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Effect of urtica dioica agglutinin on breast cancer cell growth

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Abstract

Background: Breast cancer remains a leading cause of mortality among women globally, necessitating the exploration of novel therapies with minimal side effects. Urtica dioica agglutinin (UDA), a lectin derived from stinging nettle, exhibits antiproliferative properties in various cancers; however, its effects on breast cancer cells remain underexplored. This study evaluates the cytotoxic potential of UDA against MCF-7 breast cancer cells while assessing its impact on normal mammary (MCF-10A) and embryonic kidney (HEK-293) cells.

Methods: UDA was purified from Urtica dioica rhizomes by affinity chromatography and confirmed by SDS-PAGE (8.5 - 9.5 kDa) and agglutination assays. MCF-7, MCF-10A, and HEK-293 cells were treated with different concentrations of UDA (7.5 - 480 µg/mL) for 24 and 48 hours. Cytotoxicity was assessed using MTT assays to measure cell viability.

Results: UDA significantly inhibited MCF-7 proliferation in a dose- and time-dependent manner ($P < 0.01$ at 24 hours; $P < 0.0001$ at 48 hours). At 240 µg/mL (During 48 hours), viability dropped below 50%, while normal HEK-293 cells showed < 30% toxicity. MCF-10A proliferation remained unaffected, even at 480 µg/mL.

Conclusion: UDA selectively targets breast cancer cells (MCF-7) with minimal toxicity to normal cells, positioning it as a promising anticancer candidate. Further studies are needed to elucidate its mechanism of action and apoptosis-inducing potential.

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Introduction

Breast cancer is the most prevalent malignancy in women, accounting for 11.7% of all global cancer cases (1). It remains a leading cause of mortality among women, with projections estimating over 1 million annual deaths by 2040 (2). In Iran, breast cancer represents approximately 28.1% of female cancers, with incidence rates expected to rise by 63% by 2025 compared with 2016 (3,4).

Conventional therapies, including surgery, chemotherapy, and immunotherapy, face challenges such as therapy resistance and adverse side effects (5,6). Consequently, natural products, particularly herbal medicines, have gained attention for their potential to target therapy-resistant cancer stem cells (CSCs) and reduce side effects (7).

Urtica dioica (Stinging nettle) is a perennial plant with antiproliferative and antioxidant properties in different cancers (8). Previous studies have reported that its aqueous extract inhibits MCF-7 cell growth and induces apoptosis (9,10). Urtica dioica agglutinin (UDA), a lectin isolated from nettle rhizomes, has been shown to inhibit benign prostatic hyperplasia (BPH) cell proliferation (11). Lectins are carbohydrate-binding proteins with specific characteristics, such as cell agglutination, apoptosis induction, and inhibition of angiogenesis (12). Cytotoxic effects of UDA have been reported in leukemia cell lines, including Jurkat (An acute lymphoblastic leukemia cell line) (13) and HL-60 (A myeloid leukemia cell line), through marked apoptosis induction (14). This study evaluates UDA cytotoxicity against MCF-7 cells while assessing its safety in normal MCF-10A and HEK-293 cells.

Methods

Purification of UDA and agglutination assay

Stinging nettles were harvested from Tonekabon, Iran, during winter and authenticated in the herbarium of Golestan Agricultural and Natural Resources Research and Education Center (Herbarium number: 2541). Rhizomes were washed, cut into small pieces, and used immediately or stored at -20 ºC. UDA was purified by affinity chromatography on a chitin column following established protocols (15). Briefly, after homogenizing rhizomes in 0.1% HCl, extraction was carried out in several stages. Total UDA was purified from Urtica dioica extract by affinity chromatography on a chitin column equilibrated with acetate buffer. After passage of the extract, unbound proteins were washed off with 1 M NaCl. Finally, the lectin was desorbed with 0.5 N acetic acid. The lyophilized UDA was dialyzed against phosphate-buffered saline (PBS). The concentration of UDA was determined using the lectin-specific absorption method (16). Purity was confirmed by SDS-PAGE (8.5 - 9.5 kDa), and agglutination activity was tested using trypsin-treated human erythrocytes (17,18).

Cell culture

The cell lines MCF-7 (human breast cancer cell line), MCF-10A (Human normal breast epithelial cell line), and HEK-293 (Human embryonic kidney cells) were purchased from the Pasteur Institute (Tehran, Iran). The cells were cultured in RPMI-1640 medium (Gibco, USA, Cat. No. 11875093) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, USA, Cat. No. A25904DG) and penicillin-streptomycin (Gibco, USA, Cat. No. 15140122).

MTT assay

Cell growth inhibition was measured using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay. Cells (1×10^4 cells/well) were treated with UDA (7.5 - 480 µg/mL) for 24 and 48 hours at 37 ºC. Untreated cells served as the negative control. After incubation, 20 µL of MTT solution (5 mg/mL in PBS) (Sigma, USA, A101161) was added to each well, and the cells were incubated at 37 ºC for 4 hours. After DMSO was added to the wells to dissolve the formazan crystals, absorbance was measured at 570 nm. The experiment was performed three times. Cell viability (%) was calculated as (19):

$$\text{Cell viability (\%)} = \frac{A_{570nm}(sample)}{A_{570nm}(control)} \times 100$$

Results

UDA purification

Purified UDA showed agglutination activity at 20 µg/mL and a single band (8.5 - 9.5 kDa) on SDS-PAGE.

Proliferation inhibition by UDA treatment

UDA inhibited MCF-7 proliferation in a dose- and time-dependent manner, with viability dropping below 50% at 240 µg/mL (48 hours) ($P < 0.0001$). HEK-293 cells exhibited less than 30% toxicity at 480 µg/mL, while MCF-10A viability remained unaffected (Figure 1).

