mutiara
medika
Jurnal Kedokteran dan Kesehatan

Mutiara Medika: Jurnal Kedokteran dan Kesehatan
http://journal.umy.ac.id/index.php/mm

Vol 24 No 1 Page 66-70, January 2024

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

Aliye İrem Aras, Emine Arslan*, Büşra Özçay
Department of Biology, Faculty of Science, Selcuk University, Selcuk-Konya, Turkey

DATE OF ARTICLE:
Received: 27 Dec 2023
Reviewed: 11 Jan 2024
Revised: 25 Jan 2024
Accepted: 31 Jan 2024

***CORRESPONDENCE:**
earslan@selcuk.edu.tr

DOI:
10.18196/mmjkk.v24i1.20917

TYPE OF ARTICLE:
Research

Abstract: A challenging diagnosis is the outcome of pancreatic cancer's aggressivity and malignant character. This type of cancer is mostly detected in the metastatic stage. Current treatment options have various side effects, result in the survival rates and quality of life of patients are extremely decreased. Dandelion (*Taraxacum officinale* (L.) Weber ex F.H. Wigg.) is one of the natural products that has the potential to be therapeutic against a wide range of diseases. This study aimed to evaluate the potential of ethanol extract of dandelion root (DRE) as an alternative anticancer agent. Based on 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 Microtetrazolium (MTT) assay for 24, 48, and 72 hours, and half-maximal inhibitory concentration (IC)50 values for these incubation periods were found to be >10 mg/mL, 6.80 mg/mL and 6 mg/mL ($p<0.001$), respectively. As a result, DRE may reduce cell viability when applied to aggressive pancreatic cancer cells and may have the potential to be an alternative anticancer agent. This potential can be clarified with further studies.

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

INTRODUCTION

Pancreatic cancer is one of the most aggressive forms of cancer. 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 invade surrounding tissues and organs rapidly.[3] When current treatment strategies are evaluated, surgical intervention is the primary option for treating pancreatic cancer. However, for patients who are unable to undergo a surgical intervention due to the common late diagnosis of pancreatic cancer and their 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, 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 research on alternative treatments. To this extent, 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 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 water extract from the dried dandelion root on the pancreatic cancer cell line has already been established,[11] no research has been encountered in the literature about the cytotoxic impact of the ethanol extract made from the fresh dandelion 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.

MATERIAL AND 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 dH_2O, washed, had water removed after washing, 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. Furthermore, 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 to conduct culture processes. The cells were incubated at 37°C with 5% CO_2 in an incubator. The old medium was replaced, and a 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. At the end of this period, the old medium was removed, and the cells in the wells were treated with DRE (10–2.5 mg/mL) at concentrations determined by taking into account previously performed studies.[13,17] 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.[18] Negative control cells were treated with a 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 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, and 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.[19]

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

Statistical Analysis

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

RESULT

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).

