Biomedicine Advances

Biomedicine Advances. 2025;2(3):137-145
doi: 10.34172/bma.28
https://biomedad.ae

Original Article

Carvacrol and paclitaxel: Synergistic effect on apoptosis in breast cancer cell lines

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Summary

Introduction: Paclitaxel has been approved by the FDA for clinical use in cancer therapy; however, it has severe dose-related toxicity. Therefore, in the current research, carvacrol, a natural compound with anti-cancer properties, was examined as an adjuvant treatment for paclitaxel.

Methods: The MTT assay was used to investigate the cytotoxicity of carvacrol and paclitaxel against the MCF-7 and MDA-MB-231 cells. Chou and Talalay method were used for the drug-drug interactions analysis. The amount of apoptosis induction in the treated cells was determined using DAPI fluorescence staining. Finally, expression of the Bax, BCL-2, and TP53 genes at mRNA and protein levels was measured by real-time PCR and Western blot, respectively.

Findings: It was determined that the toxicity of carvacrol in both cell lines was dose- and time-dependent. Analysis of the images obtained from DAPI staining showed that the combination of carvacrol and paclitaxel resulted in the death of 77.59% and 87.24% of MCF-7 and MDA-MB-231 cells, respectively. Results from the gene expression analysis showed that the Bax and TP53 genes were overexpressed in the treated cells, while BCL-2 showed a downregulation. These results have been replicated at the protein level with a western blot test.

Conclusion: The results of this study shown that the simultaneous use of carvacrol and paclitaxel can be more effective in the treatment of breast cancer cells. This research confirms for the first time that the combined use of paclitaxel and carvacrol in the treatment of breast cancer cells can be a promising solution to reduce the limitations of chemotherapy.

Keywords: Breast cancer, Combination therapy, Carvacrol, Paclitaxel

Received: April 3, 2025, **Revised:** May 15, 2025, **Accepted:** June 18, 2025, **ePublished:** July 1, 2025

Introduction

Following cardiovascular disease, cancer is the second leading cause of death in developed countries and the third leading cause of death in developing countries.[1] By itself, it kills more people than tuberculosis, AIDS, and malaria.[2] According to statistics, cancer currently accounts for around 12% of global deaths. By it is 2030, that 13 million cancer deaths expected will be reported annually.[3] Nowadays, various methods such as chemotherapy and radiation treatment are used to treat cancer. In recent years, although considerable progress has been made in cancer treatment strategies, no definitive cure has so far been introduced.[4] Consequently, efforts to find therapeutic solutions and new medicinal compounds for the treatment of cancer are still ongoing. Providing new cancer drugs with a high level of efficacy and sufficient specificity is mandatory.[5]

Given the critical role of apoptosis in controlling cell division, new drugs should preferably have the ability to induce apoptosis in cancer cells.[6] A variety of diseases, including cancer and neurodegenerative diseases, may be associated with defects in the apoptotic process.[7] Therefore, nowadays one of the interesting strategies considered in cancer chemotherapy is drug interventions that can mediate the death of malignant cells by inducing apoptosis.[8]

Paclitaxel is a well-known drug that has been referred to in numerous studies as inducing apoptosis in cancer cells. It is the first microtubule-stabilizing agent and is recognized as the most important development in chemotherapy in the last two decades. This drug is considered one of the most widely used anti-neoplastic agents, with extensive activity in several cancers, including breast cancer, endometrial cancer, non-small cell lung cancer, bladder cancer, and cervical cancer.[9]

Systemic administration of cytotoxic chemotherapeutic agents for cancer treatment often results in toxic side effects and limits their dose. In order to increase the effectiveness of chemotherapy while reducing toxicity, it is essential to rationally design synergistic treatment regimens. Combined treatments of chemotherapy drugs and compounds with anti-cancer effects have become an

attractive field in recent cancer treatments.[10,11] Various studies have focused on the efficacy of treatment with paclitaxel and other compounds that are effective in cancer treatment. The combination of paclitaxel and 5-demethylnobiletin (DMN), a hydroxylated polymethoxyflavone from citrus fruits, has been shown to reduce dose of paclitaxel in lung cancer and consequently its reduce side effects .[12,13] Results of both in vivo and in vitro studies of the combination of paclitaxel and triptolide (TL) have proven synergistic effect loaded in lipid-polymer hybrid to have a nanoparticles (LPNs) on lung cancer xenografts with minimal systemic toxic side effects.[14] Ramucirumab is also approved both as monotherapy and in combination with paclitaxel for advanced gastric cancer in patients with progressive disease after chemotherapy.[15] Combination therapy of curcumin and paclitaxel using a cationic PEGylated liposome has been reported as a promising strategy for more effective treatment of breast cancer.[16]

Previously, we described that carvacrol induces apoptosis in the MCF-7 breast cancer cell line [17]. The aim of the current study is to investigate the effect of paclitaxel, carvacrol, and the combined treatment of paclitaxel-carvacrol on Bcl-2/Bax/P53 axis in breast cancer cells.

Methods

Cell culture

Breast cancer cell lines, including MCF-7 and MDA-MB-231, were purchased from the Pasteur Institute in Tehran. The morphology and risk of cell contamination were evaluated. The cells were cultured in RPMI 1640 culture medium and kept in an incubator at 37 °C, with 5% CO2 concentration and 95% humidity.

