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# ASSESS THE ANTIPROLIFERATIVE IMPACT OF IRAQI BLESSED THISTLE ROOTS METHANOLIC EXTRACT ON ESOPHAGEAL CANCER CELL LINE

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**Abstract**:

**Adjective**: The study aimed to evaluate the efficacy of methanolic extract from Cnicus Benedictus L. roots in eradicating esophageal cancer cells and its impact on normal cells in vitro.

**Method**: The anticancer properties of the methanolic extract of Cnicus benedicts L. routes were assessed using an esophageal cancer cell line. Concentrations of the plant extract ranging from 1 to 10000 µg/ml were tested during 24 and 72 hours of incubation to determine the most effective concentration. A normal human-derived adipose tissue cell line was used to assess the safety of plant extracts.

**Results**: The results indicate that the plant extract exhibited a significant suppressive impact on the growth of esophageal cancer cells, specifically when administered in higher concentrations and following a 72-hour incubation period. The effectiveness of the plant extract in inhibiting the growth of esophageal cancer cells was observed to be time-dependent and concentration-dependent. The plant extract exhibits a moderate level of toxicity towards human adipose tissue cells, as evidenced by the average inhibition of growth observed.

**Conclusion**: The study showed that the methanolic extract of Cnicus Benedictus L. roots effectively eliminated esophageal cancer cells. Cytotoxicity involves mechanisms that are both cell-cycle-specific and non-cell-cycle-specific. The study showed that the plant extract was selectively harmful to cancer cells, as demonstrated by the growth inhibition in normal cell lines.

**Keyword**: roots of Cnicus Benedictus L., Esophageal cancer cell line, human derived adipose tissue cell line.

## Introduction

Neoplastic diseases, generally known as cancer, remain the second most significant cause of death globally after cardiovascular diseases . **(Wang et al.,2017; Chen & Yoon, 2022)**, Cancer is responsible for 12.5% of all fatalities worldwide. The number of deaths from cancer exceeds

the combined number of deaths from malaria, HIV, and TB. **(Deo et al., 2022).** Esophageal cancer is a very aggressive type of cancer with a low survival rate, making it one of the least researched and most deadly malignancies globally. It is the sixth deadliest cancer.**(Zhang, 2013)**

The outlook for patients with locally advanced esophageal cancer who undergo traditional treatments such as surgery or radiotherapy is unfavorable. The reason for treatment failure is the combination of a high occurrence of local-regional failure and the early spread of the disease throughout the body. The necessity to tackle the early dissemination of esophageal cancer by systemic treatment has prompted research on combined-modality therapies involving chemotherapy. Simultaneous administration of chemotherapy and radiotherapy is now the established protocol for treating locally advanced esophageal cancer without surgery. **(Ilson, 2008)**

Chemotherapy medications target cancer cells but can also harm normal cells that divide rapidly, resulting in adverse effects. The effects vary depending on the specific medications and the dosage used. **(Mustapha et al.,2022)** Chemotherapy commonly causes neurotoxicity from vincristine and nephrotoxicity from methotrexate. **(Shoji etal.,2021)**

Cnicus Benedictus L. is primarily located in the northern region of Iraq and blooms between the winter and spring months. The antineoplastic and antioxidant properties of Cnicus Benedictus L are significant and are affected by the geographical location and cultivation process. **(Batziakas etal.,2020; Wied etal.,2020; Jian et al.,2020)**

Previous research have indicated that Cnicus Benedictus L functions as an antioxidant, with its efficacy influenced by environmental variables as growth duration and location. (**Batziakas, et al.,2020; Ionescu etal.,2020; Yasin et al.,2020**)

The findings regarding the antioxidant properties of C. Benedictus L. require revision. Studies have demonstrated that C. Benedictus L. exhibits scavenging properties against free radicals and nitrites. The presence of phenolic and flavonoid compounds in C. Benedictus may enhance its effectiveness due to its antioxidant qualities. (**Salim et al.,2023**Another study demonstrates that the plant's ability to combat cancer is mainly attributed to its phytochemical compounds, particularly flavonoids and their glycosides. (**Rezig et al.,2023**)

