Original Article

Antioxidant and anticancer activities of extract of *Inula helenium* (L.) in human U-87 MG glioblastoma cell line

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ABSTRACT

Aims: The aim of this study is to explore the antioxidant and antiproliferative activities of aqueous extract from aerial parts of *Inula helenium* (L.) against human U-87 MG glioma cell line.

Materials and Methods: The 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide and lactate dehydrogenase assays were used to study antiproliferative and cytotoxic activities against U-87 MG cell after 48 h exposure. In addition, to assess the oxidative effects, total antioxidant capacity and total oxidant status levels were also measured.

Results: Finally, the aqueous extracts displayed antiproliferative and cytotoxic activities at high concentrations tested, particularly at 200 μg/ml, without causing to oxidative stress.

Conclusion: The results strengthen the evidence that *I. helenium* could be considered a natural resource of potential antitumor agents for brain cancer. In addition, this study is expected to expand the existing information on the anticancer activity of *I. helenium* and to assist in a more focused design of further research as chemotherapeutic agents.

KEY WORDS: Anticancer activity, aqueous extracts, cytotoxicity, *Inula helenium*, oxidative stress, U-87 MG cell line

INTRODUCTION

Many species of the *Inula* genus, widespread in Europe, Asia, and Africa, mostly in Mediterranean, are used as traditional herbal medicines worldwide. *Inula helenium*, also known as elecampane, is one of the most important species of this genus frequently used in ethnomedicine.[1] The roots of *I. helenium* have been used in the folk medicine to treat many diseases including bronchitis, cough, lung disorder, tuberculosis, intestinal ulcers, chronic enterogastritis, indigestion, diabetes, infectious, a variety of dermatitis, and pediatric diseases.[2-5] The known predominant constituents within the *I. helenium* are sesquiterpene lactones eudesmanolides, exhibiting diverse bioactivities such as antitumor, anti-inflammatory, antimicrobial, antiproliferative, antibacterial, antistressor, and cytotoxicity.[6-13] In fact, sesquiterpenoids are the largest group of natural terpenoids and contain thousands of compounds and have been described as the active components of various medicinal plants used in traditional medicine and exhibit a wide variety of biological and pharmacological activities.[14]

A few research groups have evaluated the cytotoxic activity of *I. helenium* root in various cancer cell lines; however, aerial parts of *I. helenium* have not been yet investigated as anticancer activity *in vitro* in human glioblastoma multiforme cell line. Glioblastoma is the most frequent and malignant brain tumor, which is refractive to all treatment modalities including surgical resection, chemotherapy, and radiotherapy.[15] Indeed, glioblastoma is among the most aggressive and lethal cancers to treat due to their genetic heterogeneity, high invasive growth, and vascularization.[16] To overcome some of the therapeutic challenges in glioblastoma, new therapeutic agents have been researched to reduce the viability of cancer cells and slow tumor growth. Therefore, in this study, we have designed, for the first time, to examine *in vitro* antiproliferative and antioxidant properties of aqueous extracts from *I. helenium* aerial parts on human glioblastoma multiforme cell line, U-87 MG.

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MATERIALS AND METHODS

Plant material and extraction

The air-dried and powdered 100 g of plant material (whole aerial parts) was infused in 1 L of hot water for 15 min. The initial temperature of added water was 98°C. Infusions were left to stay at room temperature without additional heating. After the infusion had cooled, the extract was carefully decanted away from the residual solids and diluted for different concentrations.

Cell line

U-87 MG (glioblastoma multiforme) cell line used for this study was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). U-87 MG cells were grown in Dulbecco's Modified Eagle Medium supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 10% heat-inactivated fetal bovine serum.

Cell viability assay

Cell viability was monitored using 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. MTT was purchased from Sigma Chemical Company (St. Louis, Missouri, USA). The aqueous extract of *I. helenium* aerial parts was initially prepared to adjust its concentrations (0, 25, 50, 75, 100, 125, 150, 175, and 200 µg/ml) for use in experiments. Cells were plated in a 96-well plate (5×10^3 cells/well) and incubated for 48 h. Following treatment, the MTT stock solution (5 mg/ml in phosphate-buffered saline) was added to the medium and incubated with cells at 37°C for 3 h to allow cell-mediated reduction of MTT. The absorbance was measured at 570 nm in a microtiter plate reader. The viability was determined as the percentage of absorbance of *I. helenium* extract-treated cultures compared with those of untreated control cultures. In experiment used as a positive control, cells were treated with a well-known antitumoral compound, mitomycin C (MMC: 10^{-7} M, Sigma-Aldrich®). Whereas used as a negative control, cells were studied without extracts of *I. helenium*.

Lactate dehydrogenase assay

U-87 MG cells were treated with extracts from *I. helenium* for 48 h and lactate dehydrogenase (LDH) released from damaged cells in culture medium was quantified using a kit (Cayman Chemical Company, MI, USA). A volume of 120 ml cell medium was used. The rate of NADC reduction directly proportional to LDH activity was measured as an increase in absorbance at 490 nm.

