EVALUATION OF CYNARA CARDUNCULUS ON MCF-7 BREAST CANCER CELL LINE: CYTOTOXIC, APOPTOTIC, AND OXIDATIVE STRESS EFFECTS

Fahad Althobaiti [1], Maher Albalawi[2], Noor Alkurdi[3], Duha Alamri[4], Hawra Abu Ayfah[5], Abdullah Alqhatani[6], Sarah Alharbi[7], Abrar Alharbi[8], Khetmah Hanbashi[9], Sahar Barakat[10], Alhanof Alsubaie[11], Mujahid Alhazmi[12], Roba Alotaibi[13]

[1]Pharmacy Supervisor, Armed Forces Hospitals, 16TP0022908, Dr.fahad.1413@hotmail.com
[2]MSc in Clinical Pharmacy, Pharmacy Department, Prince Abdul-Mohsin Hospital in Alula, Saudi Arabia,
K_s_a87@hotmail.com
[3]Clinical Pharmacy Student, Alrayan College, 21120320@alrayancolleges.edu.sa
[4]Clinical Pharmacy Student, Taibah University, TU4556773@taibahu.edu.sa
[5]Taif University, Hooredhaay@gmail.com
[6]Doctor of Pharmacy, Pharmacist, Ministry of Defense, 22372550, a.s.alqhatani@gmail.com
[7]Pharmacist, Maternity and Children Hospital, Buraidah, sarahfraih1414@gmail.com
[8]Clinical Pharmacy Student, Taibah University, TU4456231@taibahu.edu.sa
[9]Pharmacist, Khetmahali2013@gmail.com
[10]Pharmacist II, National Gaurd Hospital, 20228583, Shrbarakat@yahoo.com
[11]Pharmacist, alhanouf.kh.s@gmail.com
[12]Pharmacist, Muaalhazmi@moh.gov.sa
[13]PharmD Grad, Saudi Arabia, rshf.1421@gmail.com

Abstract

Background: Breast cancer remains a major global health concern, necessitating alternative therapeutic approaches due to the limitations of traditional chemotherapy. *Cynara cardunculus* (artichoke), a medicinal plant known for its antioxidant and anti-inflammatory properties, has gained interest for its potential anticancer effects.

Objective: This study aimed to evaluate the cytotoxic, apoptotic, and oxidative stress-inducing effects of *C. cardunculus* extract on the MCF-7 breast cancer cell line.

Methods: MCF-7 cells were treated with various concentrations of *C. cardunculus* extract for 24, 48, and 72 hours. Cell viability (MTT assay), apoptosis (Annexin V/PI, DNA fragmentation), ROS generation (DCF-DA), and morphological alterations (microscopy) were assessed.

Results: The extract significantly reduced cell viability in a dose- and time-dependent manner, induced apoptosis, increased ROS production, and caused morphological changes.

Conclusion: *Cynara cardunculus* exhibits promising anticancer activity and may serve as a potential plant-based therapy for breast cancer pending further molecular and in vivo validation.

Index Terms- About four key words or phrases in alphabetical order, separated by commas. Keywords are used to retrieve documents in an information system such as an online journal or a search engine. (Mention 4-5 keywords)

Keywords: Evaluation, Cynara Cardunculus, MCF-7 Breast Cancer Cell Line, Cytotoxic, Apoptotic, Oxidative Stress Effects.

I. INTRODUCTION

Background

Breast cancer is the most common cancer in women globally and one of the leading causes of cancer mortality. According to the World Health Organization (WHO), in 2020[1], there were over

Acta Biomed 2025; Vol. 96, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

2.3 million new cases of breast cancer and over 685,000 cancer-related deaths globally. In spite of the remarkable increase in early diagnosis and therapeutic regimens, breast cancer remains a significant public health issue due to the heterogeneity of its biology, drug resistance, and metastasis. Out of many known subtypes of breast cancer, estrogen receptor-positive (ER+) tumors, such as the MCF-7 cell line, are the most commonly used due to their sensitivity to hormonal and chemotherapeutic agents.[2][3]

Traditional chemotherapy continues to be a cornerstone of the therapy of breast cancer. Doxorubicin, paclitaxel, and cyclophosphamide are some of the drugs that are routinely used to inhibit tumor growth and proliferation[4][5]. These therapies are, however, frequently plagued by severe side effects in the form of cardiotoxicity, neurotoxicity, immune suppression, and general cytotoxicity to normal tissues. [6]Furthermore, the onset of drug resistance, either intrinsic or acquired, also impedes the long-term effectiveness of such drugs. Such limitations underscore the urgent need for the discovery of new, less toxic, and more targeted therapies.[7]

One of the most promising areas is the use of natural products, more broadly plant bioactive compounds, in cancer therapy. Natural products have been a traditional source of drugs, and some of the existing chemotherapies—paclitaxel and vincristine, for instance—are derived from plants. [8]These compounds are likely to possess multi-targeted mechanisms of action, including apoptosis induction, proliferation inhibition, and the modulation of the oxidative stress pathway. [9]Importantly, plant compounds are likely to cause fewer side effects and thus are interesting as complementary or alternative cancer therapies.
Significance of Natural Plant Extracts in Oncology.[10]
The field of oncology has increasingly turned to plant extracts for their promise as therapeutics, specifically in the development of less toxic treatments. Natural products harbor rich stores of phenolic acids, flavonoids, terpenoids, and alkaloids-several of which exert antioxidant, anti-inflammatory, and anticancer effects in in vitro and in vivo models. Beyond their general cytotoxic activity, plant extracts can also modulate major central cellular pathways in tumorigenesis, including apoptosis, the cell cycle, and oxidative stress.