Several studies were carried out to evaluate the anticancer effectiveness of Blessed Thistle. An experiment analyzed the ethanol-based extract derived from the flowers of Cnicus Benedictus L. This extract effectively eliminated mammary adenocarcinoma cancer cells (AMN-3). The dosage and duration of exposure influence the effectiveness of the extract. **(Yasin et al.,2020; Yasin et al.,2023; Jumaa et al.,2016; Jumaa et al.,2018)**

Several studies have been conducted on a similar topic to ours, but they did not investigate the anticancer properties of Cnicus Benedictus roots on esophageal cancer cell lines. Our study aimed to evaluate the growth inhibitory effects of the methanolic extract of Cnicus Benedictus roots on esophageal cancer cell lines and human adipose tissue cell lines.

**Material and Methods:**

**1- preparation of the extract:**

**1-1- gathering of blessed thistle:** The plant was gathered from the highest mountain in northern Iraq. Between May and July.

**1-2- Preparing methanolic extracts from the roots of Cnicus Benedictus L.:**

A concentrated alcoholic solution was obtained by grinding plant roots into a fine powder and soaking 100 grams of the powder in 1000 ml of 70% methanol. The extraction was conducted using a Soxhlet apparatus and lasted for 24 hours. **(Yasin et al.,2020)**

2- **Detecting phytochemicals:**

Analyzed a variety of phytochemicals, such as flavonoids, alkaloids, carbohydrates, phenolic compounds, tannins, terpenoids, coumarin, and lignin, using specific chemical tests: Mayer's and Wagner's for alkaloids, Zinc-HCl and Alkaline reagent for flavonoids, Ferric chloride and Lead-acetate for phenolic compounds, Bontrager and Legal's test for glycosides, Trim-Hill and Liebermann-Burchard's for terpenoids, Ferric chloride for tannins, Molish's for carbohydrates, and Ninhydrin for proteins and amino acids. (**Yasin et al.,2020**)

**Cell culture:** From ICCMGR, we obtained the esophageal cancer cell line and the human-derived adipose tissue cell line. Collecting the cells was done in 75 cm2 flasks. A regulated environment is 37 degrees Celsius, accompanied by a humid atmosphere containing 5% carbon dioxide. Sigma Chemicals supplied the RPMI-1640 medium used in this investigation. There is a 10% bovine calf serum (FBS) concentration in the solution, along with 100 U/mL of penicillin and 100 g/mL of streptomycin.

**3- Assessment of growth inhibition:**

The study involved growing cells on a 96-well microtiter plate. Blessed thistle root extract at different doses was used on the cells. The number of cancer cells per well would steadily rise during the logarithmic growth phase across multiple incubation periods. We will assess the plant extract's ability to inhibit cell proliferation. It is crucial to highlight that each well initially had seven thousand cells. Cancer cells were introduced into a culture medium with 10% calf serum. The plates were placed in an incubator at 37°C for 24 hours to improve the attachment of cancer cells. Dilutions of the plant extract were prepared using a maintenance medium, with concentrations ranging from 1 to 10000 µg/ml.

Following a 24-hour incubation, the cells were subjected to six dilutions at different concentrations, with 200µl volumes for each dilution. 200µl of serum-free medium were added to the control wells on the opposite side, and the research plates were incubated for 24 and 72 hours.

A self-adhesive compound was used to seal the plates, and then they were placed in the incubator. Staining the treated and control wells with MTT dye allowed us to identify the treatment activity after incubation. At a transmission wavelength of 550 nm, the optical density of each well was measured using an ELISA reader . **(De Sousa et al., 2023; Ueda et al., 2022)**

The growth inhibition rate was determined using a mathematical formula that provides the outcome of the equation. **(Ayiomamitis et al., 2019)**

$$\text{Inhibitor rate \%} = \frac{\text{The optical density of control} \_ \text{The optical density of test.}}{\text{The optical density of control}} \times 100$$

**4- Research ethics:**

The author does not do any investigation on human issues.

