Comparative Evaluation of Aqueous and Ethanolic Extracts of *Cynara cardunculus* on Normal Cell Line HBL100 and Hepatocellular Carcinoma Cell Lines HCAM in Mice .

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Abstract :

This study aimed to investigate the cellular and biochemical effects of both aqueous and ethanolic extracts of *Cynara cardunculus* L. (artichoke) on normal and mouse hepatoma cell lines. The extracts were prepared from dried artichoke leaves and evaluated for their potential to inhibit cancer cell growth using cell viability assays.

The results revealed that both extracts exhibited a dose-dependent inhibitory effect on the proliferation of hepatoma cells. The ethanolic extract showed a stronger cytotoxic activity compared to the aqueous extract, as indicated by a more pronounced reduction in cell viability and increased apoptotic markers such as nuclear condensation and caspase-3 activation. In contrast, neither extract showed significant toxicity toward normal cells at moderate concentrations, indicating a selective antiproliferative effect against cancer cells.

These findings suggest that the bioactive phenolic and flavonoid compounds, including cynarin, luteolin, and chlorogenic acid, are responsible for the observed effects, acting through the reduction of oxidative stress and modulation of signaling pathways associated with cell proliferation and apoptosis.

In conclusion, both aqueous and ethanolic extracts of artichoke demonstrate promising anticancer potential, with the ethanolic extract showing higher efficacy.

Further in vivo studies are required to confirm these findings and clarify the molecular mechanisms involved.

Keywords: *Cynara cardunculus*, aqueous extract, ethanolic extract, hepatoma cells, antioxidants, apoptosis, cytotoxicity.

Introduction:

Cynara cardunculus L., commonly known as wild artichoke or cardoon, is a perennial plant belonging to the Asteraceae family. In recent years, it has attracted considerable scientific interest due to its rich phytochemical composition and promising biological activitie(Fuhr *et al*., 2022) , The plant is a natural source of bioactive compounds such as caffeoylquinic acids, flavonoids, and sesquiterpene lactones like cynaropicrin, which have demonstrated significant antioxidant, anti-inflammatory, and anticancer properties (Ferreira *et al*., 2023) .

Numerous studies have reported that extracts from *C. cardunculus* exhibit potent antiproliferative and pro-apoptotic effects against various human cancer cell lines For example, (Porro *et al*., 2024) showed that lipophilic extracts from *C. cardunculus* leaves significantly reduced the proliferation of colorectal cancer cells (HCT116). The study also revealed that the extracts anticancer activity was modulated by circadian clock genes such as BMAL1, PER2, and NR1D1, suggesting a molecular interplay between phytochemicals and circadian rhythm regulation in tumor progression (Neyaz , 2025) .

Moreover, phytochemical investigations of the aerial parts of cultivated cardoon (*C. cardunculus*) have led to the isolation of several potent cytotoxic sesquiterpene lactones. (Hamza *et al*., 2022) isolated grosheimin and cynaropicrin, which exhibited strong anticancer activity against hepatocellular carcinoma (HepG2) cells, with IC_{50} values of 7.49 µg/mL and 13.9 µg/mL, respectively, Molecular docking studies confirmed that these compounds interact

with the catalytic sites of caspase-3, providing a mechanistic insight into their pro-apoptotic action (Salata *et al*., 2024).

Further mechanistic analyses indicated that *C. cardunculus* extracts may arrest the cell cycle at the G2/M phase, upregulate cell-cycle inhibitors such as p21, and suppress key oncogenic pathways, including Akt and NF-κB (Acquaviva *et al*., 2023), These findings support the potential of artichoke-derived phytochemicals as multi-target anticancer agents that can modulate several cellular signaling cascades involved in proliferation, apoptosis, and oxidative stress (Philippa *et al*., 2021).

Importantly, several studies have demonstrated that *C. cardunculus* extracts exert selective cytotoxicity, affecting cancer cells more strongly than normal human fibroblasts or epithelial cells, suggesting a favorable therapeutic index (Wang *et al*., 2020), However, most available data are limited to in vitro or preliminary in vivo models, and well-designed clinical trials are still lacking. Therefore, further investigations are necessary to confirm efficacy, determine optimal dosage, and evaluate bioavailability and potential side effects in humans (Ben Kaab *et al*., 2020).

In summary, *Cynara cardunculus* appears to be a promising source of natural anticancer agents. Through its diverse phytochemical constituents—particularly phenolic acids and sesquiterpene lactones—the plant exerts antiproliferative, pro-apoptotic, and antioxidant effects via multiple mechanisms , Continued exploration of *C. cardunculus* and its active components may contribute to the development of novel therapeutic or preventive strategies against cancer (Cheng & Liu,2024) .