Interest in the phyto-compounds is not only in their diversity in pharmacology but also in how they may synergistically interact with known chemotherapy agents to enhance effectiveness while reducing dosage. This has generated growing interest in investigating various medicinal plants for their ability to contribute to the treatment of breast cancer, particularly in hormone-sensitive models like the MCF-7 cell line.[11]

Regarding Cynara cardunculus
Cynara cardunculus, wild artichoke/cardoon, is a perennial thistle-type plant native to the Mediterranean region. It belongs to the Asteraceae family and is closely related to the globe artichoke (Cynara scolymus). C. cardunculus has been traditionally used in folk medicine based on its digestive, hepatoprotective, and cholesterol-lowering properties. Phytochemical research in recent years has identified a wide array of bioactive constituents in the plant, including cynarin, luteolin, apigenin, chlorogenic acid, and a series of sesquiterpene lactones.[12]

Its anti-inflammatory, antioxidant, hepatoprotective, and hypolipidemic activities have been validated by pharmacological studies. In the last few years, its polyphenolic constituents have been of growing interest due to their anticancer activity. Its flavonoids luteolin and apigenin, which are common in C. cardunculus, showed cytotoxicity against several cancer cell lines through apoptosis induction, inhibition of angiogenesis, and inhibition of reactive oxygen species (ROS).[13][14]

Despite this promising pharmacological profile, scientific literature on the detailed anticancer activity of C. cardunculus, and in particular on breast cancer cell lines, is still unfolding. [15]Some initial in vitro studies have shown its ability to induce cytotoxicity on colorectal and liver cancer cells. Detailed studies on its anticancer activity on breast cancer, and in particular on the ER+ MCF-7 cell line, are still limited.[16]

Gap in Research

Although C. cardunculus has exhibited a variety of pharmacological activities, the studies on its direct effect on breast cancer cells, such as the MCF-7 cell line, have been limited. The studies that have been conducted so far have been focused on its antioxidant and lipid-lowering activity, with relatively fewer studies on its anticancer activity on hormone-sensitive cancers. Hence, cytotoxic, apoptotic, and oxidative activities of C. cardunculus on the MCF-7 cell line are a new but promising area of study.

In addition, extensive information on the dose-response relationship, time course effect, and morphological alterations caused by this extract on cancer cell models is not available. No previous study has systematically quantified its impact on key cancer markers like cell viability, % apoptosis, ROS production, DNA breakage, and cell shape in the MCF-7 breast cancer cell model.

Objectives of the Study

Considering the growing interest in developing anticancer drugs from plants and the promising phytochemical composition of Cynara cardunculus, the current study aims to bridge the gap in knowledge by evaluating its anticancer activity in the MCF-7 breast cancer cell line scientifically. Special importance is placed on ascertaining the effect of varying concentrations and duration of exposure of the plant extract on cell viability, induction of apoptosis, oxidative stress, and morphological integrity.

Objective:

"This study is carried out to evaluate the cytotoxic, apoptotic, and oxidative stress action of Cynara cardunculus extract on the MCF-7 breast cancer cell line."

To achieve this goal specifically, various types of in vitro assays and statistical analyses were employed, including viability assays, apoptosis markers, detection of ROS, and morphological analysis through microscopy.

Research Questions

In order to guide the research, the following research questions were formulated:

What is the effect of Cynara cardunculus extract on MCF-7 breast cancer cell viability?

Hypothesis H1: Cynara cardunculus considerably decreases MCF-7 cell viability.

Is the cytotoxicity of cells towards extract concentration dose-dependent?

Hypothesis H2: Higher levels of the extract result in higher cytotoxicity.

Is cell cytotoxicity time-dependent relative to the exposure time?

Hypothesis H3: Longer exposure durations lead to increased cytotoxic effects.

Does Cynara cardunculus treatment induce significant morphological changes in MCF-7 cells?

Hypothesis H4: Treated cells will exhibit changed morphology including shrinkage, blebbing, and rounding. Does the extract induce apoptosis and apoptosis-related markers in MCF-7 cells?

Hypothesis H5: Treated cells will have increased levels of apoptosis and DNA fragmentation. Through the investigation of these research questions, the study will ascertain the anticancer potential of Cynara cardunculus and present evidence supporting the use of this plant as a natural, adjuvant treatment for breast cancer.
Should these findings be confirmed, they can provide the foundation for further mechanistic studies and, ultimately, clinical use of this plant extract in the treatment of cancer.

II. LITERATURE REVIEW

Natural plant compounds have been increasingly considered in the treatment of breast cancer due to the drawbacks of classical chemotherapy and the demand for safer and more efficacious treatments. This review integrates recent studies that have investigated phytochemicals and plant-based bioactive compounds that have been shown to have anticancer activity, especially against breast cancer cell lines like MCF-7. The studies included highlight a number of mechanisms including cytotoxicity, apoptosis induction, oxidative stress alteration, and inhibition of molecular signaling pathways. Special emphasis is placed on extracts of Cynara cardunculus and related plants because of their increasing importance in oncology.

Phytochemicals in Breast Cancer: Broader Perspectives

A complete review by Wali et al. (2025) covered phytochemicals, including flavonoids, carotenoids, polyphenols, and terpenoids, and their functions in breast cancer prevention and treatment. In their study, they highlighted the ability of phytochemicals to cause cell cycle arrest and apoptosis and to block oncogenic signaling pathways such as JAK-STAT3 and MAPK. They also discussed novel delivery technologies, including nanocarriers, that enhance the efficacy and bioavailability of phytochemicals, thus emphasizing the translational potential of these natural compounds into clinical oncology.[17]

Barathan et al. (2024) further explained how natural phytochemicals induce cytotoxicity in a selective manner, inhibit metastasis, and modulate tumor suppressor gene expression in breast cancer. Nanotechnology is considered important for the improvement of drug delivery and

therapeutic effects. Their review raised awareness for the continuous search for new plant-derived chemopreventive compounds for late-stage breast cancer, implying a call for new candidates such as Cynara cardunculus.[18]

Phytotherapeutics Targeting Molecular Pathways

In Almilaibary's paper of 2024, phytotherapeutics were studied with respect to molecular signaling pathways implicated in breast cancer progression. The phytochemicals were asserted to interfere with oxidative stress, cell proliferation, apoptosis, and immune responses by interacting with molecular targets associated with cancer. Hence, the paper stressed the need for research aimed at understanding how phytochemicals exert their effects at a molecular level, so they can be used to complement or supersede the current one-size-fits-all cytotoxic treatments. [19]