**5- Data statistical analysis:**

This study used SAS to analyze the impact of different factors on study parameters. T-test and LSD were used to identify significant differences among means. **(Bailer,2020)**

**Results:**

**1- Detecting phytochemicals:**

The roots of Cnicus Benedictus L. were found to contain many active chemicals, including tannins, flavonoids, carbohydrates, phenolic compounds, terpenoids, coumarins, and lignins, as reported by phytochemical studies. table (1)

Various studies have linked the presence of phytochemicals in Cnicus Benedictus to its antioxidant, anticancer, and antibacterial effects. (**Yasin et al.,2020; lee etal.,2020; Sayadi et al.,2020**)

**Table (1):** The phytochemicals are derived from the methanolic extract of Cnicus Benedictus roots.

| Cnicus Benedictus roots Phytochemical contains | | |
|---|---|---|
| **Phytochemicals types** | **investigation** | **outcome** |
| carbohydrate | Molish' s test | **positive** |
| Protein and amino acid | Ninhydrin test | **negative** |
| tannins | Ferric chloride | **positive** |
| Alkaloids | Mayer's, Wagner's | **negative** |
| Flavonoids | Zinc-Hcl , Alkaline reagent , Ferric chloride , Lead-acetate | **positive** |
| **glycoside** | Borntrager's test, Legal's test | negative |
| Phenolic | Ferric chloride | **negative** |
| Terpenoids: | Trim-Hill, Liebermann-Burchard's | **positive** |

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**2- Cytotoxicity analysis**

**1-1- The inhibitory impact of Cnicus Benedictus L. extract on the division of esophageal cancer cells.**

The research results demonstrated a significant improvement in the suppression of esophageal cancer cell growth with higher concentrations of a plant extract and more extended incubation periods. The concentration and duration of exposure specifically regulated the growth inhibition. table (2)

**Table (2):** Different plant extract concentrations and incubation durations affect esophageal cancer cell proliferation.

| Plant extract concentration µg/ml | Period of incubation | | p-value |
|---|---|---|---|
| | 24 hrs. | 72 hrs. | |
| **1** | **C** 11 | **C** 17 | ***0.044** |
| **10** | **C** 13 | **BC** 26 | **N. S** |
| **100** | **B** 29 | **B** 32 | **N. S** |
| **1000** | **B** 35 | **B** 36 | **N. S** |
| **10000** | **A** 54 | **A** 72 | **N. S** |
| **LSD** | **10.64** | **10.78** | **-** |

- Significant differences (P< 0.05) between column means are indicated by large letters.
- * Significant at (P≤0.05), (**N. S**; Non- Significant).

**1-1- Cnicus Benedictus L. extract's cytotoxicity against normal cell lines:**

The research results suggest that the plant extract has minimal impact on the growth of human adipose tissue cells., Table (3),

A substantial difference was seen when comparing the impact of the plant extract on Esophageal cells with human generated adipose tissue cells. table (4,5)

**Table (3):** Variations in plant extract concentrations and incubation periods affect human derived adipose tissue cell line development.

| Plant extract concentration µg/ml | Period of incubation | | p-value |
|---|---|---|---|
| | 24 hrs. | 72 hrs. | |
| **1** | 1 | 1 | **N. S** |
| **10** | 2 | 2 | **N. S** |
| **100** | 2 | 4 | **N. S** |
| **1000** | 2 | 3 | **N. S** |
| **10000** | 3 | 5 | **N. S** |
| **LSD** | **N. S** | **N. S** | - |

- Significant differences (P< 0.05) between column means are indicated by large letters.
- * Significant at (P≤0.05), (**N. S**; Non- Significant).