Cynara cardunculus

Material and Methods:

Culturing of Cell Lines and Cell Toxicity Bioassays

Special Solutions for Primary and Secondary Tissue Culture

Stock Solution Used for Tissue Culture and Subculture

- Rosswell Park Memorial Institute (RPMI-1640) Medium
- RPMI-1640 with buffer, L-glutamine (10.4 g)
- Sodium bicarbonate (4.4%) – 15 mL
- (used to adjust the pH to 7.2)
- Penicillin – 0.5 mL
- Streptomycin – 0.5 mL
- Fetal Bovine Serum (FBS) (100 mL)

Complete the solution to one liter by adding distilled water, then sterilize with a 0.22 micron filter.

- **Antibiotics**

a- Penicillin solution containing 1 gram of penicillin in 5 mL of distilled water, then take 0.5 mL of it and add to one liter of culture medium and store the remainder at -20°C.

b- Streptomycin solution containing 1 gram of streptomycin in 5 mL of distilled water to bring the final concentration to 200 mg/mL, then take 0.5 mL of it and add to one liter of culture medium and store the remainder at -20°C.

- **Phosphate Buffer Saline (PBS)**

  Nacl 8 gm

  KCL 0.20 gm

  NaHPO4 0.92 gm

  KH2PO4 0.20 gm

  D.W. 100 µl

Sterilize in an autoclave at 121°C for 15 minutes, then store at 4°C until use.

- **Trypsin - Versene solution**

Trypsin solution 20 µl

Versene solution 10 µl

PBS 370 µl

Mix before use under sterile conditions and store at 4°C.

Cell Lines Used

Two cell lines were used in this study:

HBL-100: A normal human cell line derived from human milk epithelial cells during early lactation, showing no malignant features and maintaining a normal diploid karyotype.

HCAM (Hepatocyte carcinoma cell line): A liver cancer cell line isolated from hepatocellular carcinoma tissue.

Both cell lines were routinely maintained and subcultured to preserve their growth and viability.

Cell Culture Procedure

The HBL-100 and HCAM cell lines were obtained from the Iraqi Biotechnology Cell Bank (IRAQ Biotech) in Basrah Governorate. Upon arrival at the laboratory, the cells were subcultured at the sub-confluence stage using a 0.25% trypsin–EDTA solution for cell detachment. The cells were then transferred to sterile culture flasks containing 15 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 µg/ml of penicillin, and 100 µg/ml of streptomycin until they reached 80–90% confluence the mixture was gently mixed and then sterilely transferred to culture plates or sterile flasks, upon reaching full confluence (80-90%) , The culture medium was then aspirated, and the cell monolayer was washed with phosphate-buffered saline (PBS) Afterward, 1 ml of trypsin–EDTA was added, and the cells were incubated at 37°C for 2–5 minutes to allow complete detachment. The suspension was centrifuged, the supernatant discarded, and the cell pellet resuspended in fresh medium and redistributed into new culture flasks or plates for further subculturing , The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 (Freshney, 2010) .

Maintenance of Cell Cultures

The cell lines were routinely maintained to ensure their viability and stability The growth and morphology of the cells were monitored daily under an inverted microscope to assess cellular shape, confluence, and the absence of contamination indicators, The culture medium was replaced every 2–3 days, or whenever the color of the phenol red indicator changed, signaling nutrient depletion. Once the cells reached the optimal growth stage (subconfluence), they were subcultured following standard aseptic protocols (Al-Ali *et al*., 2022).

Cell Counting and Determination of Cell Density

Cell viability and density were estimated according to the protocol described by (Piccinini , 2017). A 20 µl aliquot of the cell suspension was mixed with 20 µl of 0.4% trypan blue stain, and viable and non-viable cells were counted using a Neubauer hemocytometer under an inverted microscope at ×10 and ×40 magnifications. Viable cells appeared colorless, whereas non-viable cells were stained blue The cells were then seeded at a density of 1×10^5 cells/ml (100 µl per well) and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 24–48 hours until a uniform monolayer was formed, suitable for subsequent .

Cytotoxicity test using MTT

(a) culture phase (Seeding)

The cells were harvested after reaching a homogeneous layer, washed twice with PBS solution, then 1 ml of trypsin was added to remove it, and the reaction was stopped by adding growth medium containing FBS. The cells were resuspended and distributed into 96-well plates at a density of 1×10^6 cells per well in 100 µL per well .

(b) Exposure Phase

Cells were exposed to aqueous and alcoholic extracts of both artichoke and wormwood at specified concentrations 2000 and 4000 µg (mL) to evaluate their toxic effects, Each compound was applied separately to cells of each cell line.