Total antioxidant capacity and total oxidant status analysis

Total antioxidant capacity (TAC) and total oxidant status (TOS) rapid and reliable automated colorimetric assays are frequently used to determine the oxidative alterations.[17] The automated Trolox equivalent antioxidant capacity and TOS assays were carried out in cellular media via using commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey).

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, version 18 (SPSS Inc., Chicago, IL, USA). Duncan's test was used to determine whether any treatment significantly differed from controls or each other. Statistical decisions were made with a significance level of 0.05.

RESULTS

Effects of aqueous extract of aerial parts of *Inula helenium* on U-87 MG cells

In this study, *in vitro* cytotoxic effect of the aqueous extract of *I. helenium* aerial parts was evaluated on U-87 MG cell lines using definite concentration ranging from 25 to 200 µg/ml by MTT assay. MTT assay, a simple and reliable technique, measures cell viability can be used for screening of antiproliferative agents. As shown in Figure 1, *I. helenium* treatment for 48 h resulted in a dose-dependent reduction in cell viability, which ranged from 16.51% to 27.31% in U-87 MG cells at concentrations of 75–200 µg/ml. However, the cells tested were more sensitive to loss of cell viability, which occurred especially with high concentrations (125, 150, 175, and 200 µg/ml) as compared to control. In addition, the cytotoxicity was determined using LDH release assay. Aqueous extracts caused a significant increase of release of LDH enzyme in a clear concentration-dependent manner [Figure 2]. Exposure of U-87 MG to *I. helenium* at the largest doses of 200 µg/ml aqueous extract seems to further enhance the release of LDH enzyme.

Oxidative damage assay

The *in vitro* antioxidant/oxidant capacity of aqueous extracts from *I. helenium* was determined by measuring TAC and TOS levels. All concentrations of aqueous extracts from *I. helenium* significantly caused a significant decrease of TAC levels on human U-87 MG cells in a concentration-dependent manner [Figure 3]. Furthermore, TAC levels in all the

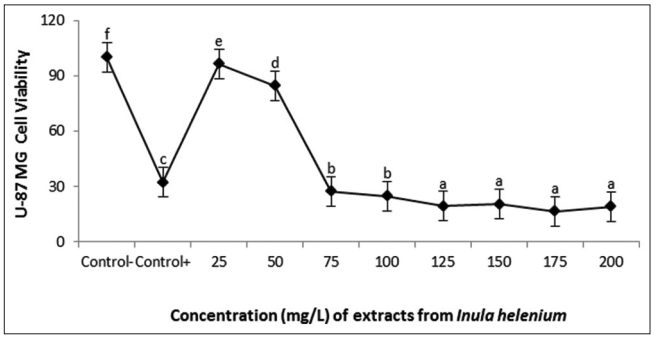

Figure 1: The cells were exposed to the specified concentrations of *Inula helenium* extracts for 48 h, and the viability of the cells was determined by the 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. (Cell viabilities are shown as percentages, and the untreated cells were regarded as 100% viable. Control−: Negative control; cultured with no treatment, Control+: Positive control; mitomycin C (10-7 M). Values are expressed as mean ± standard deviation for five cultures in each group. The bars shown by different letters are different from each other at a level of 0.05)

concentrations were statistically lower than in negative control (untreated cells). On the other hand, all the tested concentrations of *I. helenium* extracts did not lead any alterations in TOS levels on human U-87 MG cell lines [Figure 4].

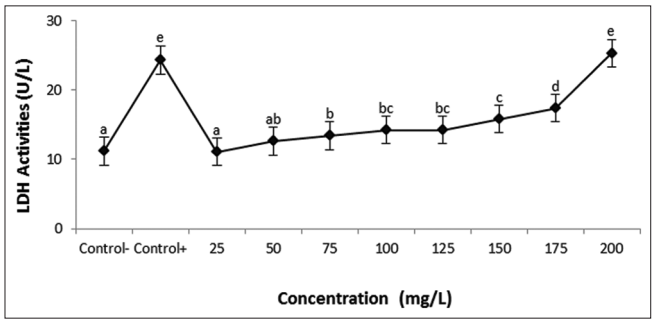

Figure 2: Level of lactate dehydrogenase on human U-87 MG cells maintained for 48 h in the presence of different concentrations of aqueous extracts from *Inula helenium*. (Control–: Negative control; cultured with no treatment, Control+: Positive control; mitomycin C (10-7 M). Values are expressed as mean ± standard deviation for five cultures in each group. The bars shown by different letters are different from each other at a level of 0.05)

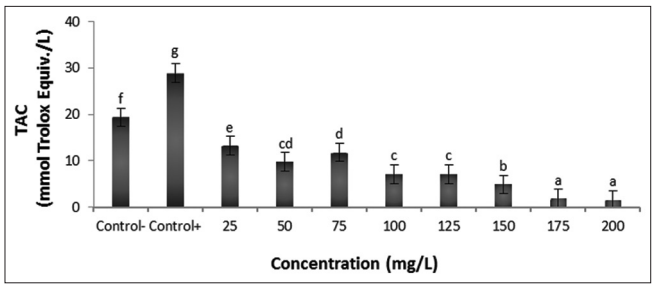

Figure 3: The effects of aqueous extracts from *Inula helenium* on total antioxidant capacity levels on human U-87 MG cells. (Different superscript letters in the same column indicate significant differences. Positive control: Ascorbic acid (10 μM) for total antioxidant capacity analysis. Values are expressed as mean ± standard deviation for five cultures in each group. The bars shown by different letters are different from each other at a level of 0.05)