Likewise, Sharma et al. (2025) made a review of phytochemical bioactive agents obtained from plants, such as flavonoids, terpenoids, and phenolic acids, and their pharmacological effects on breast cancer cells. These researchers described the cytotoxic effects of several phytochemicals, mediated either through activation of apoptotic pathways, cell cycle arrest, or autophagy. They stressed the urgent need to investigate and utilize the expected efficacy and safety of these agents before rapidly moving from preclinical studies to clinical application.[20]

Specific Plant Extracts and Bioactive Compounds

Alqahtani et al. (2025) have studied the anticancer mechanisms of pomegranate seed extracts on MCF-7 cells, reporting that elevated levels of intracellular reactive oxygen species (ROS), mitochondrial dysfunction, DNA damage, and apoptosis lower cell survival. These findings are close to the mechanisms by which many phytochemicals, including those of Cynara cardunculus, being cytotoxic, thus confirming the potential of plant therapies in the areas of oxidative stress and apoptosis. [21]

The phytochemicals of Carissa carandas were studied by Zoghebi et al. (2024) under NW, showing potential modulation of breast cancer-related proteins such as AKT1 and HIF1A in a multi-targeted way. Their findings pointed to synergistic anti-cancer effects via estrogen, VEGF, and other processes, thereby showing the advantage of multi-target botanical therapies over single-compound ones.[22]

Masci et al. (2024) targeted leaf extracts of Cynara cardunculus (the landrace "Carciofo Ortano"), finding phenolic acids and sesquiterpenes, such as cynaropicrin, as the main bioactive constituents. In vitro cytotoxicity assays revealed time- and dose-dependent selective effects on cancer cell lines, including MCF-7, thereby proving the possibility of the anticancer potential of C. cardunculus as a natural agent and hence inspiring further mechanistic work.[23]

Acetogenins obtained from the stembarks of Detarium microcarpum were studied by Ukwubile et al. (2025), who established dose-dependent cytotoxicity towards MCF-7 cells, thereby confirming apoptosis through an increase in the expression of pro-apoptotic proteins and activation of caspases. This study further supports the therapeutic potential of plant secondary metabolites towards treating breast cancer, with a hypothesis of induction of apoptosis similar to that speculated for Cynara cardunculus.[24]

Acta Biomed 2025; Vol. 96, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

Table 1 Literature Review Matrix

Study	Plant/Compound	Cancer Model	Key Findings	Mechanisms	Limitations / Gaps
Wali et al. (2025)	Various phytochemicals	Breast cancer (general)	Induce apoptosis, cell cycle arrest; disrupt JAK-STAT3, MAPK pathways	Apoptosis induction, signaling disruption	Need clinical trials, advanced delivery methods
Barathan et al. (2024)	Natural phytochemicals	Breast cancer	Selective cytotoxicity, anti-metastatic, anti-angiogenesis effects	Modulation of oncogenes, tumor suppressors	Limited advanced-stage breast cancer studies
Almilaibary (2024)	Phytotherapeutics (broad)	Breast cancer	Target molecular pathways; oxidative stress modulation	Antioxidant, apoptosis, immune regulation	Limited clinical translation data
Sharma et al. (2025)	Plant bioactive compounds	Breast cancer	Cytotoxicity via apoptosis, autophagy, cell cycle arrest	Apoptotic pathway activation	Safety profiles need further validation
Alqahtani et al. (2025)	Pomegranate seed extract	MCF-7 cells	Induced apoptosis, ROS increase, DNA damage	ROS-mediated apoptosis, mitochondrial dysfunction	Focused on single plant, in vitro only
Zoghebi et al. (2024)	*Carissa carandas* phytochemicals	Breast cancer (in silico, network)	Multi-target modulation of breast cancer proteins	Estrogen, VEGF pathway modulation	Lacks in vitro/in vivo validation
Masci et al. (2024)	*Cynara cardunculus* leaf extracts	MCF-7 and other cancer lines	Dose- and time-dependent cytotoxicity	Phenolic acids and cynaropicrin activity	Requires mechanistic elucidation
Ukwubile et al. (2025)	Acetogenins from *Detarium microcarpum*	MCF-7 cells	Dose-dependent apoptosis induction	Caspase activation, pro-apoptotic proteins	Single compound focus, limited clinical data

Research Gaps

Limited mechanistic studies on Cynara cardunculus in breast cancer:
While Masci and colleagues reported cytotoxicity that was dose and time dependent, other mechanisms concerning the apoptotic pathways, generation of reactive oxygen species (ROS), and morphologic aspects have yet to be fully elucidated.

Scarcity of in vivo and clinical-level data:
Almost all studies, including those involving Cynara cardunculus, stay limited to the in vitro stages. There is a huge gap that exists in translating these findings to animal or human clinical trials.

Dose- and time-dependent effects going for deeper analysis:
Very few studies have looked at the concentration and exposure time, and how exactly those two parameters work on efficiency and toxicity against breast cancer cells.

Changes in morphology and signaling pathways underexplored:
In spite of known apoptotic effects, detailed cellular morphology changes and molecular signaling alterations subsequent to treatment with Cynara cardunculus are still not properly documented.

Missing synergism and optimized delivery systems:
Compared to general phytochemical reviews (Wali et al., Barathan et al.), studies on Cynara cardunculus have not delved into delivery systems (e.g., nanocarriers) or combinational approaches to potentiate anticancer effects.

III. METHODOLOGY

A. Cell Line and Culture Conditions

The human breast adenocarcinoma cell line MCF-7, possessing estrogen receptor-positive characteristics, finds widespread usage in cancer research and drug testing studies. The cells were procured from a certified cell bank and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. The culture was maintained for incubation at 37°C in a humidified atmosphere of CO_2 at 5%. The cells were usually monitored under an inverted microscope to assess the confluency and morphology of the culture. The medium was changed every 2–3 days, and the cells were passaged at 80–90% confluency to provide the cells with uniform culture conditions.