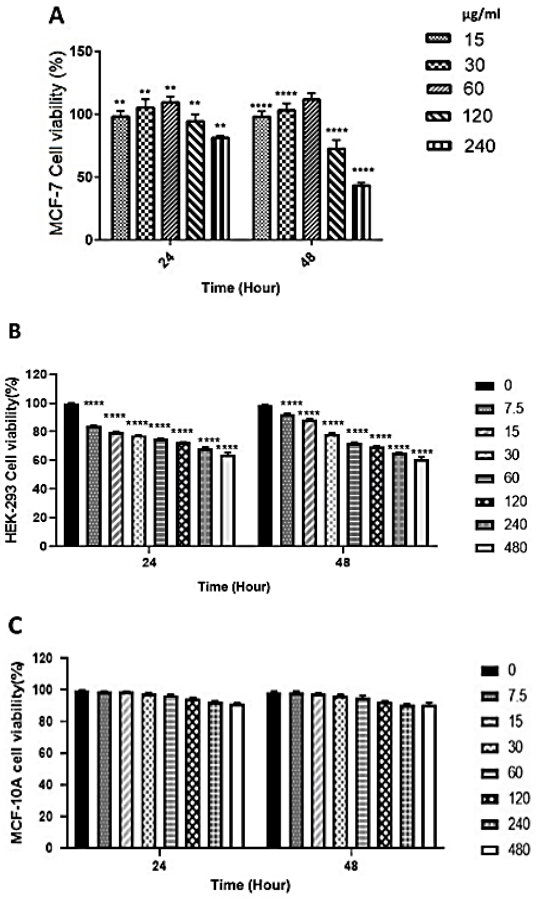

Figure 1. UDA inhibits the proliferation of MCF-7. Cell viability of (A) MCF-7, (B) MCF-10A, and (C) HEK-293 cells measured by MTT assay after 24- and 48-hour UDA treatment with different doses (15 - 240 µg/mL for MCF-7 cells and 7.5 - 480 µg/mL for MCF-10A and HEK-293 cells). Data represent mean ± SEM of three experiments. **P < 0.01, ****P < 0.0001.

Discussion

Here, we investigated the cytotoxic effect of UDA on the breast cancer cell line MCF-7. UDA significantly inhibited MCF-7 cell growth in a dose- and time-dependent manner ($P < 0.01$ at 24 hours and $P < 0.0001$ at 48 hours). The highest proliferation inhibition occurred at 240 µg/mL over 48 hours, with less than 50% inhibition. However, a much weaker effect was observed in nontumorigenic human mammary MCF-10A and normal HEK-293 cells.

As reported in previous studies, several lectins have shown anti-breast cancer activity (20-22). Valentiner et al., 2003, examined six lectins - Helix pomatia agglutinin (HPA), soybean agglutinin (SBA), peanut agglutinin (PNA), Solanum tuberosum agglutinin (STA), wheat germ agglutinin (WGA), and phytohemagglutinin (PHA-L) - on MCF-7 and other breast cancer cell lines. Three lectins inhibited MCF-7 growth during a 24-hour incubation. WGA exhibited the strongest effect, reducing cell growth with an IC50 of 70 µg/mL. At 200 µg/mL, viable cells decreased to 28%. PHA-L showed moderate dose-dependent inhibition, reducing viability to 58% at 200 µg/mL. SBA had the weakest effect, with viable cells decreasing to 77% at the same concentration (14). The inhibitory effect of UDA was most similar to that of SBA, as UDA achieved comparable inhibition at 240 µg/mL over 24 hours.

Deepa et al., 2012, demonstrated that mulberry leaf lectin (MLL) induced strong growth inhibition in MCF-7 cells after 24 hours (IC50: 8.5 µg/mL). Cell cycle analysis revealed 41% of cells in the sub-G1 phase, indicating apoptosis (21). Similarly, Savanur et al. (2014) reported that Sclerotium rolfsii lectin (SRL) significantly inhibited MCF-7 cells. Treatment with 20 µg/mL and 40 µg/mL SRL reduced viability to 50% and 39%, respectively, over 48 hours. Cell cycle analysis showed 33% of treated cells in the sub-G1 phase (indicative of apoptosis) (22). Although both MLL and SRL exhibited stronger inhibitory effects than UDA, further experiments are needed to confirm whether UDA induces apoptosis. If confirmed, the inhibitory effect of UDA could hold significant value in breast cancer treatment.

The proliferation rates of HEK-293 and MCF-10A cells treated with UDA remained approximately 70% and > 90%, respectively, at 240 µg/mL over 48 hours. In contrast, MCF-10A cells treated with SRL retained > 70% viability at a lower concentration (40 µg/mL) over the same period (22). These results suggest that UDA has fewer side effects than SRL, making it a safer candidate for therapeutic applications.

Conclusion

UDA selectively inhibits breast cancer cell proliferation with minimal impact on normal cells, supporting its potential as an anticancer agent. Future research should focus on elucidating its mechanism of action and evaluating its synergistic effects with conventional therapies.

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Ethical Statement

This study was conducted under the ethics approval code issued by Golestan University of Medical Sciences (ID: IR.GOUMS.REC.1400.422).

Conflicts of Interest

There is no conflict of interest to be declared by the authors.

Author Contributions

All the authors made substantial, direct, and intellectual contributions to the work and read and approved the final manuscript.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence

AI (DeepSeek) was used in part only to prepare the abstract and assist with translation.

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