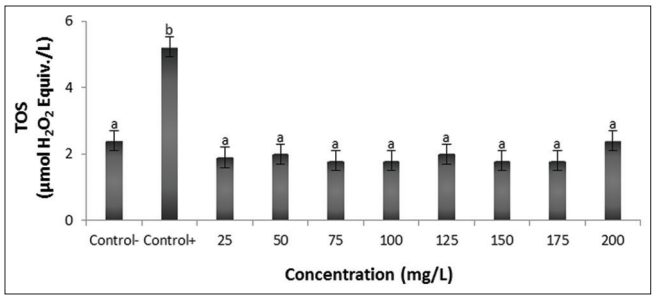

Figure 4: The effects of aqueous extracts from *Inula helenium* on total oxidant status levels on human U-87 MG cells. (Different superscript letters in the same column indicate significant differences. Positive control: Hydrogen peroxide (H_2O_2, 25 μM) for total oxidant status analysis. Values are expressed as mean ± standard deviation for five cultures in each group. The bars shown by different letters are different from each other at a level of 0.05)

DISCUSSION

Inula, from *Asteraceae* family (Compositae), is a large genus which including species commonly used in the folk herbal medicine applications. *I. helenium*, belongs to this genus, is traditionally used to treat a variety of diseases arise from inflammation, bacterial and viral infections, as well as tumors.[11,18] In a recent study, it has been revealed that the root of *I. helenium* possesses therapeutic properties such as antiseptic in exanthema, bacterial and fungal dermatitis; antipruritic in dry patches; to facilitate urinary and digestive functions; to treat symptomatic cough and bronchitis; to treat failure by the dyspeptic hepatobiliary or biliary dyskinesia; and as an adjunct in the fight against hyperglycemia and obesity.[4] Furthermore, *I. helenium* is found in commercial herbal preparations due to its important ethnomedicinal features.[19] These pharmacological properties of *I. helenium* are usually attributed to its present's important sesquiterpene lactones.[12,20-25] In the previous studies, the compounds isolated from root extract of *I. helenium* have been claimed to show cytotoxic and antiproliferative activities against many cancer cell lines. In fact, the constituents isolated from *I. helenium* display strong antiproliferative activities against MK-1, HeLa, and B16F10 cells *in vitro* was reported.[7] Similarly, the sesquiterpene lactones isolated from *I. helenium* were demonstrated to exhibit significant antigrowth activities to the cell lines of U251SP, HLE, and MM1-CB were reported.[26] In a recent study, the extract of the root *I. helenium* was reported to display a highly selective toxicity without being mutagenic toward different tumor cell lines (HT-29, MCF-7, Capan-2, and G1).[27] As far as we know, the aerial part of *I. helenium* L. has been less studied and the purpose of the present study was to evaluate some of its aerial parts' unknown activities. Our results were in accordance with these previous studies that have reported the display of antiproliferative activity in roots of *I. helenium*.

The results of the present study clearly show that the aqueous extracts of *I. helenium* aerial parts were able to trigger cell death even at low concentrations [Figure 1]. Hence, the effect of the extract on cell cycle progression may be due to its possible anticancer bioactive compounds that require isolation and further characterization. When analyzed the correlation between *I. helenium*-mediated loss of cell viability and LDH levels, a significant positive correlation was found between LDH levels and the loss of cell viability [Figures 1 and 2]. It is argued that aqueous extracts are able to exhibit strong cytotoxic activity on U-87 MG cells. Furthermore, the extracts of *I. helenium* did not induce oxidative stress at any concentration tested. The cytotoxic effects of extracts on U-87 MG cells do not seem to be mediated by oxidative stress. Thus, the lack of oxidative stress can be a great advantage toward normal cell for cell death in tumor treatment.

Over the last half-century, there has been a growing interest in the use of medicinal plants as a source of naturally bioactive

compounds for cancer chemoprevention and treatment.[28-31] Currently available data suggest that synergistic combinations of constituents present naturally in plant extracts are presumed to be highly important in the ultimate biological activity, and therefore, whole plant extracts might show greater effects than the individual constituent.[32] Interestingly, these plant extracts can also provide unique therapeutic properties with minimal or without desirable side effects.[33] Accordingly, in the present study, we used *I. helenium* as whole aerial parts for its aqueous extracts. Finally, our findings support the use of *I. helenium* to treat glioma as infusion form in traditional herbal medicine.

CONCLUSION

We provided the evidence that aqueous extracts from *I. helenium* are a potent inhibitor of the human U-87 MG glioma cells. Therefore, *I. helenium* aerial parts may be a valuable candidate source for the development of a new chemotherapeutic drug for brain cancer, and future clinical investigations on this medicinal plant should be encouraged, both *in vitro* and *in vivo*.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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