Makale Turnitin Rapor

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In vitro Cytotoxicity Evaluation of Dandelion Root Ethanol Extract on PANC-1 Cell Line

Abstract

A challenging diagnosis is the outcome of pancreatic cancer's aggressivity and malignant character. This cancer type is identified in the metastatic stage, meaning that patients have incredibly few options for treatment. Despite extensive researches and technological advancements, patients have extremely low survival rates and a declining quality of life due to the side effects of current treatment options. Dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg.) is one of the natural products that extensively is used all over the world and has the potential to be therapeutic against a wide range of diseases. Therefore, the potential of ethanol extract from dandelion root (DRE) as an anticancer agent alterntive was assessed in this study. Wherefore, in this study, the potential of ethanol extract obtained from dandelion root as an anticancer agent alterntive against heavy chemotherapy drugs that reduce the quality of life of patients with pancreatic cancer was evaluated. For this purpose, milk-rich dandelion roots were collected, cut into small pieces, and then extracted using Soxhlet in the presence of 70% ethanol. The cytotoxic effect of the DRE at certain doses (10, 5, and 2.5 mg/mL) was determined by the MTT method for 24, 48, and 72 hours.

Keywords: natural product; anti-cancer; dandelion; cytotoxicity; PANC-1

Introduction

Pancreatic cancer is one of the most aggressive forms of cancer and it is a fatal disease that develops when normal pancreatic cells proliferate and grow out of control. It occurs due to mutations in various cancer-

related genes, including tumor suppressor genes, cell cycle genes, apoptosis, and genome repair genes in germ cells and somatic cells (1, 2). This deadly form of cancer does not show early symptoms and has the potential to rapidly invade surrounding tissues and organs (3). When current treatment strategies are evaluated, surgical intervention is the primary option for treating pancreatic cancer. Although, for patients who are unable to undergo into a surgical intervention due to the common late diagnosis of pancreatic cancer and theirs metastatic ability, chemotherapy, radiotherapy, and immunotherapy constitute other treatment options. Therefore, for patients with pancreatic cancer who have few treatment options and an aggressive progression rate, the alternative treatment approaches are crucial (4, 5).

Due to their chemical diversity and reservoirs of bioactive compounds with therapeutic potential, natural products are part of a research on alternative treatments. To the extent that, according to estimates, 25% of anti-cancer drugs approved between 1981 and 2019 are natural product-related (6-8). As a natural product, the flower, leaf, and root of dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg.), a member of the *Asteraceae* family, are also used in traditional Chinese medicines and are known as a perennial medicinal plant which is widely found all over the world (9-11). Furthermore, several of earlier investigations have demonstrated the anti-tumor potential of different dandelion extracts (12-15).

It has been reported in various studies that ethanol extract has a higher content of bioactive molecules than water extract (16). Although the cytotoxicity of the dandelion water extract on the pancreatic cancer cell line has already been established (11), no research has been done on the cytotoxic impact of the ethanol extract made from the fresh plant root—which contains more bioactive molecules—on the PANC-1 cancer cell line. Therefore, in this study, the cytotoxicity of a 70% ethanol extract obtained from dandelion root against the pancreatic cancer cell line PANC-1 was determined by the MTT method.

Method

Ethanol Extraction of Dandelion Roots

The dandelion plant was collected from the parks and gardens in Konya's Meram district, in May without damaging the roots. The roots were cleaned of soil with dH2O, they were washed, dried, and cut into small pieces of approximately 0.5 mm in size. 25 g of sliced roots were placed in the Soxhlet column and extraction was performed in the presence of 110 mL of 70% ethanol. Then, the solvent was evaporated in a rotary evaporator at 60 RPM and 86 °C.