MTT Test Protocol

After 72 hours of exposure to the compounds, the medium was discarded and the cells were washed with 100 µL of PBS, The MTT solution (mg/ml) was counted in filtered PBS, and 10 µL of it was added to each well, along with 90 µL of serum-free medium, The plates were incubated for 2 hours at 37°C. The medium was then replaced with 130 µL of DMSO to dissolve the Formazan crystals, and

incubation was carried out for 20 minutes at room temperature (Al-Shammari *et al*., 2019) (Absorbance was measured at 620 nm using a plate reader (BioTek), USA), The assay was performed in triplicate, and the cell growth inhibition rate (cytotoxicity ratio) was calculated as follows:

(PR)= A/B*100

(PR) : Proliferation rate

A: Average optical density of untreated wells

B: Optical density of treated wells

The inhibition rate can be calculated from: **IR=100-PR**

Results:

The Effect of Aqueous and Ethanolic Extracts of Cynara cardunculus on the Normal Cell Line HBL100

The cytotoxic effects of the aqueous and ethanolic extracts of *Cynara cardunculus* leaves on the normal cell line HBL100 were evaluated using the MTT assay at two concentrations (2000 and 4000 µg/ml), with four replicates for each concentration. Absorbance was measured at a wavelength of 620 nm, and untreated cells were used as the control group, which recorded an absorbance value of 0.92586 nm.

The results indicated that the aqueous extract of *Cynara cardunculus* exhibited mild cytotoxic activity against the normal cell line, with cell viability percentages of 44.61% at a concentration of 2000 µg/ml and 47.80% at 4000 µg/ml.

In contrast, the ethanolic extract of *Cynara cardunculus* showed a stronger inhibitory effect on normal cells, with a cell viability of 86.93% at 2000 µg/ml, which decreased to 45.45% at 4000 µg/ml, as presented in Table (1) and Figure (1).

Table (1): The effect of Aqueous and Ethanolic extracts of *Cynara cardunculus* on normal cell lin HBL100

Extract type	Aqueous Extract		Ethanolic Extract	
Concentration	2000	4000	2000	4000
Rate of absorption	0.48325	0.51275	0.505	0.121
Rate of inhibition	47.8056	44.6194	45.4565	86.93116
SD±	0.02869	0.05924	0.0669	0.00497
Control	0.92586			
Type of test	MTT assay			
Absorbances	620 nm			
Cell line	Normal Cell Line			HBL100

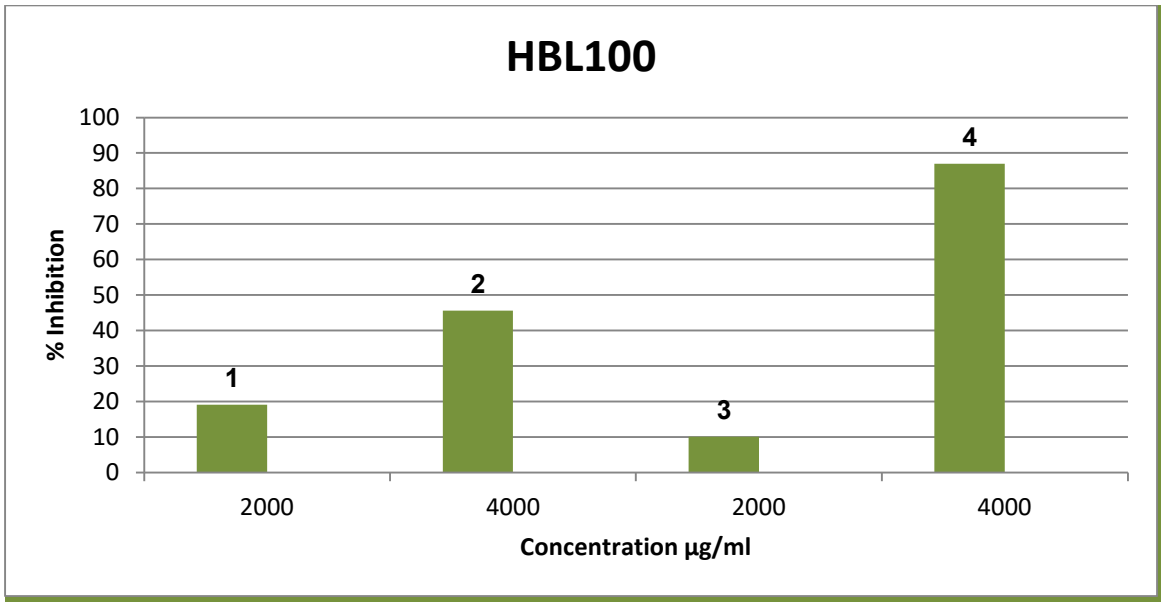

Figure (1): The effect of Aqueous and Ethanolic extracts of *Cynara cardunculus* on normal cell lin HBL100 : 1 and 2 aqueous extract , 3 and 4 ethanolic extract .