MTT cytotoxicity assay

MTT assay was used for cytotoxicity assessment. To investigate the toxicity of paclitaxel and carvacrol on the cell lines, 10^4 cells in a final 200 µL volume of complete culture medium (RPMI 1640 + 10% FBS) were added to each well of a 96-well plate and incubated at 37 °C for 24 hours. This time allowed the cells to attach to the plate and proliferate. The following day, the culture medium was drained and the cells were treated with the desired concentrations of paclitaxel (0 to 240 µM) and carvacrol (0 to 1200 µM) in the culture medium. One untreated group was also considered a control group. The plates were incubated for 24 and 48 hours and cell viability were evaluated by the MTT assay. For this purpose, the medium inside each well was discarded and placed in an incubator with 10 µL of MTT solution (5 mg/mL in PBS) for 4 hours. Afterwards, the supernatant was slowly removed so that the formed crystals were not separated from the bottom of the plate and 200 µL of DMSO were added to each well and the plates were placed on a low-speed shaker for 15 minutes until the formazan precipitates were fully resolved. Finally, the optical density (OD) of each well was read using an ELISA reader at a wavelength of 570 nm. The relative cell viability at each concentration and time was determined using the following formula.
Percentage of viability = $(OD_{test} / OD_{control})$ 100.

Drug interaction analysis

Chou-Talalay method was used for evaluation of combination effect between carvacrol and paclitaxel. Fixed ratio of 1: 50 concentrations of carvacrol and paclitaxel chosen for 24 hours treatment of each cell line. The concentration ranges were: Paclitaxel: 0.6–4.9 µM; carvacrol: 30.06–245 µM. After 24 hours, inhibition of cell growth was assessed by MTT experiment as mentioned above. Combination index (CI) was calculated via CompuSyn software version 1.0 (ComboSyn, Paramus, NJ, USA). CI > 1, CI = 1and CI < 1 indicate antagonism, additive and synergism effect, respectively. Dose Reduction Index (DRI) is the comparison of the dose of each drug alone with the degree of dose reduction of the combined drugs was another parameter that calculated with this method.[18]

Assessment of apoptosis induction

To evaluate the induction of apoptosis in cancer cells treated with paclitaxel, carvacrol, and the combination of paclitaxel and carvacrol, DAPI staining was used. For this purpose, an average of 10^5 cells were seeded in each well of a 24-well plate with 400 µL of RPMI 1640 + 10% FBS. After 24 hours, the cells were treated with different concentrations of carvacrol, paclitaxel and combined concentrations of carvacrol and paclitaxel for a 48h period. After incubation, the cells were fixed with 4% PFA for 15 minutes, followed by 10 min incubation with triton X-100. 100 µL of a 1:1000 dilution of DAPI dye was used for staining and kept for 20 minutes in the dark. Finally, cells were detected using a fluorescence microscope (Optika Microscopes, Italy).

Evaluation of the expression of genes involved in apoptosis

The expression of TP53, BCL-2 and Bax genes which are involved in apoptosis, were evaluated in cells treated with desired concentrations of paclitaxel, carvacrol, and the combination of paclitaxel and carvacrol. Total RNA was extracted from the cells using the ROJE Technologies kit (Iran) according to the manufacturer's protocol. The quantity and quality of the extracted RNA were verified by spectrophotometry and electrophoresis on agarose gel, respectively.

The cDNA synthesis kit (YTA-Iran) and oligo dT primers were used for cDNA synthesis. The reverse transcription reaction was performed at 52 °C for 50 minutes and then, the reverse transcriptase was inactivated at 85 °C for 10 seconds. The Real-time PCR was performed

Table 1. Primers used in Real Time PCR

Gene	Forward primer	Reverse primer	Amplicon size (bp)
TP53	5′-AGGCCTTGGAACTCAAGGAT-3′	5′-AGGCCTTGGAACTCAAGGAT-3′	140
BCL-2	5′-GATCAGCTCGGGCACTTTAGTG-3′	5′-GATGACTTCTCTCGTCGCTA-3′	73
Bax	5′-CGTGGTTGCCCTCTTCTACTTT-3′	5′-GATCAGCTCGGGCACTTTAGTG-3′	229
β -actin	5′-GATGACTTCTCTCGTCGCTA-3′	5′-GTAGTTTCGTGGATGCCACA-3′	131

using SYBER Green master mix and primers (Table 1). The temperature profile of the reaction consisted of one cycle of denaturation at 95 °C for 5 minutes, followed by 40 replication cycles consisting of denaturation at 95 °C for 15 seconds, annealing at 61°C for 20 seconds, and extension at 72 °C for 30 seconds.

Evaluation of proteins involved in apoptosis

To evaluate and study the effect of treatment of cells with paclitaxel, carvacrol, and their combination on the expression of P53, BCL-2, and Bax proteins, a western blot assay was used. Firstly, protein was extracted from the cell lysate. Cells were harvested and centrifuged at 1300 rpm for 10 minutes. The supernatant was discarded and 100 µL RIPA buffer was added to the cell tube. It was incubated at -20 °C for 1 hour and then centrifuged at 1300 rpm for 10 minutes at 4 °C. The supernatant containing the protein was transferred to a new microtube. SDS-PAGE was performed with a 28% resolving gel and a 22% stacking gel. The protein separated by SDS-PAGE was transferred to a PVDF membrane and the membrane was blocked with a blocking solution (5% skim milk in 0.05% PBS-Tween 20) at -4 °C for 12 hours. It was then washed three times with PBS-Tween 20 for 5 minutes and incubated for 90 minutes at 25 °C with 10 µg/mL monoclonal antibodies (Padza Co, Iran). After incubation with the primary antibody, the membrane was washed three times with PBS-Tween 20 for 5 minutes and incubated for one hour at 25°C with 0.4 µg/mL HRP secondary antibody (Padza Co, Iran) (1:2500 with 3% skim milk in 0.05% PBS/Tween). The protein bands were visualized using the ECL detection system.

Statistical analysis

The data was analyzed using GraphPad Prism 6.01 software. Considering the normality of the data distribution, the comparison of the treatment group and the control group was analyzed using the one-way ANOVA and the *P* value was calculated. The minimum level of significance was considered to be $P<0.05$. The Tukey method was used as a post hoc test.