Cell Culture

In this study, the PANC-1 (human epithelioid carcinoma) cell line was used. To cultivate the cells, a completed DMEM medium with 10% Fetal Bovine Serum and 1% penicillin/streptomycin (100 U/ml penicillin and 100 µg/ml streptomycin) was used for culturing of the cells. Sterile laminar airflow cabinets were utilized for conducting culture processes. The cells were incubated at 37°C with 5% CO_2 in an incubator. The old medium was replaced and new medium was added every 2-3 days until the cells covered the surface. When the cells covered the flask surface by 80–90%, the passage was performed with trypsin-EDTA. An inverted microscope was used to perform growth control and contamination checks on the cells.

Cytotoxicity Analysis

Approximately 10^4 cells in 200 µl of growth media were put into each well of microplate for the MTT assay. The well plate was kept in an incubator at 37°C and 5% CO_2 for 24h. After this time, the old medium was removed, and the cells in the wells were treated with certain concentrations of DRE (10–2.5 mg/mL). Each dose was administered in triplicate. During dosing, care was taken to keep the solvent DMSO ratio constant at 0.1% for each dose application (17). Negative control cells were treated with medium containing DMSO at the same concentration. After application, cells were incubated in a 37°C, 5% CO_2 incubator for 24, 48 and 72 hours. At the end of the time periods, the media was removed and 20 µl of Thiazolyl Blue Tetrazolium Bromide (MTT) solution was added to the wells and incubated for 3 hours at 37°C, 5% CO_2. At the end of the 3 hours, MTT dye was removed from the wells, 200 µl DMSO was added and shaken in an orbital mixer for 1 hour. After incubation, the absorbance of the wells was measured at a wavelength of 570 nm on a microplate reader. Cell viabilities were calculated according to the formula stated below (18).

$$\%\boldsymbol{Viability} = 100 \times \left[\frac{OD(Treated\ cells)}{OD(Untreated\ cells)}\right]$$

Statistical Analysis

Results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. Statistical analyzes obtained in this study were performed using GraphPad Prism version 8.0.2 software (GraphPad Software, Inc., San Diego, California, United States).

Results

In this study, the MTT method was used to evaluate the anticancer activity of DRE against the pancreatic cancer cell line PANC-1 at various doses and exposure times. When DRE was used at a concentration of 10 mg/mL was applied to PANC-1 cells, it inhibited cell growth by 26%, 72% and 84% for 24, 48 and 72 hours (Figure 1 and Figure 2A).