These findings indicate that both extracts of *Cynara cardunculus* influence the viability of normal cells in a dose-dependent manner, with the ethanolic extract showing comparatively stronger cytotoxic potential.

The Effect of Aqueous and Ethanolic Extracts of *Cynara cardunculus* on the Hepatocellular Carcinoma Cells HCAM .

The cytotoxic effects of the aqueous and ethanolic extracts of *Cynara cardunculus* leaves on the hepatocellular carcinoma cell line (HCAM) were evaluated using the MTT assay at two concentrations (2000 and 4000 µg/ml), with four replicates for each concentration. Absorbance was measured at a wavelength of 620 nm, and untreated cells were used as the control group, which recorded an absorbance value of 0.54368 nm.

The results indicated that the aqueous extract of Cynara cardunculus exhibited a strong cytotoxic effect against hepatocellular carcinoma cells, with cell viability decreasing to 49.08% at 2000 µg/ml and to 24.71% at 4000 µg/ml. The cytotoxicity increased with higher extract concentrations.

Similarly, the ethanolic extract showed a comparable cytotoxic trend, with cell viability recorded at 42.55% at 2000 µg/ml and decreasing to 20.73% at 4000 µg/ml, as shown in Table (2) and Figure (2).

Table (2): The effect of Aqueous and Ethanolic extracts of *Cynara cardunculus* on Hepatocyt Cancer Cell Line HCAM.

Extract type	Aqueous Extract		Ethanolic Extract	
Concentration	2000	4000	2000	4000
Rate of absorption	0.53025	0.51700	0.31233	0.10475
Rate of inhibition	24.7158	49.0861	42.5527	80.7334
SD±	0.0043	0.0120	0.090	0.0099
Control	0.54368			
Type of test	MTT assay			
Absorbances	620 nm			
Cell line	HEPATOCYT Cancer cell line			HCAM

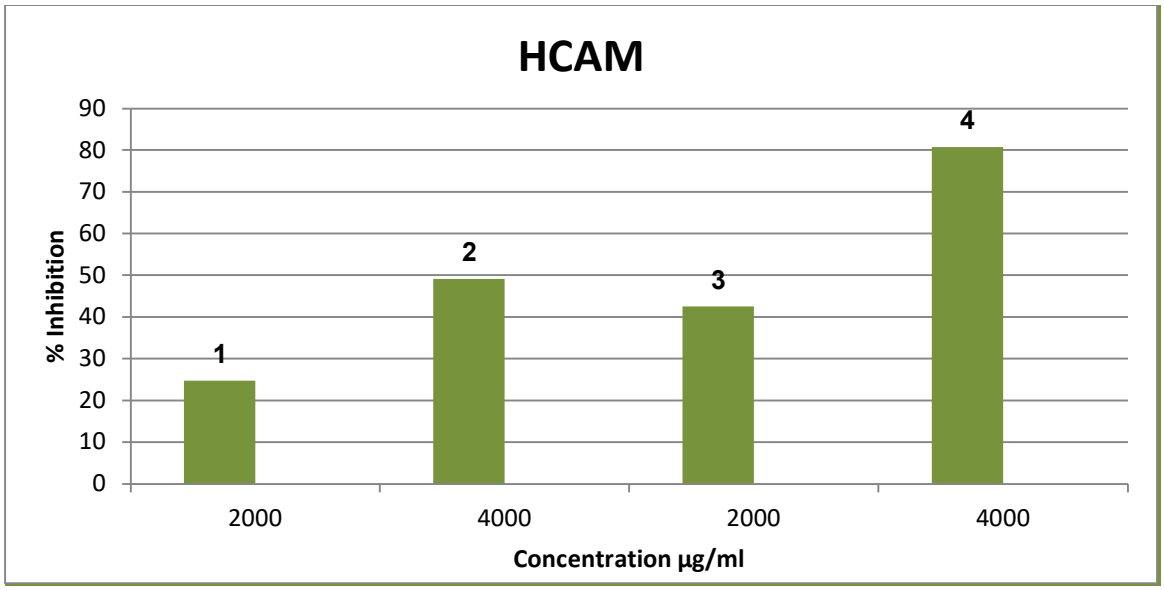

Figure (2): The effect of Aqueous and Ethanolic extracts of *Cynara cardunculus* on Hepatocyt cancer cell line HCAM : 1 and 2 aqueous extract , 3 and 4 ethanolic extract .

