In Vitro Radiosensitizing Effects of Urtica Dioica Leaf Aqueous Extract on the Killing of U87MG Glioblastoma Cell Line

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ABSTRACT

Background: Glioblastoma Multiforme (GBM) is a highly aggressive brain tumor with a poor prognosis. Despite advancements in radiotherapy, its effectiveness is limited due to challenges in delivering high doses without harming healthy tissues.

Objective: The current study aimed to determine whether Urtica dioica extract could enhance the cytotoxic effects of radiation on U87MG glioma cells and explore the underlying mechanisms.

Material and Methods: This *in-vitro* study was conducted on the U87MG glioma cell line and investigated the effects of Urtica dioica extract (at various concentrations) and irradiation (2 Gy) on cell viability, cell cycle distribution, and apoptosis.

Results: Urtica dioica extract exhibited a concentration and time-dependent cytotoxic effect on U87MG cells. Notably, combining the extract with radiotherapy resulted in a significantly greater reduction in cell viability compared to either treatment alone. Cell cycle analysis revealed that the combination treatment induced G2/M phase arrest more effectively than either treatment alone. Additionally, Urtica dioica extract enhanced the pro-apoptotic effects of radiation, indicated by a significant increase in the late apoptotic cell population.

Conclusion: This study demonstrates the radiosensitizing properties of Urtica dioica extract in U87MG glioma cells. The extract promotes cell cycle arrest and apoptosis, potentially leading to improved radiotherapy efficacy. These findings suggest Urtica dioica as a promising complementary therapy for GBM treatment.

Keywords

Apoptosis; Cell Cycle Arrest; Glioblastoma Multiforme (GBM); Radiosensitizer; Radiotherapy; Urtica Dioica Extract; U87MG Glioma Cells; Complementary Therapies

Introduction

Glioblastoma Multiforme (GBM) is an extremely aggressive type of brain tumor that originates in the central nervous system. The prognosis for individuals diagnosed with GBM is usually poor, with a survival rate of only about 12 to 15 months after diagnosis [1].

The primary approach to treating GBM is surgery aimed at removing as much of the tumor as possible. However, this method can have its limitations if the tumor is located in critical brain regions, such as

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those responsible for language control [2]. After surgery, the combination of temozolomide with radiation has become the gold standard of treatment as it offers a significant improvement in survival rates [3, 4].

While advances have been achieved in 3D conformal radiotherapy and Intensity-Modulated Radiotherapy (IMRT), glioblastoma still shows a poor response to radiotherapy [5]. One of the reasons for the ineffectiveness of radiation therapy is the challenge of delivering high doses because nearby healthy tissue suffers collateral damage [6]. To increase the radiation dose in cancer cells without exceeding the permissible limit for healthy tissues and organs, the use of radiosensitizers has emerged as a practical option [7].

Over the last two decades, consideration of treatment with natural products has gained importance due to their lower toxicity and effectiveness than radiosensitizers [8]. Previous research has shown that herbal remedies can stop the development and progression of tumors, producing satisfactory results while minimizing damage to healthy cells [9, 10]. Induction of apoptosis serves as a crucial indicator of cytotoxic anticancer factors. Numerous natural compounds, including those from plants, have been documented to modulate apoptosis pathways that were previously inactive in cancer cells. The ability to induce apoptosis in cancer cells and to prevent uncontrolled cell proliferation is a crucial aspect of immunopharmacology [5, 9].

Among the famous medicinal plants, Urtica dioica has valuable active ingredients with some species (30-45). However, three main species are predominant and observed as plants, including the large, the small, and the Greek Urtica dioica in Iran. Urtica dioica, previously consumed as a food with recognized medicinal properties, has been incorporated into traditional Iranian medicine, particularly for its blood sugar-lowering effects [11].