**Table (4): A 24-hour study comparing the effects of various doses of plant extracts on the growth of Esophageal and human adipose tissue cell lines.**

| Plant extract concentration µg/ml | Cell line | | p-value |
|---|---|---|---|
| | Esophageal | human derived adipose tissue | |
| 1 | **C** 11 | 1 | **0.015*** |
| 10 | **C** 13 | 2 | **0.008*** |
| 100 | **B** 29 | 2 | **0.006*** |
| 1000 | **B** 35 | 2 | **0.006*** |
| 10000 | **A** 54 | 3 | **0.0001*** |
| LSD | **10.64** | **N. S** | - |

- Significant differences (P< 0.05) between column means are indicated by large letters.
- * Significant at (P≤0.05), (**N. S**; Non- Significant).

**Table (5): A 72-hour study comparing the effects of various doses of plant extracts on the growth of Esophageal and human adipose tissue cell lines.**

| Plant extract concentration µg/ml | Cell line | | p-value |
|---|---|---|---|
| | Esophageal | human derived adipose tissue | |
| **1** | **C** 17 | 1 | **0.014*** |
| **10** | **BC** 26 | 2 | **0.008*** |
| **100** | **B** 32 | 4 | **0.003*** |
| **1000** | **B** 36 | 3 | **0.007*** |
| **10000** | **A** 72 | 5 | **0.005*** |
| **LSD** | **10.78** | **N. S** | |

- Significant differences (P< 0.05) between column means are indicated by large letters.
- * Significant at (P≤0.05), (**N. S**; Non- Significant).

**Discussion:**

Several investigations were conducted to assess the anticancer properties of various components of Cnicus Benedictus L. A study demonstrated that the alcoholic extract from Cnicus Benedictus flowers effectively suppresses the growth of human breast cancer cells in vivo experiments. **(Yasin etal.,2020)**, Some studies have shown that the alcoholic extract of Cnicus Benedictus L can hinder the proliferation of Dalton's lymphoma ascites cells. **(Human ,2023).**

Flavonoids are the primary factor influencing the anticancer properties of a plant extract. Flavonoids inhibit the growth of certain types of cancer cells. It includes components in the blood, brain, lung, uterus, salivary glands, and melanoma. Cells with a high mitotic index show a more noticeable effect than cells with a low mitotic index. **(Khan et al.,2021)**

Terpenoid compounds in Cnicus Benedictus contribute to its anticancer effects. The chemicals induce autophagy by activating signaling pathways and reactive oxygen species. It is leading to the elimination of cancer cells. (**El-Baba et al., 2021**)

Tannins enhance the plant's anticancer properties by interfering with DNA replication and preventing the growth of cancer cells . (**Yıldırım et al.,2015**)

The root of Cnicus Benedictus L. is believed to have anticancer properties because of its influence on osmolality. Extracts contain proteins, carbohydrates, minerals, and other elements that establish a hypertonic environment within cancer cells. Depending on the dose, the cancer cells are exposed to an unfavorable environment, potentially leading to an osmotic shock. (**Ijarotimi et al.,2021).**

We omitted cytotoxicity data beyond 48 hours in our research. The brief duration does not allow us to accurately determine the cytotoxicity pattern, namely whether it is concentration-dependent or time-dependent.

**Conclusion**:

The research found that the methanolic extract from the roots of Cnicus Benedictus L. can suppress the growth of esophageal cancer cells through both cycle-specific and non-cycle-specific cytotoxic effects. The extract's lack of cytotoxicity on regular cell lines demonstrated its safety.

**Author Contributions:**

design and development: Marwan I. Al-Zuhairi
Data collection and organization: Marwan I. Al-Zuhairi
Data analysis and interpretation: Marwan I. Al-Zuhairi
Composition of the article: Marwan I. Al-Zuhairi
Reviewing the essay critically for key conceptual points: Marwan I. Al-Zuhairi
Proficiency in statistical analysis : Marwan I. Al-Zuhairi
Ultimate endorsement and guarantee of the article: Marwan I. Al-Zuhairi

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**Conflicts of interest** :

There is total neutrality.

**Abbreviations:**

**(ICCMGR):** The Iraqi Centre for Cancer and Medical Genetics Research.
**MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stain
**RPMI:** roswell park memorial institute medium
**SAS:** Statistical Analysis System
**LSD:** Least Significant Difference

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