Results

Cell viability and drug interactions

The viability of MCF-7 (Figure 1A) and MDA-MB-231 (Figure 1G) cells was evaluated under the influence of carvacrol concentrations ranging from 1 to 1200 µM and it was found that the toxicity of carvacrol in both cell lines was dose and time dependent. The IC50 in 24-hour treatment of MCF-7 and MDA-MB-231 cell lines was calculated to be 246.13 and 178.52 µM, respectively. In 48-hour treatment, the IC50 of carvacrol for MCF-7 and MDA-MB-231 cell lines was 187 and 154.2 µM, respectively. The survival of MCF-7 (Figure 1B) and MDA-MB-231 (Figure 1H) cell lines was investigated under the treatment with concentrations ranging from 1 to 240 µM paclitaxel and the results showed that with the increase of paclitaxel concentration, cell viability decreased for both 24 hours and 48 hours of treatment. The amount of IC50 in MCF-7 and MDA-MB-231 cells during 24 hours was 20 and 15.8 µM, respectively, and in 48 hours IC50 was 18.6 and 12.3 µM, respectively.

CI for different inhibition fractions (Fa) of growth of MCF-7 and MDA-MB-231 cells evaluated based on the Chou-Talalay method and Compusyn 1.0 software. CI results and isobologram diagrams are shown as a function of the effect level (Figures 1D, B, F and J, K, L). Results confirmed that carvacrol synergistically enhanced the cytotoxicity of paclitaxel against both MCF-7 and MDA-MB-231 breast cancer cell lines. The most robust synergy between the two agents (0.25) was calculated at 0.75 fractional inhibition of growth of MCF-7 and 0.26 was calculated at 0.75 fractional inhibition of growth of MDA-MB-231. At the same time, the dose reduction index (DRI) values indicated that the effective paclitaxel dose in synergistic combination with carvacrol could be significantly reduced (Table 2).

Apoptosis induction and DNA condensation

The induction of apoptosis by carvacrol (IC50), paclitaxel (IC50), and the combination of both agents in MCF-7 and MDA-MB-231 cell lines was investigated using DAPI staining. In this regard, the percentage of cells in the control group was considered 100%, and the other groups were measured against it. As shown in Figure 2, carvacrol and paclitaxel reduced the number of living cells, but the impact of paclitaxel was stronger. On the other hand, the treatment of MCF-7 cells with carvacrol and paclitaxel caused an increased induction of apoptosis and death of 77.59% of cells. Moreover, the combination of the two agents induced apoptosis in 87.24% of MDA-MB-231 cells, indicating that these cells were more sensitive than MCF-7 cells. Results shown in Figures 2D and 2H, exhibit that the combination of carvacrol and paclitaxel significantly induced DNA condensation in both cell lines.