Several *in-vivo* and *in-vitro* studies have highlighted the effective anti-cancer properties of Urtica dioica extract in various cancers, such as breast [12], colon, gastric [9], and lung [13] and its effectiveness in inducing glioblastoma cell death through cell cycle arrest and initiation of apoptosis underlined [3, 6].

To the best of our knowledge, no research has been published on the anticancer effects of Urtica dioica combined with radiation on glioblastoma cancer cells. Given the resistance of this cancer to conventional chemotherapy and radiotherapy and the need for new treatment strategies for glioblastoma, this study aimed to investigate the anticancer potential of Urtica dioica in improving the effectiveness of radiotherapy.

Material and Methods

This is an *in-vitro* study to evaluate the effect of Urtica dioica leaf aqueous extract and irradiation on high-grade glioma cells.

Preparation of herbal extract

The leaves of Urtica dioica were collected from the wild in the plains of Chogha Zanbil in Shush City, Khuzestan Province, Iran. Ethical approval was obtained from the Research Ethical Committee (REC) to collect Urtica dioica Dezful University of Medical Sciences, Dezful, Iran. First, the plants were examined by Ms. Marzieh Anaam (botanist) and Dr. Behnaz Deihim, a taxonomist from the Department of Bacteriology and Virology, Dezful University of Medical Sciences, evaluated and approved. A specimen of the plant was then deposited in the herbarium collection of the Dezful Medical Faculty. These plants were dried in a dark environment with 10-15% humidity and ground in a hand mill. A total of 20 grams of nettle leaves were boiled for 15 minutes and separated from the waste using a filter press, and the solution was extracted under the filter containing the active ingredient. For better filtration, Whatman filter paper No. 400 was used and the solution was then distilled under reduced pressure and removed. Later, it

was filtered with a 0.2 µm filter [11].

Cell Culture

The U87MG glioma cell line (IBRC C10982) obtained from the Iranian Biological Resource Center was cultured in Minimum Essential Medium (MEM) supplemented with Earl's Balanced Salt Solution (DMEM), 10% heat-inactivated Fetal Bovine Serum (FBS), 1% non-essential Amino acids, 2 mM L-glutamine, 100 µg/ml streptomycin, and 100 U/ml penicillin. Cells were maintained at 37 °C in a 5% CO_2 environment. When approximately 80% confluence was reached, the cells were detached with 0.25% trypsin-EDTA and subcultured.

Cell viability assay

The MTT assay, a widely used method for assessing cell viability, was used. Approximately 15×10^3 cells were seeded into each well of a 96-well plate and allowed to adhere for 48 h in a 5% CO_2 humidified atmosphere at 37 °C. Cells were then exposed to different concentrations of Urtica dioica extract (20, 40, 60, 80, 125, 250, 500, 1000, and 2000 µg/ml) for 24 and 48 h with or without irradiation. After exposure, cells were subjected to MTT solution incubation for 4 h, and the resulting formazan crystals were dissolved in DMSO. The absorbance at 570 nm was measured with an ELISA reader [9]. The IC50 (50% inhibitory concentration) was determined as the concentration causing a 50% reduction in cell viability, extrapolated from cytotoxicity curves [13] (each concentration: three times).

Cell Cycle Analysis

Cells with a total number of 5×10^5 were distributed in the wells of a tissue culture plate and divided into different groups, including control, radiation exposure without treatment, Urtica dioica extract treatment without radiation, and the combined Urtica dioica extract with radiation treatment category. After a 24-hour interval, the surface-attached cells were exposed to the IC50 dose of the extract and/or 2 Gy irradiation and cultured for 48 hours. After treatment, cells were fixed in cold 70% ethanol. After washing twice in Phosphate Buffered Saline (PBS), cells were treated with 50 µL of a 100 µg/mL stock solution of RNase-free DNase and incubated for 30 minutes, then 20 µL of 1 mg/mL PI was added, followed by cell cycle distribution analysis using a Fluorescence Activated Cell Sorting (FACS) scanning flow cytometer (Attune NxT).