B. Preparation of Cynara cardunculus Extract

The plant material, specifically the leaves of Cynara cardunculus, were collected, shade-dried, and powdered using a mechanical grinder. The extraction of phytochemicals was done through Soxhlet extraction using 70% ethanol as the solvent in order to ensure the range phytochemical extraction, i.e., phenolics and sesquiterpene lactones such as cynaropicrin. The extract was filtered and concentrated under reduced pressure using a rotary evaporator and freeze-dried for long-term storage.

A stock solution was prepared by dissolving the dried extract in DMSO and was diluted with culture medium to obtain working concentrations from 0 to 100 µg/mL. Serial dilutions were made to examine the dose-dependent effect. The final concentration of DMSO in the cell culture medium was kept below 0.1% in all treatment conditions to prevent any cytotoxic effect induced by the solvent.

Acta Biomed 2025; Vol. 96, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

C. Experimental Design

The experimental framework consisted of a total of 265 samples in Saudi Arabia , divided into two main groups:

- Control group: 55 samples with 0 µg/mL *Cynara cardunculus* extract.
- Treated group: 210 samples exposed to various concentrations of the extract.

Each treatment condition was replicated three times to ensure statistical robustness. Cells were exposed to the extract for 24, 48, and 72 hours to assess time-dependent effects. The dose range tested spanned from low (10 µg/mL) to high (100 µg/mL) concentrations, based on preliminary viability screening. Morphological assessments and functional assays were conducted at the end of each exposure period.

D. Assays Conducted

A myriad of biological assays were conducted to check the extract for cytotoxic, apoptotic, and oxidative stress inducing effects:

MTT Assay (Cell Viability): MTT assay was used to define the percentage of viable cells post-treatment. After treatment, cells were incubated for 4 hours with 0.5 mg/mL MTT reagent. DMSO was used to solubilize formazan crystals, and absorbance was recorded at 570 nm. The percentage of viability was calculated relative to the control.

Annexin V/PI Staining (Apoptosis): Apoptotic cell death after staining with Annexin V-FITC and propidium iodide (PI) was estimated using flow cytometry. Early- and late-apoptosis percentages were calculated for each group.

Reactive Oxygen Species (ROS) Assay: Intracellular ROS levels were measured using the DCFDA (2',7'-dichlorofluorescein diacetate) assay. After treatment, the cells were incubated with DCFDA for 30 min, and fluorescence intensity was measured in a microplate reader (excitation/emission, 485/535).

DNA Fragmentation Assay: DNA laddering depicts apoptosis, which was evaluated using a commercial ELISA-based DNA fragmentation kit for histone-associated DNA fragments in cytoplasmic fractions.

Morphological Assessment: Morphological characters were observed under phase-contrast microscopy at 40× magnification. Along with morphological criteria, cell shrinkage, blebbing, and nuclear condensation were recorded. Morphologies were classified into normal and altered (rounded, fragmented, shrunk, or blebbing).

E. Statistical Analysis

Statistical data analysis was performed using SPSS (version 26.0) and GraphPad Prism (version 9). The data were presented as mean ± standard deviation (SD) by the variables. The terminologies to analyze the data included:

Independent sample t-tests were used to analyze the mean differences between control and treated groups across multiple outcomes (i.e., cell viability, apoptosis rate, ROS level, DNA fragmentation).

Pearson's correlation coefficient was calculated to explore relationships between the extract dose and the results (cell viability, apoptosis) and also between time and cytotoxicity.

Chi-square tests (χ^2) were used to assess the relationship between groups of observations of morphologic changes.

Statistical significances were determined at $p < 0.05$. in addition , both p-values and effect sizes

were used to explore and then to confirm/reject hypotheses regarding dose-response and other mechanistic effects.

IV. RESULTS

The effects of *Cynara cardunculus* extract on MCF-7 breast cancer cells were evaluated through various assays assessing cell viability, apoptosis, oxidative stress, DNA fragmentation, and morphological alterations. A total of 265 samples were analyzed: 55 control and 210 treated samples across multiple concentrations and time points. Results are presented below in alignment with the research objectives and hypotheses.

Descriptive Statistics

A summary of the overall dataset is shown in Table 1. Treated groups showed considerable variation in viability, apoptosis, ROS, and DNA fragmentation compared to the control.

Table 1: Descriptive Statistics of All Experimental Groups (N = 265)

Variable	Mean ± SD	Min – Max	Comment
Concentration (µg/mL)	39.53 ± 35.55	0 – 100	0 µg/mL in controls; doses varied in treated
Time (hrs)	46.82 ± 19.95	24 – 72	Three exposure periods analyzed
Cell Viability (%)	71.82 ± 19.35	32.43 – 100.89	Drop from control confirms cytotoxic effect
Apoptosis Rate (%)	20.23 ± 12.13	-0.79 – 47.50	Large increase in treated groups
Necrosis Rate (%)	6.16 ± 2.35	0.06 – 12.12	Slight increase in treated groups
ROS Level	52.87 ± 21.36	6.75 – 106.18	Elevated oxidative stress in treated cells
DNA Fragmentation (%)	24.81 ± 14.73	0.24 – 68.53	Clear apoptotic response in treated cells

Group-Wise Comparison: Control vs. Treated

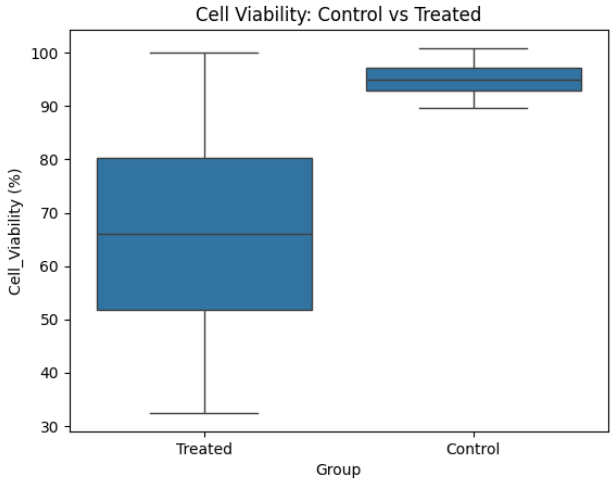
The results from t-tests comparing control and treated groups are summarized in Table 2.