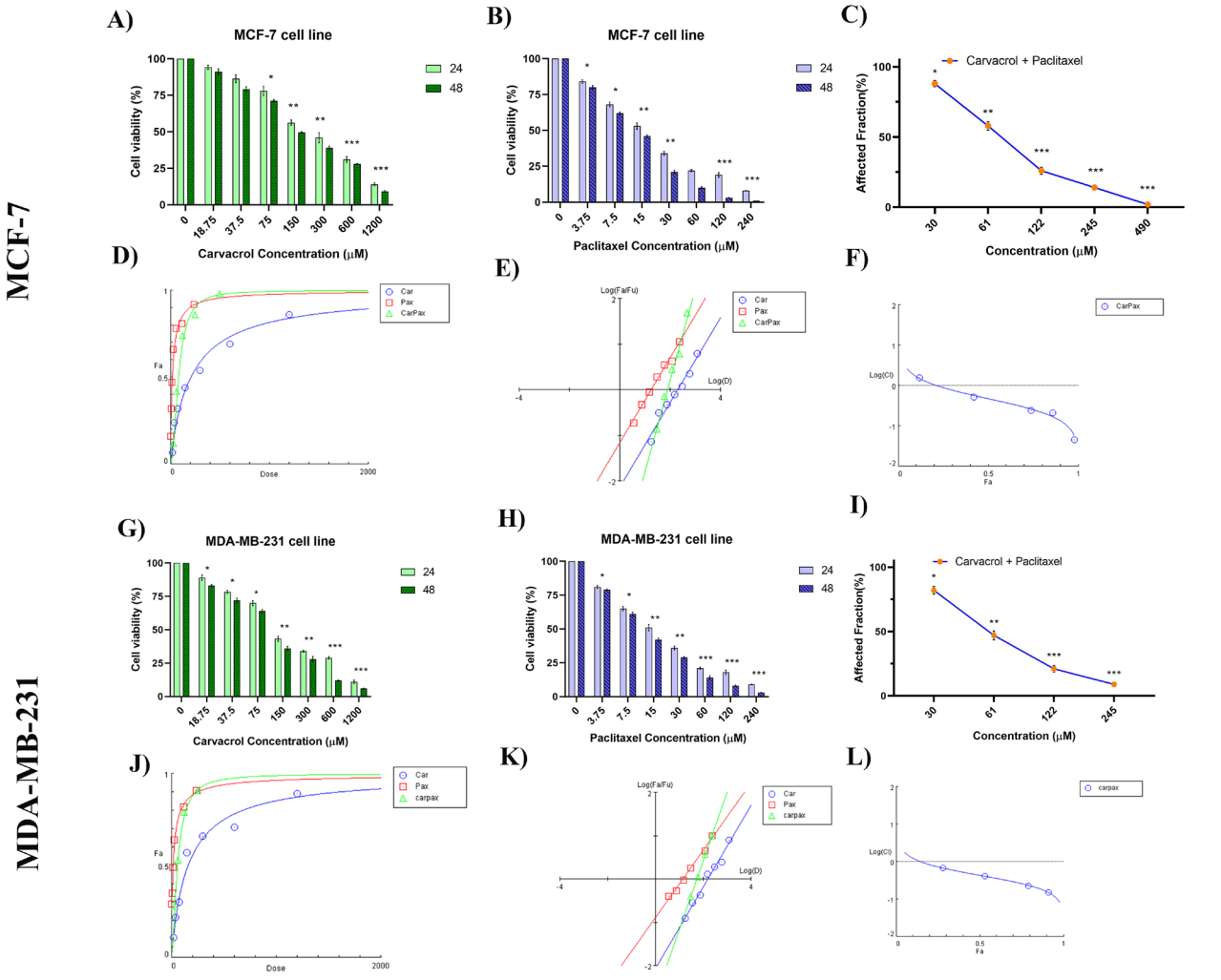

Figure 1. Concentration–response effects of Carvacrol and Paclitaxel on MCF-7 and MDA-MB-231 cells using A, B, G and H MTT assay following 24 and 48-h treatment with each agent. D–F and J-L analysis of synergy between carvacrol and Paclitaxel combination after 24 h post treatment against MCF-7 and MDA-MB-231cells represent dose effect-plot, median-effect plot and combination index plot, respectively. (*$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with control)

Table 2. Dose-response association of Paclitaxel and Carvacrol alone or in combination.

Compound	**IC50(μM)**	**r**	**CI Values**			**DRI**		
MCF-7								
			Fa 0.25	Fa 0.5	Fa 0.75	Fa 0.25	Fa 0.5	Fa 0.75
Paclitaxel	20	0.98				6.12	11.5	21.6
Carvacrol	246.13	0.98				1.37	2.58	4.84
Pax/Car	80.31	0.99	0.88	0.47	0.25			
Compound	**IC50(μM)**	**r**	**CI Values**			**DRI**		
MDA-MB-231								
			Fa 0.25	Fa 0.5	Fa 0.75	Fa 0.25	Fa 0.5	Fa 0.75
Paclitaxel	15.8	0.99				6.31	13.02	26.86
Carvacrol	178.52	0.94				1.76	2.81	4.48
Pax/Car	56.68	0.99	0.72	0.43	0.26			

DRI, dose reduction index; r, correlation coefficient.

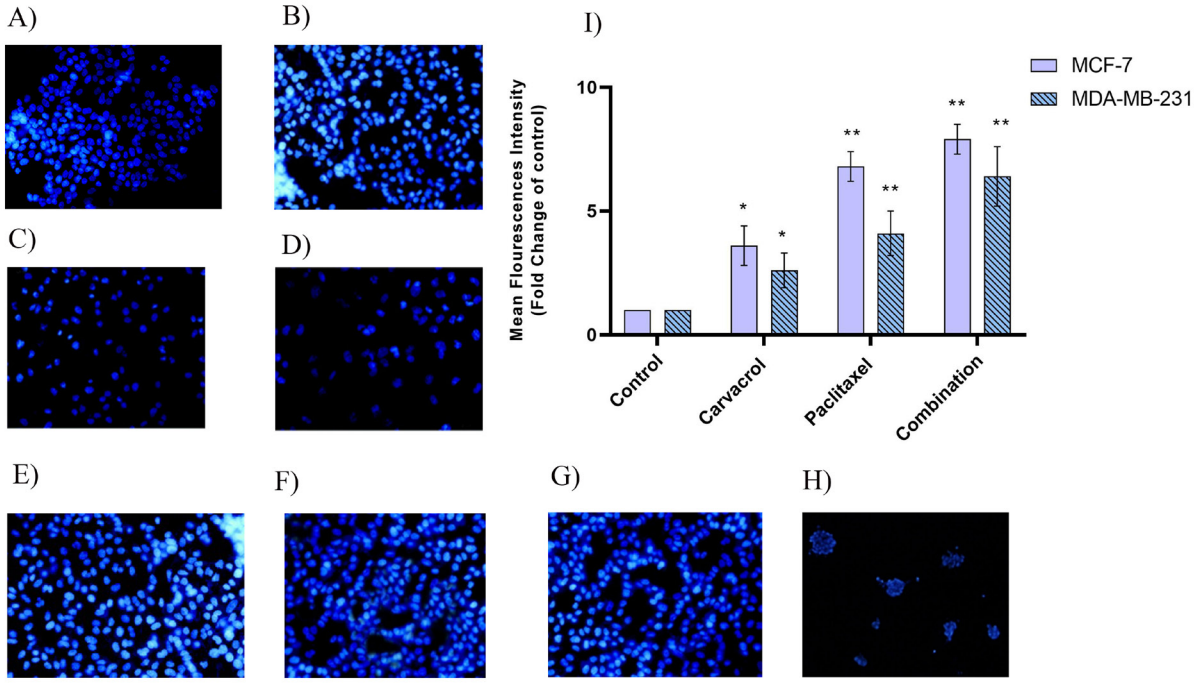

Figure 2. Effects of carvacrol, paclitaxel, and their combination on apoptotic response in MCF-7 and MDA-MB-231 cells. 10,000 cells of MCF-7 and MDA-MB-231 were seeded in 24-well plates and treated with carvacrol, paclitaxel, and their combination for 48 hours. (A) control, (B) carvacrol IC50 (C) paclitaxcel IC50 and (D) combination of two drugs IC50 in MCF-7 plate. (E) control, (F) carvacrol IC50 (G) paclitaxcel IC50 and (H) combination of two dugs IC50 in MDA-MB-231 plate. (I) depicted histogram chart represent the number of apoptotic cells which shown as mean ± SD calculated from counting a minimum of 4×10^2 cells in random fields of triplicate wells from three independent experiments. (*$P < 0.05$, **$P < 0.01$, *** $P < 0.001$ vs. untreated control).