Apoptosis Assay

After an initial seeding (5×10^5 cells in all wells of the tissue plate), the apoptosis was assessed, following similar grouping parameters as previously described. 48 hours after treatment with the IC50 dose of the extract and/or 2 Gy irradiation, cells were stained with Annexin VFITC and propidium iodide using the Annexin VFITC Apoptosis Detection Kit (MabTag) to detect apoptotic cells using a flow cytometer.

Irradiation Conditions

Irradiation was performed using a clinical linear accelerator (Artist; Siemens Company, Germany) that generated a 6 MV photon beam. Solid water plates were used for backscattering and setup conditions, with the plates irradiated with a field size of 20×20 cm^2 with a dose of 2 Gy.

Statistical analysis

Results were presented as mean ± standard deviation from three independent experiments. The IC50 was calculated using Graphpad Prism 5. Statistical significance between groups was assessed using a Student's t-test and one-way ANOVA (P-value<0.05).

Results

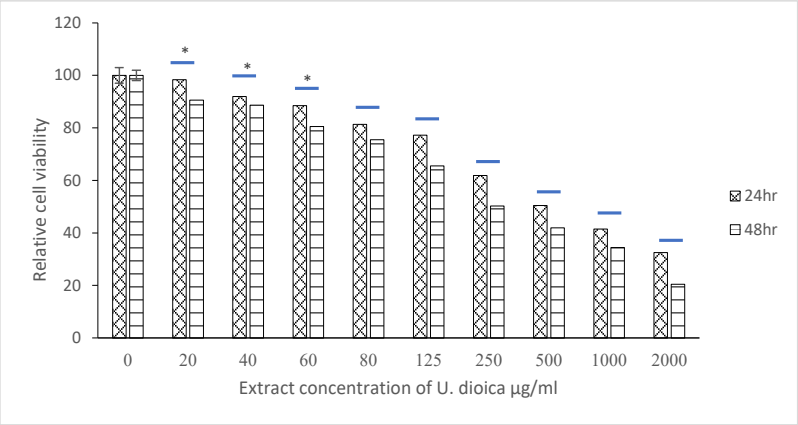
Cytotoxic and radiosensitivity effects of Urtica dioica aqueous extract on U87MG cells

To evaluate the cytotoxic potential of Urtica dioica extract on U87MG glioblastoma cells, cell viability was extracted after treatment with different concentrations of the extract (0–2000 µg/mL) for 24 and 48 hours.

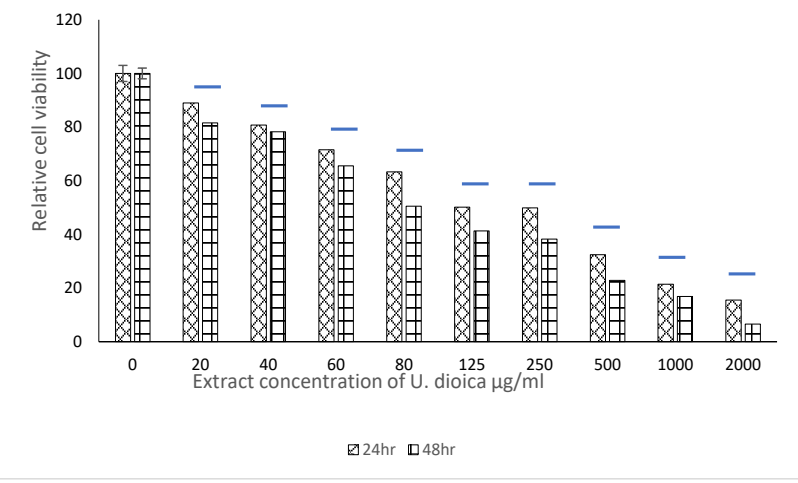
Treatment with Urtica dioica extract resulted in a significant decrease (*P*-value=0.00) in the viability of U87MG cells compared to the untreated control group (Figure 1a). This effect was concentration-dependent, with higher extract concentrations, resulting in greater reductions in cell viability. Furthermore, a time-dependent effect was observed, in which cells exposed to the extract for 48 hours showed a more significant decrease in viability compared to those for 24 hours (Figure 1). These results demonstrate the antiproliferative potential of Urtica dioica extract against U87MG glioma cells.