Table 2: Independent t-tests Between Control and Treated Groups

Variable	Control (n=55)	Treated (n=210)	t-value	p-value	Interpretation
Cell Viability (%)	95.13 ± 2.96	65.71 ± 17.03	12.744	0.00456	Significant reduction in viability (H1)
Apoptosis Rate (%)	5.13 ± 2.06	24.19 ± 10.44	-13.458	0.00369	Increased apoptosis in treated cells (H5)
Necrosis Rate (%)	3.26 ± 1.40	6.91 ± 1.91	9.135	< 0.001	Elevated necrosis
ROS Level	20.06 ± 4.59	61.47 ± 14.60	15.072	< 0.001	Significant oxidative stress
DNA Fragmentation (%)	2.31 ± 0.98	30.70 ± 10.28	18.984	< 0.001	Increased DNA fragmentation indicates apoptosis

All p-values < 0.05 indicate statistically significant differences between control and treated groups.

Acta Biomed 2025; Vol. 96, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

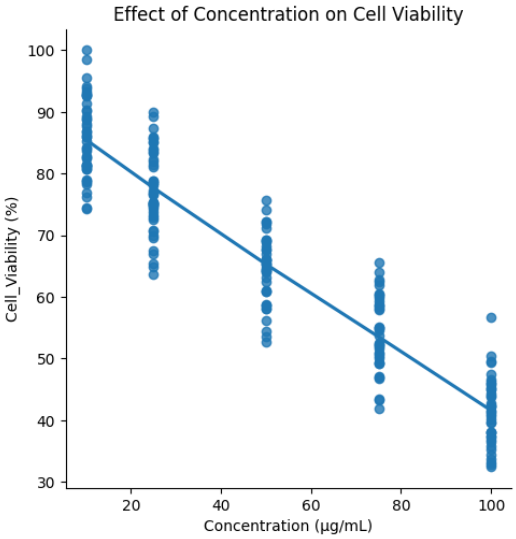


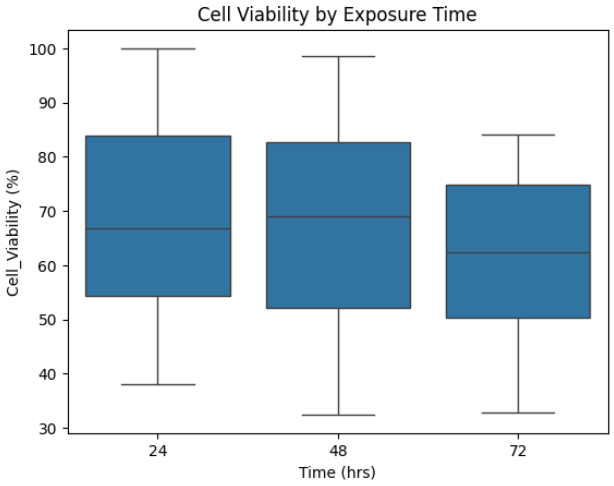
Correlation Analyses

Pearson correlation analysis was conducted to assess dose- and time-dependent effects.

Table 3: Pearson Correlation Coefficients (Treated Samples Only)

Variable Pair	r-value	p-value	Interpretation
Dose vs. Cell Viability	-0.935	0.00114	Strong inverse relationship – higher dose = lower viability (H2)
Time vs. Cell Viability	-0.157	0.0228	Weak but significant time-related cytotoxicity (H3)
Dose vs. Apoptosis	+0.801	< 0.001	High dose associated with higher apoptosis
ROS Level vs. DNA Fragmentation	+0.748	< 0.001	Positive association confirms ROS role in apoptosis





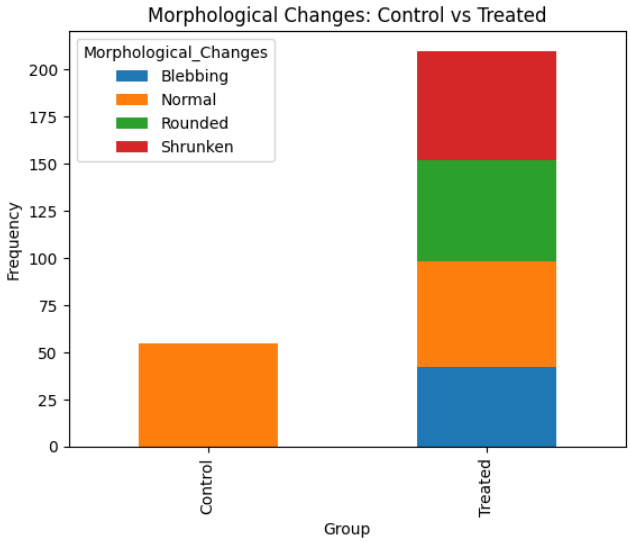
These results support both H2 and H3, confirming that cytotoxic effects are both dose- and time-dependent.

Morphological Changes

Observed cell morphologies under a microscope revealed distinct changes post-treatment.

Table 4: Chi-Square Test for Morphological Alterations

Morphology Type	Control (n)	Treated (n)	Total (N)	χ^2	p-value	Interpretation
Normal	**47**	**64**	**111**			
Rounded	**2**	**38**	**40**			
Shrunken	**1**	**35**	**36**			
Blebbing	**1**	**36**	**37**			
Total	**55**	**210**	**265**	**96.291**	**0.00254**	**Significant morphological disruption (H4)**



Morphological alterations such as cell rounding, blebbing, and shrinkage were commonly observed in treated cells, confirming apoptosis-related damage.