Gene expression analysis

The expression of the Bax, TP53, and BCL-2 genes was assessed as effective apoptotic genes using the real-time PCR method (Figure 3). This was implemented in two cell lines, MCF-7 and MDA-MB-231, and with carvacrol, paclitaxel, and combination treatments. The results showed that in both cell lines, all treatments reduced the expression of the anti-apoptotic BCL-2 gene. Conversely, the expression of two pro-apoptotic genes, TP53 and Bax, showed a significant increase in all treatments and in both cell lines ($P < 0.05$). Furthermore, simultaneous treatment with carvacrol and paclitaxel was more effective in gene regulation in both cell lines.

Protein expression assay

Protein expression of BCL-2, Bax, and P53 was evaluated using Western blot (Figure 4). Western Blot analysis, in line with real-time PCR analysis, showed that BCL-2 had downregulation and Bax and P53 had upregulation in the two cell lines studied. Furthermore, the simultaneous use of carvacrol and paclitaxel further modified protein expression.

Discussion

While paclitaxel has been approved by the FDA for clinical use in cancer treatment, its severe dose-related toxicity is considered a major drawback in its use. Of course the disadvantages of current treatment can be overcome by combining paclitaxel with other chemotherapy medications or compounds with anticancer properties to increase treatment effectiveness.[19] The results demonstrated the cytotoxicity of the two compounds for MCF-7 and MDA-MB-231 cell lines.

One of the cytotoxic mechanisms of anticancer compounds is the induction of apoptosis, therefore, in the present study, the induction of apoptosis by paclitaxel, carvacrol and the combination of the both agents in the cell lines was investigated by different methods. The first method was the direct evaluation of apoptosis using DAPI staining, which showed that both compounds cause a significant increase in apoptotic cells. Furthermore, the results of this assessment demonstrated the synergistic effect of paclitaxel and carvacrol on apoptosis.

On the other hand, the expression of three genes involved in apoptosis including Bax, BCL-2 and TP53, was evaluated at both mRNA (by real-time PCR) and protein (by Western blot) levels. The results of the gene expression analysis showed that the Bax and TP53 genes were overexpressed in the treated cells, while the expression of the BCL-2 gene was reduced. These results were replicated at the protein level with the western blot. The results demonstrated that the combination of carvacrol and paclitaxel is more effective in upregulation of pro-apoptotic genes Bax and TP53 and downregulation of anti-apoptotic gene BCL-2. Additionally, these results suggest that using combined treatment with carvacrol, lower doses of paclitaxel are needed, and as a result, the side effects of paclitaxel can be reduced using carvacrol.

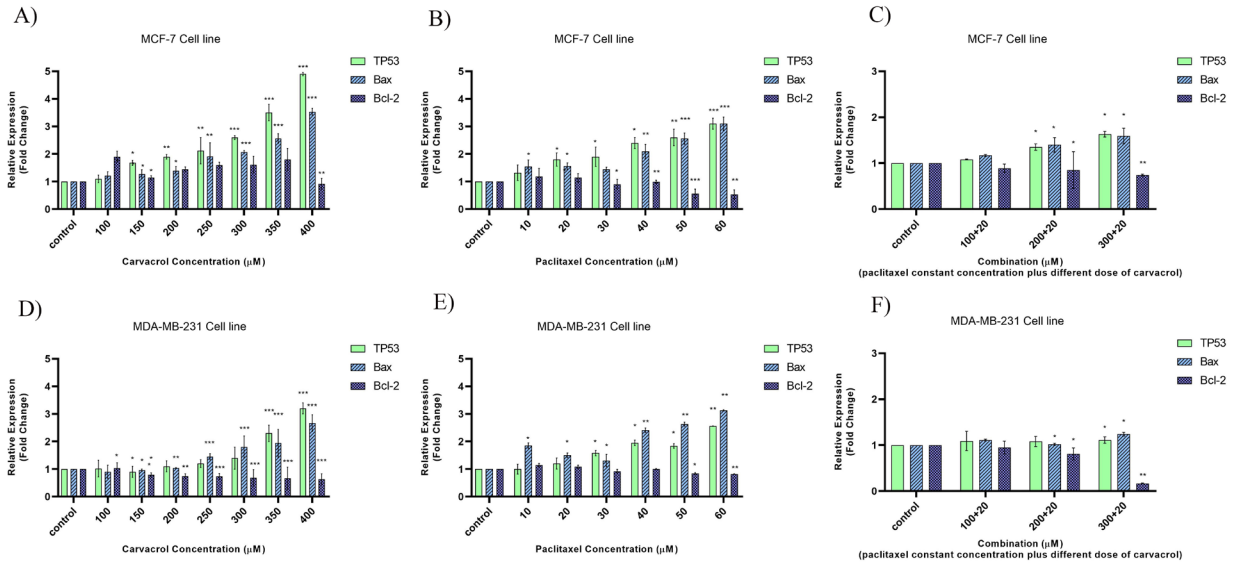

Figure 3. Inhibitory effects of carvacrol, paclitaxel and their combination for 48h treatment on expression of TP53, BCL-2 and Bax genes mRNA evaluated by qPCR assay. Data of qPCR were normalized against β- actin. Bars represent fold differences of the mean of normalized expression values ± SD against β- actin. A, B and C shows gene expression in MCF-7 cell line and D, E and F shows gene expression in MDA-MB-231 cell line (*$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. untreated control)