To evaluate the potential synergistic effect between Urtica dioica extract and radiotherapy, cells were treated for 24 or 48 hours (Figure 1b). Notably, combination treatment resulted in a significantly greater reduction (*P*-value<0.0001) in U87MG cell viability at both time points compared to treatment with either extract or irradiation alone.

Table 1 presents the IC50 concentrations of



(a)



(b)

Figure 1: The cytotoxic effect of the Urtica dioica extract on U87MG at 24 and 48 hours cells a. with or b. without radiation *P<0.05 was considered significant compared to the untreated control group

Table 1: The IC50 dose of Urtica dioica extracts on U87MG cells at 24 and 48 hours with or without radiation.

	Without radiation		With radiation	
Time(h)	24	48	24	48
IC50(µg/mL)	500.03	249.7	125.21	80.42

Urtica dioica extract. The IC50 values for Urtica dioica in U87MG cells were 500.03 µg/mL and 249.7 µg/mL after 24 and 48 hours of treatment in the absence of radiation, respectively. These values were in contrast to 125.21 µg/mL and 80.42 µg/mL for the group treated with radiation at the same time points (24 and 48 hours, respectively).

Effect of Urtica dioica aqueous extract on Cell Cycle Distribution

Flow cytometry analysis with Propidium Iodide (PI) staining was employed to investigate the influence of Urtica dioica on the cell cycle distribution of U87MG glioblastoma cells. Figure 2A presents the cell cycle histograms for the control group (a), the radiation-only group (b), the Urtica dioica-treated group without radiation (c), and the Urtica dioica-treated group with radiation (d). A more detailed analysis of the cell distribution across the various cell cycle phases is depicted in Figure 2B.

A significant increase in the G2/M phase population of U87MG cells was observed following treatment with Urtica dioica. Compared to the control group (15.85%±1.4%), the percentage of cells in the G2/M phase increased to 25.9%±1.44% with Urtica dioica treatment alone. This effect was further amplified when Urtica dioica treatment was combined with radiation. The G2/M population in the Urtica dioica and radiation group (55.60%±0.74%) displayed a significant rise compared to both the control and the Urtica dioica-only groups (*P*-value<0.05).

No statistically significant difference (*P*-value>0.05) was observed in the percentage of G2/M phase cells between the radiation-only group (20.70%±1.3%) and the control group or the Urtica dioica-treated group alone.

The FACS analysis also indicated a decrease in the G1 population of U87MG cells following treatment with both Urtica dioica and