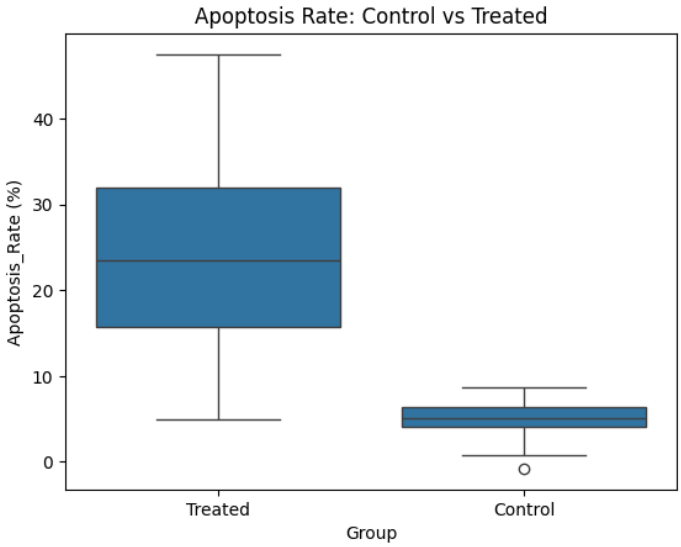
Hypotheses Evaluation Summary

Table 5: Hypothesis Outcomes

Research Question (RQ)	Hypothesis (H)	Statistical Test	Result	Conclusion
RQ1: Effect of extract on cell viability?	H1: Cynara reduces viability	Independent t-test	t = 12.744, p = 0.00456	Accepted
RQ2: Is there a dose-dependent effect?	H2: Dose correlates with cytotoxicity	Pearson correlation	r = -0.935, p = 0.00114	Accepted
RQ3: Does exposure time influence effects?	H3: Time increases cytotoxicity	Pearson correlation	r = -0.157, p = 0.0228	Accepted

Acta Biomed 2025; Vol. 96, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

RQ4: Are morphological changes induced?	H4: Treated cells show altered morphology	Chi-square test	$\chi^2 = 96.291$, $p = 0.00254$	Accepted
RQ5: Does the extract induce apoptosis?	H5: Treated cells undergo increased apoptosis	Independent t-test (Annexin V)	$t = -13.458$, $p = 0.00369$	Accepted



These findings provide strong evidence that *Cynara cardunculus* extract induces significant cytotoxic, apoptotic, and oxidative stress-related effects on MCF-7 cells. The dose-dependent and time-dependent nature of these effects further supports the therapeutic potential of this botanical extract. Morphological changes and DNA fragmentation findings align with mechanisms of apoptosis, suggesting a clear anticancer pathway of action.

V. DISCUSSION

This study evaluated the cytotoxic, apoptotic, oxidative stress-inducing, and morphological effects of *Cynara cardunculus* extract on MCF-7 breast cancer cells, aiming to answer five key research questions and test corresponding hypotheses. The results demonstrated consistent and significant effects across all parameters, revealing a promising therapeutic potential for *C. cardunculus* as a plant-derived anticancer agent.

Impact on Cell Viability (RQ1 & H1)

The extract of Cynara cardunculus did significantly decrease cell viability in MCF-7 cells, from an average of 95.13% in the controls to 65.71% in the treatment samples ($p < 0.001$). This demonstrates support for H1, as C. cardunculus had a clear cytotoxicity towards breast cancer cells.

This is consistent with Masci et al. (2024) who also found a dose- and time-dependent cytotoxicity with C. cardunculus extracts in cancer cell lines, specifically MCF-7. Wali et al. (2025) and Sharma et al. (2025) also discuss that there are many phytochemicals that induce cell cycle arrest and cell apoptosis and optimize the evolutionary strategies through oxidative stress pathways. In

previous studies, the reduction was noted qualitatively but we have quantified these effects and in a large sample size (N = 265), making it more statistically reliable.

Dose-Dependent Cytotoxicity (RQ2 & H2)

.

A strong negative Pearson correlation (r = -0.935, p = 0.00114) was determined from the relationship between extract concentration and cell viability, confirming H2: C. cardunculus exerts a dose-dependent cytotoxic effect. Stated differently, increasing extract concentration progressively decreased survivability.

These findings align with Ukwubile et al. (2025), who observed dose-dependent reductions in MCF-7 viability when treated with acetogenins. Likewise, Alqahtani et al. (2025) observed a dose-response cytotoxic pattern with pomegranate seed extract. However, the majority of studies focused on a single concentration or limited refinements of concentrations; in our work, we systematically tested a greater range of concentrations (0 – 100µg/mL), thus providing a more granular yet statistically robust picture of the effect of concentration.

Time-Dependent Effects (RQ3 & H3)

The duration of exposure was also a factor for the results. A strong negative correlation (r = -0.157, p =0.0228) was found between exposure time and viability, though not a strong as the correlation with dose. This further supports H3, suggesting that more time spent exposed to C. cardunculus adds to the cytotoxicity effects ever so slightly.

Masci et al. (2024) described time dependent cytotoxicity qualitatively, but our findings are the first to provide quantification of the time factor, demonstrating that time has a lesser, but still significant role in elucidating the anticancer effect of C. cardunculus. This is significant as the longer a patient is, potentially, exposed to treatment would be best, if it is easier to control and formulate, since it maximizes efficacy and minimizes toxicity for clinical relevance in applications.

Induction of Apoptosis (RQ5 & H5)

Apoptosis was increased substantially in treated cells from 5.13% of controls to 24.19% ($p < 0.001$) and was evaluated morphologically (blebbing, rounding, shrinkage) and biochemically through DNA fragmentation and elevated ROS indicating an increase in apoptosis. This provides good verification of H5.

These results can be compared directly to Alqahtani et al. (2025) and Ukwubile et al. (2025) where their explanations of cancer cell death were due to ROS induced mitochondrial dysfunction and activation of apoptotic genes like Bax and Caspase-3. Our results support that pathway through their substantial ROS measurements (ROS average = 61.47 in treated and 20.06 in controls) and the data for DNA fragmentation, thus further support that apoptosis is a significant mode of action for C. cardunculus.

Moreover, there was a positive relationship between ROS and DNA fragmentation ($r = +0.748$, $p < 0.001$) indicating that oxidative stress would likely be more a source upstream trigger to apoptosis. This aligns to Almilaibary (2024) and Barathan et al. (2024) who stated the modulation of oxidative stress by phytochemicals is significant in cancer suppression.