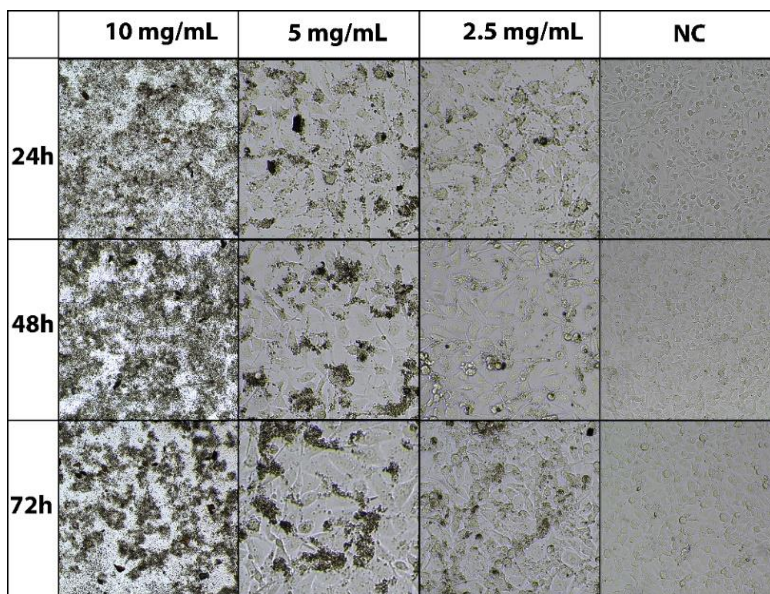

Figure 1. Cell Morphology after treatment with the DRE for 24 h, 48 h, and 72 h

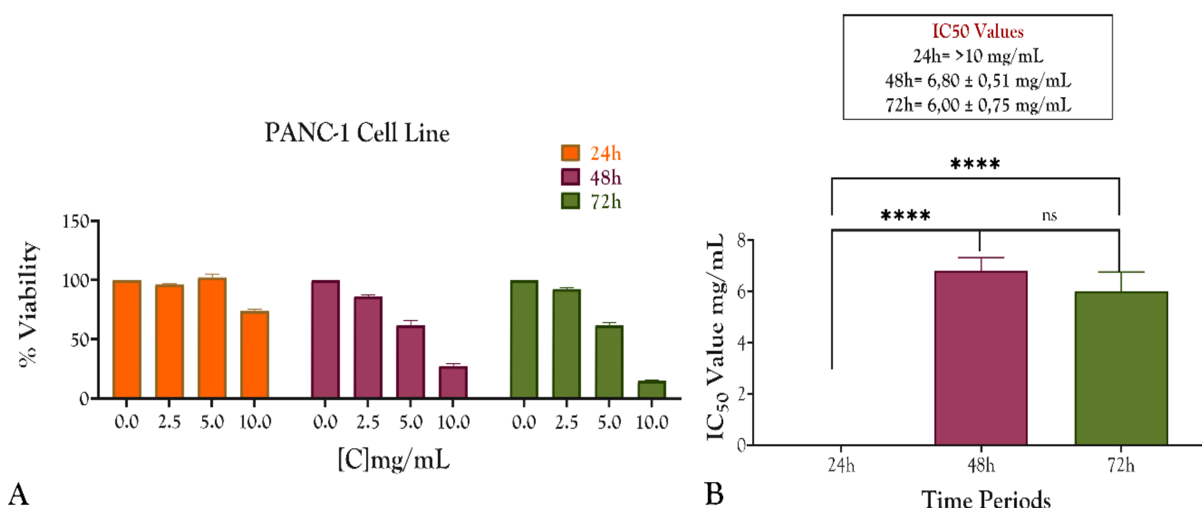

Figure 2. Graph of Cell Viability Percentage (A) and IC_{50} Values (B) after treatment with the DRE for 24 h, 48 h, and 72 h and analysis by MTT (n=3). (P < 0.05) represents significant results, ****(P < 0.0001)

The evaluation was carried out for three separate incubation periods, 24, 48, and 72 hours, and IC_{50} 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. The treatment of PANC-1 cells with 10 mg/mL of DRE produced the maximum inhibition rate, and the lowest IC50 value was reached during the 72-hour treatment period. As a result, cytotoxic activity increased with longer exposure periods and higher DRE concentrations. It 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 dosages of water extract used.[11] When different literature data are evaluated together with our results, the anticancer effects of extracts made with different solvents acquired from dandelion roots confirm each other. When different literature data are evaluated collectively with our results, the anticancer

effects of extracts made with different solvents were obtained from dandelion roots that confirmed each other. When different literature data are evaluated collectively with our results, the anticancer effects of extracts made with different solvents were obtained from dandelion roots that confirmed each other. In one of these investigations, Rehman et al. (2017) documented the efficaciousness of dandelion root methanol extract on HepG2, HCT116, and MCF7 cancer cell lines.[20] In a different study, the anti-cancer properties of dandelion root extract were assessed in gastric cancer cell lines (SGC7901 and BGC823), and the results showed that the extract inhibited the cells' ability to proliferate and migrate.[21] Further studies are needed to analyze the compounds found in dandelion roots and evaluate their selective anticancer potential. However, current results are established from the literature, and the data reported in this study revealed the potential of dandelion root extracts as cytotoxic anticancer agents.

CONCLUSION

The findings on the cytotoxic concentration range of ethanol extract obtained from fresh latex-filled dandelion roots presented here demonstrated that even on highly aggressive pancreatic cancer cell lines, it had cytotoxic effects. These results might support the potential of dandelion root extract as an anticancer agent.

Therefore, we suppose that with 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 is 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. Based on this reason, analyzing data from various cancer cell lines and extracts made using various solvents is crucial to the development of anticancer drugs.

ACKNOWLEDGEMENT

This study was supported financially under a project (Project No: 1919B012206061) from the Scientific and Technological Research Council of Türkiye (TÜBİTAK). The research abstract was also presented orally at the 2nd International Selçuk Health Sciences Congress, Selçuk University/KONYA, and published in the abstract book of the congress.

CONFLICT OF INTEREST

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

REFERENCES

1. Gnoni A, Licchetta A, Scarpa A, Azzariti A, Brunetti AE, Simone G, et al. Carcinogenesis of pancreatic adenocarcinoma: precursor lesions. Int J Mol Sci. 2013;14(10):19731-62. http://dx.doi.org/10.3390/ijms141019731
2. Jamieson NB, Morran DC, Morton JP, Ali A, Dickson EJ, Carter CR, et al. MicroRNA Molecular Profiles Associated with Diagnosis, Clinicopathologic Criteria, and Overall Survival in Patients with Resectable Pancreatic Ductal Adenocarcinoma. Clinical Cancer Research. 2012;18(2):534-45. http://dx.doi.org/10.1158/1078-0432.ccr-11-0679
3. Zhao Z, Liu W. Pancreatic Cancer: A Review of Risk Factors, Diagnosis, and Treatment. Technology in Cancer Research & Treatment. 2020;19:1533033820962117. http://dx.doi.org/10.1177/1533033820962117
4. Duan H, Li L, He S. Advances and Prospects in the Treatment of Pancreatic Cancer. International Journal of Nanomedicine. 2023;18(null):3973-88. http://dx.doi.org/10.2147/ijn.s413496
5. Neoptolemos JP, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future perspectives. Nature Reviews Gastroenterology & Hepatology. 2018;15(6):333-48. http://dx.doi.org/10.1038/s41575-018-0005-x
6. Huang M, Lu J-J, Ding J. Natural Products in Cancer Therapy: Past, Present and Future. Natural Products and Bioprospecting. 2021;11(1):5-13. http://dx.doi.org/10.1007/s13659-020-00293-7
7. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803. http://dx.doi.org/10.1021/acs.jnatprod.9b01285