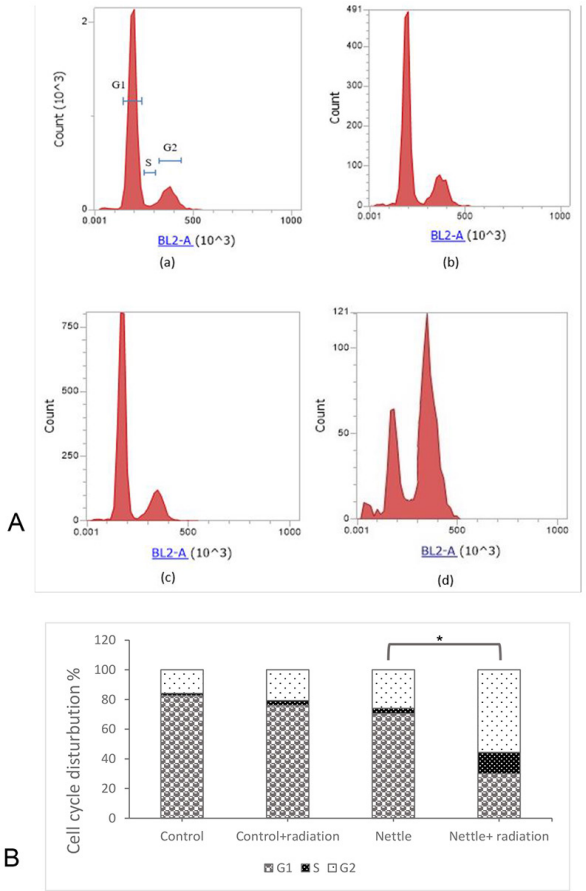


Figure 2: **A**) Displays flow cytometry histograms illustrating cell cycle distribution using the propidium iodide (PI) method on U87MG cells (a), the radiation-only group (b), the Urtica dioica-treated group without radiation (c), and the Urtica dioica-treated group with radiation (d). **B**) The graph represents the cell distribution across various phases of the cell cycle. Statistical significance was considered when *P*<0.05 compared to the Urtica dioica group.

radiation compared to Urtica dioica treatment alone (*P*-value<0.05). This decrease, along with the increase in the G2/M population, suggests that the combined treatment may be hindering cell progression through the G1 phase and promoting cell cycle arrest at the G2/M checkpoint (Figure 2).

Effect of Urtica dioica aqueous extract on Apoptotic Cell

The influence of Urtica dioica extracts, with or without radiation, on the induction of apoptosis in U87MG glioblastoma cells was investigated using flow cytometry analysis with Annexin V/PI staining. This technique differentiates between viable, early apoptotic, late (secondary) apoptotic, and necrotic cell populations.

The percentage of cells undergoing early and late apoptosis increased following treatment with Urtica dioica. The Urtica dioica and radiation group exhibited a significantly higher percentage of early apoptotic cells (16.0±1.9%) compared to the Urtica dioica-only group (7.91±1.4%) (*P*-value<0.05). Similarly, the late apoptotic cell population was also significantly higher in the Urtica dioica and radiation group (50.52±2.1%) compared to the Urtica dioica-only group (22.15±1.7%) (*P*-value<0.05) (Figure 3).

Discussion

The current study investigated the radiosensitizing effect of Urtica dioica leaf extract on U87MG glioma cancer cells. The results revealed that the extract exerts a cytotoxic effect on these cells, with the potency increasing in a concentration-dependent manner. This aligns with previous research demonstrating the antiproliferative properties of Urtica dioica extracts in various cancer cell lines [10, 11, 13].

Furthermore, a time-dependent effect with longer exposure durations was observed, leading to greater reductions in cell viability, showing Urtica dioica extract disrupts cellular processes essential for long-term survival in U87MG cells.

The most significant finding of this study is the synergistic effect between Urtica dioica extract and radiotherapy. Treatment with both agents resulted in a considerably greater reduction in cell viability compared to either treatment alone. This indicates that Urtica dioica extract can enhance the effectiveness of radiotherapy in eliminating U87MG glioma cells.

This study provides the IC50 value, which quantifies the agent's potency in suppressing the targeted process. The IC50, which represents the concentration required to inhibit 50% of cell growth, was significantly lower for the combined treatment group compared to the groups treated with extract or radiation alone. This shows that a lower concentration of both agents is required to achieve the same level of cell death when administered together. A limited number of studies have examined the anticancer effects of various parts of Urtica dioica on cancer cells. In the current study, the results are consistent with those of D'Abrosca et al. [13] An aqueous extract of Urtica dioica leaves reportedly resulted in a significant and dose-dependent reduction in the viability of non-small cell lung cancer H322 cells with an IC50 value of 78.33 µg/mL.