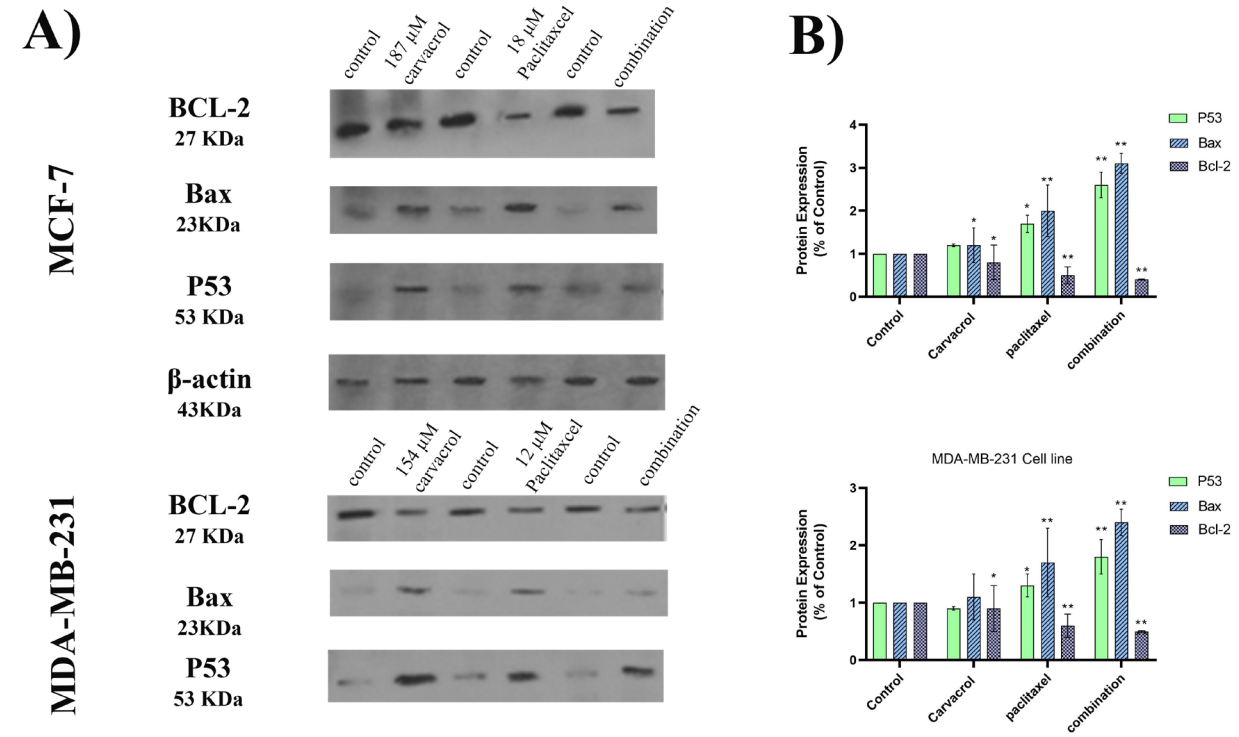

Figure 4. Quantification of P53, Bax and BCL-2 protein levels in MCF-7 and MDA-MB-231 cells incubated with carvacrol, paclitaxel and their combination. Panel A shows the expression of P53, BCL-2 and Bax in various cell lysates analyzed by Western blotting. The cells were treated with carvacrol in IC50, paclitaxel in IC50 and their IC50 combination for 48 h. β-actin protein was used as loading control. Panel B indicates the densitometric ratio of P53, BCL-2 and Bax /β-actin for each treatment. The results are representative of three independent experiments. Data were quantified using ImageJ software

Carvacrol is a phenolic monoterpene found in the essential oils of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), yellow pepperweed (*Lepidium flavum*), wild bergamot (*Citrus aurantium bergamia*), and other plants. Carvacrol has a wide range of biological activities that may be useful for clinical applications, such as antimicrobial, antioxidant and anti-cancer activities. Anticancer properties of carvacrol have been reported in clinical models of breast, liver, and lung cancer, influencing proapoptotic processes.[20] In the current study, the effect

of carvacrol alone and in combination with paclitaxel was evaluated in two breast cancer cell lines, MCF-7 and MDA-MB-231. The findings showed the cytotoxic and pro-apoptotic effect of carvacrol and paclitaxel, and synergistic effects were also observed in the simultaneous treatment of cells with these two compounds.

Li et al studied cell viability and apoptosis of the BT-483, BT-474, MCF-7, MDA-MB-231 and MDA-MB-453 cell lines treated with carvacrol. Carvacrol inhibited transient receptor potential melastatin 7 (TRPM7) in MDA-MB-231 and MCF-7 cell lines. This study showed that carvacrol suppresses breast cancer cells by regulating the cell cycle, and the TRPM7 pathway is one of the pharmacological mechanisms.[21]

Mari et al investigated the role of carvacrol in modulating PI3K/AKT signaling, which is involved in the pathogenesis of breast cancer, using MCF-7 cells. The results demonstrated a significant increase in G0/G1 phase accumulation after carvacrol treatment in MCF-7 cells. A significant decrease in the expression of p-Rb, cyclin D1, CDK4 and CDK6 proteins indicated cell cycle arrest. In addition, carvacrol treatment significantly inhibited the expression of PI3K/p-AKT proteins, which led to the induction of apoptosis through the reduction of BCL-2 and the increase of Bax protein expression.[22]

Al-Fatlawi et al conducted an experiment to investigate the anti-cancer activity of carvacrol in the MCF-7 human breast cancer cell line. Their results showed that the cytotoxicity of carvacrol against MCF-7 cancer cells was dose-dependent at 24 and 48 hours. MCF-7 cells treated with carvacrol showed induction of apoptosis through P53 and BCL-2/Bax dependent pathway. In addition, the carvacrol treatment induced gene expression of caspase-3, -9 and -6 and genomic DNA fragmentation. The study confirmed that carvacrol inhibits growth and induces apoptotic regulating genes in MCF-7 cells.[23]

As mentioned in this research, in addition to evaluating the effect of carvacrol on breast cancer cells, the effect of its combined treatment with paclitaxel was also investigated. The results showed that this combined treatment is more effective than the use of each of the compounds alone and causes the death of more cancer cells and more regulation of apoptotic genes.