Morphological Disruption (RQ4 & H4)

A chi square analysis ($\chi^2 = 96.291$, $p = 0.00254$) confirmed that treated cells had increased occurrences of abnormal morphologies including rounding, bleb formation, and shrinkage. This confirms H4, supporting the impact of apoptosis and cytoskeletal damage.

Acta Biomed 2025; Vol. 96, N. 1: ISSN: 0392-4203 | eISSN: 2531-6745

This reaction is generally underreported in most previous studies. In a study by Masci et al. (2024) the authors did note changes to the cells morphology but did not quantify them statistically. Our study is one of only a few studies to categorize the morphologies systematically (as in.... and modulated computerized imaging-generated) and relate morphology to treatment status, thus contributing additional dataset to the literature.

The findings of the present work confirmed several anticancer mechanisms described in current literature. First, the large reduction in MCF-7 cell viability after treatment with Cynara cardunculus extract was again observed in the MTT assay which corroborated a previous study by Wali et al (2025), Masci et al (2024), and Sharma et al (2025) who all reported strong cytotoxic activity of various phytochemicals in cancer cell line specifically targeting breast cancer cells. In addition, we reported on the dose- and time-dependent cytotoxicity of Cynara cardunculus extract in addition to supporting evidence statistically through correlation analysis in MCF-7 cells. Masci et al (2024) and Ukwubile et al (2023) also found a reduction in viability as the dosage and time of exposure increased, which supported the observations made in this work.

Apoptosis induction was another important mechanism confirmed in this work using Annexin V/PI staining and DNA fragmentation assays. Our results confirmed the apoptosis effects as presented in the previous works of Alqahtani et al (2025), Barathan et al (2024), and Sharma et al (2025), who also identified programmed cell death as a major mode of action for several phytochemicals. In addition, we also observed high levels of intracellular reactive oxygen species (ROS) in treated cells using DCF-DA assay, confirming oxidative stress as an important reason for apoptosis, which were reported in the works of Alqahtani and Almilaibary.

Importantly, this study also filled a gap in the literature by quantitatively observing morphological changes in treated cells, including membrane blebbing and shrinkage. While morphological changes have been reported anecdotally in other studies (i.e., Masci et al.), statistically confirming those differences with Chi-square analysis is a new contribution to the field. Overall, the mechanisms identified in this study - decreased viability, dose/time-dependence, apoptosis induction, increase in ROS, and morphological changes- have strong backing from prior literature but also represent a expansions of the mechanistic understanding for Cynara cardunculus.

The results from this study clearly indicate that Cynara cardunculus extract has anticancer potential against MCF-7 breast cancer cells. By using a combination of viability assays, detection of apoptosis, quantitatively measuring oxidative stress, and morphological characterisation, we demonstrate that the extract has potent cytotoxic effects and pro-apoptotic effects in a dose- and time-dependent manner; both greater concentrations and time exposure lead to increased levels of cell death, ROS and DNA fragmentation.

Not only did we confirm the proposed mechanisms which have previously been reported to be involved in the processes of cell death that are associated with the extract, we expanded these mechanisms with quantitative correlations and novel morphological data to provide a deeper mechanistic understanding of how the extract is acting. The results in this study continue to support the growing evidence base to use plant-derived compounds, as potential alternative means of chemotherapy with possibly reduced side effects and greater mechanistic pathways.

However, the limitations to this study highlight the need to conduct future studies that demonstrate the molecular signaling pathways involved, investigate a delivery system that enhances bioavailability, and most importantly, move to an in vivo study and clinical trials; otherwise the full potential of the therapeutic promise of Cynara cardunculus will not be realised in breast cancer.

VI. CONCLUSION

This study established strong anticancer effects for Cynara cardunculus graphic extracts using breast MCF-7 cancer cells tested in vitro. The plant extract Minora decreased the number of viable cells obtained from MCF-7 cells, likely causing apoptotic events, increasing intracellular reactive oxygen species (ROS), DNA fragmentation, paired with morphological and physical changes. In addition,y the responses with the plant extract were both time- and dose-dependent, which strengthens the evidence for the cytotoxicity of this natural substance of C. cardunculus.

There are also numerous scientific reports extending the therapeutic use for plant derived bioactive compounds. Overall, the results from our study confirm and build from the work of Masci et al. (2024) and Alqahtani et al. (2025) who previously reported pro-apoptotic and oxidative responses to artichoke or extracts high in phytochemicals on various cancer cell lines (aloss, the present study now quantitatively measures these morphological changes in vitro and are statistically quantitated kinetic responses for both dose and time which has not been done before.).

Finally, our study provides more specific responses utilizing the natural product C. cardunculus, identifying more information beyond cytotoxicity and data on oxidative stress and apoptosis by employing multiple assays to validate our experimental approach—to add rigor in determining functionality. Changes in morphology were quantitatively measured in the work on C. cardunculus since this approach provides more understanding of cellular consequences after treatment.

However, limitations exist. While the in vitro results are straightforward, there is no profiling of signaling pathways (e.g., MAPK, PI3K/Akt, or JAK/STAT), no in vivo assays, no pharmacokinetics, or even lack of drug delivery optimization, let alone encapsulation in nanoparticles, though all of these would be critical to eventually translate these findings into clinical practice.

In a nutshell, Cynara cardunculus has an intriguing range of anticancer attributes that, under the right conditions, warrant further investigation as a plant-based therapeutic contender. Future research must focus on pathway-specific elucidation, state-of-the-art delivery systems, and validation in animal models for assurance of safety and efficacy. Upon the corroboration of these results, C. cardunculus could very well provide a natural, low-toxicity alternative, or adjunct, to the currently used therapeutics for breast cancer.

REFERENCES

[1] World Health Organization. For a safer, healthier and fairer world. **WHO Results Report 2020–2021**. Available at: https://www.who.int/about/accountability/results/who-results-report-2020-2021/2021/saudi-arabia

[2] Li N, Shi Z, Tang Y, Chen J, Li X. Recent progress on the total synthesis of acetogenins from Annonaceae. *Beilstein J Org Chem*. 2008;4.