It is believed that cell cycle arrest in the G2/M phase is a crucial factor in increasing radiosensitivity [14, 15]. It is well known that radiosensitivity varies throughout the cell cycle, with cells being most sensitive during the G2/M phase [16]. This study utilized propidium iodide staining and cell cycle analysis to demonstrate that Urtica dioica extract promotes G2/M phase cell cycle arrest. Combination treatment with Urtica dioica extract and radiation resulted in a more pronounced G2/M arrest and a significant increase in the sub-G1 population compared to either treatment alone. The radiation treatment alone appeared less effective in inducing G2/M arrest compared to the combination therapy, showing that Urtica dioica extract may potentiate

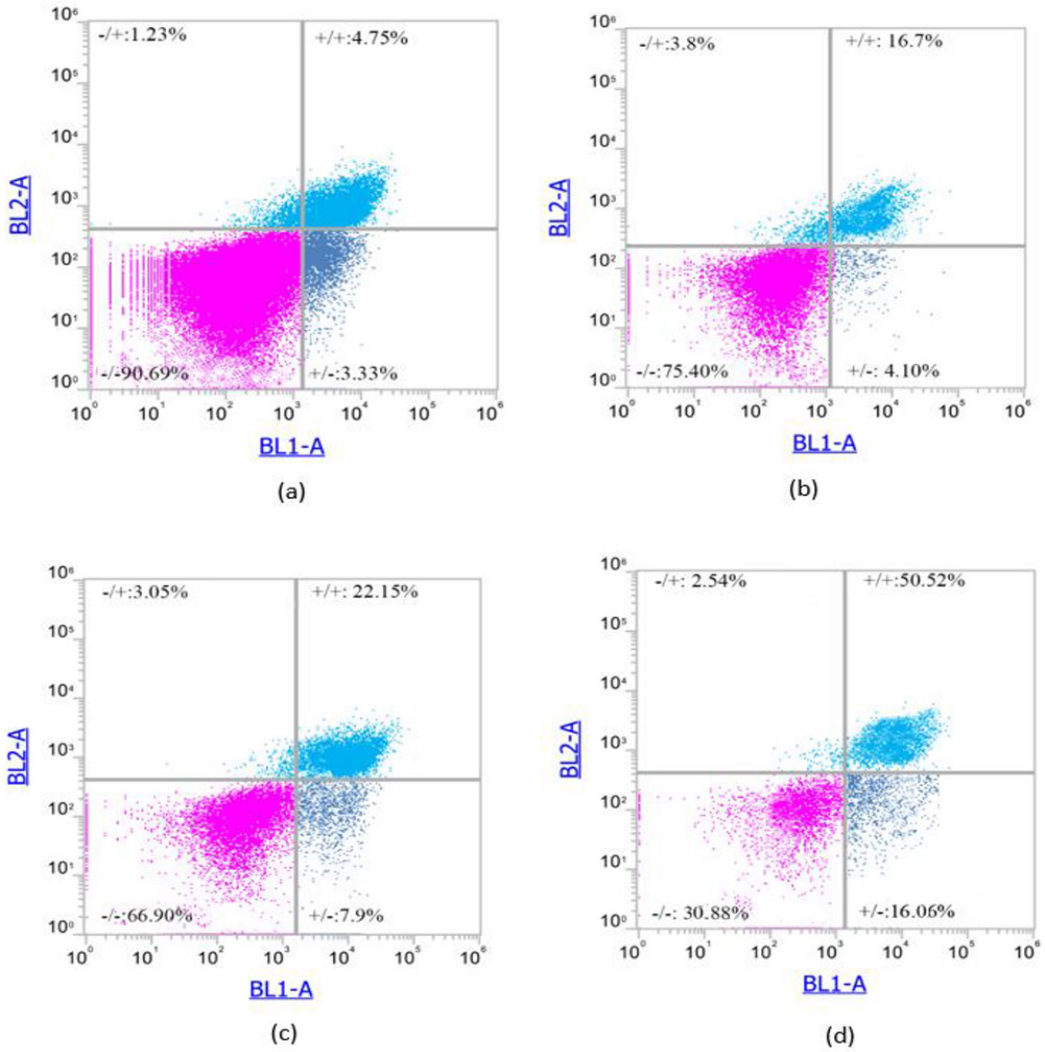


Figure 3: Illustrates the flow cytometry analysis of apoptosis in U87MG cells. Panel (**a**) represents the control group, while panel (**b**) depicts the radiation-only group. Panel (**c**) shows the Urtica dioica treatment group and panel (**d**) displays the combined radiation and Urtica dioica treatment group. In the graph, the lower left quadrant corresponds to viable cells (double negative), the lower right quadrant indicates early apoptotic cells (annexin V+: PI-), the upper right quadrant represents either late-stage apoptotic or necrotic cells (double positive), and the upper left quadrant signifies necrotic cells (annexin V-: PI+). (PI: Propidium Iodide)

the cell cycle-arresting effects of irradiation. FACS analysis revealed a noteworthy interplay between Urtica dioica extract and irradiation on the cell cycle distribution of U87MG cells. While Urtica dioica treatment alone did not significantly increase the G2/M population, the combination treatment resulted in a markedly more pronounced effect. This finding further supports a potential synergistic action between Urtica dioica extract and radiation in promoting G2/M phase cell cycle arrest. Here, the results are consistent with those of Hodroj et al. [17] who reported that Urtica dioica extract alone did not significantly alter the G2/M cell cycle population in U937 acute myeloid leukemia cells.

Annexin V/PI staining revealed a trend towards enhanced apoptosis, or programmed cell death, following treatment with Urtica dioica extract, both with and without radiation. Flow cytometry demonstrated that the extract significantly increased early-stage cell apoptosis. This finding aligns with the observed cell cycle arrest in the G0 phase, suggesting that Urtica dioica extract might induce apoptosis alongside cell cycle arrest. Supporting this finding, Hodroj et al. [17] recently reported that the extract halted the cell cycle in the G0 phase and promoted apoptosis at both early and late stages by upregulating pro-apoptotic Bax protein expression and downregulating anti-apoptotic Bcl-2 expression.