These findings demonstrate that both aqueous and ethanolic extracts of *Cynara cardunculus* possess potent inhibitory activity against hepatocellular carcinoma cells, indicating their potential as promising anticancer agents.

Discussion:

The present study demonstrates a dose-dependent and solvent-dependent cytotoxic effect of *Cynara cardunculus* extracts on malignant cells, with relatively low toxicity toward normal cells. Specifically, the ethanolic extract showed greater antiproliferative activity than the aqueous extract across comparable concentrations, while the normal cell line retained higher viability, indicating selectivity (Masci *et al*., 2024) , These findings align with recent experimental reports that compare aqueous and alcoholic artichoke extracts and document stronger anti-cancer activity for alcohol-based extracts (likely due to extraction of lipophilic bioactives).

Mechanistically, multiple lines of evidence from the literature indicate that the anticancer activity of *C. cardunculus* extracts arises from a combination of (1) induction of apoptosis (caspase activation, PARP cleavage, Annexin-V positivity), (2) disruption of mitochondrial function and redox homeostasis (increased ROS / mitochondrial depolarization), and (3) inhibition of pro-survival signaling pathways (e.g., STAT3, PI3K/AKT) (Ali *et al*.,2022) , These mechanisms have been observed in different cancer models treated either with crude extracts or with major isolated compounds such as cynaropicrin and chlorogenic acid, The ethanolic extracts tend to concentrate lipophilic sesquiterpene lactones (e.g., cynaropicrin), which have been reported to exert strong cytotoxic effects and to modulate key oncogenic pathways (Mandium *et al*., 2021) .

Differential sensitivity: cancer and normal cells

The selective inhibition of cancer cells over normal cells likely reflects intrinsic differences between malignant and non-malignant lines: cancer cells commonly exhibit higher basal oxidative stress, dysregulated cell-cycle checkpoints, and reliance on overactive survival signaling — features that render them more susceptible to phytochemical-induced apoptosis and redox perturbation (Laghezza *et al*., 2025) , Several recent in vitro studies with artichoke leaf extracts report marked decreases in viability of cancer lines (HepG2, MCF-7, SH-SY5Y, etc.) while non-tumorigenic cells (e.g., MCF10A or fibroblasts) remain comparatively unaffected within the same concentration ranges. This differential susceptibility supports the therapeutic index suggested by our data but requires quantitative confirmation (IC_{50} values and selectivity indices) (Filpa *et al*. , 2022) .

Why the ethanolic extract is often more potent

Ethanol (and hydro-alcoholic) solvents extract both polar and non-polar phytochemicals more efficiently than water alone, Consequently, ethanolic extracts of *C. cardunculus* often contain higher concentrations of sesquiterpene lactones (cynaropicrin), triterpenes, and certain flavonoid aglycones that possess documented antiproliferative activities (Acquaviva *et al*., 2023) , Recent phytochemical characterizations have shown that these lipophilic constituents correlate with in vitro cytotoxic potency, Therefore, the greater activity of

ethanolic extracts in our experiments is consistent with their enriched content of these bioactives, For translational research, fractionation and compound-level quantification are recommended to identify the principal active constituents (Barbosa *et al*., 2024) .

Integration with hepatocyte (HCAM) findings and metabolic effects

Beyond direct cytotoxicity, aqueous artichoke extracts have demonstrated modulation of metabolic and oxidative pathways in hepatocyte models (e.g., protection against palmitate-induced insulin resistance via IRS1/PI3K/AKT/FoxO1 signaling) (Deng *et al*., 2023) , Such metabolic effects indicate that water-soluble phenolics (chlorogenic acid, cynarin, luteolin glycosides) can exert cell-protective or modulatory actions that might explain the milder impact of aqueous extracts on normal hepatic-derived lines while still affecting cancer cells through metabolic interference. Hence, both extract types may be acting through complementary mechanisms: ethanolic extracts more strongly trigger apoptotic pathways, while aqueous extracts modulate oxidative/metabolic responses.

Final synthesis

In sum, the combined evidence — including the present results — supports that Cynara cardunculus extracts have selective anticancer potential with solvent polarity strongly shaping bioactivity (Feiden *et al*., 2023) , Ethanolic extracts, enriched in lipophilic sesquiterpene lactones and triterpenes, are generally more cytotoxic to cancer cells, whereas aqueous extracts exert modulatory and sometimes protective metabolic effects. Future work must quantify potency, identify active constituents, and perform in vivo validation to determine translational potential.

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