8. Huang MY, Zhang LL, Ding J, Lu JJ. Anticancer drug discovery from Chinese medicinal herbs. Chin Med. 2018;13:35. http://dx.doi.org/10.1186/s13020-018-0192-y
9. Chen P, Ding S, Yan Z, Liu H, Tu J, Chen Y, et al. Structural Characteristic and In-Vitro Anticancer Activities of Dandelion Leaf Polysaccharides from Pressurized Hot Water Extraction. Nutrients. 2023;15(1):80. http://dx.doi.org/10.3390/nu15010080
10. Koo HN, Hong SH, Song BK, Kim CH, Yoo YH, Kim HM. Taraxacum officinale induces cytotoxicity through TNF-alpha and IL-1alpha secretion in Hep G2 cells. Life Sci. 2004;74(9):1149-57. http://dx.doi.org/10.1016/j.lfs.2003.07.030
11. Ovadje P, Chochkeh M, Akbari-Asl P, Hamm C, Pandey S. Selective Induction of Apoptosis and Autophagy Through Treatment With Dandelion Root Extract in Human Pancreatic Cancer Cells. Pancreas. 2012;41(7):1039-47. http://dx.doi.org/10.1097/mpa.0b013e31824b22a2
12. Li M, Zhang H, Hu X, Liu Y, Liu Y, Song M, et al. Isolation of a New Polysaccharide from Dandelion Leaves and Evaluation of Its Antioxidant, Antibacterial, and Anticancer Activities. Molecules. 2022;27(21):7641. http://dx.doi.org/10.3390/molecules27217641
13. Ovadje P, Ammar S, Guerrero JA, Arnason JT, Pandey S. Dandelion root extract affects colorectal cancer proliferation and survival through the activation of multiple death signalling pathways. Oncotarget. 2016;7(45):73080-100. http://dx.doi.org/10.18632/oncotarget.11485
14. Al-Ezzy RM, Alshanon AF, Khalaf HM. Studying some cytotoxic and cytogenetic potentials of Dandelion methanolic extract on MCF-7 cancer cell line: an in vitro study. Iraqi Journal of Science. 2023;64(3):1160-70. http://dx.doi.org/10.24996/ijs.2023.64.3.12
15. Al-Eisawi Z, Abderrahman SM, Al-khalaf IF, Al-Abbassi R, Bustanji YK. Taraxacum officinale Extracts Exhibit Safe and Selective Anticancer Activity. The Natural Products Journal. 2022;12(1):69-77. http://dx.doi.org/10.2174/2210315510999201109202255
16. Göç Rasgele P, Dönmez M, Karadeniz Ş, Yoldas T, Aydin G, Karagül P, et al. Comparison of Chemical Contents of Extracts in Different Solvents of Propolis Samples Produced in Duzce Province. International Journal of Traditional and Complementary Medicine Research. 2020;1(3):137-46.
17. Rezaie H, Alipanah-Moghadam R, Jeddi F, Clark CCT, Aghamohammadi V, Nemati A. Combined dandelion extract and all-trans retinoic acid induces cytotoxicity in human breast cancer cells. Scientific Reports. 2023;13(1):15074. http://dx.doi.org/10.1038/s41598-023-42177-z
18. Demir S ADE, Aliyazicioğlu Y. In vitro Cytotoxic Effect of Ethanol and Dimethyl Sulfoxide on Various Human Cell Lines. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi. 2020;23(5):1119-24. http://dx.doi.org/10.18016/ksutarimdoga.vi.702702
19. Demir S, Ayazoglu Demir E, Turan İ, Özgen U. Evaluation of Cytotoxic Effect of Onosma armeniacum Extract on Various Cancer Cells. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi. 2021;24(2):252-9. http://dx.doi.org/10.18016/ksutarimdoga.vi.729814
20. Rehman G, Hamayun M, Iqbal A, Khan SA, Khan H, Shehzad A, et al. Effect of Methanolic Extract of Dandelion Roots on Cancer Cell Lines and AMP-Activated Protein Kinase Pathway. Frontiers in Pharmacology. 2017;8. http://dx.doi.org/10.3389/fphar.2017.00875
21. Zhu H, Zhao H, Zhang L, Xu J, Zhu C, Zhao H, et al. Dandelion root extract suppressed gastric cancer cells proliferation and migration through targeting lncRNA-CCAT1. Biomedicine & Pharmacotherapy. 2017;93:1010-7. http://dx.doi.org/10.1016/j.biopha.2017.07.007