer-reviewed.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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