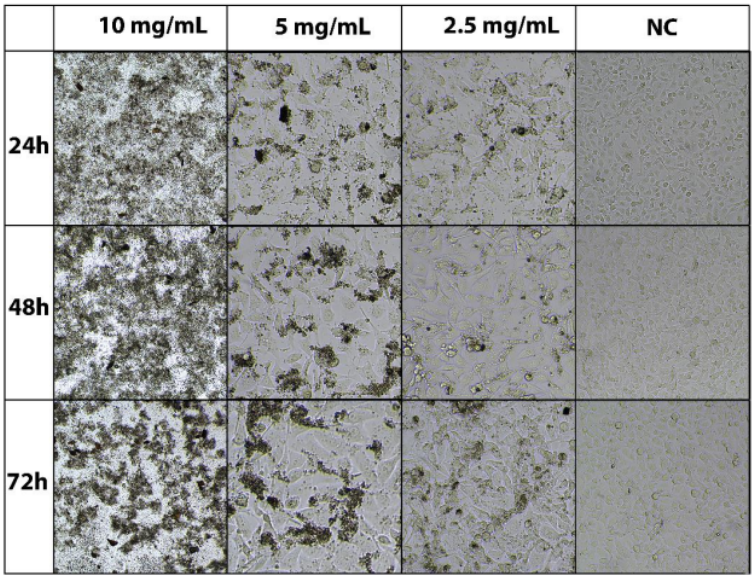

Figure 1. Cell Morphology after treatment with the DRE for 24 h, 48 h, and 72

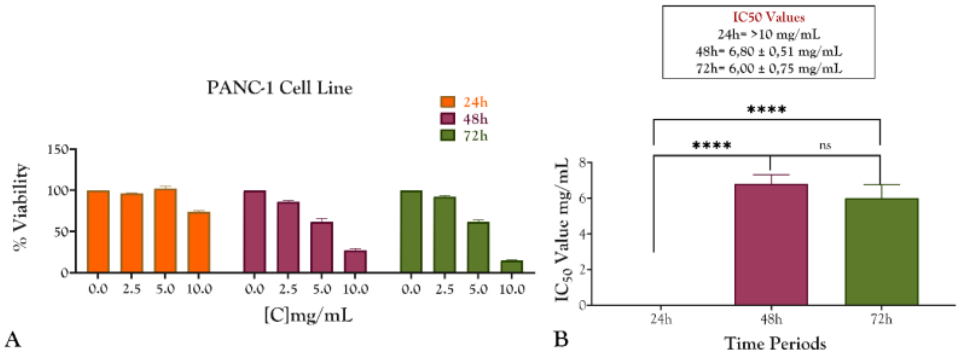

Figure 2. Graph of Cell Viability Percent (A) and IC50 Values (B) after treatment with the DRE for 24 h, 48 h, and 72 h and analysis by MTT (n=3). ($P < 0.05$) represents significant results, ****($P < 0.0001$).

The evaluation was carried out for three separate incubation periods, 24, 48 and 72 hours, and IC50 values for these incubation periods were found to be >10 mg/mL, 6.80 mg/mL and 6 mg/mL ($p<0.001$), respectively (Figure 2B).

Discussion

This study investigated the cytotoxic effects of ethanol extract obtained from Dandelion root on PANC-1, human pancreatic cancer cells. According to the data, when the DRE was applied to PANC-1 cells at a concentration of 10 mg/mL, the highest inhibition rate was prevalented due to the time-dependently; it inhibited the cell growth by 84% for the 72h. When IC50 values were analyzed, they were found to be >10 mg/mL, 6.80 mg/mL, and 6 mg/mL ($p<0.001$), respectively, for 24, 48, and 72h, and the lowest IC50 value was achieved for the treatment for 72 hours. As a result, cytotoxic activity increased with longer exposure periods and higher DRE concentrations. This shows that the DRE has a cytotoxic effect against pancreatic cancer and exhibits anticancer activity. When these findings are contrasted with prior research by Ovadje et al. (2012), they agree with the IC50 values derived from the used dosages of water extract (11). When different literature data are evaluated together with our results, the anticancer effects of extracts made with different solvents acquired from dandelion roots that confirm each other. When different literature data are evaluated collectively with our results, the anticancer effects of extracts made with different solvents was obtained from dandelion roots that confirmed each other. Additionally, the anticancer activity of methanol and water extracts obtained from dandelion root after treatment of different cancer cell lines (HepG2, HCT116, MCF7, SGC7901, BGC823) was reported in two different studies by Rehman et al. (2017) and Zhu et al. (2017) (19, 20). Further studies are needed to analyze the compounds found in dandelion root and evaluate their selective anticancer potential. Although, current results are established from the literature and the data reported in this study reveal the potential of dandelion root extracts as cytotoxic anticancer agents.

Conculsion

This is the first study to show the way that cytotoxic concentration range of the ethanol extract was made from dandelion roots full of latex. The findings presented in here demonstrate that even on highly aggressive pancreatic cancer cell lines, it has cytotoxic effects. Therefore, we suppose that among more research and the ability to do both in vitro and in vivo testing, the bioactive compounds in the content can be successfully isolated for therapeutic use. Furthermore, it's important to remember that every cell type has a unique reaction to various extracts made by using various solvents and that distinct cancer cells and healthy cells may react differently to each extract. For this reason, analyzing data from various cancer cell lines and extracts made using various solvents is crucial to the development of anticancer drugs.

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Conflict of Interest

The authors have no financial or proprietary interests in any material discussed in this article.

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