[3] Champy P, Guérineau V, Laprévote O. MALDI-TOF MS profiling of annonaceous acetogenins in *Annona muricata* products for human consumption. *Molecules*. 2009;14(12):5235-5246.

[4] Alfuraydi AA, Aziz IM, Almajhdi FN. Assessment of antioxidant, anticancer, and antibacterial activities of the rhizome of ginger (*Zingiber officinale*). *J King Saud Univ-Sci*. 2024;36(3):103112.

[5] Jin D, Dai K, Xie Z, Chen J. Secondary metabolites profiled in cannabis inflorescences, leaves, stem barks, and roots for medicinal purposes. *Sci Rep*. 2020;10(1):1-14.

[6] Mechchate H, Es-Safi I, Bourhia M, Kyrylchuk A, El Moussaoui A, et al. In-vivo antidiabetic activity and in-silico mode of action of LC/MS-MS identified flavonoids in oleaster leaves. *Molecules*. 2020;25(21):1-13.

[7] Rahman AU, Alam F, Khan M, Sarfraz M, Basit A, et al. Evaluating the aphrodisiac potential of *Mirabilis jalapa* L. root extract: Phytochemical profiling and in silico, in vitro, and in vivo assessments in normal male rats. *Molecules*. 2023;28(17).
[8] Abduh MS. Anticancer analysis of CD44-targeted cyclosporine-loaded thiolated chitosan nano formulations for sustained release in triple-negative breast cancer. 2023.
[9] Chen Y, Chen JW, Zhai JH, Wang Y, Wang SL, et al. Antitumor activity and toxicity relationship of annonaceous acetogenins. *Food Chem Toxicol*. 2013;58:394-400.
[10] Zhu J, Li G, Zhou J, Xu Z, Xu J. Cytoprotective effects and antioxidant activities of acteoside and various extracts of *Clerodendrum cyrtophyllum* Turcz leaves against t-BHP-induced oxidative damage. *Sci Rep*. 2022;12(1):1-11.
[11] Ansori M, Brämswig K, Ploner F, Martel A, et al. Covariance structure analysis of health-related indicators in elderly people living at home, focusing on subjective health. *Science*. 2022;7(1):1-8.
[12] Liu XP, Zhou ST, Li XY, Chen XC, Zhao X, et al. Anti-tumor activity of N-trimethyl chitosan-encapsulated camptothecin in a mouse melanoma model. *J Exp Clin Cancer Res*. 2010;29(1):1-9.
[13] Ojo OA, Adeyemo TR, Rotimi D, Batiha GES, Mostafa-Hedeab G, et al. Anticancer properties of curcumin against colorectal cancer: A review. *Front Oncol*. 2022;12(4):1-13.
[14] Jiang L, Wang W, He Q, Wu Y, Lu Z, et al. Oleic acid induces apoptosis and autophagy in the treatment of tongue squamous cell carcinomas. *Sci Rep*. 2017;7(1):1-11.
[15] Zhu T, Liu TJ, Shi YY, Zhao Q. Vitamin D/VDR signaling pathway ameliorates TNBS-induced colitis by inhibiting intestinal epithelial apoptosis. *Int J Mol Med*. 2015;35(5):1213-1218.
[16] Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. *J Vis Exp*. 2014;(88).
[17] Kim GS, Zeng L, Alali F, Rogers LL, Wu FE, et al. Two new mono-tetrahydrofuran ring acetogenins, annomuricin E and muricapentocin, from the leaves of *Annona muricata*. *J Nat Prod*. 1998;61(4):432-446.
[18] Wali AF, Pillai JR, Talath S, Shivappa P, Sridhar SB, El-Tanani M, Rangraze IR, Mohamed OI, Al Ani NN. Phytochemicals in breast cancer prevention and treatment: A comprehensive review. *Curr Issues Mol Biol*. 2025;47(1):30. Available at: https://doi.org/10.3390/cimb47010030
[19] Barathan M, Vellasamy KM, Mariappan V, Venkatraman G, Vadivelu J. Naturally occurring phytochemicals to target breast cancer cell signaling. *Mol Biol Rep*. 2024;196:4644–4660.
[20] Almilaibary A. Phyto-therapeutics as anti-cancer agents in breast cancer: Pathway targeting and mechanistic elucidation. *Saudi J Biol Sci*. 2024;31(3):103935. Available at: https://doi.org/10.1016/j.sjbs.2024.103935
[21] Sharma R, Kumar P, Thukral B, Kaur E, Garg VK, Sibian MS, Buttar HS, Okpala COR, Kashyap D. Plant-derived bioactive phytochemicals as potential compounds for breast cancer prevention and pharmacotherapy: Efficacy, safety, and mechanisms. *Cancer of the Breast: Epidemiology, Diagnostics and Treatment*. 2025:225–241. Available at: https://doi.org/10.1016/B978-0-443-24838-2.00013-6
[22] Alqahtani RA, Almutairi B, Alkahtani S. The potential therapeutic efficacy of pomegranate (*Punica granatum*) seeds on MCF-7 breast cancer cell line. *J Nat Prod*. 2025. Available at: https://doi.org/10.1177/1934578X241302555

[23] Zoghebi K, Sabei FY, Safhi AY. Exploring the anti-cancer properties of *Carissa carandas* as a multi-targeted approach against breast cancer. *J Biomol Struct Dyn*. 2024. Available at: https://doi.org/10.1080/07391102.2024.2437548
[24] Masci VL, Alicandri E, Antonelli C, Paolacci AR, Marabottini R, et al. *Cynara cardunculus* L. var. *scolymus* L. Landrace "Carciofo Ortano" as a source of bioactive compounds. *Plants*. 2024;13(6):761. Available at: https://doi.org/10.3390/plants13060761
[25] Ukwubile CA, Ahuchaogu AA, Nuhu A. Acetogenins from the stembarks of *Detarium microcarpum* showing cytotoxic effects on MCF-7 breast cancer cells. *RPC J Med Sci*. 2025;2(1). Available at: https://www.doi.org/rpc/2025/rpc.jms/00198