The data also revealed minimal levels of necrotic cell death across all treatment groups, showing the primary mode of cell death induced by Urtica dioica and radiation in U87MG cells is likely apoptosis rather than necrosis.

These findings highlight a potential for Urtica dioica to enhance the pro-apoptotic effects of radiation therapy in U87MG cells. The significant increase in the late apoptotic population with combined treatment suggests that Urtica dioica may act synergistically with radiation to promote a more efficient apoptotic cascade in these glioblastoma cells.

The present study paves the way for further investigations into the mechanisms underlying the radiosensitizing effect of Urtica dioica extract. Future research should explore the specific molecular pathways targeted by the extract and how they interact with radiation therapy. Additionally, *in-vivo* studies using animal models are necessary to assess the efficacy and safety of Urtica dioica extract in a more complex biological system.

Conclusion

The Urtica dioica leaf extract exhibits cytotoxic properties against U87MG glioma cancer cells. Importantly, the Urtica dioica extract significantly enhances the effectiveness of radiotherapy, potentially by promoting cell cycle arrest and apoptosis. These findings can reveal that Urtica dioica extract holds promise as a complementary therapy to improve glioma cancer treatment outcomes.

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Authors' Contribution

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by M. Khorramizadeh, M. Cheshmberah, M. Anaam, and A. Kassani. The first draft of the manuscript was written by B. Deihim, and Z. Kord. with input from all authors and designed the figures. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

Our research adhered to ethical standards with approval secured from the Institutional Review Board and the Ethics Committee of Dezful University of Medical Sciences under the reference number IR.DUMS.REC.1399.005. The cell line used in our study was acquired from the Iranian Biological Resource Center without access to donor information. All experimental procedures were conducted in compliance with established guidelines and regulations to ensure ethical conduct.

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Conflict of Interest

None

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