Previous studies also declare the beneficial effect of carvacrol in combination therapy. Ahmad and Ansari investigated the effect of carvacrol alone and together with some chemotherapy drugs on HPV18+HeLa cervical cancer cells. The results of their study showed that carvacrol strongly suppresses the proliferation of cervical cancer cells through caspase-dependent apoptosis and inhibition of cell cycle progression. Furthermore, their study showed that carvacrol is synergistic with chemotherapy drugs (5-FU and carboplatin). These results suggest that natural compounds may reduce the toxic effects of chemotherapy medications.[24] Bouhtit et al investigated the effect of *Ptychotis verticillata* essential oil and its derivatives, carvacrol and thymol, on acute myeloid leukemia cell lines. They showed that the combination of carvacrol and thymol causes the death of tumor cells with low toxicity on normal cells. In this study, it was determined that different molecular pathways, including apoptosis, oxidative, autophagy and necrosis, are involved in this potential synergistic effect.[25]

The therapeutic effects of carvacrol with various medicinal and natural compounds have been investigated until now, but according to the search in the databases, the effect of the combination treatment of carvacrol and paclitaxel was evaluated for the first time in this research, and the results showed its synergistic effect in causing toxicity and inducing apoptosis in MCF-7 and MDA-MB-231 cancer cells.

On the other hand, using carvacrol along with chemotherapy drugs can protect against side effects caused by chemotherapy drugs. In this regard, Atalay et al conducted a study on the protective effects of carvacrol on paclitaxel-induced ototoxicity in an animal model. Their biochemical evidence demonstrated that paclitaxel causes oxidative stress in the cochlea. Histopathological findings showed loss of outer hair cells in the organ of Corti (CO) and moderate degenerative changes in the stria vascularis. However, in groups where carvacrol was given with paclitaxel, these biochemical, histopathological and functional changes were positively reversed. The results showed that carvacrol may have a protective effect against paclitaxel ototoxicity.[26]

While the above research differs from current research, it shows the reduction of the toxicity of paclitaxel by carvacrol, which is a valuable point in cancer treatments. A limitation of paclitaxel use is its toxicity at high doses, and strategies to reduce this toxicity are essential and important.

In the aforementioned research, it was demonstrated that carvacrol has the potential to reduce the toxicity of paclitaxel. On the other hand, carvacrol has also been shown in this study to increase the apoptotic effect of paclitaxel. These findings all together suggest that the combined treatment of carvacrol and paclitaxel should be given more attention, as it can potentially lead to an increase in the death of cancer cells while having fewer side effects.

Conclusion

In the present study, the effect of the combination treatment of carvacrol and paclitaxel on two MCF-7 and MDA-MB-231 cell lines was investigated. Generally, the results showed that the combination of carvacrol and paclitaxel may be more effective in treating cancer cells. Furthermore, the results indicated that the induction of apoptosis in the studied cells treated with carvacrol and paclitaxel at the same time was more than when the cells

were treated with the same doses of these compounds alone. Under such conditions, lower doses of paclitaxel can be used with the adjuvant treatment of carvacrol to achieve appropriate therapeutic objectives while reducing the side effects caused by paclitaxel. The present research confirms the possibility that the use of paclitaxel and carvacrol in the combination treatment of breast cancer cells may be a promising solution to reduce the limitations of chemotherapy. Further research on this issue is needed and we hope it will be addressed in the future.

Acknowledgments

With the memory of the late Dr. Alinazar Salehinia, the founder of Khorrman Pharmaceutical Company, who helped us in carrying out and improving the quality of this research with his valuable guidance.

Authors' Contribution

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Writing-original draft: Hassan Dariushnejad.
Writing-review & editing: Ayat Moradipour.

Competing Interests

The authors declare there are no conflicts of interest.

Ethical Approval

This research was approved by The Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS. REC.1399.034).

Funding

This research has not received funding from any organization.

Intelligence Use Disclosure

This article has not utilized artificial intelligence (AI) tools for research and manuscript development, as per the GAMER reporting guideline.

References

1. Kizilpinar M, Dastouri M. Therapeutic effects of NK cell-derived EVs on cancer: current advances and future treatment strategies. Biomed Adv. 2025;2(1):10-24. doi: 10.34172/bma.10.
2. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, et al. Cancer statistics for the year 2020: an overview. Int J Cancer. 2021;149(4):778-89. doi: 10.1002/ijc.33588.
3. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health. 2019;9(4):217-22. doi: 10.2991/jegh.k.191008.001.
4. Garcia-Oliveira P, Otero P, Pereira AG, Chamorro F, Carpena M, Echave J, et al. Status and challenges of plant-anticancer compounds in cancer treatment. Pharmaceuticals (Basel). 2021;14(2):157. doi: 10.3390/ph14020157.
5. Sun YS, Zhao Z, Yang ZN, Xu F, Lu HJ, Zhu ZY, et al. Risk factors and preventions of breast cancer. Int J Biol Sci. 2017;13(11):1387-97. doi: 10.7150/ijbs.21635.
6. Waks AG, Winer EP. Breast cancer treatment: a review. JAMA. 2019;321(3):288-300. doi: 10.1001/jama.2018.19323.
7. Carneiro BA, El-Deiry WS. Targeting apoptosis in cancer therapy. Nat Rev Clin Oncol. 2020;17(7):395-417. doi: 10.1038/s41571-020-0341-y.
8. Jan R, Chaudhry GE. Understanding apoptosis and apoptotic pathways targeted cancer therapeutics. Adv Pharm Bull. 2019;9(2):205-18. doi: 10.15171/apb.2019.024.
9. Alqahtani FY, Aleanizy FS, El Tahir E, Alkahtani HM, AlQuadeib BT. Paclitaxel. Profiles Drug Subst Excip Relat Methodol. 2019;44:205-38. doi: 10.1016/bs.podrm.2018.11.001.
10. Yan J, Wang Y, Jia Y, Liu S, Tian C, Pan W, et al. Co-delivery of docetaxel and curcumin prodrug via dual-targeted nanoparticles with synergistic antitumor activity against prostate cancer. Biomed Pharmacother. 2017;88:374-83. doi: 10.1016/j.biopha.2016.12.138.
11. Jiang H, Geng D, Liu H, Li Z, Cao J. Co-delivery of etoposide and curcumin by lipid nanoparticulate drug delivery system for the treatment of gastric tumors. Drug Deliv. 2016;23(9):3665-73. doi: 10.1080/10717544.2016.1217954.
12. Tan KT, Li S, Li YR, Cheng SL, Lin SH, Tung YT. Synergistic anticancer effect of a combination of paclitaxel and 5-demethylnobiletin against lung cancer cell line in vitro and in vivo. Appl Biochem Biotechnol. 2019;187(4):1328-43. doi: 10.1007/s12010-018-2869-1.
13. Mottaghitalab F, Farokhi M, Fatahi Y, Atyabi F, Dinarvand R. New insights into designing hybrid nanoparticles for lung cancer: diagnosis and treatment. J Control Release. 2019;295:250-67. doi: 10.1016/j.jconrel.2019.01.009.
14. Liu J, Cheng H, Han L, Qiang Z, Zhang X, Gao W, et al. Synergistic combination therapy of lung cancer using paclitaxel- and triptolide-coloaded lipid-polymer hybrid nanoparticles. Drug Des Devel Ther. 2018;12:3199-209. doi: 10.2147/dddt.S172199.
15. Refolo MG, Lotesoriere C, Lolli IR, Messa C, D'Alessandro R. Molecular mechanisms of synergistic action of ramucirumab and paclitaxel in gastric cancers cell lines. Sci Rep. 2020;10(1):7162. doi: 10.1038/s41598-020-64195-x.
16. Alemi A, Zavar Reza J, Haghiralsadat F, Zarei Jaliani H, Haghi Karamallah M, Hosseini SA, et al. Paclitaxel and curcumin coadministration in novel cationic PEGylated niosomal formulations exhibit enhanced synergistic antitumor efficacy. J Nanobiotechnology. 2018;16(1):28. doi: 10.1186/s12951-018-0351-4.
17. Moradipour A, Dariushnejad H, Ahmadizadeh C, Lashgarian HE. Dietary flavonoid carvacrol triggers the apoptosis of human breast cancer MCF-7 cells via the p53/Bax/Bcl-2 axis. Med Oncol. 2022;40(1):46. doi: 10.1007/s12032-022-01918-2.
18. Chou TC. Drug combination studies and their synergy quantification using the Chou-Talalay method. Cancer Res. 2010;70(2):440-6. doi: 10.1158/0008-5472.Can-09-1947.
19. Kumar DN, Chaudhuri A, Dehari D, Shekher A, Gupta SC, Majumdar S, et al. Combination therapy comprising paclitaxel and 5-fluorouracil by using folic acid functionalized bovine milk exosomes improves the therapeutic efficacy against breast cancer. Life (Basel). 2022;12(8):1143. doi: 10.3390/life12081143.
20. Sharifi-Rad M, Varoni EM, Iriti M, Martorell M, Setzer WN, Del Mar Contreras M, et al. Carvacrol and human health: a comprehensive review. Phytother Res. 2018;32(9):1675-87. doi: 10.1002/ptr.6103.
21. Li L, He L, Wu Y, Zhang Y. Carvacrol affects breast cancer cells through TRPM7 mediated cell cycle regulation. Life Sci. 2021;266:118894. doi: 10.1016/j.lfs.2020.118894.
22. Mari A, Mani G, Nagabhishek SN, Balaraman G, Subramanian N, Mirza FB, et al. Carvacrol promotes cell cycle arrest and apoptosis through PI3K/AKT signaling pathway in MCF-7

breast cancer cells. Chin J Integr Med. 2021;27(9):680-7. doi: 10.1007/s11655-020-3193-5.
23. Al-Fatlawi AA, Rahisuddin, Ahmad A. Cytotoxicity and pro-apoptotic activity of carvacrol on human breast cancer cell line MCF-7. World J Pharm Sci. 2014;2(10):1218-23.
24. Ahmad A, Ansari IA. Carvacrol exhibits chemopreventive potential against cervical cancer cells via caspase-dependent apoptosis and abrogation of cell cycle progression. Anticancer Agents Med Chem. 2021;21(16):2224-35. doi: 10.2174/1871520621999201230201258.
25. Bouhtit F, Najar M, Moussa Agha D, Melki R, Najimi M, Sadki K, et al. New anti-leukemic effect of carvacrol and thymol combination through synergistic induction of different cell death pathways. Molecules. 2021;26(2):410. doi: 10.3390/molecules26020410.
26. Atalay F, Tatar A, Dincer B, Gündoğdu B, Köyceğiz S. Protective effect of carvacrol against paclitaxel-induced ototoxicity in rat model. Turk Arch Otorhinolaryngol. 2020;58(4):241-8. doi: 10.5152/tao.2020.5714.